Published in final edited form as:

J Neurosci Res. 2018 February ; 96(2): 284–296. doi:10.1002/jnr.24129.

Neuromuscular Adaptability of Male and Female Rats to Muscle Unloading

Michael R. Deschenes^{1,2}, Matthew A. Adan¹, Maria C. Kapral¹, Kaitlin A. Kressin², Colleen M. Leathrum¹, Anna Seo, Shuhan Li, and Ellen C. Schaffrey¹

¹Department of Kinesiology & Health Sciences, The College of William & Mary, Williamsburg, VA 23187-8795 USA

²Program in Neuroscience, The College of William & Mary, Williamsburg, VA 23187-8795 USA

Abstract

Previously, it has been shown that following muscle unloading, males and females experience different maladaptations in neuromuscular function. As a follow-up, the present investigation sought to determine if male and female neuromuscular systems demonstrated similar, or disparate morphological adaptations to muscle unloading. Twenty young adult male, and 20 young adult female rats were randomly assigned to one of two treatment protocols: muscle unloading, or control conditions. Following the 2 week intervention period, immunofluorescent procedures were used to quantify pre- and post-synaptic features of neuromuscular junctions (NMJs), and to assess myofiber profiles (size and fiber type composition) of the soleus, plantaris, and EDL muscles. A 2-way ANOVA with main effects for sex and treatment was then used to identify statistically significant ($P < 0.05$) differences among structural parameters. Analysis of NMJs showed a consistent lack of differences between males and females. Overall, NMJs were also found to be resistant to the effects of unloading. When examining myofiber profiles, however, male myofibers were revealed to be significantly larger than female ones in each of the muscles examined. Unloading resulted in significant myofiber atrophy only in the primarily weight-bearing soleus muscle. Only the EDL showed unloading-induced differences in myofiber type distribution (Type II $\rightarrow$ I). These data indicate that different components of the neuromuscular system (NMJs, myofibers) respond uniquely to unloading, and that sex affects myofiber type profiles, but not NMJs. Moreover, it appears that only muscles that have their habitual activity patterns disturbed by unloading, i.e., the soleus, adapt to that intervention.

Graphical Abstract

Images in the top row show that the morphology of male and female neuromuscular junctions do not differ. Conversely, myofibers shown below demonstrate that male myofibers are larger than female ones (red = Type I, blue = Type IIA, unstained = Type IIX, and green = Type IIB fibers).

Conflict of Interest Statement

The authors have no conflicts of interest to report.

Keywords

synapse; myofiber; neuromuscular junction; sex; unweighting

INTRODUCTION

Muscle unloading has been shown to elicit a number of negative neuromuscular adaptations including diminished muscular strength, and alterations in power and fatigability (Bamman et al., 1998; Berg et al., 1993; Berg and Tesch, 1997; Dudley et al., 1992; Hackney et al., 2011). These functional modifications are associated with muscular atrophy both at the whole muscle and cellular levels (Adams et al., 1994; Deschenes et al., 2012; Widrick et al., 2002), as well as depressed electromyographic (EMG) activity of maximally contracting muscles suggesting reductions of neural drive during maximal efforts (Deschenes et al., 2009; Deschenes et al., 2012). Clearly, both morphological and functional remodeling occurs in response to muscle unloading whether that condition is presented as bed rest, crutches-assisted ambulation, limb immobilization, or the microgravity of spaceflight (Adams, 2002; Berg et al., 1991; Berg et al., 1993; Deschenes et al., 2009; Deschenes et al., 2012; Hortobagyi et al., 2000; Rozier et al., 1979).

Recently, it has been reported that when humans are subjected to muscle unloading, females experience significantly greater decrements in neuromuscular function, i.e., strength, than their male counterparts even when the same model of muscle unloading is imparted, and for the same period of time (Deschenes et al., 2012; Yasuda et al., 2005). This sex-specific response to muscle unloading has stimulated additional interest in identifying the mechanism(s) that may account for the more severe reductions in neuromuscular function evident in females compared to males as a result of muscle unloading. Accordingly, the objective of the present investigation was to assess the morphological adaptations of male and female neuromuscular systems consequent to the same, relatively brief (2 week) period of muscle unloading imparted by the same model (hindlimb suspension). Such sex-specific structural remodeling of the neuromuscular system upon exposure to muscle unloading would provide important insight into the previously observed sex-related disparities in neuromuscular function brought about by unloading.

MATERIALS AND METHODS

Subjects

Twenty young male, and twenty young female Wistar rats were purchased from Charles River Laboratories (Wilmington, MA USA). Upon reaching the age of three months, animals of both sexes were randomly assigned to either control or unloaded treatment groups. This resulted in four treatment groups (male control, male unloaded, female control, female unloaded), each with ten animals. Control rats were housed in pairs in plastic tubs with wood shavings for bedding in which they were free to move about, and were provided water and standard rat chow *ad libitum*. Rats assigned to the unloaded groups were placed in hindlimb suspension chambers (1 rat/chamber) also lined with wood shavings for bedding, with food and water provided *ad libitum*. To minimize weight loss during unloading conditions, food pellets for these animals were ground to smaller pieces with an ice crusher making food easier to handle, thus promoting eating. Regardless of treatment assignment, all rats lived in environmental conditions featuring a 12 h light/dark cycle, a temperature of 21–22°C, with relative air humidity of ~ 50%.

Intervention protocols

The model of hindlimb suspension used to impart a condition of unloading (or unweighting) of the hindlimbs was first described by Morey (1979). In this model, the animal's hindlimbs are elevated just enough to prevent contact with the floor and accordingly, weight bearing, and locomotor activity. This is achieved by securing adhesive strips along two sides of the animal's tail with a clip attached so that the clip can be placed into a swivel device that is positioned above the rat thus enabling it to move in a 360° arc using its forelimbs. Animals assigned to the unloading intervention remained in this suspended position 24 h a day for fourteen consecutive days. The animals assigned to control conditions remained in their housing tubs and were allowed to carry out normal weight bearing and ambulatory activity at will. All procedures used in the treatment and care of animals were approved by the institution's animal care and use committee which operates in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as revised in 2011.

Cytofluorescent staining of neuromuscular junctions (NMJs)

At the conclusion of the 2 week intervention period, animals were euthanized via decapitation, following anesthesia via ketamine/xylazine cocktail injection. Hindlimb muscles were then surgically removed, cleared of fat and connective tissue, quickly frozen at approximate resting length in isopentane chilled with dry ice, and stored at –85° until analysis. To visualize NMJs, 50 µm thick longitudinal sections of the middle one-third of the muscle were obtained at –20°C on a cryostat (Cryocut 1800; Reichert-Jung, NuBloch, 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). Tissue was then incubated overnight at 4°C in supernatant of the primary antibody RT97 (Developmental Studies Hybridoma Bank, University of Iowa, Cat # RT97, RRID: AB_528399), diluted 1:20 in PBS with 1% BSA. The RT97 antibody reacts with non-myelinated segments of pre-synaptic nerve terminals (Anderton et al., 1982). The next day,

sections were washed 4×15 min in PBS with 1% BSA before being incubated for 2 h at room temperature in Alexafluor 488 conjugated secondary immunoglobulin (Molecular Probes, Eugene, OR, Cat#A11001; RRID: AB_2534069) which had been diluted 1:200 in PBS with 1% BSA. Sections were then washed 4×15 min in PBS with 1% BSA before being incubated in a humidified chamber overnight at 4°C in a solution containing rhodamine conjugated α -bungarotoxin (BTX; Molecular Probes, Eugene, OR, Cat# T1175) diluted 1:600 in PBS, along with anti-synaptophysin (MP Biomedicals, Solon, OH, Cat#0810706, RRID: AB_2334188) at a dilution of 1:50. BTX recognizes post-synaptic acetylcholine (ACh) receptors, while anti-synaptophysin reacts with pre-synaptic vesicles containing ACh as synaptophysin is the most abundant protein found in the synaptic vesicular membrane (Kwon and Chapman, 2011). The next day, sections were washed 4×15 min in PBS with 1% BSA before incubating them for 2 h in a humidified chamber at room temperature in AlexaFluor 647 (Molecular Probes, Eugene, OR; RRID: AB_2535813) labeled secondary antibody diluted 1:200 in PBS to illuminate the anti-synaptophysin. Sections were then washed again for 4×15 min before being lightly coated with Pro Long (Molecular Probes, Eugene, OR Cat# P36930) 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 in the dark at -20°C until analysis. An example of this cytofluorescent staining of pre- and post-synaptic components of the NMJ is displayed in Figure 1.

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. (1990) is derived by multiplying the number of branches by the total length of those branches and dividing that figure by 100. Pre-synaptic vesicular staining was assessed as: 1) total perimeter, or the length encompassing the entire vesicular region comprised of stained vesicular clusters and non-stained regions interspersed within those clusters, 2) stained perimeter, or the composite length of tracings around individual clusters of vesicles, 3) total area, which includes stained vesicles along with non-stained regions interspersed among vesicle clusters, 4) stained area, or the cumulative areas occupied by ACh vesicular clusters, and 5) dispersion of vesicles, which was assessed by dividing the vesicular stained area by its total area and multiplying by 100. Post-synaptic 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, as well as by quantifying the percentage of the area of post-synaptic staining of receptors that was overlapped with staining of pre-synaptic ACh vesicles. Finally, to approximate the number of ACh containing vesicles supported by a given length of pre-synaptic nerve terminal

branch length, the stained area of occupied by vesicles was divided by the total length of branching for that NMJ. Figure 2 demonstrates how these measurements were acquired.

Immunofluorescent staining of myofibers

To determine myofiber profiles, 10 μm thick transverse sections were taken from the midbelly of the muscle using a cryostat set at -20°C . Once placed on a microscope slide, sections were rinsed in PBS with 1% BSA for 5 min. All primary antibodies used to assess myofiber type were obtained from the Developmental Hybridoma Bank at the University of Iowa. These immunogens were initially isolated by Dr. Stefano Schiaffino (Smerdu et al., 1994), and donated to the Hybridoma Bank. The antibody BA-D5 (Cat#BA-D5, RRID AB_2235587), diluted to a concentration of 1:10 in PBS with 1% BSA was used to identify Type I myofibers, while SC-71 (Cat# SC-71, RRID AB_2147165) used at a concentration of 1:1 reacted specifically with Type IIA myofibers, and F3 primary antibody also diluted to a concentration of 1:1 visualized Type IIB myofibers (Cat# BF-F3, RRID; AB_2266724). Type IIX myofibers were identified by their lack of immunofluorescence (although still visible). After adding properly diluted primary antibodies, muscle sections were incubated in humidified chambers at 37°C for 1 hr. Following this, sections were rinsed three times for 5 min each in PBS with 1% BSA and then incubated with fluorescently labelled secondary antibodies purchased from Molecular Probes (Eugene, OR USA) at a concentration of 1:500 in PBS containing 5% goat serum. The secondary antibodies were conjugated to Alexafluor 555 (Cat# A21147, RRID; AB_2535783) to identify Type I myofibers, Alexafluor 350 to visualize Type IIA myofibers (Cat# A21120, RRID; AB_2535763), and Alexafluor 488 (Cat# A21042, RRID; AB_2535711) to detect Type IIB myofibers (see Figure 3). Following incubation for 30 min at 37°C in humidified chambers, sections were rinsed three times, 5 min each, in PBS with 1% BSA before receiving a 3 min rinse in deionized water. Excess water was gently blotted off and Prolong Gold (Molecular Probes, Eugene, OR USA, Cat# P36930) was applied to muscle sections before applying cover slips and storing slides at -20°C in the dark.

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, and store, images of NMJs. Using a 100x oil immersion objective, it was initially established that the entire NMJ was within the longitudinal borders of the myofiber and that damage to the structure had not occurred during sectioning. A detailed image of the entire NMJ was constructed from a z-series of scans taken at 0.5 μm thick increments. 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). In each muscle, 10–12 NMJs were quantified and measurements were averaged to represent NMJ morphology within that muscle.

In the quantification of myofiber profiles, an Olympus BX41 microscope equipped with fluorescence capacity (X-Cite, Excelitas Technologies, Ottawa, Canada) was used in conjunction with Infinity Analyze software (Lumenera Corporation, Ottawa, Canada). A random sample of 125–150 myofibers from each muscle was analyzed to determine average

myofiber size (cross-sectional area), fiber type composition (% of each fiber type analyzed for that muscle), as well as percentage of total myofiber area measured that was occupied by each individual fiber type.

Statistical analysis

All results are reported as means $\pm$ SE. Pre- to post-intervention body mass data were compared with 2-tailed, dependent t-tests. All other variables were compared with a two way ANOVA (main effects of sex and treatment) performed on each NMJ variable of interest. In the event of a significant F ratio for interaction, a Fisher Protected Least Squared Difference (PLSD) post-hoc analysis procedure was conducted to identify significant pair-wise differences. No such post-hoc test was needed for significant main effects as only two levels, i.e., control/unloaded, or male/female, were present for each main effect. In all cases significance was set at $P = 0.05$. A sample size of 10/group was selected in the present investigation as numerous other studies from our lab have shown this to be effective in identifying significant between group differences in many of the same variables examined in the current study. Moreover, a power analysis based on effect size indicates that for most of the variables studied here, the desired power of 0.8 is achieved with that sample size (Lockhart, 1998). In all cases, normality of data distribution was confirmed with the Shapiro-Wilk test.

RESULTS

For the present investigation, the soleus, plantaris, and EDL muscles were studied due to their differing primary functions, and myofiber type compositions. The ankle extending soleus is comprised of 84% Type I (slow-twitch) fibers and functions as the primary postural muscle, and participates in locomotor activity (Armstrong and Laughlin, 1985a, 1985b; Delp and Duan, 1996). The plantaris is also an ankle extensor, but is made up predominantly (94%) of Type II (fast-twitch) myofibers and principally serves in locomotor activity, with little participation in maintaining posture (Armstrong and Laughlin, 1985a, 1985b; Delp & Duan, 1996; Laughlin et. al., 1984). The EDL, while also mainly a fast-twitch muscle (96% Type II), functions as an ankle flexor, and plays little role in posture and only a limited one during locomotor activity (Armstrong and Laughlin, 1985; Delp and Duan, 1996; Rasmussen et. al., 1978).

Body mass

At the onset of the intervention period, statistical analysis of body mass data disclosed a main effect for sex showing that male rats were significantly heavier than female rats ($n = 20$ /group; $F_{(1, 38)} = 1817.9$; $P = 0.0001$). At the conclusion of the 2 week intervention period, ANOVA results indicated that male rats were still heavier than female ones indicating a main effect for sex ($n = 20$ /group; $F_{(1, 38)} = 502.4$; $P = 0.0001$), while at this time period, a main effect for treatment ($n = 20$ /group; $F_{(1, 18)} = 24.1$; $P = 0.0001$) was also noted (control > unloaded) which was not detected at the pre-intervention stage. Dependent t-tests (pre- to post-intervention) revealed, however, that hindlimb suspension resulted in a significant decline in body mass among male ($n = 10$; $t = 2.64$; $P = 0.027$), but not female rats ($n = 10$; $t = 0.88$; $P = 0.399$). Moreover, in both male ($n=10$; $t = 4.19$; $P = 0.002$) and

female controls ($n = 10$; $t = 2.24$; $P = 0.05$) a significant increase in body mass was accrued during the intervention period. All body mass data are displayed in Table 1.

NMJ morphology

Soleus—Among the numerous morphological variables examined in the NMJs of this mainly slow-twitch, postural muscle, the only significant finding was that of a main effect for treatment showing that nerve terminal branch number was higher among unloaded rats than in control animals ($n = 20$ /group; $F_{(1, 38)} = 6.25$; $P = 0.017$). In no other parameter of interest, either in the pre- or post-synaptic segments of the NMJ, were significant differences noted either for sex, treatment assignment, or their interaction, in the soleus. Data concerning the effects of sex and unloading in soleus muscle NMJs are presented in Table 2.

Plantaris—In this primarily fast-twitch ankle extensor muscle, again only a single variable of interest exhibited a significant difference, namely, pre-synaptic area of vesicle staining. While the original ANOVA procedure showed a main effect indicating that unloaded plantaris muscles featured a greater number of stained vesicles per synapse ($n = 18$ /group; $F_{(1, 16)} = 4.87$; $P = 0.034$). All findings regarding the effects of sex and unloading on NMJs of the plantaris muscle can be found in Table 3.

EDL—Like the plantaris, the EDL is considered a fast-twitch (Type II) muscle, but unlike the plantaris, this anterior leg muscle functions as an ankle flexor. Although there was a paucity of significant differences in NMJ structure revealed, the EDL did feature the greatest number of synaptic differences among the three muscles examined. Indeed, four of the morphological parameters assessed were determined to have significant differences as detected by ANOVA results. This, compared to a single – of the seventeen quantified – variable demonstrating a significant difference in the NMJs of soleus and plantaris muscles. More specifically, in assessing total nerve terminal branch length in EDL synapses, a significant main effect of sex ($n = 20$ /group; $F_{(1, 18)} = 6.41$; $P = 0.015$) was noted (male > female), as well as a significant interactive effect between sex and unloading for that variable ($n = 10$ /group; $F_{(3, 36)} = 5.91$; $P = 0.02$). Post-hoc analysis showed that the total length of pre-synaptic nerve terminal branching was significantly more impressive in male control rats than among the three other treatment groups ($n = 10$ /group; $F_{(3, 36)} = 4.5$) including male HS ($P = 0.017$), female control ($P = 0.001$), and female unloaded animals ($P = 0.014$). In addition, average branch length was found to display a significant interactive effect between treatment group and sex ($n = 10$ /group; $F_{(3, 36)} = 4.47$; $P = 0.041$). Post hoc results indicated that mean branch length was greater in male control NMJs, than in female control synapses ($n = 10$ /group; $F_{(1, 18)} = 1072$; $P = 0.044$). It was also noted that nerve terminal branching complexity displayed a significant main effect for sex indicating enhanced branching complexity in male compared to female synapses ($n = 20$ /group; $F_{(1, 38)} = 3.91$; $P = 0.05$).

Along with those gender-specific differences in nerve terminal branching, ANOVA results identified a significant main effect for treatment ($n = 20$ /group; $F_{(1, 18)} = 4.71$; $P = 0.036$) when examining pre- to post-synaptic coupling. More specifically, it was ascertained that there was a greater degree of overlap of pre-synaptic ACh containing vesicles with post-

synaptic ACh receptors in unloaded rats relative to control animals. All data regarding NMJs of EDL muscles are reported in Table 4.

Myofiber profiles

Soleus—In examining myofiber profiles of our muscles of interest, the focus began with fiber size, or cross-sectional area. Initial ANOVA results on myofiber size did not distinguish among individual fiber types and instead were collapsed across fiber type to examine main effects of sex and treatment, along with interaction among those two factors. Statistical analysis revealed a significant main effect for both sex ($n = 20/\text{group}$; $F_{(1, 18)} = 6.30$; $P = 0.016$) whereby male myofibers were larger than female ones, and treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 17.23$; $P = 0.0002$) verifying that unloading induced a significant decrease in myofiber size; no significant interactive effect was observed. To evaluate the effects of unloading on myofibers in more detail, statistical analysis was also conducted on individual myofiber types. In the predominant Type I fibers of the soleus, main effects for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 6.17$; $P = 0.017$) and treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 7.18$; $P = 0.011$) were identified, (Male > Female, and Control > Unloaded, respectively). No significant interaction between sex and treatment was detected.

The initial ANOVA analysis for Type IIA muscle fibers evinced a significant effect for treatment indicating that unloading resulted in atrophy ($n = 20/\text{group}$; $F_{(1, 18)} = 10.04$; $P = 0.006$), but no effect for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.69$; $P = 0.41$), or interaction between treatment and sex was detected ($n = 10/\text{group}$; $F_{(3, 36)} = 0.106$; $P = 0.748$).

With respect to myofiber type composition of the soleus and its relationship with sex and unloading, it was discovered that the high percentage of Type I fibers expressed in the soleus (>95%) was consistent across sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.585$; $P = 0.449$) and treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.030$; $P = 0.863$), with no interaction between those variables ($n = 10/\text{group}$; $F_{(3, 36)} = 1.251$; $P = 0.270$). The percentage of various fiber types within a muscle was then combined with mean size to determine relative contribution of a fiber type to the overall cross-sectional area of the segment of muscle being examined. This variable of percentage of area occupied by a fiber type was found not to be affected by either sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.470$; $P = 0.497$), or treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.007$; $P = 0.932$) or their interaction ($n = 10/\text{group}$; $F_{(3, 36)} = 1.542$; $P = 0.222$) in the Type I myofibers of the soleus. The far more sparse (only 19 animals of the total of 40 expressed this fiber type in the soleus) type II fibers of the soleus failed to exhibit significant effects for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 1.217$; $P = 0.287$), treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.078$; $P = 0.784$), or interaction ($n = 10/\text{group}$; $F_{(3, 36)} = 0.890$; $P = 0.360$). All results from the statistical analysis of soleus muscles are located in Table 5.

Plantaris—When assessing the effects of sex and unloading on myofiber size in the plantaris, we again began with examining fiber size irrespective of fiber type. With fiber types collapsed together, initial ANOVA results showed a significant main effect of sex ($n = 18/\text{group}$; $F_{(1, 16)} = 12.074$; $P = 0.002$) indicating that the fibers of males were larger than those of females. The ANOVA results showed no significant effect for treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.001$; $P = 0.989$).

group; $F_{(1, 16)} = 0.549$; $P = 0.464$) or interaction between sex and treatment ($n = 9/\text{group}$; $F_{(3, 32)} = 1.967$; $P = 0.170$).

We then examined each individual myofiber type comprising the mainly fast-twitch (Type II) plantaris beginning with Type I fibers. The results revealed a significant main effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 8.588$; $P = 0.006$) showing that these oxidative myofibers were larger in males than females. But ANOVA results failed to detect a significant effect of treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.827$; $P = 0.371$), or interaction ($n = 9/\text{group}$; $F_{(3, 32)} = 0.702$; $P = 0.408$).

Among Type IIA myofibers of the plantaris, ANOVA results again showed a significant main effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 17.993$; $P = 0.0002$) indicating larger Type IIA fibers in male plantaris muscles than in female ones. Again, it was revealed that there was no main effect for treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.408$; $P = 0.527$), or interaction between sex and treatment ($n = 9/\text{group}$; $F_{(3, 32)} = 1.534$; $P = 0.224$).

When the statistical analysis for Type IIX myofibers was carried out, it was found that, as with Type IIA fibers, a significant main effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 18.594$; $P = 0.0001$) was present; again male fibers being larger than female ones. It was also found that no effect for treatment occurred ($n = 18/\text{group}$; $F_{(1, 16)} = 0.436$; $P = 0.514$), or interaction between sex and treatment ($n = 9/\text{group}$; $F_{(3, 32)} = 1.398$; $P = 0.245$).

Finally, statistical treatment for the cross-sectional area of Type IIB fibers again revealed a main effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 13.547$; $P = 0.0009$) showing that male myofibers were larger than those observed in females. ANOVA results from the analysis of Type IIB myofibers failed to display a significant effect for treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 1.031$; $P = 0.317$), or for the interaction between sex and treatment ($n = 9/\text{group}$; $F_{(3, 32)} = 1.987$; $P = 0.168$).

With respect to fiber type distribution of the plantaris, neither sex ($n = 18/\text{group}$; $F_{(1, 16)} = 0.118$; $P = 0.733$), nor treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.070$; $P = 0.792$), nor their interaction ($n = 9/\text{group}$; $F_{(3, 32)} = 1.463$; $P = 0.235$) affected Type I fiber type distribution in the plantaris. It was also determined that in contrast to the soleus, it was Type II (fast-twitch) fibers that dominated the composition of the plantaris accounting for at least 85% of all fibers regardless of sex, or treatment group assignment. More specifically, ANOVA results of Type IIA muscle fibers showed no significant main effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 1.814$; $P = 0.187$), or treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.028$; $P = 0.868$), or for the interaction between those two main effects ($n = 9/\text{group}$; $F_{(3, 32)} = 1.226$; $P = 0.276$). In examining the percentage of Type IIX fibers expressed in the plantaris, ANOVA results failed to display a main effect for either sex ($n = 18/\text{group}$; $F_{(1, 16)} = 1.626$; $P = 0.211$), or treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.732$; $P = 0.398$), or for the interaction between those two main effects ($n = 9/\text{group}$; $F_{(3, 32)} = 0.627$; $P = 0.434$). Similarly, when examining the relative contribution of Type IIB fibers in the plantaris, no significant main effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 1.067$; $P = 0.309$), or treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.127$; $P = 0.723$), or for their interaction ($n = 9/\text{group}$; $F_{(3, 32)} = 0.674$; $P = 0.417$) was discerned by the ANOVA analysis.

As might be expected in view of fiber type composition findings, the muscle area quantified that was occupied by Type I (and all other fiber types) remained unaffected by sex ($n = 18/\text{group}$; $F_{(1, 16)} = 0.00005$; $P = 0.994$), treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.052$; $P = 0.820$), or the interaction of those two main factors ($n = 9/\text{group}$; $F_{(3, 32)} = 1.782$; $P = 0.191$). Again, it was Type II fibers that occupied the vast majority of muscle area quantified, ranging from 87% to 92% among the four treatment groups with no differences detected among those groups. More specifically, the percentage of area occupied by Type IIA myofibers was not significantly affected by sex ($n = 18/\text{group}$; $F_{(1, 16)} = 2.545$; $P = 0.120$), or treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.005$; $P = 0.945$), or by the interaction of sex and treatment ($n = 9/\text{group}$; $F_{(3, 32)} = 0.208$; $P = 0.651$). Likewise, ANOVA results from analysis of relative proportion of area occupied by Type IIX muscle fibers failed to show a significant effect for sex ($n = 18/\text{group}$; $F_{(1, 16)} = 1.557$; $P = 0.221$), treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.423$; $P = 0.520$), or the interaction of those two main factors ($n = 9/\text{group}$; $F_{(3, 32)} = 0.068$; $P = 0.796$). Finally, the percentage of plantaris muscle cross-sectional area occupied by Type IIB fibers was not affected by sex ($n = 18/\text{group}$; $F_{(1, 16)} = 0.775$; $P = 0.385$), treatment ($n = 18/\text{group}$; $F_{(1, 16)} = 0.126$; $P = 0.724$), or interaction between sex and treatment conditions ($n = 9/\text{group}$; $F_{(3, 32)} = 0.357$; $P = 0.554$). All findings provided by our analysis of plantaris muscles are displayed in Table 6.

EDL—When determining the effects of sex and unloading on this mainly fast-twitch ankle flexing muscle, the initial ANOVA analysis revealed that with fiber types pooled together, a significant effect of sex ($n = 20/\text{group}$; $F_{(1, 18)} = 12.280$; $P = 0.001$) was established underscoring that the myofibers of male EDL muscles were larger than those of female EDL muscles. In contrast, no effect of treatment was identified ($n = 20/\text{group}$; $F_{(1, 18)} = 1.025$; $P = 0.318$). Also, no significant interaction between sex and treatment was detected ($n = 10/\text{group}$; $F_{(3, 36)} = 0.361$; $P = 0.552$).

When quantifying the size of Type I fibers exclusively, again only the effect of sex was found to be significant ($n = 20/\text{group}$; $F_{(1, 18)} = 7.917$; $P = 0.007$) and showed that male myofibers were larger than female ones. ANOVA results also indicated an absence for a main effect for treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 1.584$; $P = 0.216$), as well as for the interaction between the two main effects ($n = 10/\text{group}$; $F_{(3, 36)} = 1.109$; $P = 0.299$).

Analysis of Type IIA myofibers of the EDL again showed that male myofibers were significantly larger than those of females ($n = 20/\text{group}$; $F_{(1, 18)} = 0.037$; $P = 0.847$), and that neither treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 1.025$; $P = 0.318$), nor the interaction between sex and treatment significantly modified fiber cross-sectional area ($n = 10/\text{group}$; $F_{(3, 36)} = 0.799$; $P = 0.377$).

The assessment of Type IIX myofibers in EDL muscles showed that once again, the only significant effect was for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 18.369$; $P = 0.0001$). And as before, myofibers of male rats were larger than female ones. In contrast, no significance was observed for treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.048$; $P = 0.827$), or for the interaction between sex and treatment ($n = 10/\text{group}$; $F_{(3, 36)} = 0.491$; $P = 0.488$).

Results from the ANOVA performed on Type IIB fibers of EDL muscles again revealed a significant main effect for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 13.500$; $P = 0.0008$), without significance for treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.407$; $P = 0.527$), or interaction between sex and treatment ($n = 10/\text{group}$; $F_{(3, 36)} = 0.054$; $P = 0.817$). As observed previously, the effect of sex determined that male Type IIB fibers were significantly larger than IIB fibers in females.

Assessment of myofiber type composition of EDL muscles detected a significant treatment effect ($n = 20/\text{group}$; $F_{(1, 18)} = 10.472$; $P = 0.002$) indicating that unloaded EDL muscles expressed a greater percentage of Type I fibers than control animals. No significant effect for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.562$; $P = 0.458$), or interaction between sex and treatment ($n = 10/\text{group}$; $F_{(3, 36)} = 1.139$; $P = 0.293$) was uncovered by ANOVA. In examining the ANOVA results for the percentage of Type IIA myofibers, it was determined that neither sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.671$; $P = 0.417$), nor treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 1.750$; $P = 0.194$) displayed a significant effect. Similarly, the interaction between those two main effects also did not yield a significant finding ($n = 10/\text{group}$; $F_{(3, 36)} = 0.007$; $P = 0.931$). And in a similar manner, Type IIX myofibers also did not display a significant effect for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 1.314$; $P = 0.259$), or treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.004$; $P = 0.950$). Further, the interaction between the main effects of sex and treatment was found not to be statistically significant ($n = 10/\text{group}$; $F_{(3, 36)} = 0.051$; $P = 0.822$). The percentage of Type IIB fibers occupying the EDL was also determined to yield no differences between males and females ($n = 20/\text{group}$; $F_{(1, 18)} = 1.485$; $P = 0.230$), or as a result of treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 2.078$; $P = 0.158$). Moreover, no significant interaction between sex and treatment was identified ($n = 10/\text{group}$; $F_{(3, 36)} = 0.002$; $P = 0.967$).

When quantifying relative area occupied by individual fiber types in the EDL and how sex and treatment impacted that variable, results closely mimicked what was established for fiber type distribution. That is, only Type I myofibers exhibited sensitivity, and then only to treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 9.438$; $P = 0.004$), with analysis exhibiting that the relative area occupied by Type I myofibers in the EDL was significantly higher among unloaded muscles compared to controls. However, ANOVA results examining the relative areas occupied by Type IIA fibers in the EDL, showed no significant effect for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.428$; $P = 0.517$), treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 2.444$; $P = 0.126$), or interaction between those two variables ($n = 10/\text{group}$; $F_{(3, 36)} = 0.022$; $P = 0.882$). In examining the area occupied by Type IIX fibers in the EDL, once again ANOVA results indicated that there were no significant effects for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 1.426$; $P = 0.240$), treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 0.126$; $P = 0.724$), or interaction between those two variables ($n = 10/\text{group}$; $F_{(3, 36)} = 0.009$; $P = 0.925$). Finally, ANOVA results from the analysis of area occupied by Type IIB failed to identify a main effect for sex ($n = 20/\text{group}$; $F_{(1, 18)} = 0.926$; $P = 0.342$), treatment ($n = 20/\text{group}$; $F_{(1, 18)} = 1.486$; $P = 0.230$), or interaction between those two variables ($n = 10/\text{group}$; $F_{(3, 36)} = 0.049$; $P = 0.826$). Data concerning the effects of sex and unloading on EDL muscles can be seen in Table 7.

DISCUSSION

Recently, it has been reported that when men and women are subjected to the same period of muscle unloading, women lose muscular strength to a significantly greater degree than men (Deschenes et. al., 2012; Yasuda et. al., 2005). However, those investigations featured the assessment of neuromuscular function under *in vivo* conditions among fully conscious men and women. Accordingly, it could not be ruled out with certainty that differences in motivation during maximal voluntary muscular contractions might have contributed to sex-specific decrements in force production resulting from muscle unloading. Thus, it was determined that to better understand potential mechanisms accounting for gender-specific detrimental adaptations to muscle unloading, a small animal model employing muscle unloading would be useful. Such a model would enable the quantification of morphological adaptations, at the cellular level, of the neuromuscular system in male and female rats exposed to the same model of unloading – hindlimb suspension – for the same two week intervention period. It was also concluded that not only would male and female rats need to be age-matched, but that they would also need to be young rats so that the effects of unloading on the neuromuscular system would not be confounded by the well-established gradual decay of the neuromuscular system during the onset of senescence (Bano et. al., 2017; Gonzalez-Freire et. al., 2014; Vetrano et. al., 2014).

The design employed in the current investigation would provide, then, insight into two vital factors influencing the morphology of the neuromuscular system; sex and changes in habitual use patterns, in this case, unloading. Importantly, in pursuing our research question, the fact that a small animal model was utilized enabled us to examine the two critical components of the neuromuscular system. That is, quantification of the NMJ that delivers essential electrical impulses to the myofibers, along with the myofibers themselves which contain the contractile machinery that is responsible for muscle action and force production. Consistent with the biological sequence of events leading to muscle contraction, we first examined the morphology of the impulse transducing NMJs before quantifying the size and fiber type distribution of the myofibers generating muscular force. It is also important to note that the muscles examined in this investigation, i.e. the soleus, plantaris, and EDL, were selected based upon each's unique characteristics in function, and fiber type composition.

Since the soleus is the principal postural muscle in the rat (Armstrong and Laughlin, 1985a, 1985b), it was expected that of the three muscles examined, it would be the one that would be most adversely affected by the hindlimb suspension model of muscle unloading used. The data gleaned from NMJ analysis initially informed us that no significant sex-related differences in NMJ structure were identified among the seventeen pre- and post-synaptic variables quantified. This may be considered surprising given the close relationship between NMJ size and myofiber size previously documented (Balice-Gordon and Lichtman, 1990; Balice-Gordon et. al., 1990). Here, despite the fact that the myofibers of male rats were found to be significantly larger than female ones, the NMJs of male and female soleus myofibers showed no hint of size differences, nor any dissimilarities in the other morphological parameters examined such as pre- to post-synaptic coupling, or pre-synaptic vesicles per unit length of nerve terminal branching. The NMJs of soleus muscles did, however, exhibit one main effect in response to the treatment condition to which rats were

exposed. That is, it was revealed that nerve terminal branch number was significantly greater in unloaded soleus muscles compared to those assigned to control conditions. In reality, it is unlikely that this adaptation would directly result in alterations in neuromuscular function given that no matching post-synaptic modification was found to have occurred allowing for changed synaptic transmission.

Despite the resistance of NMJs to demonstrate gender-specific features of NMJ structure, or synaptic modifications to unloading, the myofibers examined in the same muscles evinced a number of sex-specific characteristics and significant adaptations to unloading. For example, as noted earlier (Fox et. al., 2003; Frontera et. al., 2000; Mierzejewska-Krzyzowska, 2011; Mierzejewska-Krzyzowska, 2012), our male rats displayed significantly larger myofiber cross-sectional areas than female animals, and both sexes experienced significant, and similar, atrophy as a result of unloading. Also, as previously verified (Edgerton et. al., 1995), it was Type II fibers that underwent the greatest amount of unloading-induced atrophy, despite the fact that Type I fibers are the predominant fiber type in the soleus (Delp and Duan, 1996). This was found to be true both in male and female rats. It was also of interest to note that although Type I fibers have the highest duty cycle indicating the highest amount of recruitment, within any single soleus muscle it was size and not normal patterns of activity of myofibers that determined the extent of unloading-induced atrophy. But despite the fact that male myofibers were significantly larger than female ones, neither sex was more vulnerable to unloading-induced atrophy than the other as both sexes showed similar rates of atrophy as a result of unloading.

Somewhat surprising was our finding that muscle unloading had no impact on the fiber type composition of the soleus as it had been reported earlier that this intervention led to an increase in the relative percentage of Type II fibers (Templeton et. al., 1984; Tsika et. al., 1987). Perhaps it was the relatively brief period of unloading – two weeks - that was used in the current study compared to the 4–6 week periods used in those earlier studies that explains this difference in unloading-induced fiber type conversion.

Our next challenge was to reveal neuromuscular adaptations in another ankle extensor, the plantaris, which unlike the soleus mainly functions as a locomotor muscle and does not appreciably participate in posture (Armstrong and Laughlin, 1985a, 1985b). In this mainly fast-twitch muscle the effects of sex and muscle unloading on the NMJ were largely in line with those assessed in the principally slow-twitch soleus. That is, of the seventeen parameters of the NMJ examined, not a single one showed a significant difference between males and females, again uncoupling the relationship between the size of myofibers and the size of the synapses that stimulate them. (Shortly it will be shown that fiber size differed between male and female plantaris muscles.) And again similar to findings derived from the soleus, a single parameter – pre-synaptic area of vesicle staining – was found to respond to muscle unloading by increasing the number of vesicles, although this adaptation occurred exclusively among female rats. As with the soleus, since no related post-synaptic adaptation was detected such as elevated capacity for ACh binding, it is difficult to postulate how neuromuscular transmission might have been altered.

In ascertaining myofiber profiles of plantaris muscles, the myofibers of the male muscles were found to be larger than those measured in female plantaris muscles. This result suggests that male plantaris myofibers experienced more pronounced atrophy than female ones as a result of unloading. Unfortunately, due to the research design used, assessment of fiber size prior to unloading was not possible thus precluding direct measurement of pre- to post-unloading alterations in fiber size in male compared to female animals. When assessing the effects of sex and muscle unloading on percent fiber type composition, and relative area of cross-sectional area occupied by individual fiber types, it was discerned that neither of these variables of myofiber profile individually demonstrated significant effects for sex or unloading, nor a significant interaction between them. This is inconsistent with previous studies indicating that even relatively brief periods, i.e., 15 days, of unloading elicit increases in the relative expression of the Type IIB isoform of myosin heavy chain in plantaris muscles (Jee et. al., 2014).

Much like the plantaris, in the similarly fast-twitch EDL muscle, NMJs were consistently resistant to the effects of sex and unloading, although this muscle displayed the greatest number of synaptic parameters affected – four – by those two independent variables. Again, these measures were pre-synaptic in nature, with nerve terminal branching being longer and more complex in architecture among male rats, which, in turn, decreased the degree of overlap between ACh vesicles and receptors.

As with synaptic measures, myofiber profiles of EDL muscles resembled those of plantaris muscles in that the EDL fibers of male rats were larger than female EDL myofibers, regardless of which treatment group animals had been assigned. Also similar to what was established in plantaris myofibers, unloading failed to induce significant atrophy of EDL myofibers. This supports earlier data indicating that even four weeks of unloading failed to result in myofiber atrophy in the EDL (Deschenes et. al., 2001). This was true not only when fiber types were pooled together, but also when individual fiber types were analyzed separately. This resilience of the EDL to unloading related atrophy is reasonable given the fact that this ankle flexor typically is not markedly recruited during the weight bearing or locomotor activities that are disturbed during hindlimb suspension (Armstrong and Laughlin, 1985a; Laughlin et. al., 1984). In short, the normal activities of the EDL are not interfered with during hindlimb suspension, and accordingly, no deleterious adaptations occur in this muscle as they did in the weight-bearing and ambulatory active soleus, which was the only muscle examined that experienced overarching negative adaptations to unloading. This also suggests that impairment of weight-bearing activity is a more potent stimulus to trigger atrophic responses than impaired ambulation, since the plantaris which participates in locomotor activities, but not maintenance of upright posture (Armstrong and Laughlin, 1985a, 1985b), also was resistant to unloading-related atrophy.

When assessing fiber type distribution of the EDL, it was determined that over 90% of the fibers quantified were fast-twitch, i.e., Type II. This was noted in both male and female rats such that sex had no impact on myofiber type composition of the EDL. When considering the other independent variable of interest, it was discovered that unloading resulted in an increase in the percentage of Type I myofibers present in the EDL. And by extension, it was

also noted that unloading was associated with a greater relative area of EDL muscle area occupied by Type I fibers.

In forming meaningful, overarching messages from the data generated by this study, it is noteworthy that NMJ structure was found to be remarkably similar between males and females despite sex-related differences in underlying myofiber size, and that male and female NMJs were equally resistant to the condition of muscle unloading. And although the unloading intervention employed here was brief (2 weeks), earlier investigation has revealed that in young adult rats, the failure of unloading to evoke morphological remodeling of the NMJ is also evident with a longer (4 weeks) unloading intervention among young adult rats (Deschenes et. al., 2003).

The present findings also revealed that the sensitivity of the neuromuscular system to changes in activity levels depends upon the normal, habitual activity patterns of the muscles involved and whether such activity is disrupted with interventions that eliminate weight-bearing and locomotor behavior. Here, the postural soleus muscle experienced a far greater degree of remodeling, i.e. atrophy, than the non-postural plantaris and EDL muscles.

The morphological findings reported here are wholly consistent with physiological ones reported earlier indicating that synaptic transmission across the NMJ is equally effective in men and women, and that muscle unloading, at least for brief periods, does not interfere with synaptic transmission of the peripheral motor nervous system (Deschenes et. al., 2012). Importantly, the data presented here show that myofiber atrophy is not necessarily precipitated by NMJ degeneration, as has been suggested to occur during the process of sarcopenia, or age-related muscle atrophy (Alchin, 2014; Rudolf et. al., 2014). Also clearly demonstrated in the present investigation was that the two different components of the neuromuscular system, i.e., myofibers and NMJs, can display properties independent of each other both during normal, routine conditions, as well as during periods of altered use. To that point, myofiber profiles exhibited sex-related differences – mainly expressed as larger myofibers among male soleus, plantaris, and EDL muscles – during normal conditions of activity seen among our control animals. Myofibers, at least those of soleus muscles, were also found to be responsive to the effect of muscle unloading, while NMJs of all three muscles examined displayed a robust resistance to unloading-induced remodeling. This contrasts with previous reports indicating that changes in myofiber size were necessarily associated with concomitant adjustments in NMJ size (Balice-Gordon and Lichtman, 1990; Balice-Gordon et. al., 1990). The disassociation of the sensitivity of NMJs to unloading from that of myofibers may well be rooted in how unloading directly affects the function of those two structures. That is, the principal function of the NMJ is to transduce electrical impulses from motor neurons to connected myofibers. On the other hand, myofibers are mainly designed to develop contractile force. It has been found that following a brief, significant depression immediately following the onset of unloading, within seven days neuromuscular electrical transmission activity operates at ~ 80% of that which occurs during control, or normal weight-bearing conditions (Alford et. al., 1987). In contrast, with hindlimb suspension, muscle fibers are relieved of their need to perform normal contractile activities for weight-bearing and ambulatory work. Thus the normal activities of myofibers are, indeed, deeply interrupted leading to atrophy.

With respect to the question that initially drove this investigation, the data presented here indicate that under normal (control) conditions, the neuromuscular system both does, and does not, display sex-specific morphology. That is, when examining the synapses of that system, male and female NMJs were found to be structurally similar. However, when quantifying myofiber profiles, it was determined that male muscle cells were significantly larger than female ones in each of the three muscles assessed (soleus, plantaris, EDL). And regarding the question of whether neuromuscular systems exhibit sex-specific sensitivities to the state of muscle unloading, the data refute that notion. In both male and female systems NMJs were unaffected by two weeks of unloading. And while that same intervention resulted in significant myofiber remodeling, the atrophy detected in males and females was similar. The uncoupling phenomenon detected here as it pertains to the two main components of the neuromuscular system, i.e. NMJs and myofibers, must be taken into consideration during the planning and execution of rehabilitative efforts following periods of disuse as a result of injuries, surgery, and bed rest.

Acknowledgments

This work was supported by the National Institutes of Health, grant number R15 AR060637-04, and The Foundation for Aging Studies and Exercise Science Research, to Michael R. Deschenes.

Role of Authors

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: MRD. Acquisition of data: MAA, MCK, KAK, CML, ECS AS, SL. Analysis and interpretation of data: MRD. Drafting of the manuscript: MRD, MAA. Critical revision of the manuscript: MRD. Statistical analysis: MRD. Obtained funding: MRD. Administrative, technical support: MAA, MCK, KAK. Study supervision: MRD.

References

- Adams GR. Human unilateral lower limb suspension as a model for spaceflight effects on skeletal muscle. *J Appl Physiol* (1985). 2002; 93:1563–5. author reply 1565–6. [PubMed: 12369570]
- Adams GR, Hather BM, Dudley GA. Effect of short-term unweighting on human skeletal muscle strength and size. *Aviat Space Environ Med*. 1994; 65:1116–1121. [PubMed: 7872913]
- Alchin DR. Sarcopenia: describing rather than defining a condition. *J Cachexia Sarcopenia Muscle*. 2014; 5:265–268. [PubMed: 25092476]
- Alford EK, Roy RR, Hodgson JA, Edgerton VR. Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. *Exp Neurol*. 1987; 96:635–649. [PubMed: 3582549]
- 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]
- Armstrong RB, Laughlin MH. Metabolic indicators of fibre recruitment in mammalian muscles during locomotion. *J Exp Biol*. 1985; 115:201–213. [PubMed: 4031765]
- Armstrong RB, Laughlin MH. Rat muscle blood flows during high-speed locomotion. *J Appl Physiol* (1985). 1985; 59:1322–1328. [PubMed: 4055609]
- Balice-Gordon RJ, Breedlove SM, Bernstein S, Lichtman JW. Neuromuscular junctions shrink and expand as muscle fiber size is manipulated: in vivo observations in the androgen-sensitive bulbocavernosus muscle of mice. *J Neurosci*. 1990; 10:2660–2671. [PubMed: 2388082]
- Balice-Gordon RJ, Lichtman JW. In vivo visualization of the growth of pre- and postsynaptic elements of neuromuscular junctions in the mouse. *J Neurosci*. 1990; 10:894–908. [PubMed: 2156964]

- Bamman MM, Clarke MS, Feeback DL, Talmadge RJ, Stevens BR, Lieberman SA, Greenisen MC. Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. *J Appl Physiol* (1985). 1998; 84:157–163. [PubMed: 9451630]
- Bano G, Trevisan C, Carraro S, Solmi M, Luchini C, Stubbs B, Manzato E, Sergi G, Veronese N. Inflammation and sarcopenia: A systematic review and meta-analysis. *Maturitas*. 2017; 96:10–15. [PubMed: 28041587]
- Berg HE, Dudley GA, Haggmark T, Ohlsen H, Tesch PA. Effects of lower limb unloading on skeletal muscle mass and function in humans. *J Appl Physiol* (1985). 1991; 70:1882–1885. [PubMed: 2055867]
- Berg HE, Dudley GA, Hather B, Tesch PA. Work capacity and metabolic and morphologic characteristics of the human quadriceps muscle in response to unloading. *Clin Physiol*. 1993; 13:337–347. [PubMed: 8370234]
- Berg HE, Larsson L, Tesch PA. Lower limb skeletal muscle function after 6 wk of bed rest. *J Appl Physiol* (1985). 1997; 82:182–188. [PubMed: 9029214]
- Delp MD, Duan C. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. *J Appl Physiol* (1985). 1996; 80:261–270. [PubMed: 8847313]
- 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, McCoy RW, Holdren AN, Eason MK. Gender influences neuromuscular adaptations to muscle unloading. *Eur J Appl Physiol*. 2009; 105:889–897. [PubMed: 19125276]
- Deschenes MR, McCoy RW, Mangis KA. Factors relating to gender specificity of unloading-induced declines in strength. *Muscle Nerve*. 2012; 46:210–217. [PubMed: 22806370]
- Deschenes MR, Wilson MH. Age-related differences in synaptic plasticity following muscle unloading. *J Neurobiol*. 2003; 57:246–256. [PubMed: 14608661]
- Dudley GA, Duvoisin MR, Adams GR, Meyer RA, Belew AH, Buchanan P. Adaptations to unilateral lower limb suspension in humans. *Aviat Space Environ Med*. 1992; 63:678–683. [PubMed: 1510640]
- Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jiang B, Bell G, Harris B, Saltin B, Gollnick PD, Roy RR. Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. *J Appl Physiol* (1985). 1995; 78:1733–1739. [PubMed: 7649906]
- Fox J, Garber P, Hoffman M, Johnson D, Schaefer P, Vien J, Zeaton C, Thompson LV. Morphological characteristics of skeletal muscles in relation to gender. *Aging Clin Exp Res*. 2003; 15:264–269. [PubMed: 14582690]
- Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, Roubenoff R. Skeletal muscle fiber quality in older men and women. *Am J Physiol Cell Physiol*. 2000; 279:C611–8. [PubMed: 10942711]
- Gonzalez-Freire M, de Cabo R, Studenski SA, Ferrucci L. The Neuromuscular Junction: Aging at the Crossroad between Nerves and Muscle. *Front Aging Neurosci*. 2014; 6:208. [PubMed: 25157231]
- Hackney KJ, Cook SB, Ploutz-Snyder LL. Nutrition and resistance exercise during reconditioning from unloading. *Aviat Space Environ Med*. 2011; 82:805–809. [PubMed: 21853859]
- Hortobagyi T, Dempsey L, Fraser D, Zheng D, Hamilton G, Lambert J, Dohm L. Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization and retraining in humans. *J Physiol*. 2000; 524(Pt 1):293–304. [PubMed: 10747199]
- Jee H, Sakurai T, Lim JY, Hatta H. Changes in alphaB-crystallin, tubulin, and MHC isoforms by hindlimb unloading show different expression patterns in various hindlimb muscles. *J Exerc Nutrition Biochem*. 2014; 18:161–168.
- Kwon SE, Chapman ER. Glycosylation is dispensable for sorting of synaptotagmin 1 but is critical for targeting of SV2 and synaptophysin to recycling synaptic vesicles. *J Biol Chem*. 2012; 287:35658–35668. [PubMed: 22908222]
- Laughlin MH, Mohrman SJ, Armstrong RB. Muscular blood flow distribution patterns in the hindlimb of swimming rats. *Am J Physiol*. 1984; 246:H398–403. [PubMed: 6703075]
- Lockhart, RS. Introduction to Statistics and Data Analysis for the Behavioral Sciences. W.H. Freeman and Company; New York: 1998.

- Mierzejewska-Krzyzowska B, Drzymala-Celichowska H, Bukowska D, Celichowski J. Gender differences in morphometric properties of muscle fibres measured on cross-sections of rat hindlimb muscles. *Anat Histol Embryol.* 2012; 41:122–129. [PubMed: 21981810]
- Mierzejewska-Krzyzowska B, Drzymala-Celichowska H, Celichowski J. Gender differences in the morphometric properties of muscle fibres and the innervation ratio of motor units in rat medial gastrocnemius muscle. *Anat Histol Embryol.* 2011; 40:249–255. [PubMed: 21426379]
- Morey ER, Sabelman EE, Turner RT, Baylink DJ. A new rat model simulating some aspects of space flight. *Physiologist.* 1979; 22:S23–4. [PubMed: 545372]
- 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]
- Rasmussen S, Chan AK, Goslow GE Jr. The cat step cycle: electromyographic patterns for hindlimb muscles during posture and unrestrained locomotion. *J Morphol.* 1978; 155:253–269. [PubMed: 633374]
- Rozier CK, Elder JD, Brown M. Prevention of atrophy by isometric exercise of a casted leg. *J Sports Med Phys Fitness.* 1979; 19:191–194. [PubMed: 502534]
- Rudolf R, Khan MM, Labeit S, Deschenes MR. Degeneration of neuromuscular junction in age and dystrophy. *Front Aging Neurosci.* 2014; 6:99–110. [PubMed: 24904412]
- Smerdu V, Karsch-Mizrachi I, Campione M, Leinwand L, Schiaffino S. Type IIX myosin heavy chain transcripts are expressed in type IIB fibers of human skeletal muscle. *Am J Physiol.* 1994; 267:C1723–C1728. [PubMed: 7545970]
- Templeton GH, Padalino M, Manton J, Glasberg M, Silver CJ, Silvwer P, DeMartino G, Leconey T, Klug G, Hagler H, et al. Influence of suspension hypokinesia on rat soleus muscle. *J Appl Physiol.* 1984; 56:278–286. [PubMed: 6368494]
- Tomas J, Fenoll R, Mayayo E, Santafe M. Branching pattern of motor nerve endings in a skeletal muscle of the adult rat. *J Anat.* 1990; 168:123–135. [PubMed: 2323988]
- Tsika RW, Herrick RE, Baldwin KM. Interaction of compensatory overload and hindlimb suspension on myosin isoform expression. *J Appl Physiol* (1985). 1987; 62:2180–2186. [PubMed: 2956233]
- Vetrano DL, Landi F, Volpato S, Corsonello A, Meloni E, Bernabei R, Onder G. Association of sarcopenia with short- and long-term mortality in older adults admitted to acute care wards: results from the CRIME study. *J Gerontol A Biol Sci Med Sci.* 2014; 69:1154–1161. [PubMed: 24744390]
- Widrick JJ, Trappe SW, Romatowski JG, Riley DA, Costill DL, Fitts RH. Unilateral lower limb suspension does not mimic bed rest or spaceflight effects on human muscle fiber function. *J Appl Physiol* (1985). 2002; 93:354–360. [PubMed: 12070225]
- Yasuda N, Glover EI, Phillips SM, Isfort RJ, Tarnopolsky MA. Sex-based differences in skeletal muscle function and morphology with short-term limb immobilization. *J Appl Physiol* (1985). 2005; 99:1085–1092. [PubMed: 15860685]

Significance Statement

This investigation demonstrated that neuromuscular junctions (NMJs) do not exhibit sex-specific morphological features, nor are they sensitive to brief (2 weeks) periods of muscle unloading. In contrast, the myofibers of those same neuromuscular systems show pre-existing sex-related differences – male fibers being larger than female ones – while both sexes showed that unloading results in myofiber atrophy, but only among muscles that are commonly recruited for weight-bearing activity, i.e., the soleus. These findings should help us develop optimally focused rehabilitative efforts following medical conditions, or clinical procedures that result in limb immobilization (casting), bed rest, or crutches assisted ambulation.

Figure 1.

Representative panel of images of the same fluorescently stained neuromuscular junction from young, control plantaris muscle. Presynaptic nerve terminal branches are stained in green (top, right), post-synaptic ACh receptors are stained in red (top, left), and pre-synaptic ACh vesicles are stained in blue (bottom, left). An overlay of all NMJ components is shown in bottom, right (scale bar = 20 μ m).

Figure 2.

Representative image of tracings used to quantify morphological aspects of neuromuscular junction from aged, control plantaris muscle. The left panel depicts tracings used to quantify presynaptic nerve terminal branches. The middle panel shows tracings (both manually and drawn and generated by software) used to quantify presynaptic ACh vesicles. The right panel illustrates tracings (both manually drawn and generated by software) used to quantify postsynaptic ACh receptors (scale bar = 20 μ m).

Figure 3. Representative image of immunofluorescent staining to assess myofiber profiles. Type I fibers are stained in red, Type IIA fibers are stained in blue, and Type IIB fibers are stained in green while Type IIX fibers remain unstained (scale bar = 100 μ m).

Table 1

Body Mass (g) of Male and Female Rats Before and After 2-wk Intervention Period.

	Male Control	Male Unloaded	Female Control	Female Unloaded
Pre-intervention *	358.7 ± 3.4	362.3 ± 4.2	221.4 ± 2.4	216.6 ± 2.9
Post-intervention * [†]	382.0 ± 5.9 [#]	340.8 ± 7.3	231.6 ± 4.5 [#]	211.6 ± 6.8

Values are means ± SE, N=10/group.

* indicates significant (P = 0.05) main effect of sex (Male > Female).

[†] indicates significant (P = 0.05) main effect of treatment (Control > Unloaded).[#] indicates significant (P = 0.05) pre- to post-intervention difference (Pre > Post).

Table 2

Effects of Muscle Unloading on Neuromuscular Junctions of Soleus Muscles in Male and Female Rats.

<i>Pre-synaptic</i>	Male Control	Male Unloaded	Female Control	Female Unloaded
Branch number *	12.0 ± 0.5	14.0 ± 1.2	10.6 ± 0.7	12.5 ± 0.5
Total branch length (μm)	133.7 ± 12.4	149.6 ± 14.1	123.1 ± 13.3	123.6 ± 8.5
Average branch length (μm)	11.8 ± 1.3	11.0 ± 0.8	12.3 ± 0.8	10.5 ± 0.8
Branching complexity	18.4 ± 2.2	25.1 ± 4.5	16.4 ± 2.8	18.3 ± 1.8
Vesicle/Branching	1.0 ± 0.2	0.8 ± 0.1	1.2 ± 0.3	1.1 ± 0.2
Total vesicle perimeter (μm)	143.2 ± 12.5	147.3 ± 10.3	139.1 ± 7.4	137.5 ± 9.4
Stained vesicle perimeter (μm)	231.2 ± 35.3	235.4 ± 25.3	201.3 ± 19.1	217.3 ± 21.1
Total vesicle area (μm ²)	558.5 ± 78.8	575.4 ± 62.1	498.2 ± 54.0	498.4 ± 61.8
Stained vesicle area (μm ²)	132.4 ± 37.4	113.2 ± 20.8	102.7 ± 16.5	120.7 ± 19.2
Vesicle dispersion (%)	26.4 ± 4.6	20.3 ± 1.5	21.6 ± 2.0	30.4 ± 5.4
<i>Post-synaptic</i>				
Total endplate perimeter (μm)	151.4 ± 15.4	144.7 ± 8.7	136.9 ± 7.7	137.4 ± 8.8
Stained endplate perimeter (μm)	290.9 ± 28.0	308.9 ± 29.7	255.7 ± 20.5	283.0 ± 48.4
Total endplate area (μm ²)	620.9 ± 66.2	653.2 ± 61.6	567.4 ± 62.8	585.4 ± 74.4
Stained endplate area (μm ²)	232.1 ± 33.6	232.0 ± 27.8	182.0 ± 23.9	210.7 ± 21.6
Endplate/Branching	2.0 ± 0.1	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.1
Pre- to postsynaptic coupling	61.6 ± 9.1	54.5 ± 7.3	76.1 ± 16.0	65.0 ± 10.1
Endplate dispersion (%)	41.9 ± 4.2	37.0 ± 1.0	33.7 ± 2.5	39.6 ± 2.7

Values are means ± SE, N=10/group.

* indicates significant (P < 0.05) main effect for treatment (Unloaded > Control).

Branching complexity = branch number x total branch length ÷ 100; Vesicle/branching = stained vesicle area ÷ total branch length; Vesicle dispersion = stained vesicle area ÷ total vesicle area x 100; Endplate/branching = stained endplate area ÷ total branch length; Pre- to post-synaptic coupling = stained endplate area ÷ stained vesicle area x 100; Endplate dispersion = stained endplate area ÷ total endplate area x 100.

Table 3

Effects of Muscle Unloading on Neuromuscular Junctions of Plantaris muscles in Male and Female Rats.

<i>Pre-synaptic</i>	Male Control	Male Unloaded	Female Control	Female Unloaded
Branch number	5.9 ± 0.5	6.5 ± 0.7	5.4 ± 0.5	5.8 ± 0.7
Total branch length (µm)	100.3 ± 5.9	102.8 ± 8.2	85.8 ± 6.5	96.2 ± 9.7
Average branch length (µm)	18.9 ± 1.4	16.9 ± 0.7	17.1 ± 0.7	18.2 ± 1.1
Branching complexity	7.1 ± 1.3	7.9 ± 1.8	5.6 ± 0.8	7.2 ± 1.8
Vesicle/Branching	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.2
Total vesicle perimeter (µm)	143.7 ± 9.5	149.8 ± 11.8	131.3 ± 7.5	148.0 ± 10.3
Stained vesicle perimeter (µm)	160.3 ± 8.0	173.2 ± 9.6	155.3 ± 10.7	175.7 ± 8.4
Total vesicle area (µm ²)	351.5 ± 21.5	345.5 ± 18.4	316.6 ± 22.1	368.5 ± 31.4
Stained vesicle area (µm ²) [*]	77.1 ± 6.0	88.4 ± 8.3	74.3 ± 7.8	92.5 ± 3.2
Vesicle dispersion (%)	24.8 ± 2.7	28.1 ± 1.8	24.1 ± 1.3	27.8 ± 2.3
<i>Post-synaptic</i>				
Total endplate perimeter (µm)	126.3 ± 6.1	130.8 ± 9.3	117.0 ± 6.6	130.8 ± 8.1
Stained endplate perimeter (µm)	220.5 ± 19.4	230.8 ± 16.9	195.3 ± 13.9	213.1 ± 22.2
Total endplate area (µm ²)	482.3 ± 57.6	484.9 ± 41.4	388.2 ± 32.9	485.9 ± 62.8
Stained endplate area (µm ²)	144.5 ± 14.7	151.2 ± 12.0	119.8 ± 13.0	155.6 ± 19.2
Endplate/Branching	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.2	1.8 ± 0.2
Pre- to postsynaptic coupling	66.6 ± 9.0	68.1 ± 6.3	72.2 ± 5.4	74.2 ± 8.0
Endplate dispersion (%)	31.9 ± 0.6	32.5 ± 1.3	31.7 ± 1.3	33.8 ± 1.8

Values are means ± SE, N=9/group.

^{*} indicates significant (P = 0.05) main effect for treatment (Unloaded > Control).

Table 4

Effects of Muscle Unloading on Neuromuscular Junctions of EDL muscles in Male and Female Rats.

<i>Pre-synaptic</i>	Male Control	Male Unloaded	Female Control	Female Unloaded
Branch number	5.9 ± 0.3	5.7 ± 0.2	5.6 ± 0.3	5.7 ± 0.3
Total branch length (μm) *	128.8 ± 5.2 [§]	111.5 ± 5.4	104.5 ± 5.1	111.0 ± 3.4
Average branch length (μm) *	22.6 ± 0.9 [‡]	20.4 ± 0.5	19.6 ± 0.9	21.7 ± 1.4
Branching complexity [‡]	8.4 ± 0.7	7.0 ± 0.7	6.3 ± 0.6	6.6 ± 0.5
Vesicle/Branching	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Total vesicle perimeter (μm)	137.7 ± 5.6	134.3 ± 8.5	124.9 ± 6.9	130.3 ± 6.4
Stained vesicle perimeter (μm)	178.4 ± 12.6	177.4 ± 15.6	173.3 ± 9.2	179.9 ± 8.6
Total vesicle area (μm ²)	411.8 ± 47.7	323.6 ± 30.1	347.3 ± 16.7	354.8 ± 15.6
Stained vesicle area (μm ²)	80.1 ± 8.1	85.9 ± 10.4	79.1 ± 6.5	88.1 ± 5.8
Vesicle dispersion (%)	23.1 ± 2.3	26.7 ± 1.2	24.3 ± 1.4	25.9 ± 1.2
<i>Post-synaptic</i>				
Total endplate perimeter (μm)	128.9 ± 6.1	116.7 ± 4.5	115.9 ± 5.1	113.6 ± 3.4
Stained endplate perimeter (μm)	250.2 ± 32.5	201.3 ± 15.9	204.3 ± 10.0	204.1 ± 8.0
Total endplate area (μm ²)	605.0 ± 59.0	467.2 ± 41.1	471.0 ± 34.9	492.4 ± 30.2
Stained endplate area (μm ²)	270.6 ± 21.8	208.6 ± 23.6	206.2 ± 19.5	202.5 ± 16.1
Endplate/Branching	2.1 ± 0.2	1.8 ± 0.1	2.1 ± 0.2	1.8 ± 0.1
Pre- to postsynaptic coupling [#]	34.1 ± 3.2	45.6 ± 4.4	44.1 ± 4.3	51.1 ± 4.8
Endplate dispersion (%)	46.5 ± 2.1	44.4 ± 2.0	45.1 ± 2.6	42.1 ± 2.7

Values are means ± SE, N=10/group.

* indicates significant (P = 0.05) effect for interaction between sex and treatment.

[§] indicates significant (P = 0.05) difference from all other groups.[‡] indicates significant (P = 0.05) difference from Female Control.[‡] indicates significant (P = 0.05) main effect for sex (Male > Female).[#] indicates significant (P = 0.05) main effect for treatment (Unloaded > Control).

Table 5

Effects of Unloading on Myofiber Profiles of Soleus Muscles in Male and Female Rats.

<i>Cross-sectional area (μm^2)</i>	Male Control	Male Unloaded	Female Control	Female Unloaded
Types combined [*] [†]	3300 $\pm$ 151	2616 $\pm$ 191	2892 $\pm$ 135	2179 $\pm$ 189
Type I [*] [†]	3320 $\pm$ 151	2631 $\pm$ 197	2674 $\pm$ 300	2205 $\pm$ 187
Type IIA [†]	2368 $\pm$ 266	1531 $\pm$ 421	2218 $\pm$ 230	1189 $\pm$ 236
<i>Myofiber type composition (%)</i>				
Type I	96.4 $\pm$ 1.1	97.4 $\pm$ 0.6	95.4 $\pm$ 1.0	92.7 $\pm$ 2.4
Type IIA	3.6 $\pm$ 1.3	2.6 $\pm$ 1.1	4.6 $\pm$ 0.8	7.3 $\pm$ 4.0
<i>Percentage area occupied by fiber type</i>				
Type I	97.8 $\pm$ 1.0	99.1 $\pm$ 0.4	98.5 $\pm$ 0.7	96.9 $\pm$ 1.8
Type IIA	2.2 $\pm$ 0.2	0.9 $\pm$ 0.9	1.5 $\pm$ 0.7	2.1 $\pm$ 1.3

Values are means $\pm$ SE, N=10/group.^{*} indicates significant (P = 0.05) main effect for sex (Male > Female).[†] indicates significant (P = 0.05) main effect for treatment (Control > Unloaded).

Table 6

Effect of Unloading on Myofiber Profiles of Plantaris Muscles in Male and Female Rats.

<i>Cross-sectional area (μm^2)</i>	Male Control	Male Unloaded	Female Control	Female Unloaded
Types combined *	3194 $\pm$ 284	2730 $\pm$ 180	2138 $\pm$ 284	2281 $\pm$ 166
Type I *	2144 $\pm$ 249	1858 $\pm$ 166	1527 $\pm$ 75	1516 $\pm$ 83
Type IIA *	2351 $\pm$ 252	2052 $\pm$ 154	1476 $\pm$ 82	1572 $\pm$ 90
Type IIX *	3485 $\pm$ 367	3059 $\pm$ 200	2215 $\pm$ 146	2335 $\pm$ 132
Type IIB *	4623 $\pm$ 436	3841 $\pm$ 328	2983 $\pm$ 284	3109 $\pm$ 193
<i>Myofiber type composition (%)</i>				
Type I	12.8 $\pm$ 1.8	15.5 $\pm$ 4.2	15.2 $\pm$ 2.6	15.6 $\pm$ 2.1
Type IIA	31.1 $\pm$ 1.7	33.4 $\pm$ 2.0	30.5 $\pm$ 3.2	27.4 $\pm$ 2.6
Type IIX	36.3 $\pm$ 2.2	32.2 $\pm$ 2.3	31.2 $\pm$ 2.6	31.1 $\pm$ 2.6
Type IIB	19.8 $\pm$ 4.5	18.9 $\pm$ 3.1	24.1 $\pm$ 5.3	25.9 $\pm$ 5.6
<i>Percentage area occupied by fiber type</i>				
Type I	10.6 $\pm$ 2.0	12.1 $\pm$ 4.5	12.8 $\pm$ 3.5	7.9 $\pm$ 1.6
Type IIA	24.2 $\pm$ 2.0	25.1 $\pm$ 1.9	21.3 $\pm$ 3.3	24.5 $\pm$ 2.6
Type IIX	39.2 $\pm$ 3.2	36.3 $\pm$ 2.8	37.4 $\pm$ 3.2	33.2 $\pm$ 3.4
Type IIB	26.0 $\pm$ 6.4	26.5 $\pm$ 4.8	28.5 $\pm$ 7.1	34.4 $\pm$ 6.3

Values are means $\pm$ SE, N=9/group.* indicates significant ($P < 0.05$) main effect of sex (Male > Female).

Table 7

Effect of Unloading on Myofiber Profiles of EDL Muscles in Male and Female Rats.

<i>Cross-sectional area (μm^2)</i>	Male Control	Male Unloaded	Female Control	Female Unloaded
Types combined *	2316 $\pm$ 193	2098 $\pm$ 161	1761 $\pm$ 78	1705 $\pm$ 63
Type I *	1282 $\pm$ 54	1298 $\pm$ 103	987 $\pm$ 50	1163 $\pm$ 85
Type IIA *	1297 $\pm$ 78	1252 $\pm$ 84	988 $\pm$ 34	1058 $\pm$ 52
Type IIX *	2083 $\pm$ 124	1984 $\pm$ 132	1546 $\pm$ 80	1598 $\pm$ 84
Type IIB *	3248 $\pm$ 286	3073 $\pm$ 242	2461 $\pm$ 125	2379 $\pm$ 81
<i>Myofiber type composition (%)</i>				
Type I $\dagger$	6.5 $\pm$ 0.8	8.1 $\pm$ 0.6	6.0 $\pm$ 1.0	9.6 $\pm$ 0.9
Type IIA	26.8 $\pm$ 2.9	30.0 $\pm$ 1.9	25.1 $\pm$ 2.1	27.9 $\pm$ 2.1
Type IIX	29.8 $\pm$ 2.0	30.3 $\pm$ 3.3	27.2 $\pm$ 3.1	26.4 $\pm$ 2.6
Type IIB	36.9 $\pm$ 4.3	31.6 $\pm$ 3.5	41.7 $\pm$ 3.9	36.1 $\pm$ 3.2
<i>Percentage area occupied by fiber type</i>				
Type I $\dagger$	3.8 $\pm$ 0.7	5.2 $\pm$ 0.6	3.6 $\pm$ 0.7	6.7 $\pm$ 0.9
Type IIA	15.9 $\pm$ 2.1	18.3 $\pm$ 1.4	14.5 $\pm$ 1.7	17.4 $\pm$ 1.4
Type IIX	28.8 $\pm$ 3.1	29.7 $\pm$ 4.2	24.5 $\pm$ 3.2	25.4 $\pm$ 2.9
Type IIB	51.5 $\pm$ 5.7	46.8 $\pm$ 5.4	57.4 $\pm$ 4.5	50.5 $\pm$ 4.2

Values are means $\pm$ SE, N=10/group.

* indicates significant (P = 0.05) main effect for sex (Male > Female).

 $\dagger$ indicates significant (P = 0.05) main effect for treatment (Unloaded > Control).