2016

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Recommended Citation

Deschenes, M. R., & Leathrum, C. M. (2016). Gender-specific neuromuscular adaptations to unloading in isolated rat soleus muscles. Muscle & nerve, 54(2), 300-307.

Published in final edited form as:

Muscle Nerve. 2016 August ; 54(2): 300–307. doi:10.1002/mus.25038.

Gender-specific Neuromuscular Adaptations to Unloading in Isolated Rat Soleus Muscles

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Abstract

Introduction—The potential of gender to affect unloading-induced neuromuscular adaptations was investigated.

Methods—Twenty male and 20 female rats were assigned to control (CTL), or unloaded (UL) conditions. After 2 weeks of unloading, soleus muscles were removed, and neuromuscular function was assessed during a train of alternating indirect (neural) and direct (muscle) stimuli.

Results—In rested muscle, strength showed significant ($P \leq 0.05$) main effects for gender (male > female) and treatment (CTL > UL). By the end of the testing protocol, when muscles showed fatigue, gender-related and treatment-related differences in strength had disappeared. Neuromuscular transmission efficiency and strength suffered a greater decline during the testing protocol in males than females. Unloaded male muscles displayed greater contractile velocity than female muscles both when rested and fatigued.

Discussion—Gender did affect unloading-induced neuromuscular adaptations. The greater strength of rested male muscles was due to greater muscle mass and neuromuscular transmission efficiency.

Keywords

neuromuscular junction; myofiber; unweighting; disuse; strength

INTRODUCTION

Muscle unloading has been shown to elicit a number of detrimental effects on the neuromuscular system. These maladaptations include strength decrement[1,2], decline in power[3], altered muscle fatigability[4], decreased motor drive to contracting muscle tissue[5,6], and atrophy of the whole muscle and its constituent myofibers.[7,8] More recently, it has been reported that gender may play a role in the susceptibility of the neuromuscular system to unloading-related remodeling.[9,10]

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The authors have no financial disclosures, or conflicts of interest to report.

Although the impact of unloading is manifested in the muscle and its myofibers in terms of strength and power decrements, the actual mechanism involved may reside further upstream of the muscle tissue displaying alterations. More specifically, it is possible that muscle performance is impaired following unloading due to alterations in neuromuscular transmission efficiency, i.e., dampened communication between motor nerve terminals and the myofibers that they innervate. Indeed, it has been demonstrated that during a train of stimuli, neuromuscular transmission can be compromised resulting in decrements in muscle endurance, or ability to sustain contractile force over time.[11,12] How muscle unloading may influence neuromuscular transmission efficiency is not fully understood at this time, nor is the impact of gender on synaptic function of the neuromuscular system. This is an important variable to consider, since it has been reported that in humans, women suffer greater strength decrements than men as a result of muscle unloading[10,13], and spaceflight crews (who are exposed to muscle unloading) are increasingly comprised of women.[14] Thus, the aim of this investigation was 2-fold: 1) to determine the effects of gender on unloading-induced changes in neuromuscular function, and 2) to assess the role of neuromuscular transmission efficiency in unloading-related alterations in muscle performance. We studied an animal model of unloading and performance assessment along with an isolated muscle arrangement to determine neuromuscular function. The main benefit of testing isolated muscles in an *ex vivo* setting was that the potentially confounding effects of motivation and pain tolerance were eliminated during contractions so that physiological properties solely determined neuromuscular performance.

MATERIALS AND METHODS

Subjects

Twenty male and 20 female Wistar rats (6 mo old) purchased from Charles River Laboratories (Wilmington, MA, USA) were assigned randomly to either control (CTL) or unloaded (UL) treatment groups, resulting in 4 groups of 10 rats each (Male-CTL, Male-UL, Female-CTL, Female-UL). Animals assigned to the UL groups were subjected to a 2-week period of hindlimb suspension as previously described.[15] The 2-week intervention periods for male and female rats did not occur simultaneously, but rather occurred in subsequent months. Previous work has shown that 2 weeks of hindlimb suspension produces significant alterations in muscle performance and muscle fiber atrophy.[16,17] During the unloading intervention, the animal's hindlimbs were elevated from contact with the floor, thus preventing weight-bearing and ambulatory activity, using an adhesive strip placed along the length of the tail and attached to a clip. The clip was then secured into a swivel device suspended above the rat to allow it to move in a 360° arc using its forelimbs, which remain in contact with the floor. Animals remain in this condition of hindlimb suspension 24 h a day. To facilitate eating in an attempt to maintain body mass, food pellets are fractured into smaller pieces by being placed in an ice crusher. In contrast, rats assigned to the CTL groups were placed in tubs lined with wood shavings and allowed free ambulatory and weight bearing activities. All animals, regardless of treatment group, were provided standard rat chow and water *ad libitum*. All rats were housed in a 21–22° C environment on a 12-hour light-dark cycle. All experimental procedures were approved by the institution's animal care

and use committee which operates in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Neuromuscular Performance

Following the 2 week intervention period, neuromuscular performance of the soleus muscle was quantified with an *ex vivo* muscle stimulation and recording device (system 1205A, Aurora Scientific Inc., Aurora, ON, Canada) as depicted in Figure 1. The soleus was chosen for study, because it functions as the main postural muscle[18] and the model of unloading employed here causes severe disruption of its normal pattern of neuromuscular activity. In preparation for performance analysis, muscles were removed surgically from living, anesthetized rats (ketamine/xylazine cocktail of 50/10 mg/kg body mass). Following a 15 min incubation in Ringer solution (137 mM NaCl, 4.7 mM KCl, 3.4 mM $CaCl_2$, 1.2 MM $MgSO_4$, 1mM NaH_2PO_4, 112 D-glucose, pH = 7.4) that was vigorously aerated with gas (95% oxygen, 5% carbon dioxide) and maintained at 21–22° C. The muscle was then subjected to an electrical stimulation protocol that first established optimal muscle length in order to determine peak isometric force. The stimulation procedures utilized emulated those of Lomo and Rosenthal[19] by alternating indirect and direct stimulation to quantify neuromuscular transmission efficiency. The stimulation parameters consisted of a series of sets featuring 9 pulses at a constant 37 V and ~25 Hz for a duration of 0.2 msec (indirect or neural stimulation via nerve terminal endings) followed by a single 2 msec duration pulse (direct muscle stimulation) so that the duration of each set was 30 sec. Sets were repeated continuously for a total protocol duration of 5 min. Contractile recordings were collected with the software accompanying the stimulation/recording system, and recordings were later analyzed using the same software. Contractile variables quantified were peak tension (highest single measures from indirect and direct stimulation), specific tension (tension/whole muscle wet weight), and time to peak tension (onset of force development determined manually). Each of these parameters was quantified during both indirect (nerve) and direct (muscle) stimulation. Finally, neuromuscular transmission efficiency was assessed by dividing peak tension produced during indirect (neural) stimulation by that generated during direct (muscle) stimulation of the soleus and multiplying by 100 to be expressed as a percentage.

Statistical analysis

A 3-way analysis of variance (ANOVA) with main effects of treatment (CTL vs. UL), gender (male vs. female), and time (start vs. conclusion of protocol) was used to analyze data from each stimulation type (indirect and direct). Of primary concern, however, was evidence of significant interactive effects. In the event of a significant F-ratio, *post-hoc* assessments were employed to identify significant pairwise differences. In all analyses, significance was set at $P \leq 0.05$. All data are reported as mean ± SD. The statistical package StatView (SAS Institute, Inc, Rockville, MD) was used in all analyses.

RESULTS

Body mass and muscle wet weight

In examining body mass data collected prior to the 2 week intervention period, statistical analysis failed to reveal significant interaction, or a main effect for treatment, while a significant main effect for gender (males > females) was detected. At the conclusion of the 2 week unloading period, there was again an absence of significant interaction, although main effects for both gender (males > females) and treatment (CTL > UL) were evident. When statistical analyses were performed on wet weights of soleus muscles collected at the conclusion of the 2 week intervention, a significant interactive effect was discovered; the *post-hoc* results indicated that the muscles of male rats had undergone significantly more unloading-related atrophy than those of female rats. These data can be found in Table 1.

Neuromuscular performance

Peak torque—Strength, or peak tension, was assessed at the very beginning (Initial) and end (Final) of the 5 min train of stimuli, both by indirect (nerve) and direct (muscle) stimulation. ANOVA results showed that in the Initial time period, no significant interaction between gender and treatment occurred, but a main effect for gender (males > females) was evident along with a main effect for treatment (CTL > UL) during indirect stimulation. At the end of the stimulation session no evidence of interaction between gender and treatment was detected nor were there main effects. When examining interactive effects among gender and time, however, a significant main effect was found which showed that males lost more strength during the stimulation protocol than females. Moreover, significant interaction was established between treatment and time whereby CTL animals suffered greater declines in peak torque during the stimulation protocol than UL ones.

With direct muscle stimulation, however, ANOVA results failed to reveal a significant interactive effect between gender and treatment or a main effect for gender, although a main effect for treatment (CTL > UL) was identified. Analyses of the peak tension recordings at the end of the 5 min train of stimuli failed to reveal significant interactive or main effects. When examining changes in strength during the stimulation protocol, however, significant interaction was revealed between gender and time. Specifically, although both male and female muscles lost significant amounts of strength during the stimulation protocol, males lost significantly more than females. Significant interaction was also noted between treatment and time such that CTL muscles suffered more severe declines in force production than UL ones during the train of stimulation. Because of these interactive effects both male and female, as well as CTL and UL soleus muscles produced similar amounts of strength by the end of the testing protocol with both direct and indirect muscle stimulation. Data concerning peak tension are presented in Table 2.

Specific tension—Data collected on specific tension, or peak tension relative to whole muscle wet weight[20,21], were also analyzed. With indirect stimulation, no significant interaction between gender and treatment occurred either at the beginning or the end of the stimulation session nor were there significant main effects for those 2 variables at either the Initial or Final stages of the stimulation protocol. But as with peak tension, there was a main

effect for the variable of time, which suggests significant declines in specific tension during the indirect stimulation protocol.

Specific tension with direct muscle stimulation, however, produced 1 result that differed from those observed during indirect stimulation. With *post-hoc* analysis of the significant interaction between time and treatment, CTL muscles exhibited a greater loss of maximal force production than UL muscles. Once again the strongest muscles suffered the most during a fatigue-inducing train of electrical stimuli. Results for specific tension can be found in Table 3.

Time to peak force—As a measure of contractile velocity, time to peak force was also examined. ANOVA results showed an absence of interaction between gender and treatment, and there was no main effect for treatment. This was true whether the muscle was stimulated indirectly or directly. However, there was a significant main effect for gender in that male muscles contracted more quickly than female ones; this was true whether muscles were stimulated indirectly or directly. ANOVA results also showed a lack of interaction between the factor of time with either gender or treatment. However, there was a main effect of time, which indicated that over the course of the 5 min testing protocol the contractile velocity of soleus muscles became significantly slower. This was the case during both indirect and direct stimulation. *Post-hoc* procedures revealed that at the Initial time point, when muscles were well rested, male UL muscles took less time to reach peak force than female UL and CTL muscles, but by the Final time interval, when muscles were experiencing fatigue, those differences were no longer observed. Interestingly, these *post-hoc* results appeared during indirect stimulation only. When we examined *post-hoc* results from direct muscle stimulation, we found instead that it was at the Final time period, not the Initial one, where specific between group differences were exposed. Again, male UL muscles contracted at a faster velocity than female CTL and UL muscles. Table 4 presents data on time to peak tension.

Neuromuscular transmission efficiency—We quantified neuromuscular transmission efficiency to assess the locus of fatigue, i.e., motor nerves or muscle fibers, during repetitive maximal muscle contractions. ANOVA showed significant interaction between gender and time (i.e., Initial to Final interval changes), but not between time and treatment. *Post-hoc* analysis indicated that for all 4 treatment groups, neuromuscular transmission efficiency was significantly attenuated during the stimulation protocol. This suggests that motor nerves, rather than muscle fibers, were mainly responsible for the degradation of muscle force. Moreover, *post-hoc* findings indicated that at the outset of the testing protocol gender-related differences in neuromuscular transmission efficiency were apparent (male > female). By the end of the protocol, nerve to muscle communication was no longer different between male and female muscles, which indicates a sharper decline in neuromuscular transmission efficiency during the 5 min contractile challenge in males compared to females. Data concerning neuromuscular transmission efficacy are presented in Table 5.

DISCUSSION

The deleterious effects of muscle unloading have been well described and reviewed.[22,23,24] These effects include muscle atrophy, along with functional impairments such as compromised strength, muscle power, and neural drive to contracting muscles. More recently it has been reported that in humans subjected to brief periods of muscle unloading, women experience greater decrements in muscle strength and that this could not be ascribed to gender-specific unloading-related declines in muscle mass, or myofiber size.[10] Rather, it was demonstrated that women experienced more pronounced reductions in neural drive to maximally contracting muscle tissue as a result of unloading than men and that it was this difference in neural drive that accounted for the greater declines in strength noted among women following muscle unloading.[5,13] To further pursue this line of investigation, we decided to use an animal model of muscle unloading, hindlimb suspension, along with an *ex vivo* procedure for assessing the innate functional capacity of the neuromuscular system without the potentially confounding variable of maximal voluntary effort that can occur in human studies of neuromuscular performance.

Body and muscle mass

In this study female and male rats of the same age (6 mo) were assigned either to control or hindlimb suspension conditions for a period of 2 weeks before *ex vivo* neuromuscular capacity was quantified. Unsurprisingly, it was determined that young adult male rats had significantly greater body mass than young adult females both before and after the 2-week intervention period; this was true whether they were members of the CTL group or the UL groups. Further, we found that hindlimb suspension resulted in significant, but similar, declines in body mass in males and females (~11% and ~9%, respectively). More germane to the objectives of this project, we also determined that unloading resulted in reductions of whole muscle wet weight of both male and female soleus muscles. However, the unloading-induced muscle atrophy detected in males (~36%) was significantly greater than it was in females (~21%). Surprisingly, this did not translate to greater strength declines in males, as their unloading-induced decrement in peak tension was less than that of females. These findings served as an early indication of the vital role played by neuromuscular transmission efficiency in establishing a muscle's capacity for peak tension development.

Peak tension

The gender-related difference in strength displayed by soleus muscles (males > females) was noted only among rested muscles, i.e., at the Initial interval of the 5-min testing protocol when neuromuscular transmission efficiency was also higher in males than females. In contrast, when muscles were fatigued by the Final stage of the 5-min train of stimuli, no differences in peak tension, or neuromuscular efficiency, were noted between any of the 4 treatment groups. This shows that male muscles suffered a more severe decline in peak tension during the course of the 5 min testing protocol than did female muscles. It was obvious then, that males displayed more fatigue during the 5-min testing protocol than females and that this difference between male and female muscles was directly attributed to similar gender-specific declines in neuromuscular transmission efficiency. Similarly, these pre- to post-stimulation protocol results showed that CTL muscles of both genders, which

were stronger than UL muscles at the start of the testing procedure, suffered greater fatigue relative to UL muscles, as strength between the CTL and UL groups no longer differed by the conclusion of the train of stimuli. Moreover, *post-hoc* results revealed that resistance to fatigue during the 5-min testing protocol was significantly greater in female UL muscles than in any of the other 3 treatment groups, and this was true regardless of stimulation mode employed. Most likely this stems from the fact that those same muscles (female UL) were also the weakest of the 4 experimental groups at the onset of the testing regimen and thus had less strength to lose.

Neuromuscular transmission efficiency

In attempting to identify potential mechanisms for these differences in loss of peak tension during the 5-min testing protocol, our results from the assessment of neuromuscular transmission efficiency, or the difference between force produced by direct muscle stimulation as opposed to indirect nerve stimulation, were very informative. More specifically, similar to peak tension, we found that although neuromuscular transmission efficiency was significantly greater in males at the initial stage of contractile activity, by the end of the testing protocol no gender-related differences were evident. Thus, it appears that in a rested state when neuromuscular transmission efficiency was greater in males than females, so was strength. However, when fatigued male and female muscles displayed similar impairment of neuromuscular transmission, strength also was similar in the neuromuscular systems of the 2 genders. The impact of enhanced neuromuscular impairment during a fatiguing train of stimuli to the concurrent loss of muscle strength has been noted elsewhere.[12,25,26]

Specific tension

In addition to our inquiry into neuromuscular transmission efficiency in attempting to explain gender differences in strength as well as those between rested and fatigued muscles, we also examined specific tension, or the amount of force produced relative to muscle mass. Those results revealed that muscle quality, as assessed by specific tension, was resistant to the effects of gender and treatment, as well as their interaction. That is, both male and female fatigued solei, whether participating in CTL or UL treatment groups, exhibited equal amounts of force production relative to muscle mass as they did while they were well rested. Muscles from all 4 treatment groups, however, demonstrated significant yet similar reductions in peak tension over the course of the 5-min testing regimen. This was apparent with both indirect and direct stimulation. These findings show that specific tension could not explain gender-specific or treatment-specific differences in strength that were described above. Rather, this supports the critical role of neuromuscular transmission efficacy in determining contractile force.

Time to peak force

To gain a more comprehensive view of the impact of gender on unloading-evoked alterations in neuromuscular function, we examined time to peak force as a measure of contractile velocity. We found that gender did play a role, albeit a somewhat confusing, or at least inconsistent one. More specifically, we found that upon male muscles contracted at a faster velocity than female muscles with indirect stimulation. But *post-hoc* analysis showed that in

fact, only unloaded male muscles reached peak force faster than female muscles. Moreover, this gender-related difference was manifested only among rested muscles; by the end of the stimulation protocol gender differences could no longer be identified. Conversely, with direct stimulation, no differences between the contractile velocities of rested (i.e., early during stimulation protocol) male and female muscles were identified. Instead, it was at the final stage of the 5-min train of stimuli, that fatigued male-UL muscles contracted more rapidly than both Female-CTL and Female-UL muscles. It has previously been reported that muscle unloading promotes a faster contractile velocity in muscle and that such a change is associated with fiber type conversion with a decline in the proportion of slow-twitch (Type I) muscle fibers and an increase in the expression of fast-twitch (Type II) fibers.[27,28]

Gender-related differences in contractile velocity during rested vs. fatigued conditions, as well as during indirect vs. direct stimulation, likely were linked to changes in neuromuscular efficiency. Recall that male muscles subjected to hindlimb suspension exhibited no evidence of neuromuscular transmission blockage during the early stages of the 5 min testing protocol thus enabling stimuli delivered to motor neurons to drive unloaded muscle to its fastest rate of contraction. By the end of the stimulating protocol, however, impairment of neuromuscular transmission had become apparent, particularly in male UL muscles thus bringing them to parity with muscles from the other treatment groups in terms of rapidity of stimulation and contractile velocity. Accordingly, by the end of the stimulating protocol direct stimulation of muscles elicited differences in time to peak tension between male UL muscles and female muscles of both treatment categories (CTL and UL). It is presumed that at this time point and with direct stimulation which does not incorporate synaptic transmission, it would be the previously mentioned increased expression of fast-twitch muscle fibers typically observed in unloaded muscle that would account for elevated rate to peak tension. However, quantification of muscle fiber type profile was not featured in this study.

Overview

The results of this investigation differ from those we have previously reported which have indicated that females suffer more severe unloading-induced decrements in neuromuscular function than men.[5,13] Besides the obvious difference in species tested (humans vs. rats), we found in our earlier investigation that a more pronounced decline in motor drive produced by the central nervous system during maximal voluntary contractions explained the greater reduction in strength among unloaded female muscles compared to unloaded muscles in men.[5,13] But in this study, which used isolated muscles in an *ex vivo* testing procedure, central drive and/or maximal voluntary effort did not contribute to the expression of strength, although the peripheral nervous system (i.e., motor nerve terminals) did play a part when muscles were stimulated indirectly. Indeed, these findings suggest that when skeletal muscle is rested, neuromuscular transmission is more effective in males and females, but as fatigue sets in during the course of the 5-min testing protocol, nerve-to-muscle communication becomes an important determinant of strength and is compromised more in males than females.

In conclusion, these results show that gender plays a significant role in determining functional neuromuscular adaptations to muscle unloading. In rested muscle, before neuromuscular fatigue sets it, the greater strength expressed by male muscle can be explained by a larger muscle mass and more effective neuromuscular transmission; this is true regardless of treatment group assignment (i.e., CTL or UL). The importance of size in determining peak force production in rested muscle is confirmed by the fact that specific tension was not found to vary between genders or treatment groups in either rested or fatigued muscles, although it was diminished over the course of the 5-min testing due to the effects of fatigue. These findings also suggest that gender-related differences in the impairment of neuromuscular transmission that occur during the testing protocol (males > females) alter muscle strength such that although it is initially greater among male muscles, it no longer differs from female muscles when fatigue sets in. In short, it appears that neuromuscular synaptic communication in males is more susceptible to fatigue than it is in females. It remains to be determined whether an intervention such as exercise training would mitigate or exacerbate the gender-related differences in muscle fatigability and contractile velocity detected here. Such findings could have important applied consequences with respect to rehabilitation following periods of muscle unloading that accompany post-surgical recovery (i.e., crutch-assisted ambulation), bed rest, immobilization, or even exposure to the microgravity of spaceflight.

Acknowledgments

Grant support: This work was made possible by grant support from National Institute of Arthritis and Musculoskeletal and Skin Diseases (R15 AR060637-03) and The Foundation for Aging Studies and Exercise Science Research.

ABBREVIATIONS

CTL	control
UL	unloaded

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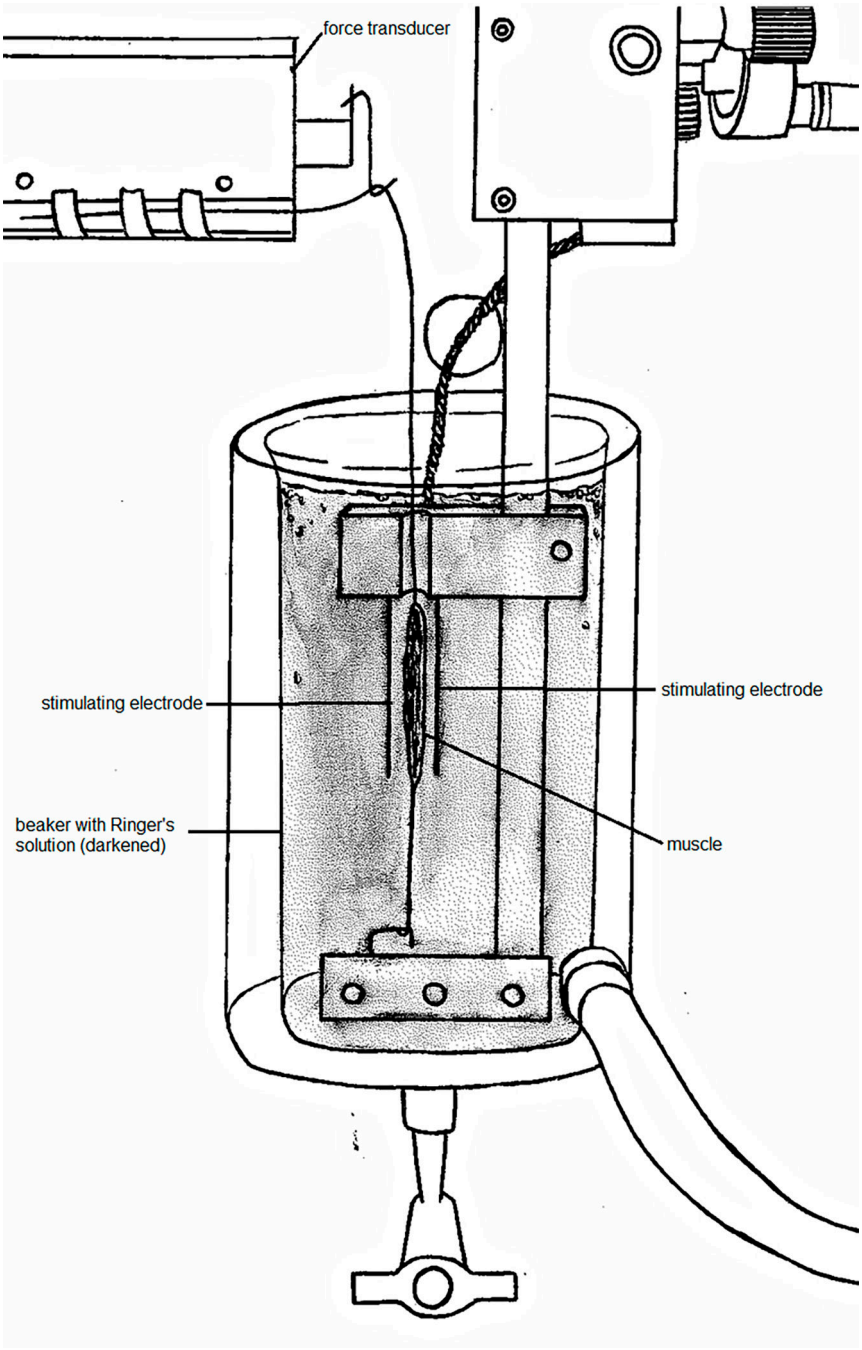

Figure 1.

Depiction of *ex vivo* muscle stimulation and recording system to collect data on neuromuscular function. Note stimulating electrodes on either side of suspended muscle bathed in Ringer solution.

Table 1

Body mass (g) before (Pre) and after (Post) the 2 wk experimental period and soleus whole muscle wet weight (mg) after the 2 wk intervention.

	Pre, body mass	Post, body mass	Soleus wet weight
Male CTL	358.7 ± 10.9 *	382.2 ± 18.3 ‡	186.1 ± 28.1 ‡
Male UL	362.3 ± 13.4 *	340.8 ± 23.1 †	119.6 ± 30.1
Female CTL	221.4 ± 7.6	231.7 ± 14.4 #	117.0 ± 14.9
Female UL	216.6 ± 9.1	211.7 ± 21.5	91.9 ± 18.1 ‡

Values are mean ± SD, N=10 for all groups except Female CTL, where N=9.

* significant ($P \leq 0.05$) difference from Female CTL and Female UL groups at that time point (Pre).

‡ significant ($P \leq 0.05$) difference from all other groups at that time point (Post).

† significant ($P \leq 0.05$) difference from Female CTL and Female UL groups at that time point (Post).

significant ($P \leq 0.05$) difference from Female UL group at that time point (Post).

Table 2

Effects of Gender and Unloading on Peak Tension (N) at Beginning (Initial) and Conclusion (Final) of 5 min Train of indirect (nerve) or direct (muscle) stimulation.

	Initial**		**Final**	
	Indirect stimulation	***Direct stimulation***	***Indirect stimulation***	***Direct stimulation***
Male CTL	45.1 ± 19.8*	48.6 ± 19.6†	12.1 ± 8.3	21.9 ± 9.5
Male UL	29.5 ± 15.5	30.1 ± 15.9	9.0 ± 7.9	15.0 ± 9.6
Female CTL	31.8 ± 11.3	38.2 ± 12.8#	10.5 ± 6.5	19.3 ± 6.7
Female UL	14.8 ± 6.9‡	20.6 ± 9.4	6.8 ± 2.6	14.8 ± 7.0

Values are mean ± SD, N=10 for all groups except Female CTL, where N=9.

** significant main effect of time (change from Initial to Final) for both Indirect ($P<0.0001$) and Direct ($P<0.0001$) stimulation.

* significant ($P\leq0.05$) difference from all other groups with indirect stimulation at the same time point (Initial).

‡ significant ($P\leq0.05$) difference from Female CTL and Female UL groups with indirect stimulation at same time point (Initial).

† significant ($P\leq0.05$) difference from Male UL and Female UL groups with direct stimulation at same time point (Initial).

significant ($P\leq0.05$) difference from Female UL with direct stimulation at same time point (Initial).

Table 3

Effects of Gender and Unloading on Specific Tension at Beginning (Initial) and Conclusion (Final) of 5 min Train of Indirect (nerve) or Direct (muscle) stimulation.

	Initial**		**Final**	
	Indirect stimulation	***Direct stimulation***	***Indirect stimulation***	***Direct stimulation***
Male CTL	24.2 ± 10.5	26.3 ± 10.5	6.4 ± 4.2	12.0 ± 5.6
Male UL	24.0 ± 10.5	24.4 ± 10.3	7.3 ± 5.3	12.4 ± 7.8
Female CTL	27.6 ± 11.9	33.1 ± 13.6	10.1 ± 5.3	17.1 ± 7.3
Female UL	17.0 ± 9.8	22.4 ± 28.2	7.5 ± 2.8	15.3 ± 5.2

Values are mean ± SD, N=10 for all groups except Female CTL, where N=9.
Specific tension calculated as peak tension (N)/muscle wet weight (mg) × 100.

** significant main effect of time (change from Initial to Final) for both Indirect ($P<0.0001$) and Direct ($P<0.0001$) stimulation.

Table 4

Effects of Gender and Unloading on Time to Peak Tension (sec) at Beginning (Initial) and Conclusion (Final) of 5 min train of Indirect (nerve) or Direct (muscle) stimulation.

	Initial**		**Final**	
	Indirect stimulation	***Direct stimulation***	***Indirect stimulation***	***Direct stimulation***
Male CTL	0.754 ± 0.123	0.751 ± 0.109	0.802 ± 0.040	0.817 ± 0.023
Male UL	0.634 ± 0.267 *	0.754 ± 0.160	0.782 ± 0.086	0.792 ± 0.046≠
Female CTL	0.802 ± 0.007	0.816 ± 0.012	0.829 ± 0.041	0.838 ± 0.038
Female UL	0.780 ± 0.067	0.804 ± 0.068	0.804 ± 0.024	0.818 ± 0.019

Values are mean ± SD, N=10 for all groups except Female CTL, where N=9.

** significant main effect of time (change from Initial to Final) for both Indirect (P=0.0039) and Direct (P=0.0208) stimulation.

* significant ($P \leq 0.05$) difference from Female CTL and Female UL groups.

Table 5

Effects of Gender and Unloading on Neuromuscular Transmission Efficacy (%) at Beginning (Initial) and Conclusion (Final) of 5 min train of Indirect (nerve) or Direct (muscle) stimulation.

	Initial**	**Final**
Male CTL	92.8 ± 36.8≠	55.3 ± 18.8
Male UL	98.0 ± 21.6*	60.0 ± 25.2
Female CTL	83.2 ± 11.7	54.4 ± 17.1
Female UL	71.8 ± 27.3	45.9 ± 23.7

Values are mean ± SD, N=10 for all groups except Female CTL, where N=9.
Neuromuscular Efficacy calculated as indirect Peak Tension/Direct Peak Tension × 100.

** significant (P<0.0001) main effect of time (change from Initial to Final).

* significant (P≤0.05) difference from Female UL group at same time point (Initial).

≠ trend (P=0.08) for difference from Female UL group at same time point (Initial).