Effects of Exercise on Soleus in Severe Burn and Muscle Disuse Atrophy

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Abstract

Background:

Muscle loss is a sequela of severe burn and critical illness with bed rest contributing significantly to atrophy. We hypothesize that exercise will mitigate muscle loss after burn with bed rest.

Materials and Methods:

Male rats were assigned to sham ambulatory (S/A), burn ambulatory (B/A), sham hindlimb unloading (S/H), or burn hindlimb unloading (B/H). Rats received a 40% scald burn or sham and were ambulatory or placed in hindlimb unloading, a model of bed rest. Half performed twice-daily resistance climbing. Hindlimb isometric forces were measured on day 14.

Results:

Soleus mass and muscle function were not affected by burn alone. Mass was significantly lower in hindlimb unloading (79 vs. 139 mg, $p < 0.001$) and no exercise (103 vs. 115 mg, $p < 0.01$). Exercise significantly increased soleus mass in B/H (86 vs. 77 mg, $p < 0.01$). Hindlimb unloading significantly decreased muscle force in the twitch (31 vs. 12 g, $p < 0.001$), tetanic (55 vs. 148 g, $p < 0.001$), and specific tetanic measurements (12 vs. 22 N/cm², $p < 0.001$). Effects of exercise on force depended on other factors. In B/H, exercise significantly increased twitch (14 vs. 8 g, $p < 0.05$) and specific tetanic force (14 vs. 7 N/cm², $p < 0.01$). Fatigue index was lower in ambulatory (55%) and exercise (52%) versus hindlimb (69%, $p = 0.03$) and no exercise (73%, $p = 0.002$).

Conclusions:

Hindlimb unloading is a significant factor in muscle atrophy. Exercise increased the soleus muscle mass, twitch, and specific force in this model. However, the fatigue index decreased with exercise in all groups. This suggests exercise contributes to functional muscle change in this model of disuse and critical illness.

Key words: Muscle Atrophy, Burn, Critical Illness, Bed Rest

1. Introduction

Severe burn is accompanied by a hypermetabolic state resulting in the loss of body mass [1]. Studies of protein kinetics and DEXA imaging after severe burn have shown that protein catabolism and the resulting loss of lean muscle mass continues after wound healing and can persist 9 to 12 months after injury [2]. Severe muscle loss can increase the risks of sepsis and mortality, cause prolonged recovery times, lead to longer hospitalizations, and increase costs [3]. Given limitations in movement typically present in severe burn, the majority of patients are initially bed bound, which also has been shown to contribute to muscle atrophy [4]. Studies in healthy volunteers have demonstrated that muscle loss during bed rest is exacerbated by elevated serum cortisol, a physiological response to burn; this suggests that the effects of bed rest could be worse in burn [5]. An animal model was recently created to mimic bed rest after burn by placing severely burned rats in a tail suspension system to cause hindlimb unloading. The animals are able to move around the cage freely without bearing weight on their hindlimbs, leading to disuse. This investigation found that hindlimb disuse had an additive effect on muscle atrophy with decreased muscle mass and muscle function [6]. This experiment was the first to closely model the prolonged metabolic changes with muscle atrophy and resultant decrease in function similar to the changes observed in humans after severe burn [6,7]. This newly developed model of bed rest in burn has created the opportunity to closely study treatments aimed at diminishing muscle atrophy and loss of muscle function after severe burn.

It is well established that disuse muscle atrophy can be mitigated with concurrent resistance exercise training [4,5,8-13]. Initiation of resistance exercises at the start of bed rest in healthy volunteers was shown to be protective against loss of muscle protein synthesis [12]. The use of exercise training in burn patients is variable and incompletely evaluated. Practice patterns in the rehabilitation of burn patients typically focus on range of motion, manual muscle testing, and quality of life with less emphasis on exercise training [14]. It has been documented that a combination of aerobic and resistance

exercise training improves lean muscle mass in pediatric burn patients [15-17]. A study of burned adults randomized into control groups or exercise groups with supervised isokinetic leg exercise 3 times per week demonstrated a significant increase in strength [18]. These investigations of exercise training in burn patients have only evaluated its efficacy after recovery from the acute phase, typically as outpatients or 6 months after injury. No studies have evaluated the effects of exercise training during the initial acute phase of recovery. We hypothesize that early initiation of resistance training in burn with bed rest will mitigate muscle atrophy due to disuse. The aim of this study is to evaluate the effects of exercise on muscle mass and function in an animal model of burn with hindlimb unloading.

2. Materials and Methods

Approval was granted for all procedures from the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Health Science Center at Houston. All animal procedures were performed at this location. Forty-eight adult male Sprague-Dawley rats (Charles Rivers, Wilmington, MA, USA) were assigned to four groups ($n = 12$): sham ambulatory (S/A), burn ambulatory (B/A), sham hindlimb unloading (S/H), or burn hindlimb unloading (B/H). Animals were assigned to groups randomly after weight matching for a block design to allow for an even distribution of body mass between groups. These groups were then subdivided into exercise or no exercise ($n = 6$) for a total of eight groups. On arrival, animals were approximately 300g. Animals were placed in standard cages upon arrival and were then housed in specialized metabolic cages (144 in² of usable floor area) with a traction system for hindlimb unloading [11] for 5 days prior to burn procedures. Animals were fed a powder diet (Harlan Teklad #2018) *ad libitum* and housed in a reversed 12-hour light/dark cycle, to allow for exercise training during the animal's dark phase. Room temperature was maintained at 26 ± 2 °C, with a relative humidity of 30% to 80% to simulate, as closely as possible, the ambient temperature maintained in a standard burn unit.

2.1 Thermal Injury

All animals were anesthetized with isoflurane (2-3% in 100% O₂). Each rat was shaved on the dorsal and ventral surface. Rats randomly assigned to the burn treatment groups (B/A or B/H with and without exercise) received a full-thickness scald burn of 40% total body surface area as previously described [19]. The burned animals were resuscitated with 20 ml of intraperitoneal Lactated Ringer's [20]. Animals randomized to the sham treatment groups (S/A or S/H with or without exercise) were submerged in room temperature water after shaving. All animals were given buprenorphine (0.05 mg/kg subcutaneous) at 8 hours after injury for analgesia after burn.

2.2 Hindlimb Unloading

Animals randomized to the S/H or B/H (with and without exercise) groups were placed in a tail harness and attached to a hindlimb unloading system described by Morey-Holton and Globus [11]. After animals fully recovered from anesthesia, the harness was attached to a hook and pulley traction system on top of the cage with a fish-line swivel device. The rats were positioned at a 30° head-down angle from the floor unloading the hindlimbs only. The system rides along two parallel sides of the cage and allows the animals to have 360° access within the cage and to move on an x-y axis. Rats were able to freely access food and water without their hindlimbs contacting the walls of the cage. Animals were observed for signs of distress immediately after unloading and several times a day throughout the study period. Animals in the ambulatory groups were housed in similar cages but without hindlimb unloading.

2.3 Exercise

All animals were pre-trained 10 days prior to injury. Animals were trained to climb 1 meter at an 80° incline. The 6-inch climbing lanes were covered in ½ inch plastic mesh. During the pre-training period, rats were trained daily (a.m. only) for the first 6 days and then twice daily (a.m./p.m.) once acclimated. Each session consisted of 5 climbs from bottom to top with a 10 second rest between repetitions. On the day of injury, rats were

randomly assigned to exercise groups, with the exclusion of rats that outright refused to climb (n=14) during the pre-training period. After injury, weights in a plastic bag were attached with Velcro to the base of the tail during exercise. Weights were calculated as percent body mass of each rat and gradually increased by 10% every few days as tolerated with a maximum weight of 50% body mass.

2.4 Muscle Function

On day fourteen after burn or sham, all animals were anesthetized with isoflurane (2-3% in 100% O₂). The soleus and plantaris on the left leg were isolated and prepared for *in situ* isometric muscle function as previously described by Wu *et al.* [21]. The plantaris tendon was attached with a suture loop to the lever arm of a dual-mode servo muscle lever system (Aurora Scientific, Inc., model 305c). The isometric force of the plantaris muscle was then measured and recorded using the ASI muscle lever system with dynamic muscle control and analysis software (Aurora Scientific, Inc., 615A). The optimal length of the muscle was determined with a series of twitches and interval stretches. Initially, the muscle was put on 20 g of stretch. A single 200 Hz twitch was stimulated with impulse duration of 0.2 msec at 10 mA. The muscle was then stretched 0.3 mm and stimulated again with 25 seconds between stimulations. This pattern was continued until there was less than 2% change in force between twitches, indicating the optimal length (Lo) of the muscle. The length of the muscle at optimal stretch was measured with calipers. The maximum twitch (Pt) and tetanic (Po) parameters were then measured at optimal length. Isometric tetanic function was stimulated at 150 Hz with impulse duration of 0.2 msec, 75 pulses per train, at 10 mA, for a total of 1 second. Po was measured three times with an off-tension recovery period of 2 minutes between stimulations. This same procedure was then performed on the soleus; however, initial tension was started at 2 g of stretch. After completion of a rest period, following the third tetanic force measurement, the soleus fatigue function was measured for a total of 240 seconds at 40 Hz with impulse duration of 0.2 msec, 14 pulses per train, at 10 mA..

High-throughput Dynamic Muscle Analysis software (Aurora Scientific, Inc., 615A) was used to determine the time to peak tension (TPT) and half-relaxation time (1/2 RT) from the twitch response curves. The three tetanic force (P_o) measurements were averaged. The tetanic force was converted to specific force (N/cm^2) using the physiologic cross sectional area (CSA) with the formula $CSA = (\text{muscle mass} \times \cos \theta) / (\text{coefficient of muscle density} \times \text{muscle fiber length})$ [22]. The coefficient of muscle density is 1.06, and θ is the muscle fiber pennation angle of 16.4 and 3.9 for the plantaris and soleus in rats [22]. Muscle fiber length was calculated from measured muscle length at L_o using the reference ratio of 0.34 and 0.69 for plantaris and soleus in rats, respectively. Reference pennation angle, muscle density, and fiber-to-muscle length ratios were based on measurements from Eng *et al.* [22]. The fatigue minimum was considered the absolute force after 4 minutes of the fatigue function test. The fatigue index is the minimum reported as a percentage of the maximum force during the fatigue test.

2.5 Tissue Analysis

Following muscle function analysis, animals were euthanized by exsanguination. The hindlimb muscles on the right were harvested, weighed, snap frozen in liquid nitrogen, and stored at -80°C for protein analysis. The left hindlimb muscles were harvested, weighed, placed in 10% neutral-buffered formalin for 24-48 hours, and stored in 70% ethanol. Samples were paraffin embedded prior to microtome sectioning. Hematoxylin and eosin staining was performed. Quantitative analysis of soleus muscle fiber size was performed with blinded random cluster sampling of the muscle fiber cross-sectional area. Image analysis was performed using ImageJ software.

2.6 Statistical Analysis

Statistical analysis was performed with one-tailed or two-tailed Student's t-test, Mann Whitney U test, or three-way ANOVA using the Holm-Sidak method for pairwise multiple comparisons where appropriate using Systat Sigma Plot®. Numbers reported as mean $\pm$ SEM.

3. Results

3.1 Animal Body Mass

We found a significant difference in animal body mass between treatment groups at day 14 ($p < 0.001$). Using pairwise multiple comparison procedures and controlling for the other treatment levels, we found that body mass was lower in the burn (284 ± 4 vs. 309 ± 5 g, $p < 0.001$), hindlimb-unloaded (283 ± 3 vs. 311 ± 5 g, $p < 0.001$), and exercise treatments (290 ± 4 vs. 303 ± 5 , $p = 0.004$). Evaluating the effects of exercise within groups showed a significantly ($p < 0.05$) lower body mass in B/A and S/A compared to no exercise with a two-tailed and one-tailed t-test, respectively (Fig.1). The decrease in body mass with exercise was not seen in the B/H and S/H groups.

3.2 Plantaris Mass and Protein Content

We found decreased right plantaris muscle mass in burn (B/A, 358 ± 11 mg) and hindlimb-unloaded (S/H, 292 ± 8 mg) compared to sham (S/A, 401 ± 14 mg); hindlimb (B/H, 293 ± 6 mg) was lower than B/A ($p < 0.01$, all comparisons). Within hindlimb unloading, there was no difference between burn and sham. There was no significant difference between groups with or without exercise. When plantaris tissue weight was normalized to body mass, a significant difference was seen only between the ambulatory and hindlimb treatments ($p < 0.001$). There was no difference in plantaris total protein content between groups. (Table 1)

3.3 Soleus Mass and Protein Content

The right soleus mass was significantly lower in hindlimb-unloaded (79 ± 13 mg) versus ambulatory (139 ± 19 mg, $p < 0.001$) and no exercise (103 ± 7 mg) versus exercise (115 ± 7 , $p < 0.01$). There was no difference between burn and sham. This difference persisted when normalized to body mass. There was a significantly lower soleus whole muscle

protein content in the hindlimb compared to the ambulatory groups (4.5 ± 1.1 vs. 8.4 ± 1.1 mg, $p < 0.05$). (Table 1)

3.4 Plantaris Muscle Function

The left plantaris muscle function demonstrated a significantly lower maximum twitch force (Pt) in the hindlimb-unloaded group (89 ± 6.5 g) versus the ambulatory group (111 ± 6.8 g, $p < 0.05$). The absolute tetanic force (Po) was also lower in the hindlimb group (505 ± 20 g vs. 565 ± 21 g, $p < 0.05$). There was no significant effect in the burn or exercise treatments. There was no significant difference between groups in the plantaris specific tetanic force (Po/CSA), the half relaxation time ($1/2$ RT), the time to peak tension (TPT), or the twitch to tetanic ratio (Pt/Po). We also found no difference in the plantaris physiologic cross sectional area (CSA) or optimal muscle length (Lo). (Table 2)

3.5 Soleus Muscle Function

The left soleus isometric twitch force was significantly lower in the hindlimb-unloaded group (31 ± 1.5 vs. 12 ± 1.5 g, $p < 0.001$). Compared to no exercise, the B/H exercise group had a significantly higher twitch force (14 ± 2.1 g vs. 8 ± 1.9 g, $p < 0.05$). Across all other factors, there was no significant difference in the soleus twitch force between exercise and no exercise. There was a longer $1/2$ RT in the B/A versus B/H groups (83 ± 9 ms vs. 48 ± 8 ms, $p < 0.05$). There was a longer soleus TPT in the ambulatory versus hindlimb group (61 ± 4 ms vs. 49 ± 5 ms, $p < 0.05$). (Table 3)

There was a significantly lower soleus tetanic force in the hindlimb-unloaded group (55 ± 5 vs. 148 ± 6 g, $p < 0.001$). B/A had a lower tetanic force in the exercise group versus no exercise (118 ± 14 vs. 165 ± 9 g, $p = 0.02$). In B/H, no difference in soleus absolute tetanic force was seen with or without exercise. All hindlimb groups had significantly lower specific tetanic force than ambulatory (12 ± 1.2 vs. 22 ± 1.3 N/cm², $p < 0.001$). The specific tetanic force in B/H was significantly higher in

exercise versus no exercise (14 ± 1.7 vs. 7 ± 1.3 N/cm², $p < 0.01$); this effect was not seen in S/H or B/A groups. There was no difference in soleus Pt/Po between groups.

The soleus fatigue index was significantly lower in the ambulatory (55%) and exercise (52%) groups versus hindlimb-unloaded (69%) and no exercise (73%) groups ($p = 0.03$, $p = 0.002$ respectively). The soleus fatigue minimum was significantly lower in the hindlimb groups and in the ambulatory exercise group. The soleus CSA was significantly lower in the exercise (5.1 ± 0.3 vs. 6.1 ± 0.3 mm², $p < 0.05$) and hindlimb (4.8 ± 0.3 vs. 6.4 ± 0.3 , $p < 0.001$) compared to no exercise and ambulatory respectively.

3.6 Soleus Myofiber Size

Myofiber size of the soleus was significantly decreased in the hindlimb unloaded versus ambulatory (1552 ± 36 μm^2 vs. 2866 ± 38 μm^2 , $p < 0.001$) and no exercise versus exercise (1790 ± 36 μm^2 vs. 2630 ± 38 μm^2 , $p < 0.001$) groups. There was not a significant difference in soleus myofiber size between the burn and sham groups in the ambulatory animals. However, within the no exercise group, soleus myofiber size was significantly decreased in B/H compared to S/H (means reported in Table 1, $p < 0.05$). Exercise had a greater impact on soleus muscle fiber size recovery in B/H compared to S/H (Fig. 2).

4. Discussion

In our investigation of muscle function after burn and hindlimb disuse, we found that resistance exercise in the acute recovery period significantly increased the mass, myofiber size, and function of the soleus. We did not find significant changes after exercise in the predominately fast-twitch plantaris muscle. These findings are important given that the majority of critically injured patients are subject to prolonged muscle disuse due to bed rest. This is especially true of severely burned patients, who typically require prolonged ventilation, multiple surgical procedures, and decreased mobility as a result of injury patterns.

It is established that muscle loss after burn is a result of a net increase in protein breakdown [2]. Studies in rodents have found decreased fiber size [23] with a greater impact on the fast-twitch muscle groups after burn [21]. It is also known that muscle disuse results in significant atrophy leading to a smaller fiber size with a greater effect on slow-twitch muscle groups [8]. In burn patients, it is unknown what degree of muscle atrophy is caused by disuse compared to burn alone. An animal model of thermal injury with hindlimb unloading was developed to mimic prolonged bed rest after burn. This model was found to demonstrate a closer approximation of the metabolic response and muscle atrophy after severe burn in humans [6,7]. These findings suggest that disuse is a contributing factor to the loss of muscle mass in burn patients. Many investigations have established that exercise diminishes atrophy due to muscle disuse. [4,5,8-13][14][15-17][18] Various forms of exercise have been studied in rat models of hindlimb unloading including treadmill [24], direct sciatic stimulation [25,26], isometric resistance training [25,27], flywheel resistance training [28], and climbing [29]. Dynamic resistance training in hindlimb suspension has been shown to attenuate soleus atrophy with increased mass and muscle protein [30]. Resistance training activates the PI3-AKT-mTOR pathway increasing protein synthesis [9].

Our aim was to evaluate the effects of a resistance exercise program initiated immediately following thermal injury in an animal model of muscle disuse with hindlimb unloading. We chose to evaluate a climbing protocol after burn because it is a voluntary dynamic resistance training model previously shown by Herbert *et al.* to attenuate muscle atrophy in hindlimb-unloaded rats [29,30]. We measured the change in muscle properties and function of the plantaris and soleus muscles because both are plantar flexor muscles, one predominantly fast-twitch and the other slow-twitch, respectively. Plantar flexor muscles and slow-twitch muscles have been shown to have a greater response to hindlimb unloading; whereas fast-twitch muscles have been shown to have a greater loss of mass in response to burn [10,21,31].

4.1 Animal Body Mass and Muscle Characteristics

Consistent with prior findings, animal mass was lower with burn, hindlimb unloading [25,29,32] and decreased further in B/H [6,7]. Exercise did not significantly alter body mass in the hindlimb groups [27,33,34]. B/A was found to have the greatest decrease in body mass with exercise. However, the maintenance of relative soleus and plantaris mass in this group suggests the decrease in mass is not from the loss of lean muscle.

Our findings of decreased plantaris but not soleus mass after burn are consistent with prior experiments identifying a loss of fast-twitch muscle mass after burn [6,21,35]. The plantaris mass was further reduced with hindlimb unloading, similar to findings by Wu *et al.* using this model [6]. However, we did not find a difference between the plantaris mass in S/H and B/H groups as was previously reported by Wu *et al.* [6]. It is possible that pre-training could have contributed to this difference. When the plantaris was normalized to body mass, there was only a significant decrease in the hindlimb treatments compared to ambulatory. We did not observe an effect of exercise on plantaris mass in any treatments. These findings are not surprising given that prior investigations found little effect of exercise on fast-twitch muscles in hindlimb unloading [27,29]. There was no difference in the plantaris total protein content between groups. These findings suggest that hindlimb unloading has a greater impact on plantaris mass than burn and the current exercise protocol utilized does not compensate for the loss of plantaris mass.

There was a significant decrease in the soleus absolute and normalized mass as well as protein content with hindlimb unloading consistent with prior findings [10,27,29,32]. Similarly Herbert *et al.* and other groups found exercise remediated some of the soleus muscle mass loss but did not recover to ambulatory levels in the hindlimb groups [27,29,36]. Similar to prior reports, the soleus myofiber size was decreased with hindlimb unloading [37]. We also found that the soleus myofiber size was lowest in B/H. Exercise partially recovered fiber size; this effect was greater on B/H but did not return to normal levels. Exercise increased myofiber size across all conditions.

4.2 Muscle Function

The maximum twitch and absolute tetanic forces were lower in the plantaris of the hindlimb-unloaded groups, similar to prior findings [38]. We did not observe a difference in plantaris muscle function between ambulatory burn and sham. This is similar to findings by Wu *et al.*, except that we did not appreciate a difference between B/H and S/H absolute tetanic (P_o) and the twitch/tetanic (P_t/P_o) ratio [6]. We did not identify differences in any other plantaris muscle function parameters. These findings are consistent with the changes observed in the plantaris muscle mass. The lack of changes in specific force is consistent with prior findings [6,38] and suggests that decline in plantaris function is related to a decrease in quantity of muscle tissue predominantly as a result of hindlimb unloading. Exercise did not appear to impact muscle atrophy from hindlimb disuse in the plantaris. Prior experiments have shown a mild improvement in muscle function of fast-twitch muscle groups with exercise training during hindlimb suspension [27,29,33]. It is possible that we did not see an effect on the plantaris with exercise training due to differences in duration, frequency, and type of training.

The soleus demonstrated lower twitch force in the hindlimb-unloaded groups as previously reported [6,10,27,29,38]. Exercise increased the twitch force only in the burn hindlimb group but not to control levels. The increased speed to peak (TPT) and relaxation time ($1/2$ RT) observed in the soleus of hindlimb groups is similar to prior findings and could reflect an increased relative proportion of fast-twitch fibers [6,38].

The absolute and specific tetanic soleus forces were lower across all hindlimb groups consistent with prior findings [6,10,27,32]. The soleus specific tetanic force was doubled with exercise in the burn hindlimb treatment only; however, this increase was not sufficient to return to ambulatory levels. Prior studies of exercise in hindlimb unloading have demonstrated variable results, but many did show improvement in the soleus [27]. The lack of improvement in the S/H group after exercise suggests that a more rigorous regimen could be used and may demonstrate an even larger change in the B/H group. There was no difference in the twitch to tetanic ratio (P_t/P_o) across treatments; this is in contrast to prior findings by Wu *et al.* [6]. A possible explanation for the differences in

Pt/Po could be differences in the protocol of isometric muscle function testing such as sequential instead of simultaneous muscle testing and a truncated protocol to obtain Lo. This difference in protocols could have led to altered muscle ATP levels between the two methods at the time of tetanic force testing. Also the pre-training period could have created a protective effect accounting for differences in our findings.

The soleus fatigue index observed in the hindlimb-unloaded group was higher than the ambulatory group, but the minimum force after 4 minutes was significantly lower than the ambulatory groups. The fatigue index in the exercise hindlimb groups was similar to ambulatory levels. The alteration in the contraction times and fatigue properties is suggestive of a change in the relative fiber types in the soleus muscle with hindlimb unloading. We did find a relatively increased percentage of fast-twitch fibers in the soleus muscle after hindlimb unloading. Exercise in B/H further increased the ratio of fast-twitch fibers (data not shown). The soleus is known to have a decrease in the percentage of slow-twitch fibers with unloading [32], and our histological and functional findings suggest that resistance exercise does not diminish the loss of slow-twitch fibers.

5. Conclusions

Consistent with prior investigations of this burn animal model, we found that hindlimb unloading contributed to the loss of muscle mass, myofiber size, and function in severe burn [6]. We have shown that exercise did not impact changes in the plantaris characteristics or muscle function in this model. We did demonstrate some changes in the soleus with an exercise regimen; however, this did not result in a return to normal levels of function. We found that exercise increased soleus force only in the burn hindlimb group. Exercise remediated the loss of muscle mass from hindlimb unloading across groups but was not fully compensatory. Similarly, exercise correlated with an increase in soleus myofiber size across all treatments, with the greatest impact on the burn hindlimb group. These findings suggest that resistance exercise can be protective in sparing some muscle function in burn injury with muscle disuse. Exercise appears to have an increased compensatory effect on hindlimb unloading in the presence of burn. Our histological and

functional findings in the soleus suggest that resistance exercise increases myofiber size but does not prevent the loss of slow-twitch fibers. Our current findings suggest that resistance training does not completely ameliorate the loss of function and will likely need to be part of a regimen aimed at reducing muscle atrophy. Further investigations with exercise regimens that include aerobic and/or pharmacological components are needed.

Legends

Table 1.
Muscle Mass, Myofiber Size, and Protein Content (Right)

Abbreviations used: S/A = sham ambulatory, B/A = burn ambulatory, S/H = sham hindlimb unloading, B/H = burn hindlimb unloading. All numbers reported as mean $\pm$ standard error of the mean. Please refer to results section for statistical significance.

Table 2.
Plantaris Dimensions and Isometric Muscle Function (Left)

Abbreviations used: S/A = sham ambulatory, B/A = burn ambulatory, S/H = sham hindlimb unloading, B/H = burn hindlimb unloading, Lo = optimal muscle length, PCSA = physiologic cross sectional area, Pt = twitch force, TPT = time to peak twitch, $\frac{1}{2}$ RT = half relaxation time, Po = absolute tetanic force, Po/CSA = specific tetanic force, Pt/Po = twitch/tetanic ratio. All numbers reported as mean $\pm$ standard error of the mean. Please refer to results section for statistical significance.

Table 3.
Soleus Dimensions and Isometric Muscle Function (Left)

Abbreviations used: S/A = sham ambulatory, B/A = burn ambulatory, S/H = sham hindlimb unloading, B/H = burn hindlimb unloading, Lo = optimal muscle length, PCSA = physiologic cross sectional area, Pt = twitch force, TPT = time to peak twitch, $\frac{1}{2}$ RT = half relaxation time, Po = absolute tetanic force, Po/CSA = specific tetanic force, Pt/Po = twitch/tetanic ratio. All numbers reported as mean $\pm$ standard error of the mean. Please refer to results section for statistical significance.

Figure 1.
Animal Body Mass

Abbreviations used: S/A = sham ambulatory, B/A = burn ambulatory, S/H = sham hindlimb unloading, B/H = burn hindlimb unloading. Animals were weighed on day 14 after burn or sham. Exercise was compared to No Exercise within treatment groups. # $p <$

0.05 with one-tailed t-test and * $p < 0.05$ with two-tailed t-test. Please refer to the results section for significant differences between other factors.

Figure 2.

Soleus Myofiber Size

Abbreviations used: S/A = sham ambulatory, B/A = burn ambulatory, S/H = sham hindlimb unloading, B/H = burn hindlimb unloading. Exercise was compared to No Exercise within treatment groups. # $p < 0.05$ with one-tailed t-test and * $p < 0.001$ with two-tailed t-test. Please refer to the results section for significant differences between other factors.

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Table 1

Muscle Mass, Myofiber Size, and Protein Content (Right)								
Group		Plantaris			Soleus			
		Wet Weight (mg)	Weight (mg) per 100g Body Mass	Protein Content (µg)	Wet Weight (mg)	Weight (mg) per 100g Body Mass	Protein Content (µg)	Myofiber Size (µm ²)
No Exercise	S/A	394 ± 24	117 ± 7	24.6 ± 7.4	142 ± 5	43 ± 2	7.13 ± 0.72	2187 ± 488
	B/A	365 ± 17	119 ± 4	24.3 ± 2.4	122 ± 7	40 ± 2	2.95 ± 0.60	2395 ± 291
	S/H	290 ± 14	98 ± 6	28.4 ± 3.6	69 ± 5	23 ± 2	11.7 ± 4.62	1499 ± 89
	B/H	289 ± 9	106 ± 3	20.3 ± 3.7	77 ± 7	28 ± 3	3.82 ± 1.67	1079 ± 79
Exercise	S/A	408 ± 25	130 ± 6	24.1 ± 3.1	147 ± 9	47 ± 2	8.55 ± 0.93	3739 ± 43
	B/A	349 ± 13	123 ± 5	19.8 ± 0.7	143 ± 7	50 ± 2	4.24 ± 0.75	3146 ± 305
	S/H	294 ± 14	102 ± 2	31.2 ± 15	84 ± 2	29 ± 1	9.93 ± 0.52	1648 ± 130
	B/H	296 ± 10	109 ± 4	22.9 ± 3.0	86 ± 4	32 ± 2	4.59 ± 0.93	1985 ± 170

Table 2

Plantaris Dimensions and Isometric Muscle Function (Left)

Parameter		No Exercise				Exercise			
		S/A	B/A	S/H	B/H	S/A	B/A	S/H	B/H
Muscle	Wet weight (mg)	459 ± 22	383 ± 22	341 ± 16	337 ± 13	405 ± 27	410 ± 24	347 ± 14	390 ± 61
	Lo (mm)	39 ± 0.9	39 ± 1.0	37 ± 1.1	35 ± 0.7	39 ± 1.0	40 ± 1.8	38 ± 0.8	40 ± 1.4
	PCSA (mm ²)	31 ± 0.9	26 ± 1.9	25 ± 1.4	26 ± 1.3	28 ± 2.1	29 ± 2.7	24 ± 1.1	27 ± 5.6
Twitch Force	Pt (g)	102 ± 12	117 ± 15	93 ± 7.3	89 ± 9.3	129 ± 27	97 ± 13	95 ± 11	80 ± 7.8
	TPT (ms)	28 ± 1.3	30 ± 0.9	30 ± 1.3	29 ± 1.2	30 ± 0.6	31 ± 1.0	30 ± 1.8	32 ± 0.7
	1/2 RT (ms)	18 ± 1.7	20 ± 1.0	19 ± 1.2	18 ± 1.4	18 ± 2.1	16 ± 2.1	17 ± 1.2	18 ± 1.2
Tetanic Force	Po (g)	565 ± 42	602 ± 39	539 ± 30	517 ± 37	603 ± 72	492 ± 54	511 ± 13	452 ± 29
	Po/CSA (N/cm ²)	18.2 ± 1.4	23.2 ± 2.3	21.9 ± 1.6	20.2 ± 1.8	20.3 ± 1.7	17.9 ± 2.3	21.4 ± 0.5	18.6 ± 2.4
Pt/Po (%)		19 ± 1.7	20 ± 2.5	17 ± 0.5	17 ± 1.2	21 ± 2.1	20 ± 1.4	18 ± 1.8	18 ± 2.6

Table 3

Soleus Dimensions and Isometric Muscle Function (Left)

Parameter		No Exercise				Exercise			
		S/A	B/A	S/H	B/H	S/A	B/A	S/H	B/H
Muscle	Wet weight (mg)	204 ± 16	205 ± 11	139 ± 15	174 ± 36	175 ± 13	197 ± 12	127 ± 10	114 ± 7.0
	Lo (mm)	44 ± 1.3	43 ± 2.0	39 ± 1.7	37 ± 0.4	41 ± 1.9	44 ± 2.2	40 ± 1.0	41 ± 1.1
	PCSA (mm ²)	6.3 ± 0.4	6.5 ± 0.4	5.0 ± 0.6	6.3 ± 1.4	6.0 ± 0.6	6.0 ± 0.4	4.3 ± 0.3	3.8 ± 0.4
Twitch Force	Pt (g)	31 ± 2.8	36 ± 4.4	14 ± 2.1	8.0 ± 2.0	30 ± 2.6	27 ± 2.0	11 ± 0.6	14 ± 2.1
	TPT (ms)	58 ± 8.0	66 ± 5.5	50 ± 6.6	48 ± 7.0	49 ± 11	73 ± 5.3	48 ± 14	49 ± 4.5
	1/2 RT (ms)	57 ± 12	90 ± 6.0	65 ± 11	51 ± 11	58 ± 17	80 ± 4.0	60 ± 17	45 ± 5.1
Tetanic Force	Po (g)	159 ± 18	165 ± 9.3	56 ± 4.4	42 ± 5.5	150 ± 16	118 ± 14	66 ± 8.3	54 ± 4.9
	Po/CSA (N/cm ²)	25 ± 2.8	25 ± 1.7	12 ± 2.2	7.0 ± 1.3	23 ± 2.4	20 ± 1.9	17 ± 2.0	14 ± 1.7
Pt/Po (%)		20 ± 1.4	22 ± 1.5	24 ± 6.7	19 ± 2.5	26 ± 5.2	23 ± 1.8	21 ± 3.7	26 ± 4.9
Fatigue	Minimum	105 ± 15	100 ± 10	35 ± 4.0	24 ± 2.8	76 ± 21	48 ± 16	31 ± 10	32 ± 11
	Index (%)	66 ± 5.9	69 ± 6.3	82 ± 11	76 ± 3.0	52 ± 11	37 ± 11	63 ± 6.3	58 ± 13

Figure 1

Figure 2