

Pre-habilitation and rehabilitation for attenuating hindlimb unweighting
effects on skeletal muscle and gait in adult and old rats

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Key words: skeletal muscle force mass, muscle force, aging, exercise, reloading

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Key words: stride length, muscle force, aging, exercise, reloading

Objective: To compare the effectiveness of pre-habilitation, exercise prior to hindlimb unweighting (HLU), versus rehabilitation, exercise given following HLU, to no exercise, on skeletal muscle mass and force, and

gait function.

Design: Randomized control trial.

Setting: Animal Laboratory.

Participants: Male SPF Fisher344/Brown Norway rats (n=149). Groups consisted of adult and old controls, HLU, pre-habilitation, rehabilitation, natural cage recovery (reloading) and exercise without HLU

Interventions: 10 days of general conditioning exercise was given to 6 mos adult and 30 mos old rats before or after a week of HLU.

Main outcome measures: gait stride length and width. Soleus, plantaris, EDL and P. Longus mass and peak contractile force. Whole gastrocnemius (GAST) mass. Total protein concentration soleus and GAST.

Results: Muscle mass (~30%) and force (24-36%) declined with age in all muscles studied. In adult rats declines in muscle mass occurred with HLU in soleus, plantaris and GAST. Pre-habilitation did not prevent the loss of muscle mass in adult rats. Rehabilitation and natural recovery effectively restored soleus and GAST muscle mass in adult rats but not soleus peak force. Old rats had a significant 23% HLU effect only on GAST mass (1670±129 control, 1274±184 HLU). Pre-habilitation did not prevent the decline in GAST mass. Rehabilitation in old rats restored GAST mass to within 13% of control. Pre-habilitation was effective for preventing and rehabilitation was effective for restoring soleus contractile force in old rats (Control 114±9, HLU 67±22. Prehab 106±31, Rehab 120±26g), compared to recovery without exercise (86±29g). A significant reduction in stride length was observed with aging (136±18 vs. 98±10 mm), which decreased further with HLU (78±14mm). Pre-habilitation attenuated HLU-related reductions in stride length and rehabilitation was effective for stride length restoration in old rats.

Conclusions: Exercise, particularly rehabilitation, was more effective for old than young rats. Pre-habilitation and rehabilitation diminished some of the detrimental effects of HLU on skeletal muscle mass and force, and gait function in old rats.

INTRODUCTION

In humans and in animals, a significant age-related loss in muscle mass and strength occurs, particularly in the lower extremities¹⁻⁶. Age-related strength loss in the lower extremities of humans is associated with declines in walking function, particularly the shortening of stride, and an increase in gait asymmetry⁴. Old rats also exhibit a reduction in stride length, an increase in stride width, and more gait asymmetry with age, that is associated with muscle atrophy in the lower extremities^{7,8}.

Many older adults experience periods of inactivity (e.g., bed rest) which, when superimposed on age-related decline, results in additional loss in muscle mass and strength that often leads to compromised functional capacity^{9,10}. The effects of reduced physical activity on the musculoskeletal system have been studied extensively in animal models¹¹⁻¹⁵. For several decades the hindlimb unweighting (HLU) animal model has been used to document the significant loss in muscle mass and function that occurs when weight-bearing is removed from the lower extremities. In young and in old rats, HLU results in skeletal muscle atrophy that bears similarity to the decline that occurs with bed rest in humans^{9,16,17}.

Resistance exercise has been shown to improve lower extremity strength in older adults^{18,19}. These strength increases have been associated with improved gait function, suggesting 1) that part of the decline in gait and strength with age is due to inactivity and, 2) that a healthy older adult is able to respond to an exercise stimulus designed to increase strength¹⁹. Rehabilitation exercise is effective for the successful remediation of inactivity-induced losses in strength and has been shown to restore lost function, or at least improve function, in older adults^{20,21}. Although pre-habilitation is hypothesized to be effective^{22,23}, little is understood about its potential for modifying change in skeletal muscle mass and strength or gait function in an aging organism. We chose the HLU animal model to explore the potential of pre-habilitation exercise to attenuate the declines in muscle mass and strength and function observed with imposed inactivity. The primary purpose of this investigation was to determine pre-habilitation versus rehabilitation effectiveness for the remediation of HLU effects on skeletal muscle mass and force, and on gait function, in adult and old animals. We hypothesized that either pre-habilitation or rehabilitation would be effective for attenuating HLU-related declines. A secondary intent of this study was to evaluate pre-habilitation versus rehabilitation effectiveness compared to spontaneous recovery (no exercise intervention) following HLU.

METHODS

Animals. Specific pathogen-free Fisher 344/Brown Norway males were procured from the aging colony maintained by the National Institute on Aging. Pathogen-free animals were chosen to ensure the study of age-related decline without the potential confound of disease. Rats were 6 and 30 months of age when they arrived and upon receipt they were placed in a barrier facility for one week of acclimatization. Six month old rats are stable and mature (slow growing). Thirty month old animals exhibit age-related sarcopenia but are not yet pre-morbid. After acclimatization, six month old rats were randomly assigned to adult control, adult hind limb unweighted, adult pre-habilitation, adult rehabilitation, adult recovery (reloading) or adult exercise. Thirty month old rats were assigned to old control, old HLU, old pre-habilitation, old rehabilitation, old cage recovery, or old exercise. Between 10 and 15 rats were in each of the 12 groups. Power analyses of data collected from previous studies suggested that 9-10 rats were needed per group for a HLU effect size of 0.8, $\alpha=0.5$. Additional animals were used to enhance the robustness of the data and to accommodate for potential loss of data or rats.

Vivarium temperature was 22-23°C and a 12:12 haour light-dark cycle extending from 7 am to 7 pm was maintained. Rats were individually housed in 18x18" plexiglass cages and all rats, control and unweighted, had free access to food and water *ad libitum*. All protocols were performed in accordance with the *Guide for the Care and Use of Laboratory Animal* as approved by the American Physiological Society and by the Animal Use Boards of both universities conducting these studies.

Hindlimb unweighting (HLU). All rats were familiarized to the hindlimb unweighting procedure for an hour a day for 5 days preceding suspension. We have found that rats acclimated to HLU adjust to the unweighting experience without an increase in adrenal gland weight. HLU rats were put into a harness system that suspended the rat at an angle of ~30° to remove weight from the lower limbs. This method is a modification of the procedure described in detail by Morey-Holton and Globus²⁴. To accomplish the unweighting, two small casts were applied, one to the tail and another around the thorax to eliminate excessive lumbar lordosis. Wires from the tail and body casts were fed through a leader that was attached to a swivel hook at the top of the cage, thus allowing animals access to all parts of the cage. Lower limbs were free to move in all directions and weight was borne on the forelimbs. The duration of HLU was 7 days. This was chosen as a significant HLU-related decline in hindlimb muscle mass and force occurs in that time frame ^{14,15,25}.

Pre-habilitation. For 10 days prior to unweighting rats were taken from their cages and placed in a

'gymnasium' where there were toys to climb upon, around, and through. In addition to spontaneous ambulation activity, all animals were obligated to perform each of the following: jump out of a 4" square box that was 6" deep a total of 20x, walk up and down a three foot long 30° ramp 6x in each direction. Rats also had to hold onto a mesh screen for ~1 minute while the screen was tilted sideways in both directions, backwards and head down during each exercise bout. Finally, rats were obligated to balance for 5 minutes within an 8" clear plastic ball that was rolled from side to side. Holding onto the wire mesh and balancing inside the ball required lower extremity musculature to brace. All exercises were intended to recruit lower extremity musculature using body weight as resistance. Each individual rat received the same exercise protocol and the same number of exercise repetitions, thus the amount of exercise was standardized as much as possible for each animal. Between bouts of ramp walking and other specific tasks, rats were in an environment that encouraged climbing up on objects, exploring their surroundings, and standing on their hind limbs.

The pre-habilitation program was given for 10 days prior to HLU and each group session (3-4 rats/group) lasted ~45 minutes. The exercise program was chosen because it was less likely to cause injury to old rats (compared with resistance exercise), it requires little equipment, and was consistent with the capabilities of old rats. This form of activity and this type of exercise has been shown to improve function in old rats⁸.

Rehabilitation. The rehabilitation protocol was identical to that employed for pre-habilitation except that 10 days of exercise was given following 7 days of HLU rather than before.

Gait analysis. Walking records were obtained on all rats as an index of functional capability. Older rats, like older humans, exhibit a reduction in stride length and an increase in stride width with age, when compared to young animals⁷. Gait stride is also sensitive to change with running exercise and with alterations in lower extremity strength^{7,8}. To obtain reliable and consistent data, rats were acclimatized during the week of HLU training to a 3 foot long plexiglass square tube. Rats were taught to walk the entire length of the tube at their preferred or 'normal' gait speed without stopping or turning. Once rats walked the entire length of the tube without stopping with consistent gaits, 3-5 permanent records of stride length and stride width were obtained which yielded ~40 strides for each rat. To be sure that gait values were consistent we tested 6 young and 6 old rats on two different days, with 25 strides analyzed for each day. Reliability of data collection (stride length and width) was demonstrated with ICC values of 0.98 for young and 0.95 for old

animals. Our coefficient of variation for young rats was a little less than 2% whereas for old rats our coefficient of variation was ~3%. To obtain a permanent record a 3 foot long sheet of paper was inserted into the gait tube and the paws of each rat were coated with blue (fore paws) or black (rear paws) ink. The distance from the heel strike of the left foot to the next heel strike on the same side was measured as stride length. For stride width, a perpendicular line was drawn from a line bisecting each paw and the distance of that line was measured to the nearest millimeter.

Contractile tension. The ultimate reflection of muscle quality is its contractile tension generating capability. Thus, peak tetanic tension or P_o values were obtained for four muscles of interest in all rats. The day following rehabilitation exercise or at the end of the final day of unweighting, rats were anesthetized with an IP injection of sodium pentobarbital (65 mg/kg) with 0.05 ml injections given as needed to maintain deep anesthesia. Body temperature was maintained by keeping the anesthetized rat on a water-jacketed heating pad. A small incision through the skin on the lateral side of the left leg exposed the distal tendons of the soleus, plantaris, peroneus longus, and extensor digitorum longus (EDL). These muscles were chosen because of their discreet attachments (ensuring accurate removal and weighing) and for their differences in fiber type and function. Tendons were attached in turn to a Grass force transducer with 2.0 silk. The tibial and common peroneal nerves were isolated in turn and placed on a bipolar stimulation electrode. The exposed portion of each nerve and muscle were bathed in 37°C mineral oil or rat Ringer's solution, respectively. After determination of optimal muscle length (L_o), peak tetanic tension was elicited by 0.5 ms supramaximal pulses (1.5 volts) delivered at 100 Hz for soleus (400 ms duration), and 150Hz for the EDL (250 ms duration), peroneus longus (300 ms duration) and plantaris (350 ms duration). Several contractions were elicited for each muscle to ensure maximal P_o values were obtained. Two minutes of rest were given between each maximal contraction.

Muscle mass, total protein concentration. Once experiments were complete, animals were given an overdose of pentobarbital, and the 4 muscles of interest plus GAST were dissected out bilaterally and weighed. Values for total protein concentration were assessed for the locomotor GAST and postural soleus, using the bicinchoninic acid method (Sigma Chemical, St. Louis MO).

Contractile tension/muscle mass. To provide a general indication of muscle quality, contractile tension values were divided by muscle mass to create a ratio of P_o /mass.

Data Management: Values for muscle mass, muscle mass/body mass ratios, total protein concentration, contractile tension, P_o /mass, and gait stride length and width, were analyzed using SPSS, version 11.5. A 2x6 ANOVA was performed initially to identify age, group and age * group interactions. One by six ANOVA also was performed to identify differences within the adult groups and within the old groups of rats. If significance was achieved ($p < 0.05$) a Scheffe post-hoc test was performed to determine where significant differences existed. All data are presented as means \pm standard deviation.

RESULTS

Body mass and Muscle mass: adult rats. Exercise rats and all unweighted groups weighed less than the controls (Table 1), There was a significant loss of muscle mass with unweighting in the adult soleus (Group effect $p < 0.001$, $F = 15.474$, $Eta^2 = 0.402$), plantaris (Group effect $p < 0.001$, $F = 10.035$, $Eta^2 = 0.304$), EDL (Group effect $p < 0.001$, F value = 11.09, $Eta^2 = 0.537$), and gastrocnemius (Group effect $p < 0.001$, $F = 39.619$, $Eta^2 = 0.646$, Figure 1a). P. Longus was unaffected (data not shown). To accommodate for changes in body mass, muscle mass was normalized to body mass. Muscle mass/body mass ratios were significant only for HLU-related declines in GAST (Table 2). Differences between pre-habilitation and rehabilitation rats were apparent for plantaris and GAST wet mass but when normalized to body weight the pre-habilitation ratio for plantaris was higher than the ratio for rehabilitation rats. There was no apparent advantage of exercise over spontaneous recovery.

Body mass and Muscle mass: old rats. Old rats lost ~10% of their body mass with unweighting (Table 1). As expected, muscle mass declined significantly with age in all five muscles examined (Main Age effect: F values = 15.044 for soleus, 71.654 for plantaris, 62.615 for EDL, 20.44 for P. Longus and 186.351 for GAST, Figure 1b). A significant age x group interaction existed for soleus ($F = 15.94$, $Eta^2 = 0.139$), EDL ($F = 4.101$, $Eta^2 = 0.155$), and plantaris ($F = 4.789$, $Eta^2 = 0.172$, Figure 1b). For old rats, unweighting resulted in a smaller absolute decline in muscle mass than what was seen in the adult unweighted animals. Only GAST mass declined significantly with HLU (24%) which pre-habilitation did not prevent. Rehabilitation and recovery were effective for restoring GAST mass to normalized control values (Table 2). Soleus mass normalized to body mass did not improve in rats allowed to spontaneously cage recover (Table 2).

Contractile tension (P_o): adult rats. HLU resulted in a significant loss in soleus (Main Group effect $p < 0.001$, F value = 22.59, $Eta^2 = 0.699$) and P. Longus muscle force ($P = 0.008$, $F = 3.055$, $Eta^2 = 0.122$). A strong trend

toward loss in P_o ($p=0.062$) with HLU was observed in plantaris (Table 3). Pre-habilitation did not prevent the loss in soleus muscle force with HLU nor did rehabilitation result in full recovery. When contractile tension was expressed as a ratio of tension to muscle mass (P_o/mass) differences between the control group, HLU, recovery and exercise groups remained for soleus ($F=15.94$, $\text{Eta}^2=0.404$, Table 4).

Contractile tension: old rats. The expected age-related decline in P_o was observed in all four muscles tested (Main Age effects: Sol $p<0.001$, $F=14.437$, $\text{Eta}^2=0.175$, Plantaris $p<0.001$, F value 47.946, $\text{Eta}^2=0.407$, P. Longus $p<0.001$, F value=62.639, $\text{Eta}^2=0.486$, EDL $p<0.001$, F value=73.445, $\text{Eta}^2=0.534$, Table 3). HLU resulted in a significant reduction in peak tetanic tension in soleus in old rats but the absolute decline in P_o for old rats was less than that observed for young animals (Table 3). In contrast to young rats, pre-habilitation was effective for attenuating the HLU-related loss in muscle force in soleus, and rehabilitation was effective for restoring soleus force. Differences in soleus peak tetanic tension between pre-habilitation and rehabilitation rats and controls were not significant. Ten days of cage recovery did not increase soleus peak muscle force. For P. Longus, a significant decline in P_o was observed with HLU which was attenuated by pre-habilitation but rehabilitation did not enhance recovery in force. The ratio of $P_o/\text{muscle mass}$ revealed a significant decline with unweighting only for P. Longus ($F=19.094$, $\text{Eta}^2=0.224$, Table 4). An Age x Group interaction was apparent for soleus only ($p<0.001$, $F=10.596$, $\text{Eta}^2=0.319$)

Gait characteristics: adult rats. There was a trend toward a shortening of stride length with unweighting and an increase in stride length with pre-habilitation and rehabilitation exercise but differences were not significant at any time point. Exercise rats had a significant increase in stride length. There were no HLU or exercise effects on stride width (Table 5).

Gait characteristics: old rats. An age-associated reduction in stride length and an increase in stride width were observed in old rats ($p<0.01$, Table 5). Half of the increase in stride width was due to the proportionate increase in body size with age ($R^2=0.50$). HLU resulted in a significant reduction in stride length that was attenuated by pre-habilitation and eliminated by rehabilitation. Normal cage activity was also effective for attenuating the HLU-related decline in stride length but rehabilitation was more effective. There was no effect of exercise or unweighting on stride width.

Total protein concentration: adult rats. No significant differences in GAST protein concentration were noted for any of the conditions studied (Table 6). A significant reduction in protein concentration was seen in the

soleus with unweighting that was offset with either pre-habilitation or rehabilitation exercise.

Total protein concentration: old rats. There was no significant decline in total protein concentration in either muscle with aging (Table 6). Unweighting had no significant effect on the GAST. Soleus, however, had a loss in total protein concentration with unweighting that was not restored by either rehabilitation or pre-habilitation.

DISCUSSION

It was hypothesized that both pre-habilitation and rehabilitation would be effective for modifying the detrimental effects of simulated bed rest and results lend partial support for the hypothesis. Major findings include: 1) absolute HLU-related losses in muscle mass and force were greater in adult than old rats, 2) pre-habilitation and rehabilitation were more effective in old than adult rats and effectiveness differed on a muscle to muscle basis, 3) although pre-habilitation helped to attenuate decline, rehabilitation exercise restored most of the decline associated with 7 days of HLU and 4) for soleus, pre-habilitation and rehabilitation were more effective in old than adult rats and more effective than spontaneous recovery alone.

As noted by other investigators, HLU resulted in a loss of body mass^{12-14,26} which in part, contributed to the decline in muscle mass. Also consistent with previous reports, 7 days of HLU resulted in significant atrophy (~25%) of the calf muscles in adult rats but had little effect on the non-postural muscles^{14,27}. In contrast to adult rats, the magnitude of atrophy response in the old rats was ~10% less. There is evidence that muscles from old rats are more sensitive to inactivity-related decline than young rats^{13,26}. For example, Deschenes et al reported that HLU-related atrophy of the soleus and EDL occurred only in their 22 month rats compared to their 8 month old animals¹³. However, there was no age-associated atrophy in their 22 month old rats prior to HLU and Fisher 344 rats are not considered 'old' until the age of 24 months, when mortality reaches 50%²⁸. Chen and Alway²⁹ unweighted senescent rats (38 months) and found the magnitude of absolute soleus and plantaris decline to be greater than that of their 3 month animals. Three month old rats are growing rapidly however, and when Chen and Alway accounted for differences in body mass by creating ratios of muscle mass/body mass, muscle loss was actually greater in the young animals, Our rats were old but not senescent and it is possible that 38 month old rats lose body mass at a more rapid rate than 30 month animals. Chen and Alway unweighted rats for 3 weeks as opposed to our one week which may also contribute to the difference in findings.

Of greater concern than atrophy *per se* in an aged organism is the magnitude of force decline in relation to muscle mass loss, particularly with reduced physical activity. Old rats in this study had already undergone a 25% loss in soleus P_0 /muscle mass prior to unweighting and had an additional 25% decline with one week of HLU. P. Longus had not shown a decline in P_0 /muscle mass with aging but the week of HLU rapidly degraded the ratio of muscle force to muscle mass in old rats by 22%. The disproportionate loss of muscle force with inactivity in old animals may provide insight as to why the functional capacity of older adults deteriorates so rapidly with bed rest^{9,10,17}. Strategies to prevent the marked loss of contractile force in an aged organism are vitally important, particularly given the findings of a failure to recover lost soleus mass and tension in old rats that have been immobilized^{30,31}. Failure to regain muscle mass and force following a period of inactivity is observed only in old animals, not young rats, suggesting that old muscle has limited restorative potential. Satellite cell function is altered in old rats and likely contributes to the impaired soleus response to reloading³².

Our old rats also failed to recover soleus mass and contractile tension with 10 days of reloading (spontaneous cage activity) following 7 days of HLU. The lack of recovery in this muscle may be due to several factors including damage due to excessive overload and failure to upregulate the necessary signaling pathways required for protein synthesis³¹⁻³³. Reloading the soleus after a bout of HLU has been shown to result in muscle damage³⁴ which may persist long after the resumption of weight-bearing³⁵. Our old rats weighed ~100g more than the adult rats, had significant age-associated soleus muscle atrophy before HLU, and sustained additional atrophy following HLU. It is possible the large body mass imposed on a very atrophied soleus during recovery resulted in more damage in old rats than in adult rats, preventing the recovery of muscle mass and force. However, rehabilitation exercise was effective for enhancing muscle mass and force after HLU, suggesting that an aging soleus, even if damaged, is able to recover with exercise.

Exercise alone significantly increased muscle mass and contractile force in the old but not adult rats. The success of the exercise program in the old rats may have been due to the nature of the exercise stimulus. Because old rats weighed more and had smaller muscles, the exercises given were more challenging for the old than adult animals. The improvements in muscle mass and force in the old rats supports the concept that old muscle is adaptable if the stimulus is adequate as suggested by studies in

older adults¹⁸⁻²¹. The findings from our study are in contrast to those of Blough and Linderman³⁵ and others^{36,37} who reported a limited hypertrophic response in old rats. In these studies overload of the plantaris muscle was achieved either through ablation or denervation of the synergists (e.g., gastrocnemius). Although rats in the Blough and Linderman³⁵ and Degens and Alway³⁷ studies were older (33 and 38 months, respectively) than the animals used in this investigation, rats studied by Thomson and Gordon³³ were the same age as those in our study, suggesting that age is not the only factor associated with the lack of hypertrophy. The observation of an increase in muscle mass and force in this study compared to the lack of hypertrophy with overload suggests that improvement in muscle mass and strength can occur in an aged organism if the stimulus is not excessive.

The exercise program used in this study used a combination of isometric, concentric and eccentric activities and some of the exercises challenged end range of motion at the ankle (e.g., ball rolling, ramp walking). There is evidence to indicate that eccentric exercise prior to HLU exerts a protective effect, attenuating soleus damage²⁵. Pre-habilitation may have been effective in this study for the partial preservation of soleus mass and force because eccentric contractions helped protect the muscle from HLU-induced damage. There is also evidence that passive stretch attenuates soleus muscle damage during HLU³⁸. Possibly in this study pre-habilitation attenuated soleus decline by preventing excessive muscle shortening. Isometric exercise has also been shown to prevent soleus muscle mass and force decline and to attenuate plantaris mass and force decline during HLU³⁹. Our findings indicate that exercise that includes isometrics, given prior to HLU, was effective for attenuating soleus muscle loss. Old rats in the exercise group had larger muscles than the controls and thus pre-habilitation may have exerted protective effects on the old soleus simply by increasing muscle size and force prior to unweighting.

HLU resulted in a significant decline in total protein concentration in adult rats that was eliminated by either pre-habilitation or rehabilitation exercise. Old rats also exhibited a significant decline in total soleus protein concentration in response to unweighting, and neither form of exercise restored total protein concentration to control values. Contractile tension improved in old rats to the extent that differences between old control and pre-habilitation and rehabilitation rats were not apparent. This finding suggests that the protein lost with unweighting was not confined to contractile proteins such as myosin. It is possible that mitochondrial protein showed a disproportionate loss in old rats. There is evidence for loss of mitochondrial

protein with unweighting but only in young rats⁴⁰.

Age-associated declines in strength are associated with loss of gait function in older humans⁴¹ and rats⁸ and it is probable in this study that changes in stride length with unweighting and with intervention were related to the losses and increases in muscle force that occurred with HLU and exercise, respectively. In humans, age-related deficits in lower extremity strength, sensation, balance and range of motion are all reflected in a deterioration of gait. Gait stride (a major determinant of velocity) and width (determinant of balance) in the rat were sensitive to change with just 7 days of HLU and with 10 days of exercise and thus, gait provides an easy method to obtain an index of functional capacity in this animal model.

In summary, HLU resulted in a greater loss of muscle mass and contractile tension in adult rats compared to old rats. Pre-habilitation attenuated soleus decline and rehabilitation was effective for soleus restoring muscle mass and contractile function in old but not adult rat soleus. Pre-habilitation or rehabilitation for the soleus were more effective than no intervention suggesting that exercise is necessary for the reduction of HLU-related declines in old skeletal muscle.

References

1. Hepple RT, Ross KD, Rempfer A. Fiber atrophy and hypertrophy in skeletal muscles of late middle-aged Fisher 344 x Brown Norway F1- Hybrid rats. *J Gerontol: Biol Sci* 2004;59A: 108-117
2. Evans WJ. What is sarcopenia? *J Gerontol: Biol Sci* 1995;50A:5-14.
3. Taaffe DR, Cauley JA, Danielson M, Nevitt MC, Lang TF, Bauer DC, Harris TB. Race and sex effects on the association between muscle strength, soft tissue, and bone mineral density in healthy elders: the health, aging and body composition study. *J Bone Mineral Res* 2001;16:1343-1352.
4. Bassey EJ, Fiatarone MA, O'Neill EF, Kelly M, Evans WJ, Lipsitz LA. Leg extensor power and functional performance in very old men and women. *Clin Sci* 1992;82:321-327.
5. Gonzalez E, Messi ML, Delbono O. The specific force of single intact extensor digitorum longus and soleus mouse muscle fibers declines with aging. *J Membrane Biol* 200;178:175-183.
6. Alnaqueeb MA, Goldspink G. Changes in fibre type, number and diameter in developing and ageing skeletal muscle. *J Anat* 1987;153:31-45.
7. Dorner H, Otte P, Platt D. Training influence on age-dependent changes in the gait of rats. *Gerontology* 1996;42:7-13
8. Brown M, Taylor J, Gabriel R. Differential effectiveness of low-intensity exercise in young and old rats. *J Gerontol: Biol Sci* 2003;58A:889-894

9. Sager MA, Franke T, Inouye SK, Landefeld CS, Morgan TM, Rudberg MA, Sebens H, Winograd CH. Functional outcomes of acute medical illness and hospitalization in older persons. *Arch Intern Med* 1996;156:645-652.
10. Mahoney JE, Palta M, Johnson J, Jalaluddin M, Gray S, Park S, Sager M. Temporal association between hospitalization and rate of falls after discharge. *Arch Intern Med* 2000;160:2788-2795.
11. Edgerton VR, Roy RR, Allen DL, Monti RJ. Adaptations in skeletal muscle disuse or decreased-use atrophy. *Am J Phys Med Rehabil* 2002;81:S127-S147.
12. LeBlanc A, Marsh C, Evans H, Johnson P, Schneider V, Jhingran S. Bone and muscle atrophy with suspension of the rat. *J Appl Physiol* 1985;58:1669-1675.
13. Deschenes MR, Britt AA, Chandler WC. A comparison of the effects of unloading in young adult and aged skeletal muscle. *Med Sci Exerc Sport* 2001;33:1477-1483
14. Thompson LV, Johnson SA, Shoeman JA. Single soleus muscle fiber function after hindlimb unweighting in adult and aged rats. *J Appl Physiol* 1998;84:1937-1942
15. Hurst JE, Fitts RH. Hindlimb unloading-induced muscle atrophy and loss of function: protective effect of isometric exercise. *J Appl Physiol* 2003;95:1405-1417
16. Convertino VA, Bloomfield SA, Greenleaf JE. An overview of the issues: physiological effects of bed rest and restricted physical activity. *Med Sci Sports Exerc* 1997;29:187-190.
17. Harper CM, Lyles YM. Physiology and complications of bed rest. *J Am Geriatr Soc* 1988;36:1047-1054.
18. Binder EF, Schechtman KB, Ehsani AA, Steger-May K, Brown M, Sinacore DR, Yarasheski KE, Holloszy JO. Effects of exercise training on frailty in community-dwelling older adults: results of a randomized, controlled trial. *J Am Geriatr Soc* 2002;50:1921-1928.
19. Seynnes O, Singh MAF, Hue O, Pras P, Legros P, Bernard PL. Physiological and functional responses to low-moderate versus high-intensity progressive resistance training in frail elders. *J Gerontol: Med Sci* 2004;59A:503-509
20. Binder EF, Brown M, Sinacore DR, Steger-May K, Yarasheski KE, Schechtman KB. Effects of extended outpatient rehabilitation after hip fracture. *JAMA* 2004;292:837-846
21. Tinetti ME, Baker DI, Gottschalk M, Garrett P, McGeary S, Pollack D, Charpentier P. Systematic home-based physical and functional therapy for older persons after hip fracture. *Arch Phys Med Rehabil* 1997;78:1237-1247.
22. Topp R, Ditmyer M, King K, Coherty K, Hornyak J. The effect of bed rest and potential of prehabilitation on patients in the intensive care unit. *AACN Clin Issues* 2002;13:263-276.
23. Gill TM, Baker DI, Gottschalk M, Gahbauer EA, Charpentier PA, Regt PT, Wallace WJ. A prehabilitation program for physically frail community-living older persons. *Arch Phys Med Rehabil* 2003;84:394-404.
24. Morey-Holton ER, Globus RK. Hindlimb unloading rodent model: technical aspects. *J Appl Physiol* 2002;92:1367-1377.

25. Prisky RD, Nelson AG, Iatsch E. Eccentric exercise prior to hindlimb unloading attenuated reloading muscle damage in rats. *Aviat Space Environ Med* 2004;75:941-046
26. Alley KA, Thompson LV. Influence of simulated bed rest and intermittent weight bearing on single skeletal muscle fiber function in aged rats. *Arch Phys Med Rehabil* 1997;78:19-25
27. Gehrke AG, Krull MS, McDonald RS, et al. The effects of non-weight bearing on skeletal muscle in older rats: an interrupted bout versus an uninterrupted bout. *Biol Res Nurs* 2004;5:195-202
28. Lipman RD, Chrisp CE, Hazzard DG, Bronson RT. Pathologic characterization of brown Norway, brown Norway x Fisher 344, and Fisher 344 x Brown Norway rats with relation to age. *J Gerontol: Biol Sci* 1996;51A:B54-B59
29. Chen KD, Alway SE. A physiological level of clenbuterol does not prevent atrophy or loss of force in skeletal muscle of old rats. *J Appl Physiol* 2000;89:606-612
30. Zarzhevsky N, Carmeli E, Fuchs D, Coleman R, Stein J, Reznick AZ. Recovery of muscles of old rats after hindlimb immobilization by external fixation is impaired compared with those of young rats. *Exp Gerontol* 2001;36:125-140
31. Machida S, Booth FW. Regrowth of skeletal muscle atrophied from inactivity. *Med Sci Exerc Sport* 2003;36:52-59
32. Gallegly JC, Turesky NA, Strotman BA, Gurley CM, Peterson CA, Dupont-Versteegden EE. Satellite cell regulation of muscle mass is altered at old age. *J Appl Physiol* 2004;97:1082-1900.
33. Thomson DM, Gordon SE. Diminished overload-induced hypertrophy in aged gast-twitch skeletal muscle is associated with AMPK hyperphosphorylation. *J Appl Physiol* 2004;98:557-564
34. Kasper CE. Sarcolemmal disruption in reloaded atrophic skeletal muscle. *J Appl Physiol* 1995;79:607-614
35. Blough ER, Linderman JK. Lack of skeletal muscle hypertrophy in very aged male Fischer 344 x Brown Norway rats. *J Appl Physiol* 2000;88:1265-1270
36. Mozdziak PE, Pulvermacher PM, Schultz E. Muscle reduction during hindlimb unloading results in a reduction in muscle size after reloading. *J Appl Physiol* 2001;91:183-190
37. Degens H, Alway SE. Skeletal muscle function and hypertrophy are diminished in old age. *Muscle Nerve* 2002;27:339-347
38. Baewer DV, Hoffman M, Romatowski JG, Bain JLW, Fitts RH, Riley DA. Passive stretch inhibits central corelike lesion formation in the soleus muscles of hindlimb-suspended unloaded rats. *J Appl Physiol* 2004;97:930-934
39. Hurst JE, Fitts RH. Hindlimb unloading-induced muscle atrophy and loss of function: protective effect of isometric exercise. *J Appl Physiol* 2003;95:1405-1417
40. Goto YI, Komaki H, Igarashi F, Nonaka I. Muscle mitochondrial changes by experimental immobility and hindlimb suspension. *J Gravit Physiol* 2000;7:109-110
41. Ringsberg K, Gerdhem P, Johansson J, Obrant KJ. Is there a relationship between balance, gait performance and

muscular strength in 75-year-old women? Age and Ageing 1999;28:289-293

Table 1. Body mass (g).

	Prior to intervention	Post intervention
Adult rats		
Control	460±39	
HLU	391±24	345±30
Pre-hab	368±10	344±21
Rehab	---	384±30
Recovery	---	365±16
Exercise	---	382±23
Old rats		
Control	522±56	
HLU	526±52	481±59
Pre-hab	505±26	466±39
Rehab	559±35	478±64
Recovery	547±20	482±48
Exercise	---	549±40

Note: body weights were not taken on all groups prior to intervention (e.g., HLU, exercise).

Table 2. Muscle weight/body weight ratios for adult and old rats ($\times 10^3$)

	Postural and Locomotor			Anti-gravity	
	Soleus	Plantaris	Gastroc.	P. Longus	EDL
<u>Adult Rats</u>					
Controls (15)	0.467 \pm 0.04	0.959 \pm 0.10	4.91 \pm 0.27	0.500 \pm 0.11	0.437 \pm 0.03
HLU (12)	0.445 \pm 0.12	0.904 \pm 0.26 ^a	4.72 \pm 0.19 ^a	0.505 \pm 0.14	0.451 \pm 0.12
Prehab. (11)	0.511 \pm 0.07	1.010 \pm 0.09	4.91 \pm 0.27	0.498 \pm 0.07	0.510 \pm 0.05
Rehab (12)	0.500 \pm 0.05	0.824 \pm 0.09 ^a	5.14 \pm 0.48	0.509 \pm 0.04	0.477 \pm 0.03
Recovery (11)	0.492 \pm 0.05	1.076 \pm 0.12	5.13 \pm 0.61	0.517 \pm 0.54	0.485 \pm 0.04
Exercise (12)	0.493 \pm 0.05	1.021 \pm 0.04	5.33 \pm 0.33	0.508 \pm 0.07	0.467 \pm 0.02
<u>Old Rats</u>					
Controls (15)	0.316 \pm 0.05 ^b	0.635 \pm 0.09 ^b	3.05 \pm 0.36 ^b	0.344 \pm 0.10 ^b	0.310 \pm 0.05 ^b
HLU (13)	0.290 \pm 0.04	0.596 \pm 0.07	2.65 \pm 0.24 ^c	0.359 \pm 0.11	0.315 \pm 0.03
Prehab. (10)	0.329 \pm 0.04	0.541 \pm 0.16	2.50 \pm 0.31 ^c	0.365 \pm 0.12	0.319 \pm 0.03
Rehab. (15)	0.370 \pm 0.06	0.881 \pm 0.22 ^c	3.18 \pm 0.38	0.346 \pm 0.06	0.339 \pm 0.04
Recovery (10)	0.275 \pm 0.14	0.662 \pm 0.11	3.14 \pm 0.54	0.376 \pm 0.08	0.338 \pm 0.07
Exercise (12)	0.337 \pm 0.06	0.697 \pm 0.07	3.13 \pm 0.36	0.356 \pm 0.07	0.326 \pm 0.04

Note: Numbers of animals per group are provided in parentheses. See Figure 1 for abbreviations.

^aGroup (ANOVA) comparisons for adult rats were significant for soleus ($p=0.032$), plantaris ($p<0.00005$) and gastrocnemius ($p=0.005$). Scheffe post-hoc tests were significant for plantaris (recovery, exercise, prehabilitation > rehabilitation; recovery > HLU and Rehab) and gastrocnemius (exercise > HLU)

^bAll age-related declines in muscle mass/body mass ratios are highly significant

($p < 0.00001$). Age x group interaction $P < 0.001$

°Group comparisons for old rats were significant for soleus ($p = 0.004$), plantaris ($p < 0.00001$) and gastrocnemius ($p < 0.0001$). Scheffe Post-hoc tests were significant for plantaris where values for rehabilitation rats were significantly $>$ control, HLU, recovery and prehabilitation groups and gastrocnemius where control, recovery, rehabilitation and exercise $>$ prehabilitation

Table 3. Peak tetanic tension (g)

	Soleus	Plantaris	P. Longus	EDL
<u>Adult Animals</u>				
Controls	189±27	523±78	444±17	266±43
HLU	83±24 _a	440±82	376±9 _a	280±49
Prehab	93±21 _a	459±38	391±16	292±45
Rehab	128±23 _a		431±108	381±10 301±44
Recovery	110±26 _a		459±56	365±46 274±38
Exercise	164±36	542±63	391±45	307±25
<u>Old Animals</u>				
Controls	114±19 _{b,c}		328±73 _b	321±8 _b 184±42 _b
HLU	67±22 _a	318±81	282±16 _a	181±18
Prehab	106±31	356±73	312±8	198±41
Rehab	120±26	335±88	271±16 _a	219±54
Recovery	86±29 _a		323±87	307±58 191±38
Exercise	136±12 _d		398±48 _d	370±14 _d 230±21 _d

_aMain group effect (p<0.01 or less), _bMain age effect (p<0.01), _cAge x Group interaction (p<0.01), _dExercise effect (p<0.001 or less)

Soleus: Adult control >HLU, Prehab, Rehab and Recovery; HLU=Prehab; Rehab = Exercise > HLU and Prehab. Old Exercise > Old Control >HLU. Prehab=Rehab

Plantaris: Adult control= HLU, Prehab, Rehab, Recovery and Exercise; Old Exercise > Old Control=HLU, Prehab, Rehab and Recovery

P. Longus: Adult Control> HLU, Prehab, Rehab, Recovery and Exercise;

Old Exercise > Old Control>HLU, Rehab

EDL: Adult control=HLU and Recovery; Prehab, Rehab and Exercise >Control.

Old Exercise > Old Control=HLU, Prehab, Rehab and Recovery

Note: a subset of the adult and old exercise results have been presented in publication number #8

Table 4. Ratios of contractile tension to muscle mass (P_o /mass $\times 10^3$)

	Soleus	Plantaris	P. Longus	EDL
ADULT				
Control	916±160	1197±135	2075±174	1353±226
HLU	523±116 _a	1333±201	2054±183	1691±287
Prehab	568±156 _a	1332±153	2179±181	1691±283
Rehab	669±101 _a	1151±264	2139±190	1680±274
Recovery	623±103 _a	1276±117	2075±179	1599±258
Exercise	914±177	1287±204	1943±349	1696±187
OLD				
Control	703±135 _b	1105±195	2049±192	1278±276
HLU	459±168 _a	1091±293	1591±103 _a	1244±142
Prehab	671±173	1044±220	1771±271	1288±242
Rehab	661±96	1035±161	1685±100 _a	1446±278
Recovery	531±91	985±182	1634±182 _a	1168±132
Exercise	729±36	1149±114	2792±138	1347±146

_aMain Group effect $p < 0.01$), _bMain Age effect (< 0.01), _cAge x Group effect ($p < 0.01$)

Soleus: Adult control, Exercise > HLU, Prehab, Rehab and Recovery; Prehab=Rehab= Recovery >HLU. Old Control and Exercise > HLU, Prehab, Rehab, and Recovery. Prehab= Rehab > HLU = Recovery

Plantaris: Adult control: No group differences. Old rats: No group differences.

P. Longus: Adult rats: no group differences. Old Control and Exercise > HLU, Prehab, Rehab, Recovery.

Exercise > Control

EDL: Adult rats: no group differences. Old rats: no group differences.

Table 5. Stride length and width (mm).

STRIDE LENGTH	Pre-HLU	Post Prehab Or Exercise	Post HLU	Post Rehab or Recovery
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Adult Animals

Controls	136±18			
HLU	137±8		127±17	
Prehab	131±20	143±15		132±20
Rehab	128±12		118±17	140±19
Recovery	123±10		114±18	133±21
Exercise	118±14	133±14 _a		

Old Animals_b

Controls	98±10			
HLU	92±7		78±14 _a	
Prehab	92±12	102±16	88±17	
Rehab	96±9		87±11	103±10 _a
Recovery	96±14		77±5 _a	93±16
Exercise	88±9		101±14 _a	

Table continues next page.....

Table 5 continued. Stride length and width (mm)

STRIDE WIDTH	Pre-HLU	Post Prehab or Exercise	Post HLU	Post Rehab or Recovery
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Adult Animals

Controls	37±5			
HLU	39±4		40±11	
Prehab	39±4	38±5	37±10	
Rehab	40±4		38±11	39±7
Recovery	38±3		42±7	41±4
Exercise	40±4	38±4		

Old Animals_b

Controls	56±6			
HLU	61±4		60±10	
Prehab	59±3	58±5	64±12	
Rehab	61±4		58±7	55±6
Recovery	59±6		59±10	57±7
Exercise	59±4	56±7		

_aMain Group effect (p<0.01, F=548.64, Eta²=0.507); _bMain age effect (p<0.01, F=356.30, Eta²=0.278)

Table 6. Total protein concentration values (g/g of muscle tissue).

<u>Adult animals</u>	Control	HLU	Rehabilitation	Pre-habilitation
Gastrocnemius	187±5	186±8	204±8	202±5
Soleus	175±7	157±9 _a	164±6	188±6
<u>Old animals</u>				
Gastrocnemius	201±5	198±4	183±7	189±6
Soleus	174±5	151±6 _a	152±5 _a	149±5 _a

_a Main Group effect (F value=6.721, Eta²=0.283, p<0.05)

Figure Legend

Values are means \pm sd. Abbreviations are HLU= Hindlmb unweighting, Prehab= prehabilitation, Rehab= Rehabilitation, EDL= extensor digitorum longus,

_aMain effect unweighting ($p < 0.01$ or less), _bSignificant age effect ($p < 0.01$ or less), _cAge x Group interaction ($p < 0.01$)

Soleus: Adult Control > HLU, Prehab; Control = Rehab, Recovery and Exercise;

HLU = Prehab

Old Control > HLU, Control = Prehab, Rehab, Recovery and Exercise

Plantaris: Adult Control > HLU, Prehab; Control = Rehab, Recovery and Exercise;

HLU = Prehab.

Old Control > HLU; Prehab = Rehab = Recovery, Exercise > all groups

Gastrocnemius: Adult Control > HLU, Prehab, Rehab and Recovery; Rehab and Exercise >

HLU and Prehab

Old Control, Rehab, Recovery and Exercise > HLU = Prehab.

EDL: Adult control > HLU and Prehab; Control = Prehab, Rehab, Recovery and Exercise

Old Control = HLU, Prehab, Rehab, Recovery and Exercise

Figure 1a.

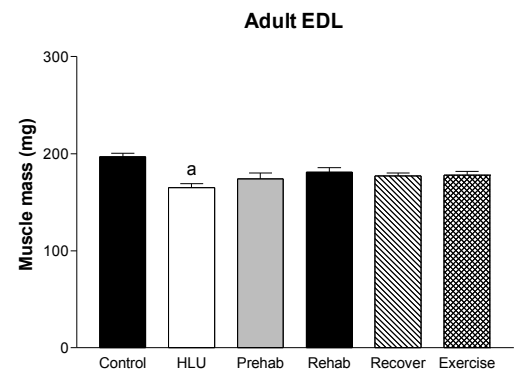
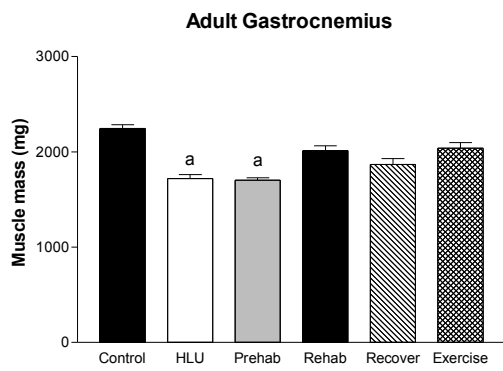
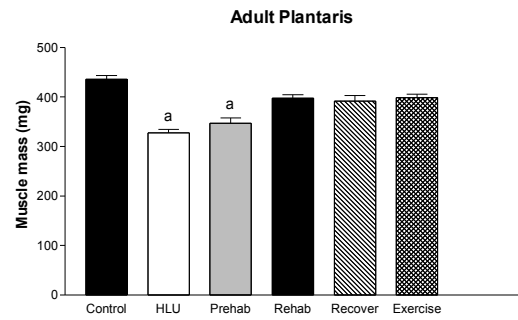
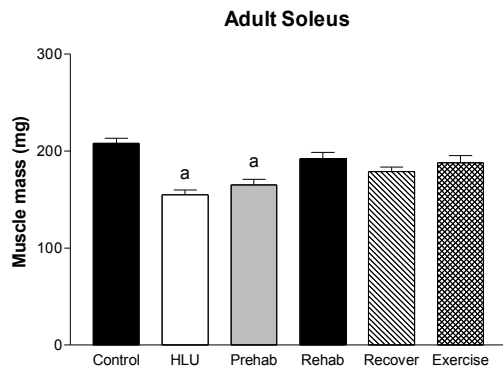


Figure 1b.

