Dynamic foot stimulation attenuates soleus muscle atrophy induced by hindlimb unloading in rats

Antonios Kyparos,[1,2] Daniel L. Feeback,[4] Charles S. Layne,[3]
Daniel A. Martinez,[2] and Mark S.F. Clarke.[3]

[1]Institute for Space Systems Operations, [2]Connective Tissue Physiology Laboratory, Department of Biology & Biochemistry, [3]Laboratory of Integrated Physiology, Department of Health & Human Performance, University of Houston, Houston, TX 77204, [4]Muscle Research Laboratory, Space Life Science Directorate, NASA/Johnson Space Center, Houston, TX 77058

Send all correspondence to:

Mark S.F. Clarke, Ph.D.
Associate Professor
Department of Health & Human Performance
University of Houston
3855 Holman Street, Garrison-Rm 104D
Houston, TX 77204-6015

713-743-9854 (office phone)
713-743-9860 (FAX)
mclarke@mail.uh.edu (email)

ABSTRACT

Unloading-induced myofiber atrophy is a phenomenon that occurs in the aging population, bed-ridden patients and astronauts. The objective of this study was to determine whether or not dynamic foot stimulation (DFS) applied to the plantar surface of the rat foot can serve as a countermeasure to the soleus muscle atrophy normally observed in hindlimb unloaded (HU) rats. Thirty mature adult (6-month-old) male Wistar rats were randomly assigned into ambulatory control (AMB), hindlimb unloaded alone (HU), or hindlimb unloaded with the application of DFS (HU+DFS) groups. A dynamic pattern of pressure was applied to the right foot of each HU animal using a specially fabricated boot containing an inflatable air bladder connected to a solenoid air pump controlled by a laptop computer. The anti-atrophic effects of DFS were quantified morphometrically in frozen cross-sections of soleus muscle stained using the metachromatic-ATPase fiber typing technique. Application of DFS during HU significantly counteracted the atrophic response observed in the soleus by preventing approximately 85% of the reduction in Type I myofiber cross-sectional area (CSA) observed during HU. However, DFS did not protect type II fibers of the soleus from HU-induced atrophy or any fiber type in the soleus muscle of the contralateral control leg of the DFS-treated HU animals. These results illustrate that the application of DFS to the rat foot is an effective countermeasure to soleus muscle atrophy induced by HU.

Key words: dynamic foot stimulation, sensory receptors, proprioception, skeletal muscle atrophy, soleus, mechanical unloading, unweighting, rat

INTRODUCTION

Mechanical unloading of skeletal muscle (SKM) during space flight or ground-based analogues, such as human bedrest and rodent hindlimb unloading (HU) models, induces SKM atrophy particularly affecting the anti-gravity musculature of the lower limbs (9, 10). Atrophy is characterized by a decrease in muscle volume, mass and strength, alterations in histochemical and protein expression characteristics as well as a decrease in neuromuscular function (4, 5, 14, 23, 24).

The effects of SKM atrophy have serious implications for various and diverse populations. Astronauts need to maintain optimal physical performance to deal with the demanding tasks and unexpected situations that they may encounter in space. Bed-ridden patients require effective rehabilitation techniques in order to counteract inactivity-induced atrophy and facilitate the recovery process. The elderly require novel interventions to supplement existing physical activity approaches designed to retard the detrimental effects of the aging process on the neuromuscular system. Therefore, designing and validating a simple and efficient countermeasure to inactivity-induced neuromuscular decrements is of paramount importance.

In the terrestrial environment, the maintenance of normal muscle function in the lower limbs partially depends on the interaction between ground reaction forces and activation of specific sensory receptors that transmit these stimuli to the central nervous system (6). Under unloaded conditions, this interaction is no longer present resulting in a disruption of the signals normal transmitted along the neural pathways between the sensory receptors, central nervous system and effectors. Previous research conducted during space flight in humans (17) and on the ground in both humans (16) and rats (7) has demonstrated that increasing sensory input by applying pressure to the soles of the feet results in an increase in neuromuscular activation of the lower limb muscles. These studies suggest that sensory input can initiate or enhance motor output even under unloaded conditions. More importantly foot pressure-induced neuromuscular activation initiated during unloading has been shown to produce a significant attenuation of soleus muscle atrophy (7).

Although the characteristics and the spatial localization of sensory receptors (i.e. cutaneous mechanoreceptors) in both the human (26, 31) and the rat foot have been adequately described (18), information regarding the potential utility of stimulating these receptors in order to ameliorate unloading induced SKM atrophy is scarce. In the study of De-Doncker et al. (7), a rat HU model was used to examine the potential implications of cutaneous mechanoreceptor stimulation in the prevention of muscle atrophy. This study utilized a simple experimental setup consisting of a balloon inflated by a sphygmomanometer in contact with the soles of both hindlimb feet in animals undergoing HU. This approach was carried out in anesthetized animals that had been immobilized in a support frame. Considering the integral role played by both peripheral and spinal neurons in the proprioceptive pathways activated by plantar stimulation, the use of a general anesthetic in this model may have confounded the proprioceptive response, as has been shown to previously to be the case for a variety of anesthetic agents during neuromuscular activation (12). As such, this study did not address the issue of whether or not providing such stimulation is consistent with the development of a practical countermeasure to unloading-induced muscle atrophy in otherwise healthy humans.

Therefore, the present study was designed to investigate whether or not the use of a novel stimulation paradigm/technology known as dynamic foot stimulation (DFS), capable of applying a dynamic pattern of pressure to the plantar surface of the rat foot in fully conscious animals, would counteract the soleus muscle atrophy normally observed as a consequence of HU. Utilizing a miniaturized version of the DFS technology previously shown to induce neuromuscular activation of the lower limb musculature in humans (15), a dynamic pattern of pressure was applied to the rat foot using a specially fabricated boot containing a microprocessor-controlled inflatable air bladder worn by a conscious, alert animal. We hypothesized that such patterned mechanical stimulation of the plantar surface of the rat foot during HU would attenuate unloading-induced SKM atrophy due to enhanced levels of neuromuscular activation in the hindlimb. Information gained from this study will have direct implications for the development of a novel countermeasure designed to prevent the neuromuscular degradation induced by gravitational unloading.

EXPERIMENTAL DESIGN AND METHODS

Animal care

Thirty mature adult (6-month-old) male Wistar rats (Harlan, Indianapolis, IN) were used in the study. The animals were individually housed in a 12-hour light/dark cycle animal facility with controlled temperature and humidity. Access to standard laboratory rodent chow (Tekland, Harlan, Indianapolis, IN) and tap water were unrestricted throughout the study. Animals were acclimated to the animal care facility seven days before the experiment began. Rats were then randomly divided into three groups as follows: a) ambulatory controls (AMB), b) hindlimb unloaded alone (HU), and c) hindlimb unloaded with the application of DFS (HU+DFS). All use of animals was approved by both the Committee for Animal Use for Research and Education (CAURE) at NASA/Johnson Space Center and the Institutional Animal Care and Use Committee at University of Houston, prior to the initiation of the study. All procedures were in accordance with the guidelines established by the Public Health Service Policy on humane care and use of laboratory animals.

Hindlimb Unloading (HU) procedure

Unloading of the rat hindlimbs was achieved using a modified version of a previously described tail suspension protocol (19). Rats were anaesthetised utilizing a 5% isoflurane gas/air mixture, placed supine and their tails lightly cleaned with 10% povidone iodine. For protection against adhesive irritation, rat-tails were lightly coated with tincture of benzoin spray and when dry the tails were covered with a thin foam pre-wrap material. Soft and breathable adhesive first aid tape strips (Nexcare, 3M) were applied to the front and rear side of the tail along the tail's surface, starting just above the hairline and covering about 2/3 the length of the tail. The two ends of the strips were threaded through a reformed vinyl-coated paper clip loop and adhered to each other. Approximately 1 cm of the proximal end and 10 cm of the distal end of the tail remained uncovered to visually assure adequate blood flow within the tail. Rats were observed every 2 hours during the first 12 hours of suspension and at no less than 8-hour intervals thereafter. The suspension device consisted in an aluminium bar placed laterally across the top of the cage on two vertical supporters fixed to the sides of the cage. A brass-fishing swivel was attached to the bar by a metal hook allowing movement in all directions within the cage. The rat was unloaded by attaching the paper clip to the swivel. These polycarbonate modified cages allow the animals to move freely and to access all areas in the cage using their forelimbs as their only mechanism of movement, while leaving the hindlimbs unsupported. Rats were suspended at a 25° angle from the cage floor by adjusting the bar height. The animals were suspended in this fashion for a total of 10 days. After termination of the 10-day HU period, rats were deeply anesthetized and the soleus muscles were harvested for frozen cross-sectioning followed by morphometric analysis as described below. Animals were then euthanized by intravenous (i.v.) injection of Euthasol.

Dynamic Foot Stimulation (DFS)

A custom-built boot with a bladder that contacted the sole of the foot when inflated (Fig. 1) was used to stimulate the sensory receptors in the soles of the rat's foot in conscious alert animals undergoing HU. Due to the "cuff" design of the boot and the means by which it was attached to the animals foot (i.e. a Velcro strap), collateral stimulation of pressure receptors located on the upper part of the foot could not be prevented. Without removing the animal from the HU position, the DFS boot was attached to the foot of the right leg under isoflurane gas anaesthesia (5% isoflurane / 95% air mixture). The animals where then placed back in the HU cage and allowed to fully recover from anaesthesia for a period of 20 min. Pressure was applied to the foot by inflation/deflation of the latex bladder in contact with the sole of the foot using an electronically controlled air pump (WPI, Saratosa, FL) attached to a hose leading to the bladder. The pressure stimulation protocol consisted of a 5 sec inflation/5 sec deflation of the air bladder for a total of 20 min followed by a 10-min rest interval. This cycle was repeated 8 times over a four-hour period during each day of the 10 day HU period. The pressure in the bladder during the inflation was 104 mmHg. Pump cycling time and duration were controlled by a microprocessor. The boot was maintained on the foot only during the application of the pressure and was removed every day after the termination of the protocol.

It has been suggested that to stimulate all types of sensory receptors present within the sole of the rat foot, pressure that exceeds their mechanical threshold (i.e. >8 mN) needs to be applied (18). In general, a pressure of 1 kN/m^2 (1 mN/mm^2) corresponds to a pressure of 7.5 mmHg. Thus, a pressure of 8 mN/mm^2 is equal to 60 mmHg. Given that the mean sole area of the 6-month old male rats used in the study is between 450-500 mm^2, the pressure required to stimulate the entire plantar surface was calculated as 13.9 mN/mm^2 (6255-6950 mN) or 104 mmHg. The specific inflation pressure used in this study was chosen because it met the calculated mechanical threshold needed to stimulate the rat's foot sensory receptors, yet it did not induce a nocioceptive reaction in the animals. The total time during which pressure was applied to the foot of the HU rat corresponded to 5.6% of the entire 10-day HU period.

Tissue Collection and Processing

Rats were deeply anesthetized with an intraperitoneal injection of an anaesthesia mixture (ketamine 40-80 mg/kg body wt and xylazine 5-10 mg/kg body wt at a ratio of 1:1). The hair of the lower limbs was shaved up to the knee joint and a small incision was made on the backside of the ankle uncovering the Achilles tendon. Skin was gently reflected using blunt-tip forceps to expose the calf muscles. The soleus muscle was then carefully separated and excised. The excised muscles were attached to wooden rods by pins inserted through the tendon attachments so that the muscle remained elongated without being stretched. In preparation for histochemical analysis, the muscle samples from the midbelly of the soleus were immersed in TissueTek OCT mounting medium (Sakura Finetek, Torrance, CA) frozen in liquid nitrogen-cooled isopentane and stored at –80° C. Upon analysis, frozen cross sections (5 μm) from the soleus muscle were cut using a Zeiss Microm HM 500 OM microtome cryostat and picked up onto Superfrost Plus glass slides (Erie Scientific, Portsmouth, NH).

Histochemical-Morphometric analysis

Fiber typing on frozen sections was performed utilizing the metachromatic dye-ATPase myofibrillar stain method originally described by Ogilvie and Feeback (20) as modified by Konishi *et. al.* (13). This staining method allows identification of four major fiber types (type I, IIA, IIB and IIC) in a single muscle cross-section based on selective color production in each individual fiber type. The colors produced by each myofiber type using this method were as follows: type I (turquoise), type IIA (light pink), type IIB (violet) and type IIC (blue) (Fig. 2). Three consecutive cross sections were taken from the mid-belly of the soleus muscle for each rat in this study. Two photo frames were taken from each section with a digital camera (DCS 420 Kodak) attached to an Axiophot light microscope (Zeiss, Germany) so that the complete cross-section of the soleus was imaged. Each image was then imported into Adobe Photoshop software (Adobe Systems Inc., San Jose, CA) and the perimeter of each myofiber was delineated by drawing around the perimeter in order to produce a digital overlay mask. Each individual myofiber type was then assigned a separate color scheme by filling in the interior area of the outlined myofibers using a defined 256-level color spectrum in Adobe Photoshop. The cross sectional area (CSA) of the four different fiber types in all three sections were then separately calculated using Object-Image software (NIH, Bethesda, MD) by utilizing a color thresholding approach to quantify the individually colored digital representation of the different myofiber types in the muscle section. Myofiber CSA and fiber type distribution in the soleus muscles were evaluated after analyzing a total of at least 600 myofibers for each muscle.

STATISTICAL ANALYSIS

To evaluate any differences in mean myofiber CSA of different fiber types in the soleus muscle among the experimental groups, one-way analysis of variance (ANOVA) was carried out using the SPSS statistical analysis program. When the univariate F test was significant, Scheffe's *post hoc* test was used to further identify significant differences in myofiber CSA between the experimental group means (i.e. ambulatory control group, HU group and HU+DFS group) for individual myofiber types. To evaluate any differences in myofiber CSA of DFS-treated and contralateral control soleus muscle in the same HU+DFS animal, a paired Student's t-test was applied. Statistical significance level was set at $P < 0.05$.

RESULTS

No significant differences in myofiber CSA of any myofiber type (i.e. Type I, IIA, IIB or IIC) was detected between the right and left soleus muscles of AMB control or HU animals (Table 1). As the DFS apparatus was placed on the right hindlimb of the animal, all subsequent comparisons between experimental groups (i.e. AMB, HU and HU+DFS) were carried out on soleus myofiber CSA values obtained from the right hindlimb of the animals only. In addition, Type IIC myofibers were encountered very rarely in any of the experimental groups (i.e. Type IIC myofibers were detected in less than four animals per experimental group). Therefore, Type IIC CSA data from soleus muscle obtained from AMB, HU and HU+DFS animals is not included in our statistical analysis scheme (i.e. One-Way ANOVA), rather the mean myofiber CSA data for each group are displayed as a descriptive measure in Table 1.

As expected, after 10 days of HU a significant decrease ($P < 0.0001$) of approximately 42% (*4,128* $\mu m^2 \pm 537\ \mu m^2$ *vs. 2,396* $\mu m^2 \pm 479\ \mu m^2$) in Type I myofiber CSA in the soleus muscle of HU animals was seen as compared to the soleus muscle of the AMB control group (Fig. 3). However, no significant difference in Type I soleus CSA was observed between the AMB control and the HU + DFS group (*4,128* $\mu m^2 \pm 537\ \mu m^2$ *vs. 3,717* $\mu m^2 \pm 609\ \mu m^2$) (Fig. 3). Our results indicate that the DFS protocol was responsible for the prevention of almost all (i.e. over 85% of the atrophy response in HU alone) of the myofiber atrophy normally observed in Type I myofibers of the soleus muscle after 10 days of HU. However, DFS did not prevent the HU-induced atrophy observed in either Type IIA or Type IIB myofibers in the rat soleus (Fig. 3).

When the Type I myofiber CSA in the soleus muscle from the DFS treated right leg and the contralateral control, non-DFS treated left leg from the same HU animals were compared (Fig. 4), a significant difference ($P < 0.001$; paired Student's t-test) was found. The average Type I fiber CSA in the untreated contralateral leg ($2{,}499 \pm 447\ \mu m^2$) was significantly smaller than that observed in the DFS-treated leg ($3{,}717 \pm 609\ \mu m^2$). Unlike Type I fibers however, the CSA of Type IIA and IIB myofibers in DFS-treated animals were not significantly different ($P > 0.05$) from the values observed in HU rats (Fig. 4). These data indicate that the effects of DFS are limb-specific and that DFS does not appear to induce any systemic anti-atrophic effects on unloaded muscle tissue.

In addition, no significant differences ($P > 0.05$) were found among experimental groups with regard to soleus muscle composition in terms of percent fiber type, indicating that HU alone nor HU+DFS induced a fiber type shift that could be resolved using the metachromatic ATPase staining technique (Fig. 5).

DISCUSSION

The specific aim of this study was to determine whether or not dynamic foot stimulation (DFS) applied to the plantar surface of the rat foot would counteract the atrophy in the soleus muscle normally induced by hindlimb unloading.

Rat HU is an animal model that is widely used to study skeletal muscle (SKM) atrophy and other physiological modifications associated with muscle inactivity and disuse. It has been demonstrated that HU induces rapid decreases in SKM mass within the first week of unloading particularly affecting the antigravity soleus muscle (29). The 10-day suspension protocol used in our study induced significant atrophy in the soleus muscle. When suspended rats were compared to ambulatory controls, the CSA decreased by 42% in Type I fibers, 32% in Type IIA and 43% in Type IIB (Fig. 3). These data clearly demonstrate that the application of DFS during unloading is a highly efficient means of preventing the significant Type I myofiber atrophy normally induced in the soleus muscle as a consequence of unloading. However, DFS did not appear to protect Type II fibers in the unloaded soleus from atrophy (Figs. 3 & 4).

There is consensus in the literature that HU induces a reduction in CSA of soleus Type I fibers. However contradictory results have been reported as to the degree to which HU also induces atrophy in Type II myofibers and whether or not there is myofiber type conversion (from slow Type I to fast Type II) associated with unloading. This discrepancy in the literature is mainly attributed to the differences in the age and gender of the animals used in the studies as well as the length of the suspension period. The average life span of the rat is about 28 months. From the data available in the literature it is apparent that both very young rats (up to 3 months of age) and aged rats (older that 20 months of age) are more susceptible to unloading-induced SKM atrophy (3, 8). Deschenes et al. (8) suggested that in young rats the alterations in fiber size and type might be due to the interference of the unloading condition with the developmental process of the muscle that naturally occurs during the young age (27), while in aged rats this might be the result of an increased sensitivity to the adverse effects of disuse associated with the aging process (30).

Significant reduction in soleus CSA for both Type I and Type II fibers as well as slow to fast twitch transformation of fibers have been demonstrated in 6-week and 3-month old male rats after two and three weeks of unloading, respectively (2, 22). Other investigators, using 17-week old male rats tail-suspended for two weeks (28) or 3-months and 22-month old female rats suspended for three weeks (25) also found a significant decrease in CSA for both Type I and Type II fibers, yet no change in soleus muscle fiber type composition. Deschenes et al. (8) showed that after 4 weeks of unloading in 22-month old male rats, soleus myofiber CSA was decreased by 48% in Type I fibers, 40% in Type IIA and 44% in Type IIB fibers, while in younger adult 8-month old rats Type I myofiber CSA decreased by only 20%. A conversion of fibers from Type I to Type II also occurred in the aged animals, yet there was no fiber type alteration detected in the younger rats. In this context, the results of our study with respect to soleus muscle fiber type composition and fiber CSA using 6-month old male rats, are in agreement with the findings previously reported in the literature for the animals of this particular age (i.e. skeletal muscle from mature adult animals)

The basic concept that mechanical stimulation applied to the soles of the feet during unloading could ameliorate muscle atrophy has in part been previously validated.

De-Doncker et al. (7) showed that foot pressure to the soles of the rat feet partially prevented soleus muscle atrophy normally induced by 14 days of unloading. In this study a pressure of 40 mmHg was applied to the plantar surface of both hindlimbs using a latex balloon manually inflated by a sphygmomanometer. Unlike our findings however, a partial prevention of SKM atrophy was found not only in Type I but also in Type II myofibers. This discrepancy may be explained by the use of a less descriptive histochemical method (11) for fiber type classification compared to our histochemical methodology (20). It was also found that soleus myofiber CSA was significantly protected in both Type I and Type II myofibers but that foot pressure did not prevent the transformation of Type I to Type II fiber types in the soleus muscle. An efficient preservation of Type I myofiber CSA observed in our present study may be explained by the longer duration of our protocol and/or the higher stimulation pressure applied to the rat foot using the DFS technology.

A second ancillary hypothesis tested in the present study was whether there was a systemic effect with regard to muscle CSA preservation associated with the application of DFS. While the "treatment leg" that experienced DFS in the HU animals showed significant preservation of Type I myofiber CSA in the soleus, no such protective effects on Type I myofiber CSA was observed in the contralateral leg of the same animal (Fig. 4). Rather, Type I myofibers of the soleus in the HU-DFS contralateral control leg atrophied to the same degree as Type I myofibers of the soleus muscle in the HU-alone group (Figs. 3 & 4). These data suggest that DFS has no systemic effect on SKM mass preservation, but rather it is confined to those muscles within the leg undergoing the DFS stimulus in contrast to other studies which stimulate both feet simultaneously (7).

The underlying concept behind our study is the well-established motor control principle that sensory input (i.e. pressure application) can modify motor output (i.e. neuromuscular activation). Previous research has demonstrated that rat soleus muscle electromyographic (EMG) activity was significantly decreased by ~85% (7) during the first days of unloading and it was gradually restored after 7-10 days of unloading (1, 21). Interestingly a significant increase in the soleus EMG activity has been observed when pressure was applied to the plantar surface of the feet in the suspended rats (7). As a possible explanation for this increased EMG activity the authors proposed that stimulation of the cutaneous mechanoreceptors (i.e. Merkel discs, Meissner corpuscles, Ruffini endings, Pacinian corpuscles) located in the plantar surface of the rat's feet induced an increase in neuromuscular activity (7), as has also been reported in human subject's under-going similar plantar stimulation (15). Although there was no direct relationship established between increased EMG activity and SKM atrophy attenuation it is possible that the application of DFS facilitated interactions between nerve and muscle, thus maintaining neuromuscular interactions between the sensory and motor systems and muscle tissue.

In conclusion, the results of the present study illustrate that external mechanical stimulus applied to the rat foot is capable of counteracting unloading-induced soleus muscle atrophy. It is postulated that this effect is achieved via stimulation of proprioceptive pathways that in turn activate appropriate motoneurons to generate motor unit contraction mimicking the neuromuscular activity patterns normally induced by load bearing in the terrestrial environment. This underlying concept promises to serve as the basis for the development of a novel supplement to currently utilized in-flight exercise countermeasures for astronauts during space flight, as well as an effective rehabilitation tool for clinical populations such as the bed-ridden or elderly patients.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Institute for Space Systems Operations, under a joint program between University of Houston and NASA/Johnson Space Center. Our special thanks to the staff of the NASA/Johnson Space Center Animal Care Facility for their tireless efforts in supporting this project

REFERENCES

1. **Alford EK, Roy RR, Hodgson JA, and Edgerton VR.** Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hindlimb suspension. *Exp Neurol* 96: 635-649, 1987.

2. **Anderson J, Almeida-Silveira MI, and Perot C.** Reflex and muscular adaptations in rat soleus muscle after hindlimb suspension. *J Exp Biol* 202 (Pt 19): 2701-2707, 1999.

3. **Asmussen G, Miersch H, and Soukup T.** The influence of suspension hypokinesia on contractile properties of slow and fast twitch muscles of young growing and adult rats. *Biomed Biochim Acta* 48: S426-431, 1989.

4. **Baldwin KM.** Effects of altered loading states on muscle plasticity: what have we learned from rodents? *Med Sci Sports Exerc* 28: S101-106, 1996.

5. **Bamman MM, Clarke MS, Feeback DL, Talmadge RJ, Stevens BR, Lieberman SA, and Greenisen MC.** Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. *J Appl Physiol* 84: 157-163, 1998.

6. **Bock O.** Problems of sensorimotor coordination in weightlessness. *Brain Res Brain Res Rev* 28: 155-160, 1998.

7. **De-Doncker L, Picquet F, and Falempin M.** Effects of cutaneous receptor stimulation on muscular atrophy developed in hindlimb unloading condition. *J Appl Physiol* 89: 2344-2351, 2000.

8. **Deschenes MR, Britt AA, and Chandler WC.** A comparison of the effects of unloading in young adult and aged skeletal muscle. *Med Sci Sports Exerc* 33: 1477-1483, 2001.

9. **Edgerton VRaR, R. R.** Neuromuscular adaptations to actual and simulated spaceflight. In: *Hanbook of Physiology*, edited by (Ed.) IAPS. Baltimore, MD: Williams & Wilkins, 1996, p. 721-763.

10. **Fitts RH, Riley DR, and Widrick JJ.** Physiology of a microgravity environment invited review: microgravity and skeletal muscle. *J Appl Physiol* 89: 823-839, 2000.

11. **Guth L and Samaha FJ.** Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exp Neurol* 25: 138-152, 1969.

12. **Ingalls CP, Warren GL, Lowe DA, Boorstein DB, and Armstrong RB.** Differential effects of anesthetics on in vivo skeletal muscle contractile function in the mouse. *J Appl Physiol* 80: 332-340, 1996.

13. **Konishi M, Iwamoto S, Ohara H, and Shimada M.** Two-dimensional changes of muscle fiber types in growing rat hindlimb. *Acta Anat Nippon* 75: 267-273, 2000.

14. **Kourtidou-Papadeli C, Kyparos A, Albani M, Frossinis A, Papdelis CL, Bamidis P, Vivas A, and Guiba-Tziampiri O.** Electrophysiological, histochemical, and hormonal adaptation of rat muscle after prolonged hindlimb suspension. *Acta Astronaut* 54: 737-747, 2004.

15. **Layne CS, Forth KE, Baxter MF, and Houser JJ.** Voluntary neuromuscular activation is enhanced when paired with a mechanical stimulus to human plantar soles. *Neurosci Lett* 334: 75-78, 2002.

16. **Layne CS, Lange GW, Pruett CJ, McDonald PV, Merkle LA, Mulavara AP, Smith SL, Kozlovskaya IB, and Bloomberg JJ.** Adaptation of neuromuscular activation patterns during treadmill walking after long-duration space flight. *Acta Astronaut* 43: 107-119, 1998.
17. **Layne CS, Mulavara AP, Pruett CJ, McDonald PV, Kozlovskaya IB, and Bloomberg JJ.** The use of in-flight foot pressure as a countermeasure to neuromuscular degradation. *Acta Astronaut* 42: 231-246, 1998.
18. **Leem JW, Willis WD, and Chung JM.** Cutaneous sensory receptors in the rat foot. *J Neurophysiol* 69: 1684-1699, 1993.
19. **Morey-Holton ER and Globus RK.** Hindlimb unloading rodent model: technical aspects. *J Appl Physiol* 92: 1367-1377, 2002.
20. **Ogilvie RW and Feeback DL.** A metachromatic dye-ATPase method for the simultaneous identification of skeletal muscle fiber types I, IIA, IIB and IIC. *Stain Technol* 65: 231-241, 1990.
21. **Ohira Y, Nomura T, Kawano F, Sato Y, Ishihara A, and Nonaka I.** Effects of nine weeks of unloading on neuromuscular activities in adult rats. *J Gravit Physiol* 9: 49-59, 2002.
22. **Picquet F, Canu MH, and Falempin M.** Phenotypic changes in the composition of muscular fibres in rat soleus motor units after 14 days of hindlimb unloading. *Pflugers Arch* 440: 229-235, 2000.
23. **Recktenwald MR, Hodgson JA, Roy RR, Riazanski S, McCall GE, Kozlovskaya I, Washburn DA, Fanton JW, and Edgerton VR.** Effects of spaceflight on rhesus quadrupedal locomotion after return to 1G. *J Neurophysiol* 81: 2451-2463, 1999.
24. **Riley DA, Ellis S, Slocum GR, Sedlak FR, Bain JL, Krippendorf BB, Lehman CT, Macias MY, Thompson JL, Vijayan K, and De Bruin JA.** In-flight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. *J Appl Physiol* 81: 133-144, 1996.
25. **Simard C, Lacaille M, and Vallieres J.** Effects of hypokinesia/hypodynamia on contractile and histochemical properties of young and old rat soleus muscle. *Exp Neurol* 97: 106-114, 1987.
26. **Sonnenborg FA, Andersen, O. K., and Arendt-Nielsen, L.** Modular organization of excitatory and inhibitory reflex receptive fields elicited by electrical stimulation of the foot sole in man. *Clin Neurophysiol* 111: 2160-2169, 2000.
27. **Stump CS, Tipton CM, and Henriksen EJ.** Muscle adaptations to hindlimb suspension in mature and old Fischer 344 rats. *J Appl Physiol* 82: 1875-1881, 1997.
28. **Taguchi S, Morii H, and Ishihara A.** Effects of body suspension on soleus muscle fibres and spinal motoneurones in the rat. *Comp Biochem Physiol A* 100: 801-803, 1991.
29. **Thomason DB and Booth FW.** Atrophy of the soleus muscle by hindlimb unweighting. *J Appl Physiol* 68: 1-12, 1990.
30. **Thompson LV, Johnson SA, and Shoeman JA.** Single soleus muscle fiber function after hindlimb unweighting in adult and aged rats. *J Appl Physiol* 84: 1937-1942, 1998.
31. **Trulsson M.** Mechanoreceptive afferents in the human sural nerve. *Exp Brain Res* 137: 111-116, 2001.

Table 1 Cross-sectional area (CSA) of different myofiber types identified using the metachromatic ATPase stain in the rat soleus muscle for both right and left legs in ambulatory control (AMB) and hindlimb unloaded (HU) animals.

CONDITION	Ambulatory Control (AMB)		Hindlimb Unloaded (HU)	
Myofiber Type	**Right Leg**	**Left Leg Rig**	**ht Leg**	**Left Leg**
I	4,128 ± 537	4,123 ± 370	2,396 ± 479	2,342 ± 280
IIA	4,228 ± 876	4,063 ± 432	2,873 ± 603	2,768 ± 320
IIB	3,561 ± 1,029	3,395 ± 355	2,046 ± 431	2,337 ± 367
IIC*	2,837 ± 658	2,810 ± 525	1,977 ± 349	2,403 ± 251

Values are expressed in square micrometers (μm^2) and represent means ± SD; n = 10 rats per group. AMB = ambulatory control, HU = hindlimb unloaded. No significant differences in myofiber CSA between the right and left legs of animals within the same experimental groups were detected. (*) - Type IIC myofibers were encountered very rarely in any of the experimental groups (i.e. Type IIC myofibers were detected in less than four animals per experimental group). As such, Type IIC data for AMB, HU and HU+DFS could not be included in our statistical analysis other than as a descriptive measure.

Figure 1 Dynamic Foot Stimulation (DFS) Apparatus. The external sleeve of the DFS boot was fabricated using a thin, light, yet durable plastic with an integral inflatable/deflatable air bladder (1) located beneath the sleeve surface that contacts the sole of the rat foot. Velcro restraint straps wrap around the rat foot (2) and around the ankle joint (3) so that the deflated air bladder was in close contact with the sole of the foot. The air bladder was connected by a single air line (4) to an extremely quiet air pump to prevent startling of the animal during operation. The bladder was inflated by pumping air down the line and then actively deflated by venting the pump. Inflation/deflation cycling of the bladder was controlled by a microprocessor-activated electronic valve-pressure gauge system. The boot fitted snugly on the foot without restricting the natural movement of the ankle joint, or its full range of motion.

Figure 2. **Frozen cross-section of a soleus muscle from an ambulatory control rat stained using the metachromatic ATPase stain.** Sections were pre-incubated at pH 4.35 and stained with toluidine blue as described in the Methods section. On the basis of color, fiber types were classified as Type I (turquoise), Type IIA (light pink), Type IIB (violet) and Type IIC (dark blue with dark blue edge). (Bar - 50 μm).

Figure 3. **Cross-sectional area (CSA) of different myofiber types in the soleus muscle in Ambulatory, HU and HU+DFS animals.** Panel **A** -Type I myofibers, Panel **B** -Type IIA myofibers and Panel **C** – Type IIB myofibers. The CSA of all three fiber types in the HU group was significantly smaller compared to those in the ambulatory control group ($P < 0.01$; One-Way ANOVA with *post-hoc* Scheffe test). For Type I fibers only, no

significant difference in CSA was found between the DFS group and the ambulatory control group ($P > 0.05$) in the soleus of the right hindlimb. In the DFS group, the DFS boot was attached to the right leg. Values are expressed in square micrometers (μm^2) and represent means ± SD; n = 10 rats per group. AMB = ambulatory control, HU = hindlimb unloaded, DFS = hindlimb unloaded + dynamic foot stimulation

Figure 4. Cross-sectional area (CSA) of soleus myofiber types between DFS-treated and contralateral control limb in the same animal. Values are expressed in square micrometers (μm^2) and represent means ± SD; n = 10 rats per group. Open bar = DFS treatment, right leg, Solid Bar = contralateral control, left leg. The CSA of soleus Type I myofibers in the DFS-treated right leg was significantly ($P < 0.01$; paired Student t-test) greater than the CSA of Type I myofibers in the soleus muscle of the contralateral control, left leg of the same animal. No significant differences in Type IIA and IIB fiber CSA between the right and the left legs were found ($P > 0.05$; Paired t-test).

Figure 5. Fiber type distribution in the soleus muscle among treatment groups. Values are expressed in percentage (%) and represent means ± SD; n = 10 rats per group. AMB = ambulatory control, HU = hindlimb unloaded, HU+DFS = hindlimb unloaded + dynamic foot stimulation. In the DFS group, an inflatable boot is attached to the right leg. No significant differences in fiber type distribution were found among groups ($P > 0.05$).

Figure 1

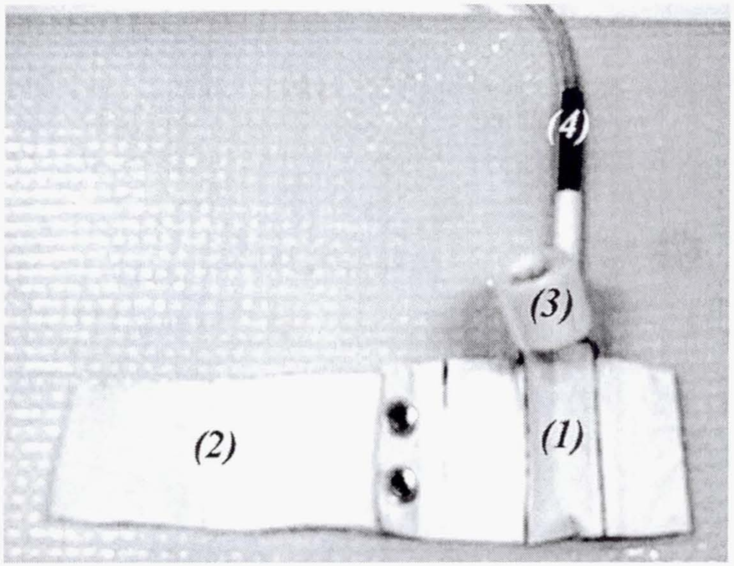


Figure 2

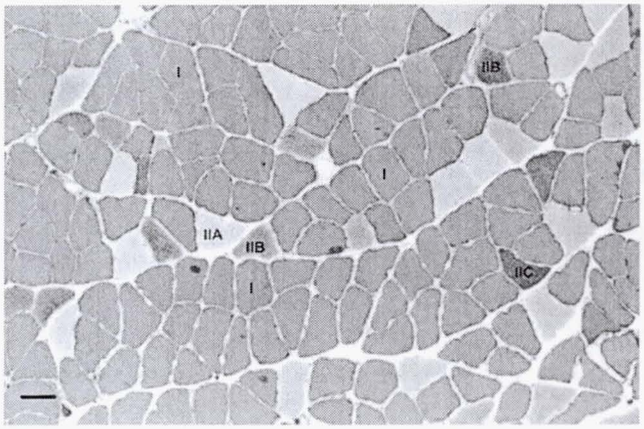


Figure 3

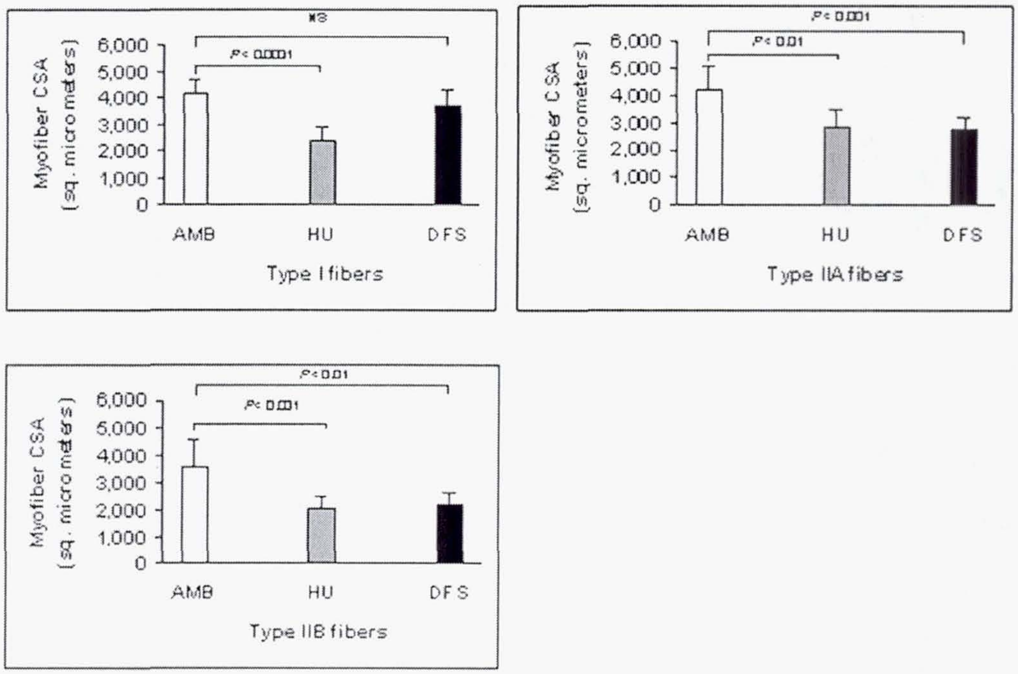

NS
P< 0.0001
Myofiber CSA
(sq. micrometers)
6,000
5,000
4,000
3,000
2,000
1,000
0
AMB
HU
DFS
Type I fibers
P< 0.001
P< 0.01
Myofiber CSA
(sq. micrometers)
6,000
5,000
4,000
3,000
2,000
1,000
0
AMB
HU
DFS
Type IIA fibers
P< 0.01
P< 0.001
Myofiber CSA
(sq. micrometers)
6,000
5,000
4,000
3,000
2,000
1,000
0
AMB
HU
DFS
Type IIB fibers

Figure 4

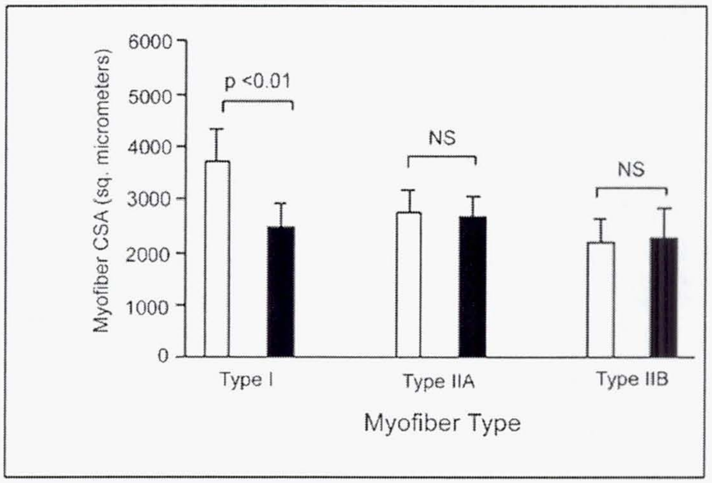
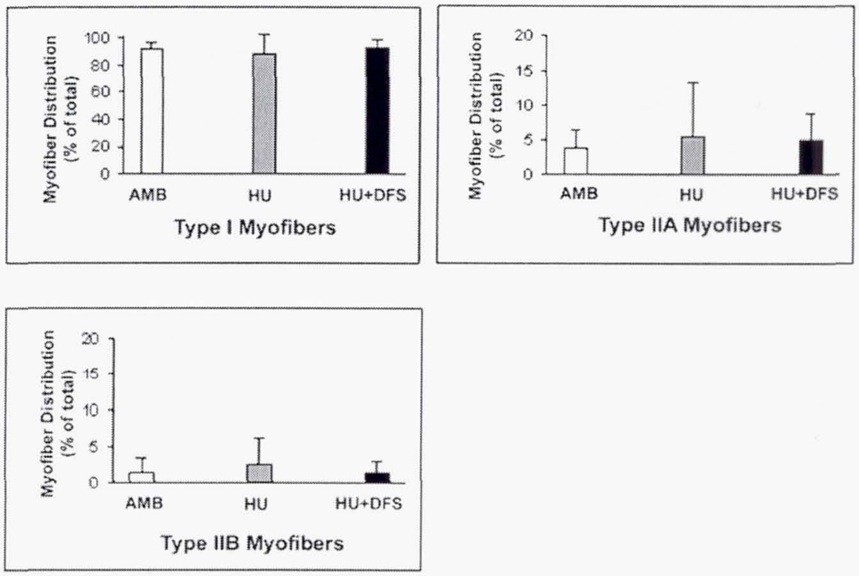

Myofiber CSA (sq. micrometers)
6000
5000
4000
3000
2000
1000
0
p <0.01
NS
NS
Type I
Type IIA
Type IIB
Myofiber Type

Figure 5


Myofiber Distribution (% of total)
100
80
60
40
20
0
AMB
HU
HU+DFS
Type I Myofibers
Myofiber Distribution (% of total)
20
15
10
5
0
AMB
HU
HU+DFS
Type IIA Myofibers
Myofiber Distribution (% of total)
20
15
10
5
0
AMB
HU
HU+DFS
Type IIB Myofibers