

## Mechanical stimulation of the plantar foot surface attenuates soleus muscle atrophy induced by hindlimb unloading in rats

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<sup>1</sup>*Institute for Space Systems Operations,* <sup>2</sup>*Connective Tissue Physiology Laboratory, Department of Biology and Biochemistry,* <sup>3</sup>*Laboratory of Integrated Physiology, Department of Health and Human Performance, University of Houston;* and <sup>4</sup>*Muscle Research Laboratory, Space Life Science Directorate, National Aeronautics and Space Administration/Johnson Space Center, Houston, Texas*

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**Kyparos, Antonios, Daniel L. Feedback, Charles S. Layne, Daniel A. Martinez, and Mark S. F. Clarke.** Mechanical stimulation of the plantar foot surface attenuates soleus muscle atrophy induced by hindlimb unloading in rats. *J Appl Physiol* 99: 739–746, 2005. First published April 7, 2005; doi:10.1152/jappphysiol.00771.2004.—Unloading-induced muscle atrophy occurs in the aging population, bed-ridden patients, and astronauts. This study was designed to determine whether dynamic foot stimulation (DFS) applied to the plantar surface of the rat foot can serve as a countermeasure to soleus muscle atrophy normally observed in hindlimb unloaded (HU) rats. Forty-four mature (6 mo old), male Wistar rats were randomly assigned to ambulatory control, HU alone, HU with active DFS (i.e., plantar contact with active inflation), HU with passive DFS (i.e., plantar contact without active inflation), and HU while wearing a DFS boot with no plantar contact groups. Application of active DFS during HU significantly counteracted the atrophic response by preventing ~85% of the reduction in type I myofiber cross-sectional area (CSA) in the soleus while preventing ~57% of the reduction in type I myofiber CSA and 43% of the reduction in type IIA myofiber CSA of the medial gastrocnemius muscle. Wearing of a DFS boot without active inflation prevented myofiber atrophy in the soleus of HU animals in a fashion similar to that observed in HU animals that wore an actively inflated DFS boot. However, when a DFS boot without plantar surface contact was worn during HU, no significant protection from HU-induced myofiber atrophy was observed. These results illustrate that the application of mechanical foot stimulation to the plantar surface of the rat foot is an effective countermeasure to muscle atrophy induced by HU.

mechanical foot stimulation; plantar stimulation; sensory receptors; proprioception; skeletal muscle atrophy; soleus; mechanical unloading; rat

MECHANICAL UNLOADING OF SKELETAL muscle (SKM) during spaceflight or ground-based analogs, such as human bed rest and rodent hindlimb unloading (HU) models, induces SKM atrophy, particularly affecting the antigravity 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

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 depends partially 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 normally transmitted along the neural pathways between the sensory receptors, central nervous system, and effectors. Previous research conducted during spaceflight 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 (27, 32) and the rat foot have been adequately described (18), information regarding the potential utility of stimulating these receptors 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

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agents during neuromuscular activation (12). As such, this study did not address the issue of whether providing such stimulation is consistent with the development of a practical countermeasure to unloading-induced muscle atrophy in otherwise healthy humans or the relative importance of mechanical stimulation of the plantar surface of the foot in eliciting this effect.

Therefore, the present study was designed to investigate whether the use of a novel stimulation paradigm/technology known as dynamic foot stimulation (DFS), capable of applying a dynamic pattern of mechanical stimulation in the form 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 during spaceflight (17), 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. In addition, by investigating the effects of wearing a boot during HU that lacked a plantar surface, the effects of passive limb loading vs. mechanical stimulation of the plantar surface on the prevention of HU-induced myofiber atrophy could be studied. 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 or disuse in terrestrial settings.

#### EXPERIMENTAL DESIGN AND METHODS

**Animal care.** Forty-four mature adult (6 mo old) male Wistar rats (Harlan, Indianapolis, IN) were used in the study. The animals were individually housed in a 12:12-h light-dark cycle animal facility with controlled temperature and humidity. Access to standard laboratory rodent chow (Tekland, Harlan) and tap water were unrestricted throughout the study. Animals were acclimated to the animal care facility 7 days before the experiment began. Rats were then randomly divided into five groups as follows: 1) ambulatory controls (AMB) ( $N = 10$ ), 2) HU alone (HU) ( $N = 10$ ), 3) HU with DFS boot with active inflation (HU+DFS) ( $N = 10$ ), 4) HU with DFS boot without active inflation (HU+NDFS) ( $N = 10$ ), and 5) HU with DFS boot without a plantar surface (HU+NPS) ( $N = 4$ ). All use of animals was approved by both the Committee for Animal Use for Research and Education at National Aeronautics and Space Administration/Johnson Space Center and the Institutional Animal Care and Use Committee at University of Houston, before 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.

**HU procedure.** Unloading of the rat hindlimbs was achieved by using a modified version of a previously described tail-suspension protocol (19). Rats were anesthetized utilizing a 5% isoflurane gas/air mixture administered to the animal by placing it head down into a partially sealed chamber into which the gas mixture was pumped. Once the animal had succumbed to anesthesia, it was removed from the chamber and placed supine on the laboratory bench adjacent to an air-extraction vent. Anesthesia was maintained during preparation for hindlimb suspension (HU) by attaching a sealed mask over the snout of the animal into which was pumped additional anesthesia. The animal's tail was lightly cleaned with 10% povidone iodine and was

then patted dry with a paper towel. 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 prewrap 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 two-thirds 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 ensure adequate blood flow within the tail. The suspension device consisted of an aluminum 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 procedure detailed above took ~10 min to perform from initial anesthesia to suspension, with the animal regaining complete consciousness from anesthesia within a period of 5 min after suspension. 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 injection of Euthasol.

**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

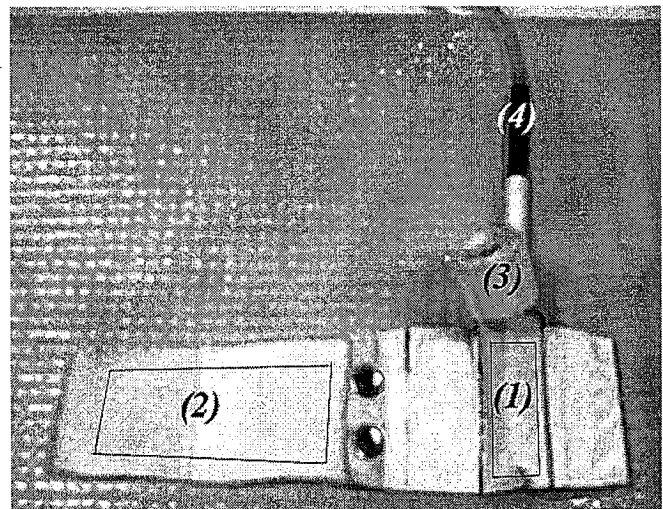


Fig. 1. Dynamic foot stimulation (DFS) apparatus. The external sleeve of the DFS boot was fabricated using a thin, light, yet durable plastic with an integral inflatable/deflatable air bladder (1) located beneath the sleeve surface that contacts the sole of the rat foot. Velcro restraint straps wrap around the rat foot (2) and around the ankle joint (3) to ensure 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 the 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. In the case of the boot without a plantar surface, the inflatable bladder and Velcro strapping overlying the plantar surface of the foot were removed (the outlined rectangular area).

by which it was attached to the animal's foot (i.e., a Velcro strap around the foot and the ankle), 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 anesthesia (5% isoflurane/95% air mixture), as described above for the initial steps used for HLS preparation, except that the animal was placed head down in the anesthesia chamber while being suspended from the tail to prevent reloading of the hindlimbs. The animals were then placed back in the HU cage and allowed to fully recover from anesthesia for a period of 20 min before the initiation of the DFS protocol. 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, Sarasota, FL) attached to a hose leading to the bladder. The pressure stimulation protocol consisted of a 5-s inflation/5-s deflation of the air bladder for a total of 20 min followed by a 10-min rest interval. This cycle was repeated eight times over a 4-h 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. In the case of animals that wore either a DFS boot without active inflation or a DFS boot without a plantar surface, an identical experimental protocol was employed. The DFS boot without a plantar surface (Fig. 1) was attached to the foot of the right leg in the same fashion as a complete DFS boot, using a Velcro strap over the top of the foot and at the ankle, except that there was no contact with the plantar surface of the foot with any of the boot material. As the vast majority of the weight of the unmodified boot was accounted for by the ankle collar and the metal connectors, rather than the inflatable air bladder constructed from 1-ml plastic sheet material and Velcro strapping from the boot resulted in a weight change of <20% between a complete and plantar surfaceless boot. All DFS animals were treated in the above manner with regard to placement of the DFS apparatus on a daily basis during the 10 days of HU, including anesthesia.

It has been suggested that, to adequately 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<sup>2</sup> (1 mN/mm<sup>2</sup>) corresponds to a pressure of 7.5 mmHg. Thus a pressure of 8 mN/mm<sup>2</sup> is equal to 60 mmHg. Given that the mean sole area of the 6-mo-old male rats used in the study is between 450 and 500 mm<sup>2</sup>, the pressure required to stimulate the entire plantar surface was calculated as 13.9 mN/mm<sup>2</sup> (6,255–6,950 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 nociceptive 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 anesthesia 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. Both the medial gastrocnemius (MG) and the soleus muscle were 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 and MG muscle were immersed in TissueTek optimum cutting temperature mounting medium (Sakura Finetek, Torrance, CA), frozen in liquid nitrogen-cooled isopentane, and stored at –80°C. Upon analysis, frozen cross sections (5  $\mu$ m) from the soleus muscle were cut using a Zeiss Microm HM

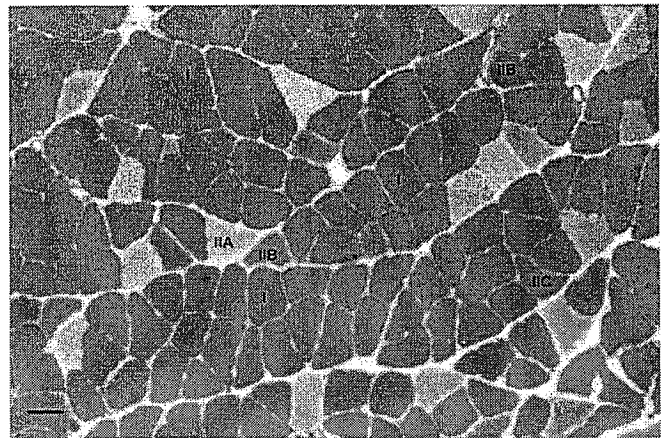


Fig. 2. Frozen cross section of a soleus muscle from an ambulatory control (AMB) rat stained using the metachromatic ATPase stain. Sections were preincubated at pH 4.35 and stained with toluidine blue, as described in EXPERIMENTAL DESIGN AND METHODS. On the basis of color, fiber types were classified as type I (turquoise), type IIA (light pink), type IIB (violet), and type IIC (dark blue with dark blue edge). Bar = 50  $\mu$ m.

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 (types 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 midbelly 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) so that the complete cross section of the soleus was imaged. Each image was then imported into Adobe Photoshop software (Adobe Systems, San Jose, CA), and the perimeter of each myofiber was delineated by drawing around the perimeter 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 was 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 MG and 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 MG and soleus muscle among the experimental groups, one-way ANOVA was carried out by using the SPSS statistical analysis program. When the univariate *F*-test was significant, Scheffé's post hoc test was used to further identify significant differences in myofiber CSA between the experimental group means (i.e., AMB group, HU group, HU+DFS group, and HU+NDFS group) for individual myofiber types. To evaluate any differences in myofiber CSA of DFS-treated and contralateral control muscles in the same HU+DFS, HU+NDFS, and HU+NPS animals, a paired Student's *t*-test was applied. Statistical significance level was set at *P* < 0.05.

Table 1. CSA of different myofiber types in the rat soleus and MG muscle for both right and left legs in AMB and HU animals

Muscle	Myofiber Type	Condition			
		AMB		HU	
		Right leg CSA, $\mu\text{m}^2$	Left leg CSA, $\mu\text{m}^2$	Right leg CSA, $\mu\text{m}^2$	Left leg CSA, $\mu\text{m}^2$
MG	I	2,478 $\pm$ 395	2,256 $\pm$ 338	1,498 $\pm$ 173	1,570 $\pm$ 224
Soleus	I	4,128 $\pm$ 537	4,123 $\pm$ 370	2,396 $\pm$ 479	2,342 $\pm$ 280
MG	IIA	2,671 $\pm$ 415	2,499 $\pm$ 324	1,669 $\pm$ 261	1,752 $\pm$ 359
Soleus	IIA	4,228 $\pm$ 876	4,063 $\pm$ 432	2,873 $\pm$ 603	2,768 $\pm$ 320
MG	IIB	3,649 $\pm$ 542	3,587 $\pm$ 324	2,507 $\pm$ 347	2,787 $\pm$ 469
Soleus	IIB	3,561 $\pm$ 1,029	3,395 $\pm$ 355	2,046 $\pm$ 431	2,337 $\pm$ 367
MG	IIC*	1,660 $\pm$ 402	1,796 $\pm$ 133	1,204 $\pm$ 365	1,289 $\pm$ 356
Soleus	IIC*	2,837 $\pm$ 658	2,810 $\pm$ 525	1,977 $\pm$ 349	2,403 $\pm$ 251

Values are means  $\pm$  SD. AMB, ambulatory control; HU, hindlimb unloaded; CSA, cross-sectional area; MG, medial gastrocnemius. 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 <4 animals per experimental group). As such, type IIC data for AMB, HU, and HU + dynamic foot stimulation could not be included in our statistical analysis other than as a descriptive measure.

## RESULTS

No significant differences in myofiber CSA of any myofiber type (i.e., types I, IIA, IIB, or IIC) were detected between the right and left soleus and MG muscles of AMB control or HU animals, respectively (Table 1). As the DFS apparatus was placed on the right hindlimb of the animal, all subsequent comparisons between experimental groups were carried out on 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 MG and soleus muscle obtained from any of the experimental groups are 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  $\sim 42\%$  ( $4,128 \pm 537$  vs.  $2,396 \pm 479 \mu\text{m}^2$ ) in type I myofiber CSA in the soleus muscle of HU animals was

seen compared with the soleus muscle of the AMB control group (Fig. 3A). However, no significant difference in type I soleus CSA was observed between the AMB control and the HU+DFS group ( $4,128 \pm 537$  vs.  $3,717 \pm 609 \mu\text{m}^2$ ). Our results indicate that the DFS protocol was responsible for the prevention of almost all (i.e.,  $>85\%$  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). In the case of the MG, after 10 days of HU, a significant decrease ( $P < 0.0001$ ) of  $\sim 42\%$  ( $2,478 \pm 395$  vs.  $1,498 \pm 173 \mu\text{m}^2$ ) in type I myofiber CSA in the MG muscle of HU animals was seen compared with the MG muscle of the AMB control group (Fig. 4). Significant differences in type I MG CSA were also observed between the AMB control, the HU, and the HU+DFS group ( $2,478 \pm 395$  vs.  $1,498 \pm 173$  vs.  $2,064 \pm 381 \mu\text{m}^2$ , respectively) (Fig. 4). Our results indicate that the DFS protocol was responsible for the prevention of a signifi-

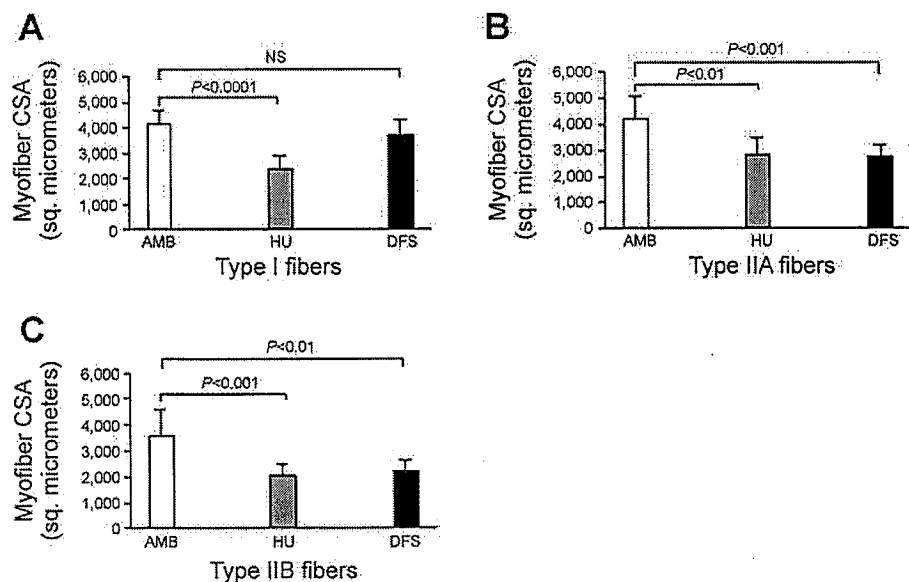


Fig. 3. Cross-sectional area (CSA) of different myofiber types in the soleus muscle in AMB, hindlimb unloaded (HU), and HU+DFS animals. A: type I myofibers; B: type IIA myofibers; C: type IIB myofibers. The CSA of all 3 fiber types in the HU group were significantly smaller compared with those in the AMB control group. For type I fibers only, no significant difference in CSA was found between the DFS group and the AMB control group in the soleus of the right hindlimb. In the DFS group, the DFS boot was attached to the right leg. Values are expressed in  $\mu\text{m}^2$  and represent means  $\pm$  SD;  $n = 10$  rats per group.

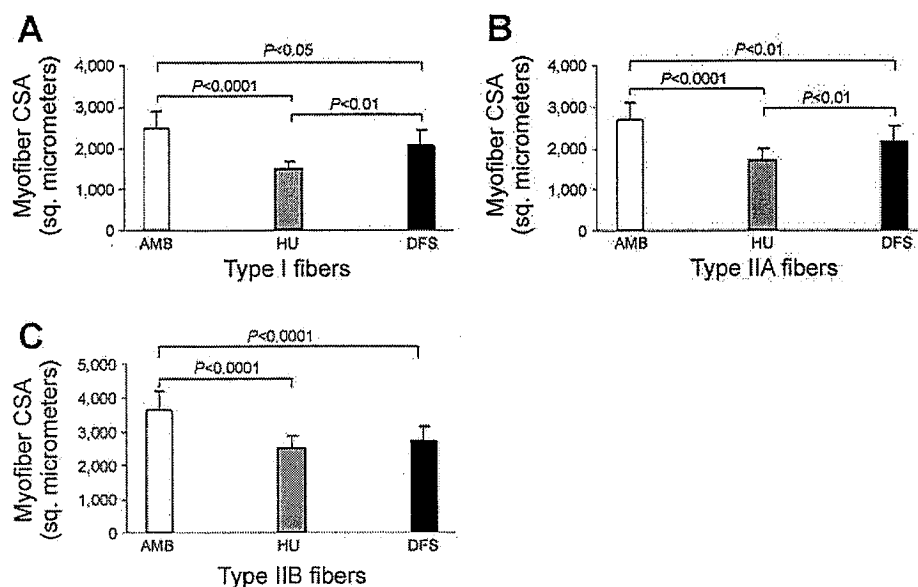


Fig. 4. CSA of different myofiber types in the medial gastrocnemius muscle in AMB, HU, and HU+DFS animals. A: type I myofibers; B: type IIA myofibers; and C: type IIB myofibers. The CSA of all 3 fiber types in the HU group were significantly smaller compared with those in the AMB group. For type I and type IIA fibers, significant difference in CSA was found between the DFS group and the HU group in the medial gastrocnemius of the right hindlimb. The CSA of type I and type IIA myofibers were also significantly different from the AMB. Values are expressed in  $\mu\text{m}^2$  and represent means  $\pm$  SD;  $n = 10$  rats per group.

cant amount (i.e., >57%) of the myofiber atrophy normally observed in type I myofibers of the MG muscle after 10 days of HU. A similar protective effect of DFS treatment was also observed in the case of type IIA myofibers in the MG, where the DFS protocol was responsible for preventing a significant amount (i.e., >46%) of the myofiber atrophy normally observed in type IIA myofibers of the MG muscle after 10 days of HU (Fig. 4). No significant protective effect of DFS was observed in type IIB myofibers of the MG muscle (Fig. 4).

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. 5), a significant difference ( $P < 0.001$ ; paired Student's *t*-test) was found. The average type I fiber CSA in the untreated contralateral leg ( $2,499 \pm 447 \mu\text{m}^2$ ) was significantly smaller than that observed in the DFS-treated leg ( $3,717 \pm 609 \mu\text{m}^2$ ). 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. 5A). In the case of MG muscles from the DFS-treated right leg and the contralateral control leg, the CSA of type I and type IIA myofibers were significantly ( $P < 0.05$ ) different (Fig. 5B). These data indicate that the effects of DFS in both the soleus and MG muscles are limb specific and that DFS does not appear to induce any systemic anti-atrophic effects on unloaded muscle tissue.

When the effect of wearing a DFS boot during HLS with and without active inflation on myofiber CSA was investigated, it was observed that wearing the DFS boot without active inflation provided a protective effect statistically indistinguishable from that observed with active inflation. Wearing of a passive boot compared with an actively inflated boot provided a similar protective effect against HU-induced type I myofiber atrophy in both the soleus (Fig. 6) and MG muscles, as well in type IIA myofibers of the MG muscle (MG data not shown). This protective effect again was confined to the hindlimb of the animal to which the DFS boot was attached (Fig. 6). One logical conclusion that could be drawn from this result was that the protective effect provided by wearing a DFS boot was

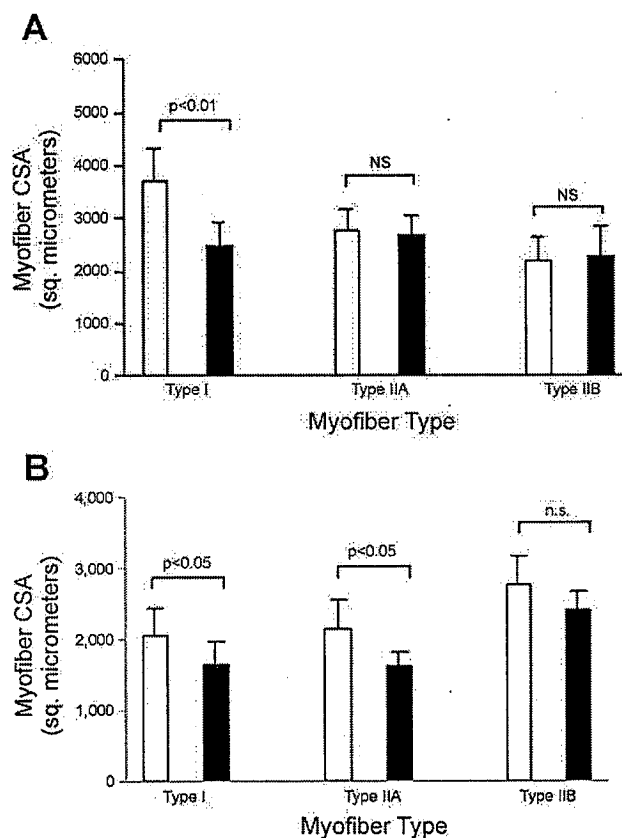


Fig. 5. CSA of soleus (A) and medial gastrocnemius (B) myofiber types between DFS-treated and contralateral control limb in the same animal. Values are expressed in  $\mu\text{m}^2$  and represent means  $\pm$  SD;  $n = 10$  rats per group. Open bars, DFS treatment, right leg; solid bars, contralateral control, left leg. The CSA of soleus type I myofibers in the DFS-treated right leg was significantly 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 soleus type IIA and IIB fiber CSA between the right and the left legs were found. Significant differences in the CSA of both type I and type IIA myofiber types in the medial gastrocnemius muscle of the DFS-treated leg were observed compared with the contralateral control, left leg of the same animal. NS, not significant.

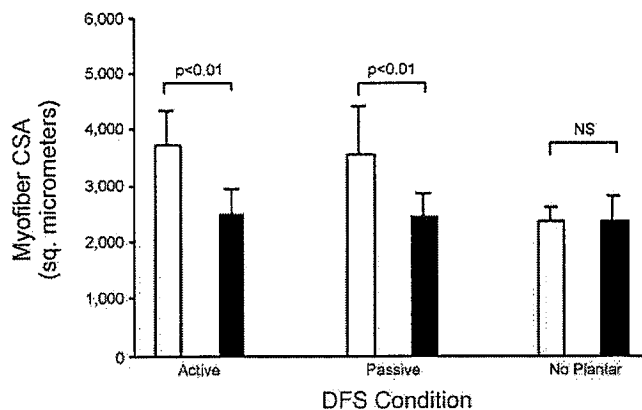


Fig. 6. CSA of soleus type I myofibers in active DFS-treated, passive DFS-treated, and no plantar surface DFS-treated animals and contralateral control limb in the same animals. Values are expressed in  $\mu\text{m}^2$  and represent means  $\pm$  SD. Open bars, DFS treatment, right leg; solid bars, contralateral control, left leg. The CSA of soleus type I myofibers in both the active and passive DFS-treated right leg was significantly greater than the CSA of type I myofibers in the soleus muscle of the contralateral control, left leg of the same animal. No significant difference in CSA of soleus type I myofibers was observed between the no plantar surface DFS-treated right leg and the contralateral control, left leg of the same animal.

solely associated with a loading effect on the limb induced by the weight of the boot. However, HLS animals that wore a DFS boot (with or without active inflation) were observed to repeatedly extend both hindlimbs during the period that they wore the DFS boot. As such, we hypothesized that, even without active inflation of the DFS boot, such movement could produce significant mechanical stimulation of the plantar surface generated by contact between the base of the rat foot and the DFS boot material. To test this hypothesis, we constructed a DFS boot that had no plantar surface contact with the rat foot, yet had all the other contact points (i.e., top of the foot, ankle). Wearing of the DFS boot without a plantar surface during HLS, even though the animals continued to extend their hindlimbs during this period, did not provide any significant protective effect with regard to protection of soleus type I myofibers from HLS-induced atrophy (Fig. 6).

In summary, our experimental data indicate that the protective effect against myofiber atrophy in the rat HLS model induced by wearing of a DFS boot is not associated with a "boot" loading effect or active recruitment of hindlimb musculature, rather is mediated via mechanical stimulation of the plantar surface. In addition, our data also indicate that this protective effect is ipsilateral in nature.

## DISCUSSION

The specific aim of this study was to determine whether DFS applied to the plantar surface of the rat foot would counteract the atrophy in the soleus and MG muscles normally induced by HU.

Rat HU is an animal model that is widely used to study 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 (30). The 10-day suspension protocol used in our study induced significant atrophy in both the soleus and MG muscles of

the rat hindlimb. Our data clearly demonstrate that the application of DFS (either active or passive) is a highly efficient means of preventing type I myofiber atrophy normally induced in the soleus muscle, and type I and type IIA myofiber atrophy normally induced in the MG muscle, as a consequence of unloading (Figs. 3 and 4). In addition, the protective effect of DFS appears to be ipsilateral and is not systemically mediated, as is indicated by a lack of a protection from myofiber atrophy in either the soleus or MG muscles of the contralateral control of the HLS DFS-treated animals (Fig. 5). Furthermore, these results indicate that mechanical stimulation of the plantar surface of the foot, rather than "loading" of the limb or indeed activation of mechanoreceptors elsewhere on the foot or ankle, appears to be the essential element required for the myofiber atrophy protective effect seen during HLS.

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 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 sex of the animals used in the studies, as well as the length of the suspension period. The average life span of the rat is ~28 mo. From the data available in the literature, it is apparent that both very young rats (up to 3 mo of age) and aged rats (older than 20 mo 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 (28), whereas in aged rats this might be the result of an increased sensitivity to the adverse effects of disuse associated with the aging process (31).

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-wk and 3-mo-old male rats after 2 and 3 wk of unloading, respectively (2, 22). Other investigators, using 17-wk-old male rats tail-suspended for 2 wk (29) or 3-mo and 22-mo-old female rats suspended for 3 wk (26) 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 wk of unloading in 22-mo-old male rats, soleus myofiber CSA was decreased by 48% in type I fibers, 40% in type IIA, and 44% in type IIB fibers, whereas in younger adult 8-mo-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-mo-old male rats are in agreement with the findings previously reported in the literature for the animals of this particular age (i.e., SKM 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 of the soleus. This discrepancy may be explained by the use of a less descriptive histochemical method (11) for fiber-type classification compared with our histochemical methodology (20). In this previous study, it was also found that foot pressure did not prevent the transformation of type I to type II fiber types in the soleus muscle. In our study, however, we did not observe any significant fiber-type shifting in the soleus muscle of our HLS animals (data not shown), an observation that may be due to the age of the animals utilized in this study, namely mature, adult animals.

A second 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. 5). 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 (Fig. 3). A similar, ipsilateral response with regard to the protective effect of DFS was also found in the MG muscles of HLS animals (Fig. 5). In contrast to the DFS effect in soleus muscle where only type I myofibers were protected, the DFS response in MG muscle, although lower in magnitude than observed in soleus muscle, appeared to protect both type I and type IIA myofibers.

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% 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 disks, 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 humans subjected to similar mechanical stimulation of the plantar surface (15).

The observation that wearing a DFS boot without plantar contact during HLS provides no protective effect against myofiber atrophy in the soleus muscle, compared with the protective effect observed during both passive and active DFS (Fig. 6), suggests that this effect is mediated via a pathway originating with activation of mechanoreceptors located specifically in the plantar surface of the foot. In addition, as the DFS boot without a plantar surface makes contact with both the ankle and dorsal surface of the foot, this suggests that other potential cutaneous or noncutaneous mechanoreceptors, such as muscle spindles and/or stretch receptors located in tissue other than the plantar region, do not play a significant role in this protective response. Furthermore, the minimal mechanical stimulation of the plantar surface produced during passive DFS (i.e., DFS

boot wearing without bladder inflation) suggests that the most likely mechanoreceptors involved are the Merkel disks and Meissner corpuscles located in the upper epidermal region of the plantar surface, rather than the Ruffini endings and Pacinian corpuscles located in the deeper dermal and subcutaneous regions of the plantar surface. Although there was no direct relationship established between increased EMG activity and SKM atrophy attenuation in this study, 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 addition, DFS treatment as applied in this study appears to provide more atrophy prevention in type I myofibers than in type II myofibers. It is interesting to speculate that this is due to greater stimulation/recruitment of specific type I neural pathways than type II neural pathways as a consequence of mechanical stimulation of the plantar surface of the foot. For example, the Merkel disks (thought to correspond to slowly adapting type I units) and the Meissner corpuscles (thought to correspond to fast-adapting type I units) are both found close to the surface of the skin, whereas Ruffini endings (thought to correspond to slowly adapting type II units) and Pacinian corpuscles (thought to correspond to fast-adapting type II units) are found in the deeper layers of the skin (25). The minimal mechanical stimulation of the plantar surface produced during passive DFS (i.e., DFS boot wearing without bladder inflation) suggests that the most likely mechanoreceptors involved are the Merkel disks and Meissner corpuscles, both of which are thought to be associated with activation of slow-adapting and fast type I units, respectively. It is interesting to speculate that studies designed to activate Ruffini endings and Pacinian corpuscles located deeper in the skin by increasing either the amplitude or frequency of DFS stimulus may yield much more significant protective effects in type II myofibers than observed in this study.

In conclusion, the results of the present study illustrate that minimal external mechanical stimulus applied to the plantar surface of the rat foot is capable of counteracting unloading-induced soleus and MG muscle atrophy. It is postulated that this effect is primarily achieved via stimulation of cutaneous pathways emanating in the plantar surface of the foot that, in turn, activate appropriate motoneurons to generate motor unit contraction mimicking the neuromuscular activity patterns normally induced by such plantar surface stimulation 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 spaceflight, as well as an effective rehabilitation tool for clinical populations such as the bed-ridden or elderly patients.

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