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Using Dynamic Foot Pressure as a Countermeasure to Muscle Atrophy

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Abstract—Microgravity during space flight induces loss of skeletal muscle (SKM) mass, strength and functionality that could jeopardize the success of a mission. One animal model commonly used to mimic the effects of microgravity on skeletal muscle is the rat hindlimb unloading (HU) model. In this model, the back legs of the rat are lifted up off the ground by a harness attached to the tail of the animal. During HU, the muscles of the back legs do not support the weight of the animal and, hence, undergo muscle atrophy. The aim of this project was to investigate whether or not mechanical pressure applied to the base of the unloaded rat foot could prevent the process of SKM atrophy by increasing neuromuscular activation in the muscles of the unloaded hindlimb. Our results indicate that the application of DFP ameliorates the SKM atrophy induced by HU in the soleus muscle of the rat. It is postulated that this effect is achieved via stimulation of proprioceptive pathways as a consequence of DFP mimicking the neuromuscular activity/contraction patterns normally induced by load bearing in specific anti-gravity muscles of the lower limbs in a terrestrial environment. This underlying concept promises to serve as the basis for the development of a novel supplement to pre-existing exercise countermeasures during space flight, as well as an effective rehabilitation tool for clinical populations such as bed-ridden or elderly patients.

MICROGRAVITY INDUCES SKM ATROPHY AS A CONSEQUENCE of mechanical unloading of the musculoskeletal system. In the context of manned space flight, the subsequent loss of muscle strength and functionality poses significant operational implications that could jeopardize the success of a mission. For example, the crew must maintain their physical condition to perform mission operations, such as extravehicular activity (EVA) and on-going International Space Station (ISS) construction tasks. Optimal muscle function is also a prerequisite to enable the flight crew to respond promptly to any emergency situations that may arise during flight or landing. Such requirements are compounded when mission duration is increased, as is the case for ISS operations and for any future manned mission to Mars. As a consequence of such operational demands, designing and validating efficient in-flight countermeasures to microgravity-induced skeletal muscle atrophy become of paramount importance for the future of manned space flight.

Background and Significance

The neuromuscular system is one of the most affected biological systems during space flight. Microgravity induces SKM atrophy particularly affecting the anti-gravity musculature of the lower limbs.^{1,2} In general in rodents slow-twitch muscles are more susceptible to space flight-induced SKM atrophy than the fast-twitch ones and extensors are more affected than flexors.³ In space, contrary to the terrestrial environment, the absence of a constant muscle loading leads to decrease in neuromuscular activation.⁴ Weightlessness has been shown to cause a decrease in muscle volume, mass and strength, alterations in fiber type and myosin heavy chain (MHC) expression, as well as a decrease in neuromuscular function and muscle capillarity.^{5,6} In addition, following the characteristics of the space flight hindlimb muscles of animals shows significant changes in muscle collagen concentration of atrophied muscles with a concomitant decrease in the concentration of mature cross-links.⁷ These data suggest that reduced load or muscle activation results in a rapid decline in non-collagenous muscle protein, which enhances the tissue concentration of collagen.

Hindlimb unloading (HU) is an accepted and a widely used model of microgravity-induced SKM atrophy since it results in many of the same basic functional, histological, and biochemical alterations detected in SKM during space flight.¹ In the HU condition the most rapid decrease in SKM mass occurs within the first week of suspension.⁸

Exercise, currently the primary on-orbit muscle degradation countermeasure, has not proven completely effective in preventing muscle atrophy. To date, the projected amount of time (as high as four hours per day) required to perform daily-prescribed exercise countermeasures to muscle atrophy on ISS will be a significant drain on productive crewmember time. Therefore, an atrophy countermeasure designed for use as an integral part of the crewmember's daily routine may prove to be of great value in maintaining muscle mass and function during long-term space flight. The purpose of this study is to investigate whether the application of mechanical

stimuli to the plantar surface of the feet can counteract microgravity-induced muscle atrophy. The basic concept behind the application of mechanical stimuli to the soles of the feet is the well-established motor control principle that sensory input (i.e., pressure application) can modify motor output (i.e., neuromuscular activation). A possible explanation of this phenomenon might be the stimulation of the cutaneous mechanoreceptors in the skin (i.e., Merkel discs, Meissner corpuscles, Ruffini endings, and Pacinian corpuscles).

Previous research conducted both during space flight⁹ and on the ground¹⁰ has demonstrated that increasing sensory input by applying pressure to the feet results in an increase in neuromuscular activation. A ground-based microgravity simulated study using hindlimb-unloaded rats showed a significant attenuation of muscle atrophy after pressure application to the soles of the rat feet.¹¹ Recently, it has been reported that providing mechanical stimulus to the legs of sheep resulted in a significant increase in bone density.¹² The aforementioned evidence provides support to the hypothesis that external mechanical stimulus applied to the feet may in part counteract the microgravity-induced muscle atrophy providing a novel and an effective in-flight countermeasure as well as an effective rehabilitation technique for bed-ridden patients.

Experimental Design and Methods

Animal care

Forty-four 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 five groups as follows: (a) ambulatory controls (AMB) (N = 10), (b) hindlimb unloaded alone (HU) (N = 10), (c) hindlimb unloaded with DFS boot with active inflation (HU+DFS) (N = 10), (d) hindlimb unloaded with DFS boot without active inflation (HU+NDFS) (N = 10), and (e) hindlimb unloaded 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 (CAURE) at the 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.¹³ Rats were anaesthetized 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 anaesthesia, it was removed from the chamber and placed supine on the lab-

oratory bench adjacent to an air extraction vent. Anaesthesia was maintained during preparation for HS by attaching a sealed mask over the snout of the animal into which was pumped additional anaesthesia. The animal's tail was lightly cleaned with 10% povidone iodine that 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 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 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 in an aluminum bar placed laterally across the top of the cage on two vertical supportors 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 approximately 10 minutes to perform from initial anaesthesia to suspension, with the animal regaining complete consciousness from anaesthesia within a period of five minutes 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 (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 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 anaesthesia (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 anaesthesia 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 anaesthesia for a period of 20 minutes before the initiation of the DFS protocol. Pressure was applied to the foot by infla-

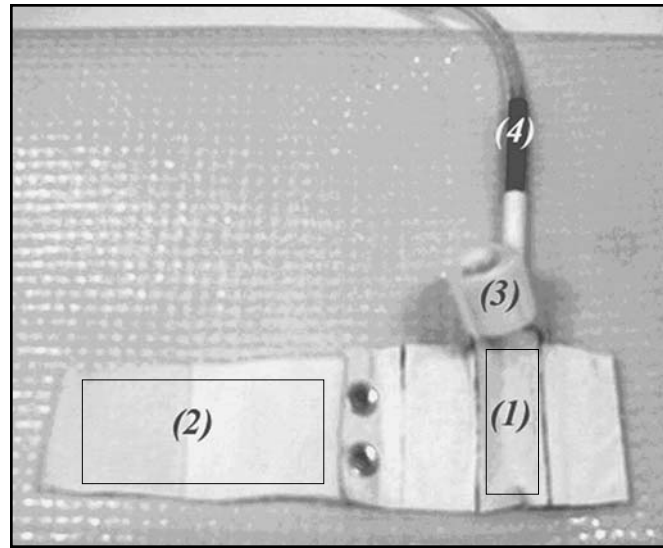


Figure 1. Dynamic Foot Stimulation (DFS) Apparatus. The external sleeve of the DFS boot was fabricated using a thin, light, yet durable plastic with an integral inflatable/deflatable air bladder (1) located beneath the sleeve surface that contacts the sole of the rat foot. Velcro restraint straps wrap around the rat foot (2) and around the ankle joint (3) 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 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 preventing 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 areas).

tion/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-second inflation/5-second deflation of the air bladder for a total of 20 minutes followed by a 10-minute rest interval. This cycle was repeated eight 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. 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 1mil plastic sheet material and Velcro material, removal of the inflatable bladder and a portion of the Velcro strapping from the boot resulted in a weight change of less than 20% between a complete and plantar surface-less 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.¹⁴ In general, a pressure of 1 kN/m² (1 mN/mm²) corresponds to a pressure of 7.5 mmHg. Thus, a pressure of 8 mN/mm² 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 mm², the pressure required to stimulate the entire plantar surface was calculated as 13.9 mN/mm² (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 of the rat's foot, 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 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. 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 mid-belly of the soleus and MG muscle were immersed in TissueTek OCT mounting medium (Sakura Finetek, Torrance, California) 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, New Hampshire).

Histochemical-Morphometric Analysis

Fiber typing on frozen sections was performed utilizing the metachromatic dye-ATPase myofibrillar stain method originally described by Ogilvie and Feedback,¹⁵ as modified by

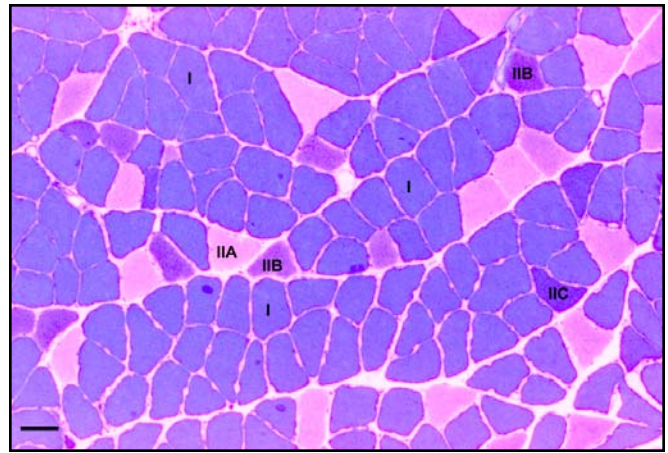


Figure 2. Frozen cross-section of a soleus muscle from an ambulatory control rat stained using the metachromatic ATPase stain. Sections were pre-incubated at pH 4.35 and stained with toluidine blue as described in the Methods section. On the basis of color, fiber types were classified as Type I, Type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

Konishi et. al.¹⁶ 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 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 analysis of variance (ANOVA) was carried out using the SPSS statistical analysis program. When

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

Muscle	Myofiber Type	Ambulatory Control (AMB)		Hindlimb Unloaded (HU)	
		Right Leg (CSA in sq. microns)	Left Leg (CSA in sq. microns)	Right Leg (CSA in sq. microns)	Left Leg (CSA in sq. microns)
MG	I	2,478 ± 395	2,256 ± 338	1,498 ± 173	1,570 ± 224
SOLEUS	I	4,128 ± 537	4,123 ± 370	2,396 ± 479	2,342 ± 280
MG	IIA	2,671 ± 415	2,499 ± 324	1,669 ± 261	1,752 ± 359
SOLEUS	IIA	4,228 ± 876	4,063 ± 432	2,873 ± 603	2,768 ± 320
MG	IIB	3,649 ± 542	3,587 ± 324	2,507 ± 347	2,787 ± 469
SOLEUS	IIB	3,561 ± 1,029	3,395 ± 355	2,046 ± 431	2,337 ± 367
MG	IIC*	1,660 ± 402	1,796 ± 133	1,204 ± 365	1,289 ± 356
SOLEUS	IIC*	2,837 ± 658	2,810 ± 525	1,977 ± 349	2,403 ± 251

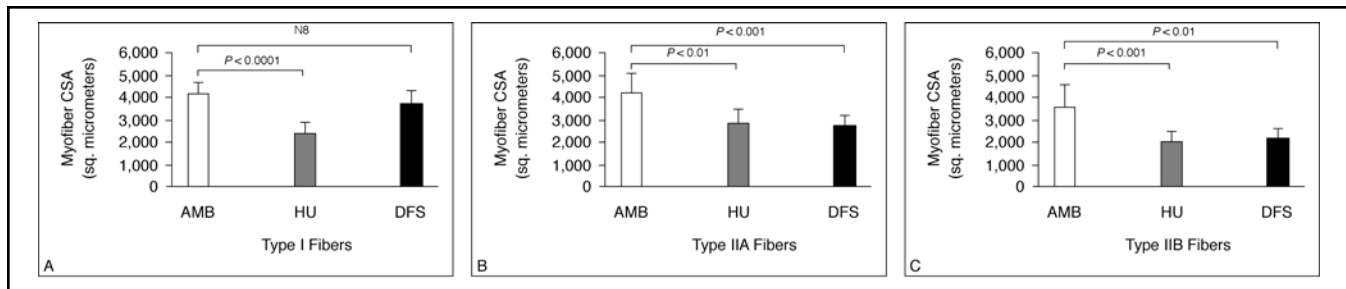


Figure 3. Cross-sectional area (CSA) of different myofiber types in the soleus muscle in Ambulatory, HU and HU+DFS animals. Panel A: Type I myofibers, Panel B: Type IIA myofibers, and Panel C: Type IIB myofibers. The CSA of all three fiber types in the HU group was significantly smaller compared to those in the ambulatory control group. For Type I fibers only, no significant difference in CSA was found between the DFS group and the ambulatory 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 square micrometers (μm^2) and represent means \pm SD; $n = 10$ rats per group. AMB = ambulatory control, HU = hindlimb unloaded, and DFS = hindlimb unloaded + dynamic foot stimulation.

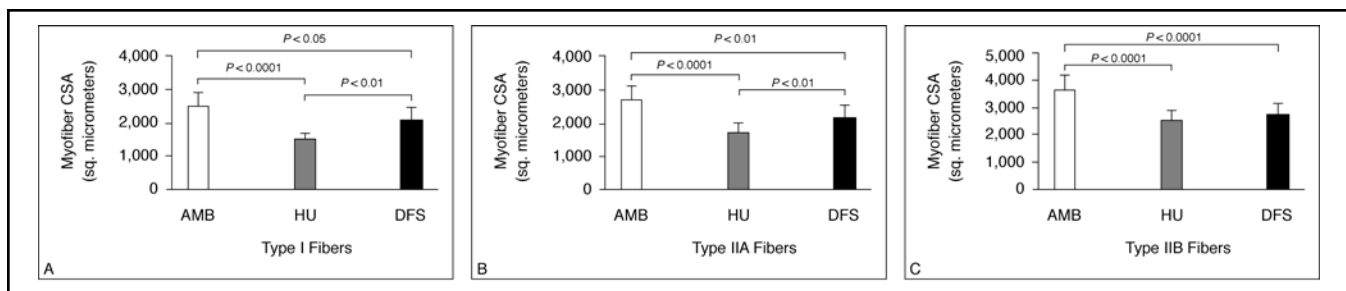


Figure 4. Cross-sectional area (CSA) of different myofiber types in the medial gastrocnemius muscle in Ambulatory, HU and HU+DFS animals. Panel A: Type I myofibers, Panel B: Type IIA myofibers, and Panel C: Type IIB myofibers. The CSA of all three fiber types in the HU group was significantly smaller compared to those in the ambulatory control group. For Type I and Type IIA fibers, significant difference in CSA was found between the DFS group and the HU group in the MG of the right hindlimb. The CSA of Type I and Type IIA myofibers was also significantly different from the ambulatory control. Values are expressed in square micrometers (μm^2) and represent means \pm SD; $n = 10$ rats per group. AMB = ambulatory control, HU = hindlimb unloaded, and DFS = hindlimb unloaded + dynamic foot stimulation.

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, 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 $P < 0.05$.

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 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 approximately 42% ($4,128 \mu\text{m}^2 \pm 537 \mu\text{m}^2$ vs. $2,396 \mu\text{m}^2 \pm 479 \mu\text{m}^2$) 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. 3A). However, no significant difference in Type I soleus CSA was observed between the AMB control and the HU + DFS group ($4,128 \mu\text{m}^2 \pm 537 \mu\text{m}^2$ vs. $3,717 \mu\text{m}^2 \pm 609 \mu\text{m}^2$). 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).

In the case of the MG, after 10 days of HU, a significant decrease ($P < 0.0001$) of approximately 42% ($2,478 \mu\text{m}^2 \pm 395 \mu\text{m}^2$ vs. $1,498 \mu\text{m}^2 \pm 173 \mu\text{m}^2$) in Type I myofiber CSA in the MG muscle of HU animals was seen as compared to 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 ($2,478 \mu\text{m}^2 \pm 395 \mu\text{m}^2$ vs. $1,498 \mu\text{m}^2 \pm 173 \mu\text{m}^2$ vs. $2,064 \mu\text{m}^2 \pm 381 \mu\text{m}^2$ respectively) (Fig. 4). Our results indicate that the DFS protocol was responsible for the prevention of a significant amount (i.e., over 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

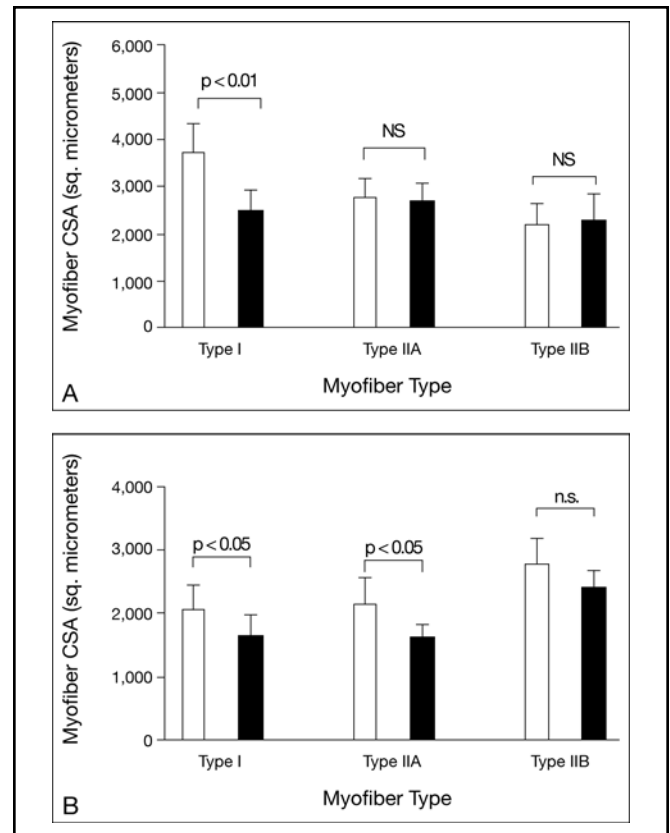


Figure 5. Cross-sectional area (CSA) of soleus (A) and medial gastrocnemius (B) myofiber types between DFS-treated and contralateral control limb in the same animal. Values are expressed in square micrometers (μm^2) and represent means \pm SD; $n = 10$ rats per group. Open bar = DFS treatment, right leg, Solid Bar = contralateral control, left leg. The CSA of soleus Type I myofibers in the DFS-treated right leg was significantly 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 MG muscle of the DFS-treated leg were observed in comparison with the contralateral control, left leg of the same animal.

amount (i.e., over 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

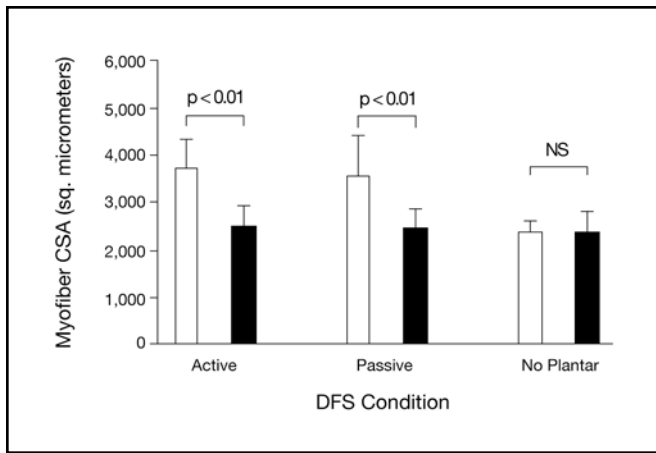


Figure 6. Cross-sectional area (CSA) of soleus Type I myofibers in active DFS-treated, passive DFS-treated and no plantar surface DFS-treated animals and contralateral control limb in the same animals. Values are expressed in square micrometers (μm^2) and represent means \pm SD. Open bar = DFS treatment, right leg, Solid Bar = 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.

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 was 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, researchers 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 as compared to 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 as 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 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 when 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. In order 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 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, it is mediated via mechanical stimulation of the plantar surface. In addition, our data also indicates that this protective effect is ipsilateral in nature.

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 and MG muscles 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.¹⁷ 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 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 the 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 in 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 than 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,¹⁸ while in aged rats this might be the result of an increased sensitivity to the adverse effects of disuse associated with the aging process.¹⁹

Significant reduction in soleus CSA for both Type I and Type II fibers as well as slow-to-fast twitch transformation of fibers has been demonstrated in 6-week and 3-month old male rats after two and three weeks of unloading, respectively.^{2,20} Other investigators, using 17-week old male rats tail-suspended for two weeks²¹ or three months and 22-month old female rats suspended for three weeks²² 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 four 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 of the soleus. This discrepancy may be explained by the use of a less descriptive histochemical method¹¹ for fiber type classification compared to our histochemical methodology.¹⁵ 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 attributed 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



MUSCLE TONE—NASA research intended to maintain muscle tone in astronauts with steady but minimal mechanical pressure has widespread potential in medical therapies. Chris Arellano, a native of Corpus Christi,, Texas, is engaged in NASA-based research in the field of kinesiology leading to his master’s degree.

CSA in the soleus, no such protective effects on Type I myofiber CSA were 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 approximately 85% during the first days of unloading, and it was gradually restored after 7-10 days of unloading.^{1,23} Interestingly, a significant increase in the soleus EMG activity had 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, and 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.²⁴

Wearing a DFS boot without plantar contact during HLS provides no protective effect against myofiber atrophy in the soleus muscle, as compared to the protective effect observed during both passive and active DFS (Fig. 6). This observation 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, it appears that other potential cutaneous or non-cutaneous 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 discs 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 discs (thought to correspond to slowly adapting type I units, SA-I) and the Meissner corpuscles (thought to correspond to fast adapting type I units, FA-I) are both found close to the surface of the skin, while Ruffini endings (thought to correspond to slowly adapting type II units, SA-II) and Pacinian corpuscles (thought to correspond to fast adapting type II units, FA-II) 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 discs 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 medial gastrocnemius muscle atrophy.²⁶ 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 space flight.²⁷⁻³² It can also serve as an effective rehabilitation tool for clinical populations such as the bed-ridden or elderly patients.

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Graduates in Health and Health Performance

Dr. Charles Layne, Dissertation Director

During the reporting period, two students completed their degree work.

Katharine E. Forth obtained her Ph.D. in Motor Control from the Department of Health and Human Performance, University of Houston. Dr. Forth had been supported by ISSO funding. Currently, she is a postdoctoral fellow in the Neurosciences Laboratory at the NASA-Johnson Space Center.

Kimthu Nguyen obtained her M.S. degree in Motor Control from the Department of Health and Human Performance, University of Houston. In Fall 2005, Ms. Nguyen intends to enter the Neuroscience doctoral program at the University of Texas at Austin.



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