



INFLATABLE RAT BOOT—Dr. Charles Layne measures pressure exerted on the muscles in a rat's foot by inserting the foot in an inflatable boot. The inflatable boot consists of a thin base made from an extremely light yet durable metal sheer with an inflatable/deflatable latex bladder adjusted on it. Velcro restraint straps secure the boot to the sole of the foot of the dominant leg during rat hindlimb suspension. The bladder is connected to an airpump by a single air/vacuum line. Pump cycling time and duration are controlled by a microprocessor.

Using Dynamic Foot Pressure as a Countermeasure to Muscle Atrophy

50-ISSO

Abstract—

In the context of human space flight, the microgravity-induced loss of muscle mass, strength and functionality jeopardize mission success. One animal model commonly used to mimic the effects of microgravity on skeletal muscle is the rat hindlimb suspension 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 hindlimb suspension, the muscles of the back legs do not support the weight of the animal and hence undergo muscle atrophy. The aim of this project is to investigate whether or not mechanical pressure applied to the base of the suspended rat foot can prevent the process of skeletal muscle atrophy by increasing neuromuscular activation in the muscles of the suspended hindlimb. Researchers expect that the application of foot pressure will decrease muscle atrophy and will serve as a supplement to exercise during space flight as well as an effective rehabilitation technique for bed-ridden patients.

THE OPERATION OF THE INTERNATIONAL SPACE STATION (ISS) constitutes a new era in space exploration with subsequent increase in the duration and frequency of the missions. Spaceflight is associated with muscle mass and strength loss, which imposes potential operational implications. To successfully complete missions objectives, the physical performance of the crewmembers is of paramount importance. The ongoing ISS construction tasks, several extravehicular activities (EVA), and possible inflight emergency situations are some of the conditions that require good physical fitness. In addition, the maintenance of the astronauts' health and physical condition upon return to earth, particularly if the exploration of Mars is to be pursued, remains one of the NASA's primary concerns.

Background and Significance

The neuromuscular system is one of the biological systems most affected during spaceflight. 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 spaceflight-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 a 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, study of spaceflight 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 and minimal muscle activation result in a rapid decline in non-collagenous muscle pro-

tein, which enhances the tissue concentration of collagen.

Hindlimb suspension (HLS) is an accepted and 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 HLS condition the most rapid decrease in SKM mass occurs within the first week of suspension.⁸

Exercise, the primary in-flight muscle degradation countermeasure, does not effectively prevent muscle atrophy. It is therefore imperative that some other forms of in-flight countermeasure be developed to supplement the prescribed exercise regimen the astronauts follow during spaceflight. 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, Pacinian corpuscles).

Previous research conducted both during spaceflight⁹ and on the ground¹⁰ have 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

Experimental plan. Mature adult male Wistar rats are randomly assigned to four groups of ten rats each as follows: sedentary controls (Ctrl), hindlimb suspended only (HLS), hindlimb suspended wearing an inflatable boot (HLS-IFL), and hindlimb suspended rats wearing a non-inflatable boot (HLS-NIFL). The stimulation of mechanoreceptors is achieved by applying pressure to the plantar surface of the foot during the 14-day period of HLS using a custom-built boot. The anti-atrophic effects of DFP application is quantified directly by morphological (muscle wet weight, myofiber cross-sectional area, neuromuscular junction size/density), histochemical (myofiber type distribution) and biochemical (myosin heavy chain-MHC isoform content, muscle collagen concentration, and maturation) analysis techniques in the soleus-Sol (predominantly slow-twitch ankle extensor muscle), medial gastrocnemius-MG (predominantly fast-twitch ankle extensor muscle), and tibialis anterior-TA (antagonist, fast twitch ankle flexor muscle) muscles.

Hindlimb suspension procedure. Unloading of the hindlimbs is achieved using a tail-suspended rat model.¹³ This model allows the animals to move freely about the cage using their forelimbs as their only mechanism of movement, while the hindlimbs are suspended at a 25° angle from the cage floor. The muscles of the hindlimbs do not support the weight of the animal and hence undergo muscle atrophy. The hindlimb suspension condition is

applied for 14 days.

Dynamic foot pressure application. A custom-built rat inflatable boot is used to stimulate the mechanoreceptors of the soles of the foot. The boot, outfitted with an inflatable/deflatable latex bladder, is attached to the foot of one leg chosen at random in HLS animals, this leg being termed the “dominant leg,” the other leg being termed the contra-lateral control leg. Pressure is applied to the foot of the dominant leg by inflation/deflation of the latex bladder using an air pump attached to a hose leading to the bladder.

EMG Recording. To validate that foot pressure using the inflatable boot induces muscle activation, a preliminary study has been conducted in anesthetized animals both recumbent and while hindlimb suspended. Bi-polar wire electrodes are placed in the Sol, MG, and TA muscles of the hindlimbs using a modified surgical procedure of that previously described.¹⁴ The electrode leads are bundled together at the base of the tail using an orthopedic tape to prevent them from being accidentally displaced. The leads are connected to an amplifier and the signals transferred through an A/D board to a data acquisition software package. Electrical activity (i.e., EMG amplitude) in the Sol, MG, and TA muscles is continuously monitored during application of foot pressure in the anesthetized recumbent and hindlimb suspended animals.

Tissue Collection and Processing. Sol, MG, and TA muscles are collected from control and hindlimb suspended animals using a procedure previously described.¹⁵ Briefly, the animals are deeply anesthetized, the hindlimbs are shaved, and the muscles are exposed and carefully dissected from the leg. The excised Sol, MG, and TA muscles are attached to wooden rods by pins inserted through the tendon attachments so that the muscle is elongated without being stretched, immersed in TissueTek OCT mounting medium, frozen in liquid nitrogen-cooled isopentane and stored at -80°C until histochemical and electrophoretic analysis.

In preparation for frozen sectioning, frozen Sol, MG, or TA muscle from pairs of control and hindlimb suspended animals are placed side by side on the same sectioning stub, mounted in embedding compound, and frozen using liquid nitrogen-cooled isopentane. This configuration ensures that the sections from control and suspended muscle is of identical thickness and is stained for the same length of time. Frozen cross sections (10 μm) are cut using a Zeiss Microm HM 500 OM microtome cryostat and picked up onto Superfrost Plus glass slides (Erie Scientific, Portsmouth, NH). Sections were allowed to air dry for one hour before histochemical staining, whereas sections destined for immuno-histochemical staining are immediately placed in a fresh D-PBS buffer.

Histochemical and Electrophoretic analysis. Investigators conduct the morphometric analysis of myofiber dimensions in frozen cross-sections of Sol, MG, and TA muscles from HLS and control animals, as previously described.¹⁵ Fiber typing on frozen sections is carried out utilizing the metachromatic dye-ATPase myofibrillar stain using the method originally developed by Ogilvie and Feedback¹⁶ as modified by Bamman et al.⁵ Immunohistochemical staining of MHC isoforms on a per myofiber basis is carried out as previously described.⁵ The relative amounts of MHC isoforms (Type I, Type IIa, Type IIb and Type IIx) are determined using glycerol/SDS-polyacrylamide gel electrophoresis as described elsewhere.⁵ Neuromuscular junction (NMJ) size/density measurements are carried out on frozen sections histochemically stained using a standard

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non-specific esterase stain followed by morphometric analysis of digitized images.

Collagen Analysis. Muscle collagen biochemistry is performed according to a previously published method.¹⁷ Briefly, skeletal muscle mid-belly cross-sections (~3-5 mg dry wt.) are hydrolyzed for 24 hours in 6M HCl, subjected to CF1 partition chromatography, and solid phase extraction prior to elution on a RP-HPLC system. Collagen cross-link analysis of HP (hydroxylysylpyridinoline) and LP (lysylpyridinoline) is monitored fluorometrically at an excitation wavelength of 295 nm and emission wavelength of 390 nm. Cross-links are expressed as moles of cross-link per moles of collagen. Skeletal muscle collagen is quantitated using an index of collagen concentration, hydroxyproline, an amino (imino) acid. Using Waters Pico-tag[®] pre-column derivatization method, hydroxyproline-PITC is eluted isocratically, monitored on an absorbance detector at 254 nm and expressed as µg Hyp/mg dry wt tissue.

Expected Results

A preliminary study has been conducting in anesthetized animals to validate whether Dynamic Foot Pressure (DFP) using the custom-built inflatable boot induces neuromuscular activation in the selected muscle examined. We have been working on developing the optimal setting for consistent EMG recording. We have established both the surgical procedure and the appropriate implantation of the fine wire electrodes to simultaneously record EMG activity from all Sol, MG, and TA muscles, while the DFP is applied. We are attempting to develop an optimal foot pressure protocol with regard to the amount and the time of pressure application. Various combinations have been examined taking into consideration the optimal activation patterns needed to stimulate the cutaneous mechanoreceptors of the plantar surface of the foot. It is expected that the application of DFP will ameliorate hindlimb-induced skeletal muscle atrophy. We postulate that this effect will be achieved via proprioceptive pathways as a consequence of DFTmimicking the neuromuscular activity/contraction patterns normally induced by load bearing in specific anti-gravity muscles of the lower limbs in a terrestrial environment.

The underlined concept promises to serve as the basis for developing a novel supplement to exercise during spaceflight as well as an effective rehabilitation technique for bed-ridden patients.

References

- ¹R. V. Edgerton and R. R. Roy. "Neuromuscular Adaptations to Actual and Simulated Spaceflight," in *American Physiological Society, Handbook of Physiology* Vol. II. Baltimore, MD: Williams & Wilkins, 1996. 721-63.
- ²R. H. Fitts, D. R. Riley, and J. J. Widrick. "Physiology of a Microgravity Environment." Invited review: "Microgravity and Skeletal Muscle," *J. Appl. Physiol.* 89 (2000): 823-39.
- ³B. Jiang, Y. Ohira, R. R. Roy, Q. Nguyen, E. I. Ilyina-Kakueva, V. Oganov, and V. R. Edgerton. "Adaptation of Fibers in Fast-Twitch Muscles of Rats to Spaceflight and Hindlimb Suspension," *J. Appl. Physiol.* 73 (1992): 58S-65S.
- ⁴M. R. Recktenwald, J. A. Hodgson, R. R. Roy, S. Riazanski, G. E. McCall, I. Kozlovskaya, D. A. Washburn, J. W. Fanton, and V. R. Edgerton. "Effect of Spaceflight on Rhesus Quadrupedal Locomotion After Return to 1G," *J. Neurophysiol.* 81 (1999): 2451-63.

⁵M. M. Bamman, M. S. F. Clarke, D. L. Feedback, R. J.

Talmadge, B. R. Stevens, S. A. Lieberman, and M. C. Greenisen. "Impact of Resistance Exercise During Bed Rest on Skeletal Muscle Sarcopenia and Myosin Isoform Distribution," *J. Appl. Physiol.* 84 (1998): 157-63.

⁶D. A. Riley, S. Ellis, G. R. Slocum, F. R. Sedlack, J. L. W. Bain, B. B. Krippendorf, C. T. Lehman, M. Y. Macias, J. L. Thompson, K. Vijayan, and J. A. De Bruin. "In-Flight and Post-Flight Changes in Skeletal Muscles of SLS-1 and SLS-2 Spaceflown Rats," *J. Appl. Physiol.* 81 (1996): 133-44.

⁷T. P. Martin, V. R. Edgerton, and R. E. Grindeland. "Influence of Spaceflight on Rat Skeletal Muscle," *J. Appl. Physiol.* 65 (1988): 2318-25.

⁸D. B. Thomason and F. W. Booth. "Atrophy of Soleus Muscle by Hindlimb Unweighting," *J. Appl. Physiol.* 68 (1990): 1-12.

⁹C. S. Layne, A. P. Mulavara, C. J. Pruett, P. V. McDonald, I. B. Kozlovskaya, and J. J. Bloomberg. "The Use of In-Flight Foot Pressure as a Countermeasure to Neuromuscular Degradation," *Acta Astronaut.* 42 (1998): 231-46.

¹⁰C. S. Layne, G. W. Lange, C. J. Pruett, P. V. McDonald, L. A. Merkle, S. L. Smith, I. B. Kozlovskaya, and J. J. Bloomberg. "Adaptation of Neuromuscular Activation Patterns During Treadmill Walking After Long-Duration Space Flight," *Acta Astronaut.* 43 (1998): 107-20.

¹¹L. De-Doncker, F. Picquet, and M. Falempin. "Effects of Cutaneous Receptor Stimulation on Muscular Atrophy Developed in Hindlimb Unloading Condition," *J. Appl. Physiol.* 89 (2000): 2344-51.

¹²C. Rubin, A. S. Turner, S. Bain, C. Mallinckrodt, and K. McLeod. "Anabolism: Low Mechanical Signals Strengthen Long Bones," *Nature* 412 (2001): 603-04.

¹³T. J. Wronski and E. R. Morey-Holton. "Skeletal Response to Simulated Weightlessness: A Comparison of Suspension Techniques," *Aviat. Space Environ. Med.* 58 (1987): 63-68.

¹⁴E. K. Alford, R. R. Roy, J. A. Hodgson, and V. R. Edgerton. "Electromyography of Rat Soleus, Medial Gastrocnemius and Tibialis Anterior During Hindlimb Suspension," *Exp. Neurol.* 96 (1987): 635-49.

¹⁵M. S. F. Clarke, R. Khakee, and P. L. McNeil. "Loss of Cytoplasmic Basic Fibroblast Growth Factor from Physiologically Wounded Myofibers of Normal and Dystrophic Muscle," *J. Cell Sci.* 106 (1993): 121-33.

¹⁶R. W. Ogilvie and D. L. Feedback. "A Metachromatic Dye-ATPase Method for Simultaneous Identification of Skeletal Muscle Fiber Types I, IIA, IIB and IIC," *Stain Technol.* 65 (1990): 231-41.

¹⁷D. A. Martinez, M. W. Orth, K. E. Carr, R. Vanderby, Jr., and A. C. Vailas. "Cortical Bone Growth and Maturational Changes in Dwarf Rats Induced by Recombinant Human Growth Hormone," *Am. J. Physiol.* 270 (1996): E51-59.

Publications

Layne, C. S., K. E. Forth, M. F. Baxter, and J. J. Houser. "Voluntary Neuromuscular Activation Is Enhanced When Paired With a Mechanical Stimulus to Human Plantar Soles," *Neuroscience Letters.* (In review.)

Presentations

Baxter, M. F., J. J. Houser, K. E. Forth, and C. S. Layne. "Timing of Somatosensory Stimulation to the Feet Modifies Human Neuromuscular Activation," Ann. Mtg., Society for Neuro-

science, San Diego, CA, Nov. 2001.

Kyparos, A., C. S. Layne, D. A. Martinez, M. S. F. Clarke, and D. L. Feedback. "Dynamic Foot Pressure as a Countermeasure to Muscle Atrophy," 2nd World Space Congress: 34th Committee on Space Research Scientific Assembly, Houston, TX, Oct. 2002. (To be presented.)

Layne, C. S., K. E. Forth, M. F. Baxter, and J. J. Houser. "Controlled Somatosensory Input Modifies Neuromuscular Activation," Ann. Mtg., North American Society for Psychology of Sport and Physical Activity, St. Louis, MO, June 2001.

Layne, C. S., K. E. Forth, M. F. Baxter, and J. J. Houser. "Enhanced Neuromuscular Activity from Mechanical Foot Stimulation," 2nd World Space Congress: 34th Committee on Space Research Scientific Assembly, Houston, TX, Oct. 2002. (To be presented.)

Layne, C. S., A. P. Mulavara, P. V. McDonald, C. J. Pruett, and J. J. Bloomberg. "Maintaining Neuromuscular Contraction Using Somatosensory Input During Long Duration Spaceflight," Bioastronautics Investigators' Workshop, Galveston, TX, Jan. 2001.

Funding and proposals

Layne, C. S., A. D. LeBlance, and Y. C. Chen. "Using Foot Somatosensory Input to Attenuate Lower Limb Muscle Atrophy During Spaceflight." National Aeronautics and Space Administration, Aug. 2001, \$399,412; *not funded*.

Layne, C. S. and M. Sabahhi. "Increasing Leg Muscle Activation Using Foot Sensory Input." Advanced Research Program, Texas Higher Education Coordinating Board, Aug. 2001, \$63,825; *not funded*.

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