

Subtractive Proteomic Profiling of Control, Atrophied, and Protected Rat Skeletal Muscle by Dynamic Foot Stimulation (DFS)

SKELETAL MUSCLE ATROPHY IS OF great concern to NASA in its daily operations due to the potential impact upon crew physical performance. To date, the single most effective muscle atrophy countermeasure that can be easily deployed on-orbit is exercise. However, the projected amount of time—as high as three hours per day—required to perform daily prescribed exercise countermeasures on the International Space Station (ISS) will be a significant drain on the productive time of crew members. Therefore, a countermeasure designed to enhance the effect of existing exercise countermeasures or one that provides an intermittent level of muscle stimulation/contraction in an unobtrusive fashion to the crew member throughout the daily routine may prove of great value in maintaining muscle mass and function during the extended periods of low or microgravity that will be encountered during the planned Lunar and Martian exploration missions.

Previous work funded, in part by, the Institute for Space Operations (ISSO) in both human and rat models has shown that mechanical stimulation of the soles of the feet (a.k.a. Dynamic Foot Stimulation—DFS) increases neuromuscular activation in the lower limb musculature of humans and, more importantly, attenuates development of muscle atrophy in the lower limb musculature of hind-limb suspended (HLS) rats, a well-accepted ground analogue of space flight-induced muscle atrophy (see Preliminary Studies Section). Based on the concept that neuromuscular activation promotes a eutrophic state in skeletal muscle tissue (e.g., maintenance of muscle mass and fiber type, maintenance of neuromuscular junction size and complexity, promotion of synaptic efficiency), we have recently demonstrated that DFS applied to the sole of the foot during HLS results in a marked attenuation of muscle atrophy in rats. These results indicate that DFS induces trophic effects in skeletal muscle capable of overcoming the atrophic effects of unloading. Most importantly, with regard to the transferability of our observations in a rodent model to astronauts, we find that both rats and humans share similar sensorimotor/proprioceptive pathways and neuromuscular activation responses to DFS.

Project Rationale

The overall aim of this project is to build on the information obtained in our rat DFS model by investigating the underlying cellular and biochemical mechanisms involved in the muscle atrophy response to mechanical unloading. To date, experimental studies aimed at understanding the biochemical and molecular events responsible for muscle atrophy have primarily been carried out using gene chip arrays that compare gene expression profiles between control and atrophied

tissue. Although an extremely powerful analytical technique, studies in gene expressions suffer from two basic limitations: (1) Not all gene expression changes between experimental conditions are directly attributable to the experimental variable of interest (e.g., mechanical unloading). (2) Even if a gene expression change is detected, this phenomenon does not necessarily mean that there is concomitant change in either the expression or activity of the gene product, the true biological effector molecule in living systems, namely the protein encoded for

by that gene. Experimental techniques which focus on the expression of actual proteins, rather than DNA/RNA in atrophied muscle, by definition directly observe biochemical changes modulated during the mechanical unloading response, rather than provide a genetic marker to those proteins that may or may not be modulated.

The second limitation noted above, specifically, the inclusion of tissue obtained from a rescue experiment, is a function of the development and availability of such rescue measures. The approach in this project is to compare protein expression profiles in skeletal muscle from control, HLS (i.e., atrophied) and DFS-treated (i.e., atrophy prevention) animals. By performing a “subtractive” proteomics approach to the experimental problem, only those proteins which are modulated by mechanical unloading and that respond in turn to the rescue measure (i.e., DFS) will be detected.

Experimental Tasks

Discovery Phase (Year 1) This task will utilize a technology known as surface enhanced laser desorption/ionization (SELDI) time of flight mass spectroscopy (TOFMS) that allows a comprehensive protein expression profile to be generated from tissues of interest. This technology has been widely used to identify potential serum biomarkers of particular disease states. It has also been employed to detect protein changes in different tissues. The UH Principal Investigator has direct experimental experience in utilizing this technology to compare protein expression profiles in a number of different tissues and model systems.

Identification Phase (Year 2) This task will focus on identifying those protein changes detected in the discovery phase. Discovery can be achieved by a number of different approaches. The simplest approach is comparison of the approximate iso-electric point (pI) value and highly accurate molecular mass value of the protein obtained using SELDI-TOFMS analysis to existing protein databases, such as the SWISS protein data base which contain (pI, Mr) values for a wide number of previously reported proteins. The second approach is to perform protein identification by means of

tryptic digestion of the protein followed by peptide mapping using SELDI-TOFMS analysis of the peptides. The peptide map is then compared to an NIH-provided peptide map database for previously reported proteins.

Facilities

The Laboratory for Integrated Physiology (LIP), housed in the Department of Health and Human Performance at the University of Houston has a fully functional biochemistry laboratory in which the ISSO Post-Doctoral Aerospace Fellow will carry out specific elements of this study includ-

ing biochemical analysis. The Muscle Research Laboratory at NASA-Johnson Space Center is also a fully equipped biochemistry laboratory. Core facilities at NASA-JSC available for use in this project are a fully staffed animal facility (currently the home of the DFS-HLS rat model of muscle atrophy) and a proteomics core facility that is equipped with a SELDI-TOFMS analysis system and robotic work station to ease the task of preparing samples.

PRINCIPAL INVESTIGATORS

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MICROORGANISMS ARE inevitable companions in human space exploration with the consequent risk of human disease. This risk increases if astronauts in space have suppressed immune systems, making them more susceptible to bacterial infection (Nefedov, Nickerson 1997).

Bacterial populations can impact manned missions in other ways. For example, the buildup of biofilms may damage or interfere with the performance of hardware, and changes in bacterial populations in advanced life support systems may interfere with biodegradation of waste or food production. The background radiation levels encountered on the International Space Station are approximately 70-100 times those seen on the Earth and the gravity vector approaches zero. A key risk is that such a doubly stressed environment may select for significant changes in the microorganisms themselves over the lifetime of an extended mission.

To begin to define the microbial risk associated with human space missions, efforts have been under way for some time to directly characterize the populations encountered in space. This characterization was first attempted on the U.S. Space Shuttle and the Russian Mir Space Station using culture-based techniques (LaRocco). More recently, researchers undertook detailed identification of culturable bacteria isolated from air, water, and surfaces on the International Space Station using 16S rRNA sequencing (Castro). The general observation is that Gram-positive bacteria such as *Staphylococcus*, *Micrococcus*, and *Bacillus* are most common in air and surface samples, whereas Gram negative bacteria are dominant in water samples. The surveys illustrate that the organisms most likely to be present are those that are routinely associated with humans and those which are found in spacecraft assembly environments. This is perhaps expected, but not comforting; as these are the organisms most like-

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ly to be able infect a human host if they develop novel virulent properties.

Preliminary Results

We have successfully employed a high aspect rotating vessel (HARV) bioreactor in the Pierson lab to study the response of *Escherichia coli* to modeled microgravity. The device minimizes fluid motion while maintaining culture aeration through a gas permeable membrane. The rotation also has the effect of randomizing the gravity vector, by rotating in the plane of gravity, producing a low shear modeled microgravity (LSMMG) environment. To obtain this environment, the HARV device is rotated at a speed sufficient to maintain cell suspension in the media and must be completely filled so that gas bubbles cannot cause solution turbulence (i.e., shear).

Our expression studies to date have revealed a substantial number of genes that are either up-regulated or down-regulated relative to controls in replicate experiments. While many of these genes are currently of unknown function, some of the genes with increased transcription in response to LSMMG are involved in the *E. coli* acid tolerance response system (transcriptional gene regulators [yhiE, yhiF] and the chaperones [hdeA, hdeB and hdeD), or are involved in cell motility (many flg and fli genes) or are chemotaxis regulating genes (cheZ and tar). The induction of acid tolerance response genes could indicate their involvement in a general *E. coli* stress response pathway. Increased transcription of the flagellar and chemotaxis genes in LSMMG, increasing cell mobility, may indicate that zones of low nutrients and high waste are occurring in the LSMMG HARV similar to those theorized to occur in space. These identified changes in bacterial LSMMG gene expression could lead to increased cell survival, virulence, and antibiotic resistances, indicating serious potential problems during long-term space flight.