Effects of Microgravity on the Passive Mechanical Properties of Murine Skeletal Muscles

Lee, K.S[1]; Lim, C[2]; Carr, J.A[1]; Gastwirt, R.F[1]; Thomopoulos S[2], Ward, S.R.[1,3,4]
Departments of [1]Orthopaedics, [3]Bioengineering, and [4]Radiology, University of California, San Diego, CA
[2]Department of Orthopaedic Surgery, Washington University, St. Louis, MO
srward@ucsd.edu

INTRODUCTION:

Previous studies have shown the effects of microgravity on the musculoskeletal system: muscle mass and force production capability decreases and muscle fiber type shifts toward a faster contracting and less endurable phenotype [1]. However, little is known about the effect of microgravity on the passive mechanical properties of skeletal muscle. This study investigated the changes of passive mechanical properties in mouse skeletal muscles after 14 days of microgravity. We measured and compared single-fiber and fiber-bundle passive mechanical properties along with titin isoform size and collagen content of trapezius, deltoid, and infraspinatus muscles from spaceflight mice and ground controls. We hypothesized that single-fiber and fiber-bundle elastic moduli would be increased along with a decrease in titin molecular mass and an increase in collagen content.

METHODS:

Adult C57BL/6 mice were exposed to either 14 days of microgravity (MG, n=4) on Space Shuttle mission STS-131 or were maintained as ground based controls (GC, n=4) for 14 days in animal enclosure modules identical to those used for spaceflight. Approximately 20gm-sized samples were harvested from trapezius (n=3), infraspinatus (n=4), and deltoid (n=4 for SF, n=3 for GC) muscles. Samples were divided into two pieces and stored in storage solution at -20°C for mechanical testing and frozen at -80°C for biochemical analysis.

Passive single-fiber and fiber-bundle mechanics were tested with a muscle mechanics set-up [2]. Immersed in chilled relaxing solution, single-fibers or fiber-bundles were dissected from samples and transferred to experimental chambers filled with chilled relaxing solution and tied to wires connected to a micromanipulator on one side and a force transducer on the other side with 10-0 sutures. After measurement of diameter and length of single-fiber or fiber-bundle using a microscope, samples were stretched in 250 µm increments. Stress relaxation was permitted for 3 minutes and then force-displacement data were collected by measuring sarcomere length and tension. Sarcomere length was measured by laser diffraction method using 7mW He-Ne laser.

To quantify titin isoforms, the molecular mass of titin in muscle samples was determined using sodium dodecyl sulfate-vertical agarose gel electrophoresis (SDS-VAGE) [3]. Briefly, frozen muscle samples were weighed and boiled in titin standard buffer at a 1:5 w/v ratio and loaded onto a gel. Human cadaver soleus (3700 kD) and rat cardiac muscle (2992 kD) were used as titin standards. Protein bands were visualized by silver staining, and relative mobility of each band was quantified using a GS-800 Calibrated Densitometer and Quantify One1-D Analysis software. Collagen percentage was determined using a colorimetric analysis for hydroxyproline content.[4]. Briefly, muscle samples were hydrolyzed in 6N HCl for 18h, neutralized, and samples were treated with a chloramine T solution for 20 min at room temperature followed by a solution of p-diaminobenzaldehyde for 30min at 60°C. The sample absorbance was read at 550nm in triplicate using a plate reader and compared to a standard curve to determine the hydroxyproline content of the tissue. Hydroxyproline content is converted to collagen content using a constant (7.46).

Three single-fiber and fiber-bundle passive mechanics experiments were performed per muscle sample. Comparisons of fiber and bundle diameter, slack sarcomere length, elastic modulus, titin molecular mass, and collagen content were made using a two way analysis of variance with microgravity (i.e., microgravity vs. ground control) and muscle (trapezius, deltoid, and infraspinatus) as the grouping variables. A linear regression was used to determine the correlation between single-fiber elastic modulus and titin molecular mass and fiber-bundle elastic moduli and collagen content. All values are presented as mean ± standard error. Statistical significance was set at $p<0.05$.

RESULTS:

Fiber-bundle elastic modulus was 89% greater ($p<0.05$) in the trapezius of SF mice (320.06±43.47 kPa) compared to that of GC mice (168.99±18.11 kPa) (Fig. 1). Single-fiber diameter, slack sarcomere length, and elastic modulus were not different between MG and GC mice. Fiber-bundle diameter and slack sarcomere length were not different between MG and GC mice. Titin molecular mass was not significantly different between MG and GC mice; however, infraspinatus had lower molecular mass titin (3557.4±4.3 kD, $p<0.05$)) compared to trapezius (3829.9±12.2 kD) and deltoid (3729.3±12.4 kD) (Fig. 2). Collagen content were not different bewteen MG and CF mice nor among muscles (Fig. 3). There was no significant correlation noted between single-fiber elastic modulus versus titin molecular mass nor between fiber-bundle elastic modulus versus collagen content.

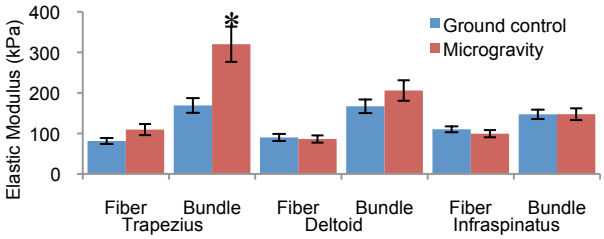

Fig. 1. Comparisons of elastic modulus. * indicates significant differences between microgravity and ground control.

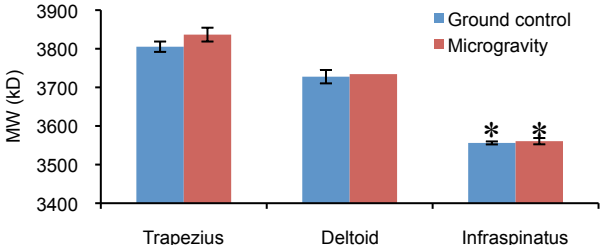

Fig. 2. Comparisons of titin molecular mass. * indicates significant differences between infraspinatus and trapezius and deltoid.

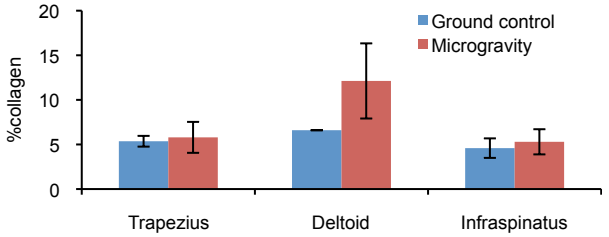

Fig. 3. Comparisons of collagen content.

DISCUSSION:

These data demonstrate that microgravity increases the elastic modulus of the trapezius muscle at the fiber-bundle level without changes in titin isoform mass nor in collagen content. This is particularly interesting because it suggests that the rules governing passive mechanical properties may vary by muscle and may be altered outside of the cell. The total content of collagen does not appear to explain these data, so perhaps the quality and/or organization of the extracellular matrix may be important. In conclusion, the results of this study suggest that weightless space environment may affect the passive mechanical properties of the extracelluar matrix in select skeletal muscles.

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