Microgravity Leads to Degeneration of Murine Rotator Cuff Muscles

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INTRODUCTION

Loading is necessary for the development and homeostatis of the rotator cuff muscles of the shoulder [1]. In the absence of loading during development and adulthood (e.g., due to a tendon rupture), the rotator cuff muscles develop significant atrophy and fatty degeneration [2-4]. Similarly, muscle degeneration has been reported due to microgravity [5]. However, the molecular mechanisms leading to these muscle changes have not been characterized. Our objective was to study the effect of microgravity on gene expression in mouse rotator cuff muscles. We hypothesized that microgravity would: 1) downregulate expression of myogenic transcription factors and markers, 2) upregulate expression of adipogenic transcription factors and markers, and 3) upregulate expression of fibrosis markers.

METHODS

Adult C57BL/6 mice were exposed to either 14 days of microgravity (MG, n=8) on Space Shuttle mission STS-131 or were maintained as ground based controls (GC, n=8) for 14 days in animal enclosure modules identical to those used for spaceflight. Supraspinatus (SS) and infraspinatus (IS) muscles were retrieved less than 30 minutes after sacrifice, placed in RNALater to stabilize mRNA and stored at -80°C. mRNA expression was quantified with real-time polymerase chain reaction using Sybr Green chemistry. The target genes examined were:

- Myogenin, MyoD1, Myf5 and Myf6 (myogenic transcription factors)
- Myostatin (a negative regulator of myogenesis)
- Myh4 (fast twitch isoform) and Myh7 (slow twitch isoform)
- CEBPalpha and PPARgamma (adipogenic transcription factors)
- Leptin (adipogenic marker)
- Collagen I (Col1a) and III (Col3a) (fibrosis markers)

GAPDH was used as a housekeeping gene. Results were expressed as fold change relative to GAPDH. Student's t-tests were used for statistical analyses with the significance level set to $p < 0.05$.

RESULTS

Myogenesis related genes: There was significant downregulation of Myf5 (SS and IS), MYf6 (IS), and MyoD (IS) in the microgravity group compared to the ground control group (Fig. 1). There was significant upregulation of myostatin (IS) in the microgravity group compared to the ground control group (Fig. 1). There were no changes in myogenin.

Muscle fiber type: There was a significant upregulation of Myh4 (SS) in the microgravity group compared to the ground control group. There was a trend towards upregulation of Myh7 (IS) in the microgravity group compared to the ground control group, but this was not statistically significant.

Adipose related genes: There was significant downregulation of PPARgamma (SS), CEBPalpha (SS), and Leptin (SS) in the microgravity group compared to the ground control group (Fig. 2).

Fibrosis related genes: There was upregulation of Col1a and Col3a (IS) in the microgravity group compared to the control group, but this was only statistically significant for collagen 3 (Fig. 3).

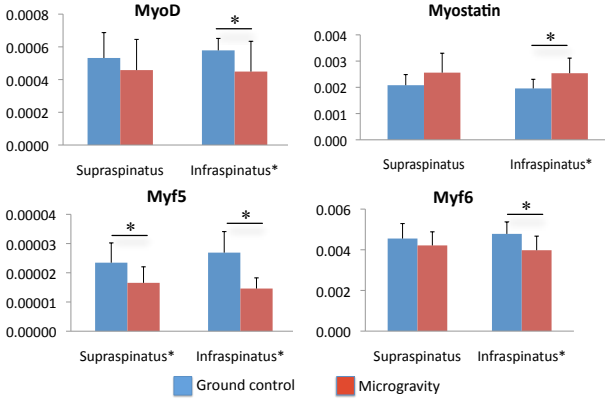

Fig. 1: Myogenesis was decreased due to microgravity (* p<0.05).

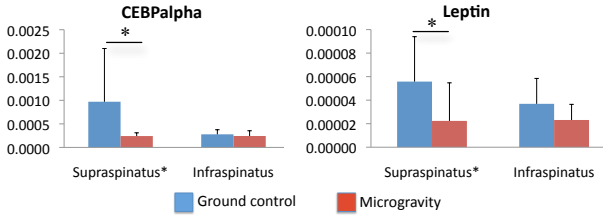

Fig. 2: Adipogenic factors were decreased due to microgravity (* p<0.05).

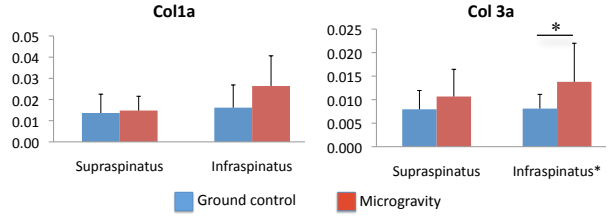

Fig. 3: Fibrosis markers were increased due to microgravity (* p<0.05).

DISCUSSION

Microgravity significantly downregulated the expression of both myogenic and adipogenic genes. MyoD is involved in initiating a myogenic differentiation program of multipotential stem cells and myostatin is a negative regulator of muscle development [6,7]. The mRNA changes reported here indicate atrophy/degeneration of the muscles due to microgravity. C/EBPα and PPARγ2 mediate transcriptional control of adipogenesis [3]. Upregulation in their expression have been observed due to muscle unloading. Leptin is manufactured in fat cells and its upregulation suggests presence of adipose tissue in the muscle [2]. Surprisingly, we found downregulation of adipogenic markers, indicating that degeneration due to microgravity may occur through different pathways than degeneration due to tendon and/or nerve injury. Microgravity also led to an increase in collagen type III, a marker of fibrosis (and hence degeneration) in muscle. Interestingly, effects were muscle specific; the infraspinatus was most sensitive to the unloading conditions in general, but only the supraspinatus showed changes in adipogenesis.

In conclusion, our results demonstrate that microgravity stimulates a catabolic response in muscles consistent with chemodenervation and surgical denervation models with regard to myogenesis and fibrosis, but inconsistent with regard to adipogenesis.

ACKNOWLEDGEMENTS

The study was funded by a grant from NASA (NNX09AP05G). The authors thanks Paula Dumars, Richard Boyle, and Vera Vizir for their support in organizing the dissections post shuttle landing.

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