THE EFFECT OF A FATIGUING BOUT OF ISOKINETIC EXERCISE ON MOLECULAR BIOMARKERS OF MYOGENESIS AND PROTEOLYSIS IN YOUNG FEMALES FOLLOWING ACL RECONSTRUCTION

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Introduction

Knee injuries are among the most common injures in adolescents. Approximately 100,000 to 250,000 of these are due to an anterior cruciate ligament (ACL) tear.[1] While most athletes transition back to sport in 6-9 months following rehabilitation, osteoarthritis (OA) remains a concern in the years following rehabilitation. 25-36% of of ACL patients will develop OA within 10 years of the initial injury.[2,3] Within 20 years, the incidence increases to 50%.[4] This is a concern for active younger adults with ACL reconstruction who may be subjected to chronic pain and degeneration from OA much earlier than age-related OA. Despite surgery and rehabilitation, many athletes return to sport with a deficit in quadriceps strength on their injured leg. ACL injury and reconstruction result in changes to the knee joint which creates dysfunction that perpetuates the inflammatory response, resulting in localized atrophy and can contribute to post-traumatic knee OA.

The purpose of this study was to determine inter- and intralimb differences of myogenic and proteolytic gene expression of the vastus lateralis (VL) at baseline and 4-hours following fatiguing bout of isokinetic resistance exercise in women following ACL reconstruction.

Methods

Nine women (24 ± 4 yr) at least one year out from ACLR (3.4 ± 1.9 yr) and currently participating in physical activity without restrictions, were recruited to participate in the study. Muscle mRNA of myogenic (MYOD, MYOG, MSTN, MYF5, and MYF6) and proteolytic (FOXO3A, MAFbx, MuRF1) genes were determined using qRT-PCR (Fig. 1). Quantification of mRNA for genes of interest was calculated using the $2^{-\Delta\Delta ct}$ method relative to stable reference genes (1.0-fold change). The geometric mean of the following three reference genes was used as the stable reference point: B2M, RPS18, and GAPDH for each participant. Results are expressed as intralimb fold-change from pre-exercise (baseline). Paired samples t-tests were used to determine within-limb differences between pre- and post-exercise and differences between limbs post-exercise. Differences in dependent variables were analyzed using principal components analysis via the *princomp* function in R version 4.2.2. The number of retained principal components was used as a solution for discrete cluster membership in a subsequent K-means clustering algorithm. Defined clusters were visualized and interpreted based on mean values. The measures associated with each cluster were compared statistically by one-way ANOVA models (α = 0.05) with Tukey post hoc comparisons. In addition, cluster pairwise differences with 95% confidence intervals for the comparisons were reported.

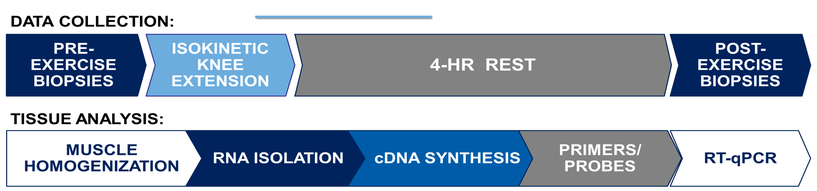

Figure 1. Order of data collection and tissue analysis methods.

Results

Both quadriceps fatigued during the exercise bout (% decline in peak torque: ACLR = -13.5 ± 16.4%; CON = -17.7 ± 13.8%). No significant differences were found within or between limbs in MYOD, MYOG, Myf6, MAFbx, MST (CON), Myf5 (CON), or FOXO3a (ACLR). ACLR expression of MST (0.48 ± 0.21-fold change, $P < 0.01$) and Myf5 (0.60 ± 0.36-fold change, $P = 0.01$), and CON expression of FOXO3A (0.71 ± 0.26-fold change, $P = 0.01$) were significantly downregulated and MuRF1 was significantly upregulated in both limbs (ACLR = 2.34 ± 1.40-fold change, $P = 0.02$; CON = 2.50 ± 1.85-fold change, $P = 0.04$) following exercise.

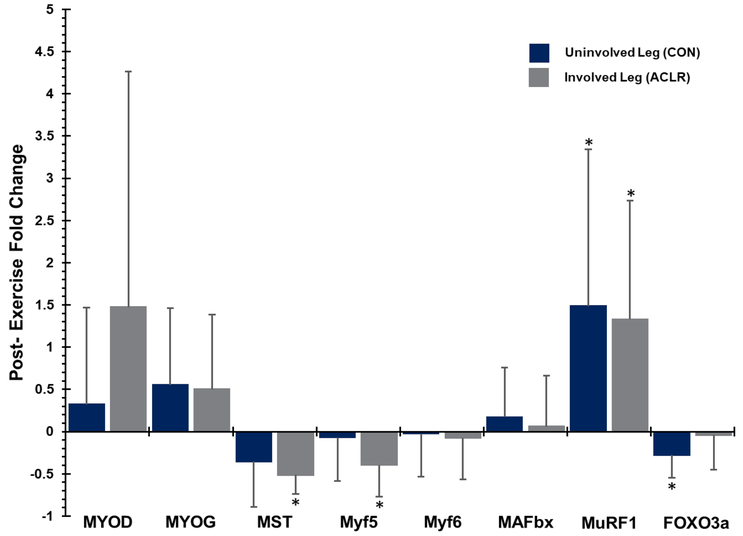

Figure 2. Fold changes in proteolytic and myogenic genes. Values are mean ± SD. *different vs. baseline, p<0.05

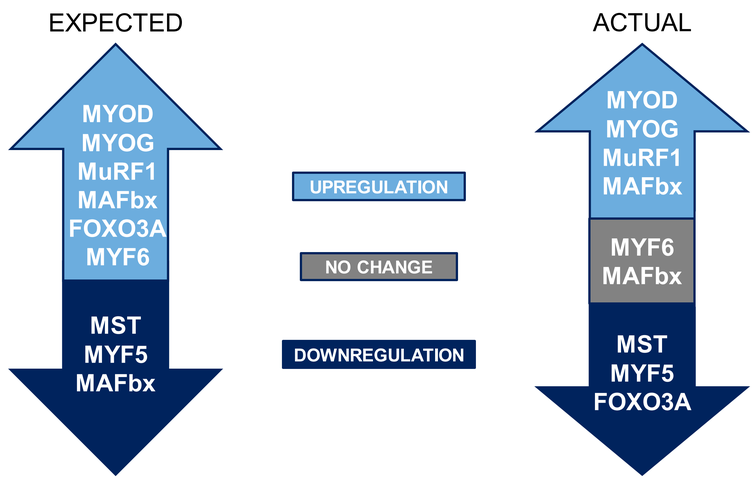

Figure 3. Expected vs. actual post-exercise response of mRNA expression of 8 muscle regulatory genes (myogenic: MYOD, MYOG, MST, MYF5, MYF6; proteolytic: MAFbx, MURF1, FOXO3A).

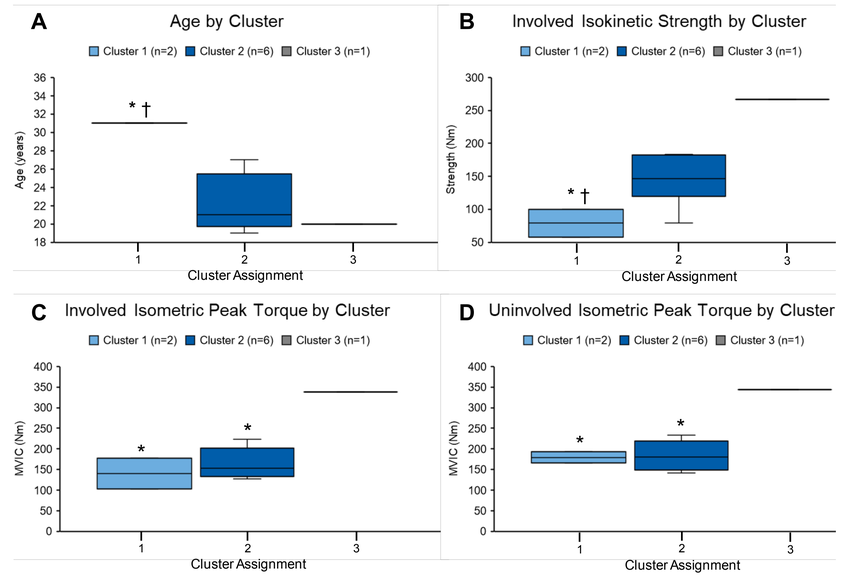

Figure 4A. K-means clusters for age B. Isokinetic Strength C,D. Peak isometric torque Values are minimum, first quartile, median, third quartile, and maximum. *different vs. Cluster 3, p<0.05; †different vs. Cluster 2, p<0.05.

Conclusions

These data demonstrate a significant upregulation of myogenic (MSTN and MuRF1) expression in ACLR post-exercise compared to pre-exercise values. However, there was not significance found in MYOD, MYOG, MYF6, and MAFBx expression. MuRF1 and FOXO3 remained unchanged. Together, the myogenic and proteolytic gene expression measured in both the involved and uninvolved limbs generated atypical signaling responses. Though our data followed expected trends, the change in expression was lower in magnitude compared to healthy young adults. These data also suggest that peak torque and isokinetic strength were greater in those that return to regular exercise post-ACLR. Future studies should tightly control time since recovery and amount of exercise post-op.

Practical Applications

For clinicians working with athletes following an ACL injury, it may be critical to focus on equalizing bilateral strength, prior to and following return to sport, to reduce their risk of developing OA.

References

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This project is supported by Dr. George F. Haddix President's Faculty Research Fund