The Cellular Response to Exercise Based on Simulated Activity Levels Amongst Tissues Involved in ACL Injury and Surgery

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INTRODUCTION: Anterior cruciate ligament (ACL) rupture is most prevalent in young active individuals, but a significant number of ACL tears occur in middle-aged to older "weekend warriors." Activity levels has been implicated in ACL injury risk as well as impacting outcomes after ACL reconstruction (ACLR). Understanding clinically relevant metabolic responses in cells of the native ACL and tendons used for autograft ACLR to activity levels may provide the first step to understanding how activity may be linked to ACL injury and impact repair and remodeling of the graft after ACLR. What is also unknown is the impact of the preconditioning of the cells in its response to a period of intense exercise stimulus. The objective of this study was to compare the native ACL and the common extensor tendons used for ACLR (patellar tendon, PT; quadriceps tendon, QT) in its responses to a simulated in vitro exercise stimulus after three different levels of preconditioning. It was hypothesized that an exercise stimulus after a preconditioning regimen to mimic sedentary and stress deprivation conditions would result in significantly higher pro-inflammatory and pro-degradative metabolic responses during the simulated exercise protocol compared to cells cultured under an active conditions.

METHODS: With ACUC approval (ACUC# 42830), ACL, PCL, PT, and QT were recovered from skeletally mature purpose bred research hounds (ACL & PCL n=10, PT & QT n=9) euthanatized for reasons unrelated to this study. PCL cells were included as an "intra-articular ligament control" based on the reported differences in injury rates and outcomes when compared to ACL. Primary cell lines were created from the tissues of each animal. Passage 2 cells (n=2/cell line/group) were plated on Collagen Type I-coated BioFlex® plates (1×10^5 cells/well), and after 24 hours were preconditioned under simulated activity levels designed to mimic a "sedentary" lifestyle (SED, 5775 steps/day), "active" lifestyle (ACT, 10,500 steps/day & a 5K run), or mechanical stress deprivation (NL, 0 steps/day). (Figure 1) A "step" was defined as one cycle of a 4% strain applied to the plate at 1.75Hz. Over the 16-hour active period, cells were loaded in 5-10 min periods with 10-240 min breaks between loaded periods until the appropriate number of steps for each profile was achieved. Cells were cultured for 6 days, and on day 6 the media was changed in ½ of the cells in all groups. Along with the media, half of the cells were collected for RNA extraction for gene expression to constitute the Before Run (BR) group. The remaining ½ of the cells were then stressed with an exercise stimulus to simulate a 5K run profile (4% strain at 2.3Hz for 33 minutes). At the end of the 5K run exercise stimulus, the media and RNA cells loaded using the 5K protocol were collected 3 hours after completion of the stimulus as the After Run (AR) group. The gene expression levels of COL I, COL III, Decorin, COMP, Elastin, MMP-2, MMP-3, ADAMTS4, TIMP-1, TIMP-2, TIMP-3, TGF-β, and COX-2 relative to GAPDH were determined from the cells of the BR and AR groups. Significant ($p<0.05$) differences between cell types for each load, and between loads within each cell type, in the BR or AR groups was determined using a one-way ANOVA with Tukey post-hoc test. Significant ($p<0.05$) differences in BR and AR were determined for each cell type in each load group using a T-Test.

RESULTS: _**Differences in Gene Expression Between Preconditioning Levels for Each Cell Population (Fig. 2):**_ **_ACL Cells:_** The SED group had significantly higher COL I gene expression than the ACT and NL groups, in the AR group but not the BR group. **_PCL Cells:_** The ACT group had significantly lower COL I gene expression and the SED group had significantly lower COMP gene expression than the NL group in the BR group, but not the AR group. **_PT Cells:_** The ACT group had significantly lower COL 1 gene expression and the SED group had significantly higher TIMP-1 gene expression than the NL group in the BR group. **_QT Cells:_** The SED group had significantly lower COL I gene expression than the NL group in the AR and BR group, and lower COL III gene expression than the NL group in the BR group. The ACT group had significantly higher COMP gene expression than the SED and NL groups in the AR group. _**Differences in After Run Gene Expression for Each Cells Population Within a Preconditioning Level (Fig. 3):**_ **_ACT group:_** COL I gene expression was significantly higher in ACL cells than QT cells in both the BR and AR groups, and in PCL cells than PT and QT cells in the AR group. COMP gene expression was significantly lower in ACL and PCL cells compared to QT cells in the AR group. TIMP-1 gene expression was significantly higher in PCL cells compared to ACL and PT cells in the AR group. **_SED group:_** COL I gene expression was significantly higher in ACL cells than QT cells in the AR group, and higher in PCL cells compared to PT and QT cells in the BR group. COX-2 gene expression was significantly lower in PCL cells than PT cells in the AR group. **_NL group:_** COL I gene expression was significantly higher in PCL cells than PT cells in the BR group. _**Differences in Cellular Gene Expression between the Before Run and After Run Groups (Fig. 2):**_ **_ACL Cells:_** The SED group had significantly higher COL I and ADAMTS4 gene expression in the AR group compared to the BR group. **_PCL Cells:_** The NL group had significantly lower COL I gene expression in the AR group compared to the BR group. **_PT Cells:_** The ACT group had significantly lower COL I and TIMP-2 gene expression in the AR group compared to the BR group. **_QT Cells:_** The NL group had significantly lower COMP gene expression in the AR group compared to the BR group.

DISCUSSION: ACL fibroblasts were the only cells that were associated with higher gene expression of an extracellular matrix molecule in response to the simulated 5K run exercise stimulus. While there were no significant differences in COL I gene expression between ACL cells in the ACT and SED groups prior to the simulated 5K run protocol, ACL cells in the SED group had significantly higher COL I gene expression in response to the simulated 5K run protocol. This was not observed for other cell types analyzed in this study. This suggests that a sudden increase in activity may be a signal to ACL fibroblasts to increase COL I expression in order to augment the extracellular matrix of the ACL in sedentary individuals who abruptly perform a higher level activity. These findings have important ramifications with respect to relative risk for ACL injury in "weekend warriors" where individuals may have a relative lower loads of physical activity mixed with intermittent periods of higher exercise activity.

<u>**SIGNIFICANCE/CLINICAL RELEVANCE:**</u> The data from this study indicate that ACL fibroblasts may have biologically relevant differences from other tissues relevant in ACL injuries and subsequent ACLR surgery based on different levels of cell preconditioning. These difference may also provide some biomechanistic insight into ACL injury as it relates to activity level as well as appropriate activity levels during early healing after ACLR. Ongoing research in our lab is aimed at further characterization of the effects of human activity level on the tissue responses in humans with ACL injury. These results may provide key insight on how activity level may impact the native ACL in terms of injury risk as well as affect tissues used to treat ACL injuries after they occur.