The Effects of Simulated Activity Levels on Cellular Metabolic Responses of the ACL and Extensor Tendon Autografts Used for ACL Reconstruction

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INTRODUCTION: Anterior cruciate ligament (ACL) rupture is the most prevalent amongst young active individuals. When surgical stabilization of the ACL-deficient knee is required, ACL reconstruction (ACLR) is often performed using extensor based autografts such as the patellar tendon (PT) or quadriceps tendon (QT). While numerous patient factors (age, sex, BMI) are associated with risk of ACL injury, reinjury, and outcomes after ACLR, patient activity level is recognized as one of the most important risk factor. There is very little work done with in vitro simulation of activity levels with cell populations relevant to ACL injury and surgery. Characterizing the cellular metabolic responses by the ACL and the tendon autografts used for ACLR to simulated activity levels may elucidate responses that may link activity level and its impact on ACL health including injury, healing, as well as graft "ligamentization" after ACLR. The objective of this study was to compare to the effects of three different in vitro activity levels on the native ACL, PT, and QT fibroblasts using a validated bioreactor. Given musculoskeletal tissues are mechanosensitive where insufficient mechanical stimulus may result in catabolic states, it was hypothesized that tendon and ligament cells cultured under stress deprivation or sedentary conditions would have higher inflammatory and degradative responses when compared to cells cultured under more 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) euthanized 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, and passage 2 cells were plated on Collagen Type I-coated BioFlex® plates (1×10^5 cells/well). After 24 hours, three simulated activity levels were then created based on a single 4% 1.75Hz strain, which was used to simulate 1 step. (Figure 1) Cells were then cultured under conditions designed to mimic 3 activity levels based on number of steps: mechanical stress deprivation (NL, 0 steps/day) and sedentary lifestyle (SED, 5775 steps/day). For the active lifestyle, both increase in steps and a period of increase frequency of steps was used to simulate a run period (ACT, 10,500 steps/day & a 5K run). Steps were applied (16-hour active period in 5-10 min periods with 10-240 min breaks between loaded periods) until the appropriate number of steps for each profile was achieved. The 5k run was simulated by applying a 4% strain at 2.3Hz for 33 minutes. Cells were cultured for 6 days. Day 3 (D3) and 6 (D6) media were tested for IL-6, IL-8, KC, MCP-1, MMP-1, MMP-2, MMP-3, and PGE2. The D6 cells were then used to determine COL I, COL III, Decorin, COMP, and MMP-2 gene expression levels. Significant ($p<0.05$) differences between cell types for each activity level, and between activity level within each cell type were determined using a one-way ANOVA with Tukey post-hoc test. Only statistically significant differences are presented in the Results.

RESULTS: _Differences in Cellular Response to Different Activity Levels (Fig. 2):_ **ACL cells:** The ACT group produced lower MMP-2 (D3) than the NL group, and higher MMP-3 (D3) than the SED and NL groups. The SED group produced higher PGE2 and IL-8, and had lower COL I expression, than the ACT and NL groups (D3 & D6). The SED group produced higher MCP-1, and lower MMP-2 (D3) and MMP-1 (D6), than the NL group. **PCL cells:** The ACT group produced higher MMP-3 (D3) than the SED and NL groups, and lower MMP-2 (D6) and COL I expression, than the NL group. The ACT and SED groups produced higher PGE2 and IL-8 than cells in the NL group (D3 & D6). The SED group produced higher MCP-1 (D3) and had lower COMP expression than the NL group. **PT cells:** The ACT group had higher MMP-2 expression and lower COL I expression than the NL group. **QT cells:** The ACT and SED groups produced higher IL-8 (D3 & D6) than the NL group. The ACT group produced higher MCP-1 than the NL group (D3). The SED group had lower Col I and Col III expression than the NL group.

Differences Between Cell Types Within a Specific Loading Regimen (Fig. 3): **ACT group:** QT produced lower MMP-2 (D3), MMP-3 (D3), and MCP-1 (D3 & D6) than PCL, and higher IL-8 on D3 (ACL and PT) and D6 (ACL, PCL, and PT). PT produced lower MCP-1 and MMP-3 (D3), and higher MMP-1 (D6), than PCL. On D3, QT produced higher PGE2 than ACL, and on D6 ACL produced lower PGE2 than PCL, PT, and QT. ACL produced higher MCP-1 (D3) than QT, MMP-2 (D6) than PT, and had higher COL I gene expression than PT and QT. **SED group:** PCL produced higher MMP-2 than PT (D3 & D6) and QT (D3); MMP-1 (D6) than ACL, PT, and QT; and COL I and MMP-2 gene expression than the PT and QT. ACL produced higher MMP-2 than QT, and IL-8 than PT (D3). ACL and PCL produced higher MCP-1 (D3) than PT and QT. **NL group:** ACL produced higher levels of MMP-1 than PT, and MMP-2 than PT and QT (D3). PCL produced higher MMP-2 (D3 & D6) than PT and QT. On D3, ACL and PCL produced lower PGE2 than PT and QT, and on D6 PT produced higher PGE2 than ACL and PCL, and QT produced higher PGE2 than PCL. On D3, the QT produced lower MCP-1 than ACL, PCL, and PT, and on D6 the PCL produced higher MCP-1 than QT.

DISCUSSION: The significant activity-based differences observed among the tested cell types were most often observed between ligament (ACL and PCL) and tendon (PT and QT) cells. ACL (ACT) and PCL (SED) cells had higher COL I gene expression levels and produced higher MMP-2 (all groups), MMP-3 (ACT) and MCP-1 (ACT & SED) compared to PT and QT cells. Very few significant differences were observed between ACL and PCL cells. Interestingly, most of the significant differences observed in this study involved ACT or SED groups in comparison to the NL group and it often involved degradative enzymes. Taken together, these differential responses have important implications for post-ACLR rehabilitation and return-to-sport protocols for patients based on the known importance of COL I, MMP-2, MMP-3, and MCP-1 in the tissue repair, remodeling, and the graft "ligamentization" processes that govern healing after ACLR. Further research is required to determine if these differences occur in vivo and would have clinically meaningful impacts with respect to objective outcomes after ACL injury and/or reinjury after ACLR.

SIGNIFICANCE/CLINICAL RELEVANCE: The data from this study indicate that ligament and tendon fibroblasts may have clinically relevant differences in their responses to activity levels of patients, which could influence risk of ACL injury as well as outcomes after ACLR. Ongoing research in our lab is aimed at further characterization of the effects of patient activity level on the metabolic responses of the ACL, PCL and common tendon autograft tissues towards development of patient-specific graft selection and post-operative rehabilitation protocols, with the goal of improving outcomes and return-to-sport metrics for patients undergoing ACLR.