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Time between Anterior Cruciate Ligament Injury and Reconstruction and Cartilage Metabolism Six-months following Reconstruction

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Abstract

Purpose—To determine the association between time from injury to ACL reconstruction ($Time^{Injury\text{-}ACLR}$) and biochemical markers of cartilage metabolism and inflammation six months following ACL reconstruction (ACLR).

Methods—Individuals with an unilateral ACL injury were enrolled at initial presentation in the orthopaedic clinic; blood was collected six months following ACLR. Enzyme-linked immunosorbent assays were used to analyze the ratio of serum concentrations of type-II collagen

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Author Contributions

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Conflicts of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

breakdown (C2C) to synthesis (CPII), plasma matrix metalloproteinase-3 (MMP-3), interleukin-6 (IL-6), and serum aggrecan neopitope (ARGS). We used separate linear regressions to assess associations between biochemical markers and $Time^{Injury\text{-}ACLR}$.

Results—Twenty-two participants (50% females, mean [SD], age 21.9 [4.5] years old; BMI 23.8 [2.6] kg/m^2) completed the study. $Time^{Injury\text{-}ACLR}$ ranged from 9 to 67 days (31.0 [14.4 days]). Greater $Time^{Injury\text{-}ACLR}$ predicted greater serum C2C:CPII ratios six months following ACLR (C2C:CPII = 0.15 [0.02], R^2 = 0.213, P = 0.030). Males (R^2 = 0.733, P = 0.001) but not females (R^2 = 0.030, P = 0.609) demonstrated a significant association between greater C2C:CPII and $Time^{Injury\text{-}ACLR}$ at the six-month follow-up exam. $Time^{Injury\text{-}ACLR}$ did not associate with IL-6, MMP-3, or ARGS at six months.

Conclusions—Greater time between injury and ACL reconstruction was associated with greater serum C2C:CPII six months following ACLR in males but not females, and IL-6, MMP-3, and ARGS levels were not associated with $Time^{Injury\text{-}ACLR}$ in males or females. The time between ACL injury and ACLR may affect collagen metabolism in males and should be further investigated in a larger study along with other patient-relevant outcomes.

Key Terms

CPII; C2C; biomarkers; ACLR; type-II collagen cleavage product; C-propeptide of type II procollagen

1. Introduction

Individuals who have sustained an anterior cruciate ligament (ACL) injury are at increased risk for developing post-traumatic osteoarthritis (PTOA).[23,26, 38,20] The development of PTOA may occur rapidly following ACL injury, as approximately one-third of individuals who sustain an ACL injury demonstrate radiographic evidence of PTOA within the first decade following injury.[23,20, 26, 38] It is hypothesized that the development of PTOA is initiated soon after macro-trauma to the joint tissues and progresses through a sequence of acute and chronic alterations to cartilage metabolism and composition.[2] Greater concentrations of both synovial and serum pro-inflammatory cytokines (IL-6),[3, 33] matrix metalloproteinases (MMP-3),[12, 19, 21] and proteoglycan breakdown (ARGS)[21, 33] have been detected within one week after ACL injury and remain elevated several months after ACL reconstruction (ACLR) in many individuals. These metabolic alterations are thought to influence early changes in tibiofemoral articular cartilage composition, consisting of decreased proteoglycan density[36] and altered collagen orientation,[7] as early as two years following ACL injury. It is critical to understand the factors that influence these alterations in metabolism, which may increase the risk of PTOA. Biochemical markers can provide information about cartilage damage[40] prior to radiographic evidence of PTOA and are critical for examining early metabolic changes that may influence future clinical outcomes. Previous studies[17, 33] have reported that concentrations of biochemical markers of cartilage metabolism are either no different or lower in patients who remain ACL deficient compared to those with an ACLR. No studies have specifically examined if the amount of time between injury and surgery is associated with cartilage metabolism in individuals who have elected to undergo ACLR early following injury. Understanding how the time between ACL

injury and reconstruction influences cartilage metabolism may be critical for determining how to best schedule ACLR for the purpose of optimizing long-term outcomes.

Sustaining a secondary meniscal or chondral injury[24,4] following ACLR may increase the already elevated risk of developing accelerated PTOA.[38,22] Furthermore, aberrant biomechanics that are perpetuated following ACL injury, such as lower peak knee extension angles during midstance[11,10] and more anteriorly shifted tibiofemoral contact patterns throughout the stance phase,[39] alter tibiofemoral contact forces during ambulation and could cause degenerative changes in the meniscus and cartilage.[1] It can be hypothesized that individuals who delay ACLR are exposed to a greater amount of aberrant loading of the injured limb (walking, running, etc.) which may hasten the progression of degenerative cartilage metabolism.[11,10]

Plasma pro-inflammatory cytokines (Interleukin-6, IL-6),[6,12, 33] plasma degenerative cartilage enzymes (matrix metalloproteinase-3, MMP-3),[12,30] serum proteoglycan breakdown (aggrecan, ARGS),[33] and serum type-II collagen breakdown (C2C) and synthesis (CPII) calculated as a ratio (C2C:CPII)[37] are elevated from one day up to one year following ACL injury compared to controls. The purpose of this preliminary study was to evaluate the association between Time $^{\text{Injury-ACLR}}$ and plasma and serum biochemical markers of cartilage metabolism six months following ACLR. We hypothesized that individuals with greater Time $^{\text{Injury-ACLR}}$ would demonstrate greater concentrations of plasma pro-inflammatory cytokines (IL-6), plasma degenerative cartilage enzymes (MMP-3), serum aggrecan breakdown (ARGS), and ratio of C2C:CPII.

2. Materials and Methods

All participants were part of a prospective longitudinal cohort study (Figure 1). Immediately following enrollment, participants completed the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire. Biochemical markers were assessed from serum and plasma samples collected at a follow-up exam approximately six months following ACLR. All time frames for data collection can be viewed in Figure 2. Study personnel communicated with participants via electronic mail and telephone to schedule the six-month follow-up exams, and recorded the dates of the ACL injury, ACLR, and follow-up appointment into an electronic system. All participants provided written informed consent and all study methods were approved by the Institutional Review Board at the University of XXXXXX prior to data collection.

2.1 Participants

Individuals between the ages of 18 and 35 years who presented to the orthopaedic clinic within a 14 day period after sustaining an ACL injury and had an MRI confirmed unilateral ACL injury were eligible for this study (Figure 1). While we did not exclude individuals who had undergone a previous ACLR, we did exclude individuals who had previous history of other major joint injury. Individuals who were currently pregnant or planned to become pregnant within 12 months of ACL injury, had a previous diagnosis of inflammatory arthritis, were in need of multi-ligament reconstruction, or declined to undergo ACLR were excluded from the study. Individuals were also disqualified if they had a previous history of

a cardiac pacemaker, cochlear implant, clinical hypertension, hepatic disease, diabetes or seizures. There are no previous studies that have evaluated the association between cartilage metabolism, biochemical markers, and Time $^{\text{Injury-ACLR}}$, further justifying the need for this preliminary study. However, moderate associations have been reported between similar biochemical markers of cartilage metabolism and knee related biomechanical outcomes at the same time point of six-months following ACLR.[27] We determined that 22 individuals with an ACLR would be needed to determine statistical significance using a two-tailed linear regression for a similar moderate association ($r=0.6$, $R^2 = 0.36$) detected between biochemical markers of cartilage metabolism and Time $^{\text{Injury-ACLR}}$ with an alpha level set at 0.05 and 80% power (G*Power, v3.1.9.2)[9].

2.2 Collection of Self-Reported Questionnaires

Immediately following enrollment, participants completed all five subscales of the KOOS questionnaire (Pain, Symptoms, Function in daily living, Function in sports and recreation, and knee related Quality of life) via a healthcare informatics system (Socrates GP, Healthcare Ltd, Sligo, Australia). The KOOS questionnaire is a valid and reliable assessment of functional status and quality of life in individuals with ACLR.[29, 31] Participants completed the Tegner Activity Scale, a self-report questionnaire that evaluates physical activity level,[35] prior to ACLR and at the six-month follow-up.

2.3 Collection and Analyses of Serum and Plasma

Blood samples were collected at the six-month follow-up exam in the orthopedic clinic and processed immediately for separation of serum or plasma. Plasma and serum were aliquoted into 1.0 mL cryovials and stored in an −80°C freezer until analysis. Serum was assessed for the ratio of type-II collagen cleavage product (C2C) to C-propeptide of type II procollagen (CPII) using commercially available enzyme-linked immunosorbent assays (ELISA) (IBEX Technologies, Inc., Canada) and serum Alanin-Arginine-Glycine-Serine (ARGS) neoepitope using a highly specific and sensitive sandwich ELISA.[16] Plasma was assessed for matrix metalloproteinase-3 (MMP-3) and interleukin-6 (IL-6) using ELISA (R & D Systems, Minneapolis, MN). Specific assay detection sensitivities were: C2C = 10 ng/ml, CPII = 50 ng/ml, MMP-3 = 0.009 ng/ml, IL-6 < 0.7 pg/ml, and ARGS = 0.025 pmoles/ml. As recommended by the manufacturer, plasma IL-6 samples were not diluted while plasma MMP-3 and serum C2C and CPII samples were diluted by 2x and 10x respectively; ARGS was assayed at final dilutions of 1:1.3. All assays were performed in duplicate for both standards and unknowns, and demonstrated inter-assay and intra-assay variability less than 10%.

2.4 Anterior Cruciate Ligament Reconstruction

One of three orthopaedic surgeons performed arthroscopically assisted single incision ACLR using an autograft harvested from the middle third of the patellar tendon via an anterior longitudinal incision. With the knee in approximately 120° of flexion, a femoral tunnel was drilled through the infra-medial arthroscopic portal on the lateral wall of the intercondylar notch of the femur. The tibial tunnel was drilled and over-reamed into the ACL footprint using a targeting guide, and the graft was attached to the femur and tibia with interference screws. All participants received structured rehabilitation supervised by a licensed physical

therapist or athletic trainer that began during the first week following ACLR and continued over the next six months.

2.5 Statistical Analysis

Means and standard deviations were calculated for all continuous variables, and frequencies were counted for all non-continuous variables. Normality was assessed for all outcomes and covariates using a Shapiro – Wilk test. Data found to be non-normally distributed were Log-10 transformed. For our primary *a priori* analyses, we conducted separate bivariable linear regression analyses with each biochemical marker as the criterion variable and Time $^{\text{Injury-ACLR}}$ as the predictor variable. Next, we conducted separate stepwise linear regression models to assess if potential covariates influenced associations between biochemical markers and Time $^{\text{Injury-ACLR}}$. Owing to the preliminary nature of this study, and due to the relatively small sample size, we conducted linear regression models separately to preserve enough statistical power to determine the association between Time $^{\text{Injury-ACLR}}$ and the biochemical marker of interest after removing variance independently associated with each covariate. The number of days between injury and the six-month follow-up exam, as well as the number of days between ACLR and six-month follow-up exam were identified as potential confounding covariates that may influence the association between Time $^{\text{Injury -ACLR}}$ and the biochemical markers of cartilage metabolism (Figure 2). Additional potential confounding covariates included body mass index (BMI), age, history of previous ACLR, concomitant meniscal and chondral injuries, patient-reported function (KOOS sub scores), and physical activity level six months following ACLR measured with the Tegner Activity Scale as potential covariates, as previous studies have reported that these measures are associated with worse outcomes following ACLR.[4, 8, 15, 35] For each stepwise linear regression, the criterion variable was the biochemical marker outcome. The first predictor variable entered was the relevant covariate followed by Time $^{\text{Injury-ACLR}}$. We evaluated the change in R^2 and the unstandardized β to determine the unique influence of Time $^{\text{Injury-ACLR}}$ on the biochemical marker of interest after accounting for the covariate of interest. We conducted separate univariate regression analyeses for males and females, as sex has previously demonstrated to influence cartilage metabolism.[25] The level of significance for all analyses was determined *a priori* at $P<0.05$ and all analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 21.0, IBM Corp., Somers, NY).

3. Results

C2C:CPII, IL-6, MMP-3 and relevant covariates were collected for 22 individuals (50% females) who underwent ACLR (means and standard deviations can be found in Table 1), and serum ARGS was collected from 21 individuals. Five individuals had undergone ACLR on the contralateral limb previous to enrollment of the study; however, all 22 participants enrolled in this study underwent primary ACLR. Fourteen individuals had a concomintant meniscal injury (1 medial, 11, lateral, 1 medial and lateral) and 13 underwent concomitant meniscal surgery (1 medial repair, 6 lateral meniscectomies, 1 lateral repair and meniscectomy, 3 lateral repairs, 1 medial and lateral meniscectomy, 1 medial repair and lateral meniscectomy, Table 1). No individuals reported an injury on the involved or

contralateral knee during the observation period. Time $^{\text{Injury-ACLR}}$ ranged from 9–67 days (mean [SD], 31.00 [14.39 days]). Biochemical markers were assessed from serum and plasma samples collected at a follow-up exam approximately six months (193.27 [19.18 days]) following ACLR. Plasma MMP-3 and IL-6 at the six-month follow-up were non-normally distributed and were Log-10 transformed prior to analysis; this transformation was successful in providing a normal distribution. Time $^{\text{Injury-ACLR}}$ and all covariates were normally distributed. All biochemical marker samples yielded results above the lower limit cutoff for each specific assay. Subject-specific values for biochemical markers and Time $^{\text{Injury-ACLR}}$ can be found in supplemental table 1. Overall, individuals who waited more days between injury and ACLR had higher ratios of C2C:CPII (0.15 [0.02]) at the six-month follow-up exam ($R^2 = 0.213$, $\beta = 0.001$, $P = 0.030$, Table 2). Associations between Time $^{\text{Injury-ACLR}}$ and ARGS (0.22 [0.05] pmol/ml), IL-6 (0.73 [1.02] pg/ml), and MMP-3 (0.71 [0.56] ng/ml) at the six-month follow-up exam were low and not statistically significant (R^2 range = 0.002 – 0.014, $\beta = -0.003 – 0.0001$, $P = 0.596 – 0.863$, Table 2).

3.1 Influence of Other Time Frames on the Association Between Time $^{\text{Injury-ACLR}}$ and C2C:CPII

Individuals with more Time $^{\text{Injury-ACLR}}$ demonstrated significantly higher C2C:CPII at the six-month follow-up exam after accounting for both days between ACL injury and the six-month follow-up exam ($\Delta R^2 = 0.194$, $\beta = 0.001$, $P = 0.043$, Figure 2, Table 3) and days between ACLR and the six-month follow-up exam ($\Delta R^2 = 0.196$, $\beta = 0.001$, $P = 0.042$, Figure 2, Table 3). There was no association between Time $^{\text{Injury-ACLR}}$ and ARGS (Table 4), MMP-3 (Table 5), and IL-6 (Table 6) after accounting for days between injury and the six-month follow-up exam and days between surgery and the six-month follow-up exam (ΔR^2 range = 0.001 – 0.021).

3.2 Influence of Relevant Covariates

BMI (23.81 [2.59] kg/m^2, $R^2 = 0.001$, $\beta = 0.0002$, $P = 0.914$, Table 3) did not predict a significant amount of variance in C2C:CPII; however, sex (50% female, $R^2 = 0.258$, $\beta = -0.023$, $P = 0.016$, Table 3) was significantly associated with C2C:CPII ratio at the six-month follow-up exam. After accounting for the influence of sex, greater Time $^{\text{Injury-ACLR}}$ ($\Delta R^2 = 0.207$, $\beta = 0.001$, $P = 0.014$, Table 3) still significantly associated with greater C2C:CPII at the six-month follow-up exam. After stratifying by sex, males with greater Time $^{\text{Injury-ACLR}}$ demonstrated greater C2C:CPII ratios at the six-month follow-up exam (n = 11, $R^2 = 0.733$, $\beta = 0.002$, $P = 0.001$, Figure 3). In females, we found no statistically significant association between Time $^{\text{Injury-ACLR}}$ and C2C:CPII ratio at the six-month follow-up exam (n = 11, $R^2 = 0.030$, $\beta = 0.0002$, $P = 0.609$, Figure 3). Age (21.95 [4.55] yrs, $R^2 = 0.029$, $\beta = 0.001$, $P = 0.452$, Table 3), history of previous ACLR (23%, $R^2 = 0.011$, $\beta = -0.006$, $P = 0.650$, Table 3), concomitant meniscal injury (64%, $R^2 = 0.056$, $\beta = -0.011$, $P = 0.290$, Table 3), concomitant chondral injury (36%, $R^2 = 0.051$, $\beta = -0.011$, $P = 0.311$, Table 3), KOOS symptoms (45.18 [16.11], $R^2 = 0.001$, $\beta = 0.00003$, $P = 0.919$, Table 3), KOOS pain (59.55 [18.38], $R^2 = 0.012$. $\beta = -0.0001$, $P = 0.634$, Table 3), KOOS daily living (64.68 [19.67], $R^2 = 0.033$, $\beta = -0.0002$, $P = 0.419$, Table 3), KOOS sports (22.64 [30.07], $R^2 = 0.035$, $\beta = 0.0001$, $P = 0.401$, Table 3), and KOOS quality of life (25.95 [20.50], $R^2 = 0.061$, $\beta = -0.0003$, $P = 0.268$, Table 3) scores, as well as physical activity level collected at

the six month follow-up exam (R^2 = 0.037, β = −0.002, P= 0.406, Table 3) did not significantly associate with the C2C:CPII ratio at the six-month follow-up exam.

History of previous ACLR predicted a significant amount of variance in ARGS at the six-month follow-up exam (R^2 = 0.217, β = 0.048, P = 0.033, Table 4), and sex predicted a significant amount of variance in MMP-3 at the six-month follow-up exam (R^2 = 0.334, β = −0.388, P = 0.005, Table 5). However, Time $^{Injury\text{-}ACLR}$ did not predict a significant amount of variance in ARGS (Table 4), MMP-3 (Table 5), or IL-6 (Table 6).

4. Discussion

Contrary to our hypothesis, there were no statistically significant associations in our entire cohort between Time $^{Injury\text{-}ACLR}$ and ARGS, MMP-3, or IL-6 collected at the six month follow-up exam. Males with greater Time $^{Injury\text{-}ACLR}$ demonstrated higher C2C:CPII ratios, while there was no association between Time $^{Injury\text{-}ACLR}$ and the C2C:CPII ratios in females. Within our entire cohort, neither days between injury and the six-month follow-up exam nor days between ACLR and the six-month follow-up exam influenced the association between Time $^{Injury\text{-}ACLR}$ and C2C:CPII. To our knowledge, our study is the first to evaluate the association between Time $^{Injury\text{-}ACLR}$ and biochemical markers of cartilage metabolism six months following ACLR, which is a common clinically relevant time point for functional evaluation and decision regarding whether to return a patient to unrestricted physical activity.[18] The sample size of the current preliminary study was small (N = 22); yet, the strength of R^2 values demonstrated between ARGS, MMP-3, IL-6 and Time $^{Injury\text{-}ACLR}$ would have necessitated a sample of approximately 558 participants to detect a statistically significant association with a power of 80% at alpha < 0.05. Therefore, the non-statistically significant low associations detected between ARGS, MMP-3, IL-6 and Time $^{Injury\text{-}ACLR}$ may lack clinical relevance. We detected a statistically significant association between C2C:CPII and Time $^{Injury\text{-}ACLR}$, which we were correctly powered to determine. Our preliminary study provides justification for conducting future studies, with larger samples sizes, to further evaluate how multiple important covariates may influence the associations between C2C:CPII and Time $^{Injury\text{-}ACLR}$.

We analyzed several biochemical markers to address a wide array of metabolic processes occurring in the knee following ACL injury including inflammation (IL-6),[12,6] degenerative enzymatic activity (MMP-3),[12,30] aggrecan breakdown (ARGS),[33] and the ratio of C2C:CPII.[37] It has been noted previously[33] that elevated synovial fluid ARGS and IL-6 decrease relative to reference values by thirty weeks following ACLR. Therefore, it is possible that the serum concentrations of ARGS and IL-6 followed similar trends in the present study and may have returned to normal values prior to the six-month follow-up exam. We did not see that Time $^{Injuyr\text{-}ACLR}$ associated with plasma MMP-3, suggesting that the MMP-3 modulated mechanisms that signal joint tissue breakdown may not have been affected by the number of days between injury and ACLR. Urine and serum markers of type-II collagen can remain elevated for years following injury, [14,19, 37] which may explain why we could detect these markers six months following ACLR within our cohort. Continued higher cartilage breakdown relative to synthesis over a period of months to years may eventually cause irreversible damage to the collagen framework within the cartilage.[13,3228]

The mechanisms influencing the association between Time $^{\text{Injury-ACLR}}$ and serum C2C:CPII in the males of our cohort, remain unclear. Future studies should evaluate if poor movement quality and the degree of cumulative joint loading influence collagen metabolism in patients that have elected to undergo ACLR and are not engaging in rehabilitation prior to ACLR. ACL deficient knees demonstrate less mechanical stability and decreased knee flexion angles during gait compared to contralateral knees in individuals with unilateral ACL injury.[39,1] It is possible that aberrant mechanics, as a result of ACL deficiency, can alter the contact location on knee articular cartilage and negatively impact homeostasis if the cartilage cannot adapt to the altered mechanical load.[2] None of the participants in our study reported a secondary acute intra-articular knee injury during Time $^{\text{Injury-ACLR}}$, which may be due to the relatively short time frame between injury and ACLR in our cohort. A history of a concomitant meniscal or chondral injury did not predict a significant amount of variance in any of our cartilage biochemical markers at the six-month follow-up exam. Therefore, no evidence exists to suggest that secondary chondral and meniscal injuries influenced the association between greater Time $^{\text{Injury-ACLR}}$ and higher C2C:CPII ratios in our cohort. When separated by sex, greater Time $^{\text{Injury-ACLR}}$ and higher C2C:CPII ratios at the six-month follow-up exam were strongly and significantly associated in males, but not in females. The sample size for this analysis was small, and we caution conclusions regarding a possible opposite C2C:CPII response based on Time $^{\text{Injury-ACLR}}$ between the sexes from this preliminary study. Yet our results should inform future research regarding the importance of examining sex differences in cartilage metabolism response following ACLR.

While our study is the first to evaluate the association between Time $^{\text{Injury-ACLR}}$ and biochemical markers of cartilage metabolism, there are limitations that should be addressed in order to inform future research. The sample size of our current study is small, which precludes us from analyzing the influence of potential covariates together in a multiple regression with Time $^{\text{Injury-ACLR}}$ and C2C:CPII at the six-month follow-up exam. Additionally we did not have the statistical power to stratify our cohort by type of meniscal injury and surgery as described in Table 1. The nature of this study was preliminary, but it demonstrates the need to conduct a larger study to determine the clinical impact of the association between Time $^{\text{Injury-ACLR}}$ and C2C:CPII following ACLR in males. Additionally, our six-month examination provides only one time-point of cartilage metabolism, and therefore may have missed significant associations that occurred prior to the six-month follow-up exam. Future studies should analyze longitudinal effects of Time $^{\text{Injury-ACLR}}$ on biochemical markers. Previous studies have interpreted C2C:CPII as a ratio of type-II collagen breakdown to type-II collagen synthesis for the purpose of measuring cartilage metabolism following ACLR;[5, 34] however, serum measurements may not specifically reflect only cartilage metabolism in the knee. Finally, we did not control for the time of day blood was drawn, and therefore we cannot account for diurnal variations that may occur with the biomarkers chosen for analysis. While we proposed that aberrant mechanical knee loading may be one explanation for the association between Time $^{\text{Injury-ACLR}}$ and C2C:CPII at the six-month follow-up exam, we were unable collect biomechanical outcomes prior to surgery to confirm this hypothesis.

Improving long term clinical outcomes is critical, and there is a dearth of research evaluating Time $^{\text{Injury-ACLR}}$ and early changes in cartilage metabolism. In this preliminary study,

biochemical markers of ARGS, IL-6, and MMP-3 at the six-month follow-up exam did not associate with Time $^{\text{Injury-ACLR}}$. Males demonstrated an association between greater Time $^{\text{Injury-ACLR}}$ and higher C2C:CPII ratios, while females did not demonstrate a statistically significant association between Time $^{\text{Injury-ACLR}}$ and C2C:CPII ratios. Although sample size is small, the results from the current study suggest that type-II collagen metabolism may respond differently in males and females based on Time $^{\text{Injury-ACLR}}$. These data suggest that future longitudinal studies should evaluate the long-term impact of time between ACL injury and ACLR in both sexes.

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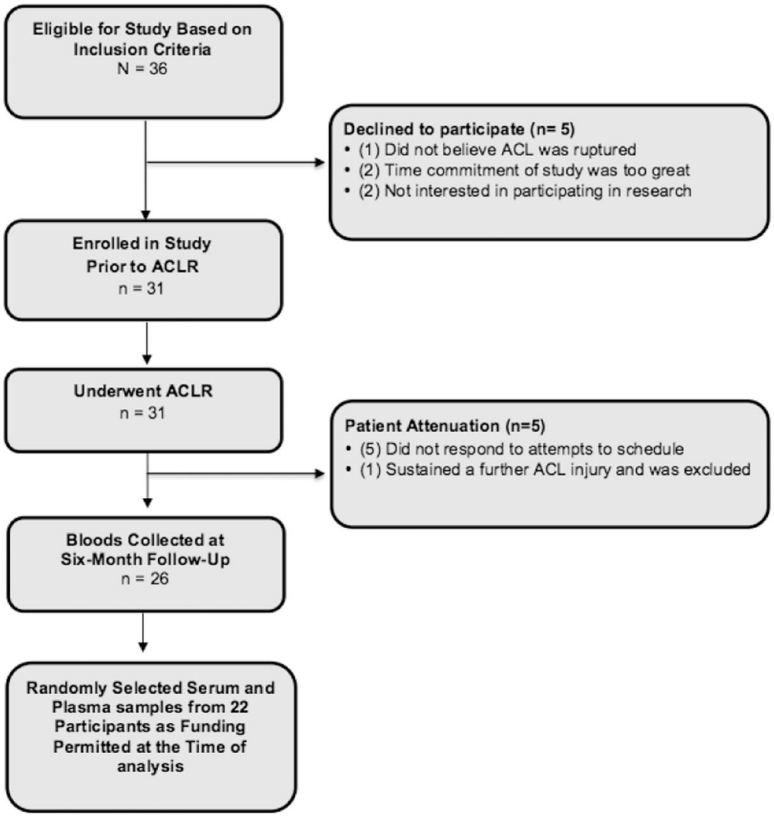

Figure 1.

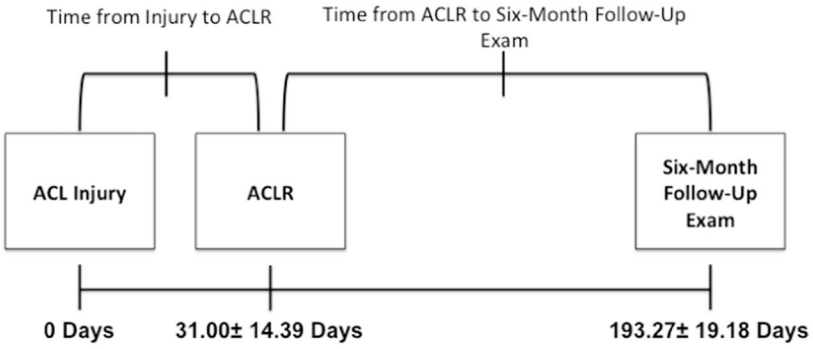

Figure 2.

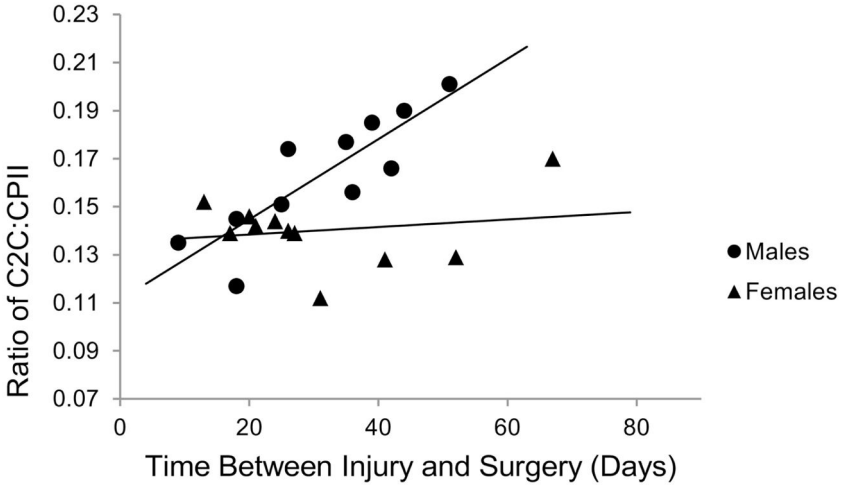

Figure 3.

Table 1

Demographics and Outcome Measure Means (mean [standard deviation])

Participants	11 (50%) Males 11 (50%) Females
Age at Six-Month Follow-Up	21.95 [4.55] yrs
BMI at Six-Month Follow-Up	23.81 [2.59] kg/m^2
History of Concomitant Meniscal Injury With ACLR	No Meniscal Injury = 8 Medial Injury = 1 (1 Repair) Lateral Injury = 11 (6 meniscectomy, 1 repair and meniscectomy, 3 repairs, 1 untreated) Medial and Lateral Injury = 2 (1 medial and lateral meniscectomy, 1 medial repair and lateral meniscectomy)
History of Concomitant Chondral Injury With ACLR	Yes = 8 No = 14
KOOS Pain Prior to Surgery	59.55 [18.38]
KOOS Symptoms Prior to Surgery	45.18 [16.11]
KOOS Function in Daily Living Prior to Surgery	64.68 [19.67]
KOOS Sport/Recreation Prior to Surgery	22.64 [30.07]
KOOS Quality of Life Prior to Surgery	25.95 [20.50]
Tegner Activity Scale at the Six-Month Follow-Up Exam	Level 1 = 3 Level 2 = 1 Level 3 = 0 Level 4 = 3 Level 5 = 7 Level 6 = 5 Level 7 = 0 Level 8 = 0 Level 9 = 0 Level 10 = 0 Missing = 3
C2C at the Six-Month Follow-Up Exam	62.10 [12.61] ng/ml
CPII at the Six-Month Follow-Up Exam	413.36 [83.95] ng/ml
C2C: CPII at the Six-Month Follow-Up Exam	0.15 [0.02]
ARGS at the Six-Month Follow-Up Exam	0.22 [0.05] pmol/ml
IL-6 at the Six-Month Follow-Up Exam	0.73 [1.02] pg/ml

MMP-3 at the Six-Month Follow-Up Exam	0.71 [0.56] ng/ml
Days Between Injury and ACLR	31.00 [14.39] days
Days Between Injury and Six-Month Follow-up Exam	228.55 [22.69] days
Days Between ACLR and Six-Month Follow-up Exam	197.45 [19.85] days

BMI – Body Mass Index, ACLR – Anterior Cruciate Ligament Reconstruction, KOOS – Knee Osteoarthritis and Outcome Score, C2C – Type-II Collagen Cleavage Product, CPII – CPropeptide of Type-II Collagen Procollagen, ARGS – Alanin-Arginine-Glycine-Serine, IL-6 – Interleukin-6, MMP-3 – Matrix Metalloproteinase-3

Table 2

Linear Regression with Biochemical Marker as the Criterion Variable and Days Between Injury and Surgery as the Predictor Variable

Biochemical Marker	R^2	Unstandardized β	P-value
C2C:CPII at the Six-Month Follow-Up Exam	.213 *	0.001 *	0.030
ARGS at the Six-Month Follow-Up Exam	0.002	0.0001	0.863
IL-6 at the Six-Month Follow-Up Exam	0.005	−0.002	0.744
MMP-3 at the Six-Month Follow-Up Exam	0.014	−0.003	0.596

C2C – Type-II Collagen Cleavage Product, CPII – C-Propeptide of Type-II Collagen Procollagen, ARGS – Alanin-Arginine-Glycine-Serine, IL-6 – Interleukin-6, MMP-3 – Matrix Metalloproteinase-3

*Significant Correlation at $p \leq 0.05$

Table 3

Influence of Time Frames and Covariates on C2C:CPII collected at the Six-Month Follow-Up Exam and Days Between Injury and Surgery

Covariate	R^2 (Unstandardized β) Between Covariate and C2C:CPII	ΔR^2 (Unstandardized β) Between Injury and Surgery and C2C:CPII after accounting for the covariate
Days Between Injury and Six-Month Follow-up	0.025 (0.0002)	0.194 (0.001) *
Days Between ACLR and Six-Month Follow-up	0.023 (0.0002)	0.196 (0.001) *
Body Mass Index	0.001 (0.0002)	0.213 (0.001) *
Sex	0.258 (−0.023) *	0.207 (0.001) *
Age	0.029 (0.001)	0.185 (0.001) *
History of previous ACLR	0.011 (−0.006)	0.219 (0.001) *
Concomitant Meniscal Injury	0.056 (−0.011)	0.201 (0.001) *
Concomitant Chondral Injury	0.051 (−0.011)	0.165 (0.001)
KOOS Symptoms at Initial Presentation	0.001 (0.00003)	0.223 (0.001) *
KOOS Pain at Initial Presentation	0.012 (−0.0001)	0.202 (0.001) *
KOOS Daily Living at Initial Presentation	0.033 (−0.0002)	0.180 (0.001) *
KOOS Sports at Initial Presentation	0.035 (0.0001)	0.266 (0.001) *
KOOS Quality of Life at Initial Presentation	0.061 (−0.0003)	0.158 (0.001)
Tegner Activity Scale at Six-Month Follow-Up	0.013 (−0.0006)	0.190 (0.001)

ACLR – Anterior Cruciate Ligament Reconstruction, KOOS – Knee Osteoarthritis and Outcome Score, C2C – Type-II Collagen Cleavage Product, CPII – C-Propeptide of Type-II Collagen Procollagen, ARGS – Alanin-Arginine-Glycine-Serine, IL-6 – Interleukin-6, MMP-3 – Matrix Metalloproteinase-3

* Significant F Change at $p \leq 0.05$

Table 4

Influence of Time Frames and Covariates on ARGS collected at the Six-Month Follow-Up Exam and Days Between Injury and Surgery

Covariate	R^2 (Unstandardized β) Between Covariate and ARGS	ΔR^2 (Unstandardized β) Between Injury and Surgery and ARGS after accounting for the covariate
Days Between Injury and Six-Month Follow-up	0.069 (−0.001)	0.008 (0.0003)
Days Between ACLR and Six-Month Follow-up	0.073 (−0.001)	0.008 (−0.0003)
Body Mass Index	0.016 (0.002)	0.005 (−0.0002)
Sex	0.003 (−0.004)	0.002 (−0.0001)
Age	0.133 (−0.004)	0.006 (0.0003)
History of previous ACLR	0.217 (0.048) *	0.002 (−0.0002)
Concomitant Meniscal Injury	0.005 (−0.007)	0.002 (−0.0001)
Concomitant Chondral Injury	0.139 (0.035)	0.007 (0.0003)
KOOS Symptoms at Initial Presentation	0.002 (−0.0001)	0.002 (−0.0001)
KOOS Pain at Initial Presentation	0.147 (−0.001)	0.015 (−0.0004)
KOOS Daily Living at Initial Presentation	0.004 (0.0001)	0.005 (−0.0002)
KOOS Sports at Initial Presentation	0.098 (−0.0005)	0.019 (−0.0005)
KOOS Quality of Life at Initial Presentation	0.037 (0.0004)	0.004 (0.0002)
Tegner Activity Scale at Six-Month Follow-Up	0.040 (−0.004)	0.00002 (−0.00001)

ACLR – Anterior Cruciate Ligament Reconstruction, KOOS – Knee Osteoarthritis and Outcome Score, C2C – Type-II Collagen Cleavage Product, CPII – C-Propeptide of Type-II Collagen Procollagen, ARGS – Alanin-Arginine-Glycine-Serine, IL-6 – Interleukin-6, MMP-3 – Matrix Metalloproteinase-3

* Significant F Change at $p \leq 0.05$

Table 5

Influence of Time Frames and Covariates on MMP-3 collected at the Six-Month Follow-Up Exam and Days Between Injury and Surgery

Covariate	R^2 (Unstandardized β) Between Covariate and MMP-3	ΔR^2 (Unstandardized β) Between Injury and Surgery and MMP-3 after accounting for the covariate
Days Between Injury and Six-Month Follow-up	0.036 (−0.003)	0.001 (−0.001)
Days Between ACLR and Six-Month Follow-up	0.017 (−0.002)	0.021 (−0.003)
Body Mass Index	0.077 (0.037)	0.016 (−0.003)
Sex	0.334 (−0.388) *	0.016 (−0.003)
Age	0.028 (0.013)	0.034 (−0.005)
History of previous ACLR	0.044 (−0.168)	0.012 (−0.003)
Concomitant Meniscal Injury	0.077 (0.194)	0.011 (−0.002)
Concomitant Chondral Injury	0.014 (0.082)	0.006 (−0.002)
KOOS Symptoms at Initial Presentation	0.108 (−0.007)	0.032 (−0.004)
KOOS Pain at Initial Presentation	0.002 (−0.001)	0.019 (−0.003)
KOOS Daily Living at Initial Presentation	0.020 (−0.002)	0.038 (−0.005)
KOOS Sports at Initial Presentation	0.021 (−0.002)	0.024 (−0.004)
KOOS Quality of Life at Initial Presentation	0.022 (−0.002)	0.037 (−0.005)
Tegner Activity Scale at Six-Month Follow-Up	0.028 (−0.027)	0.009 (−0.002)

ACLR – Anterior Cruciate Ligament Reconstruction, KOOS – Knee Osteoarthritis and Outcome Score, C2C – Type-II Collagen Cleavage Product, CPII – C-Propeptide of Type-II Collagen Procollagen, ARGS – Alanin-Arginine-Glycine-Serine, IL-6 – Interleukin-6, MMP-3 – Matrix Metalloproteinase-3

* Significant F Change at $p \leq 0.05$

Table 6

Influence of Time Frames and Covariates on IL-6 collected at the Six-Month Follow-Up Exam and Days Between Injury and Surgery

Covariate	R^2 (Unstandardized β) Between Covariate and IL-6	ΔR^2 (Unstandardized β) Between Injury and Surgery and IL-6 after accounting for the covariate
Days Between Injury and Six-Month Follow-up	0.007 (0.001)	0.017 (−0.004)
Days Between ACLR and Six-Month Follow-up	0.022 (0.003)	0.002 (−0.001)
Body Mass Index	0.068 (−0.036)	0.005 (−0.002)
Sex	0.190 (0.308)	0.005 (−0.002)
Age	0.025 (−0.013)	0.001 (−0.001)
History of previous ACLR	0.079 (0.238)	0.008 (−0.002)
Concomitant Meniscal Injury	0.069 (−0.193)	0.008 (−0.002)
Concomitant Chondral Injury	0.018 (0.099)	0.001 (−0.001)
KOOS Symptoms at Initial Presentation	0.004 (−0.001)	0.007 (−0.002)
KOOS Pain at Initial Presentation	0.0002 (−0.0002)	0.007 (−0.002)
KOOS Daily Living at Initial Presentation	0.033 (0.003)	0.000002 (0.000003)
KOOS Sports at Initial Presentation	0.044 (0.003)	0.001 (−0.001)
KOOS Quality of Life at Initial Presentation	0.002 (0.001)	0.004 (−0.002)
Tegner Activity Scale at Six-Month Follow-Up	0.058 (−0.040)	0.000002 (0.000004)

ACLR – Anterior Cruciate Ligament Reconstruction, KOOS – Knee Osteoarthritis and Outcome Score, C2C – Type-II Collagen Cleavage Product, CPII – C-Propeptide of Type-II Collagen Procollagen, ARGS – Alanin-Arginine-Glycine-Serine, IL-6 – Interleukin-6, MMP-3 – Matrix Metalloproteinase-3

*Significant F Change at $p \leq 0.05$