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The Influence of Sex, Body Mass Index, and Age on Cartilage Metabolism Biomarkers in Patients After Anterior Cruciate Ligament Injury and Reconstruction

Kazandra M. Rodriguez, MS*; Michael Thomas Curran, MEd, ATC*†; Riann M. Palmieri-Smith, PhD, ATC*

*School of Kinesiology, University of Michigan, Ann Arbor; †Department of Orthopaedic Surgery, Michigan Medicine, Ann Arbor

Context: Serum biomarkers may allow for the early identification of posttraumatic osteoarthritis after anterior cruciate ligament (ACL) injury and reconstruction. Homeostasis of matrix-metalloproteinase-3 (MMP-3) and type II collagen turnover biomarkers (C2C:CPII ratio) is believed to be compromised in individuals with ACL injury, yet the influence of sex, body mass index (BMI), and age on these biomarkers before and after ACL reconstruction remains unknown.

Objective: To determine the relationship of sex, BMI, and age with serum levels of MMP-3 and C2C:CPII before and after ACL reconstruction.

Design: Descriptive laboratory study.

Setting: Laboratory.

Patients or Other Participants: Thirty-two (females = 18, males = 14) individuals with ACL injuries.

Main Outcome Measure(s): Demographic variables and blood samples were collected before surgery and at return to activity. Serum was extracted from the blood and assays were used to quantify MMP-3 and C2C:CPII. Generalized linear mixed-effects regression models were used to assess the relationships between sex, BMI, age, time, and participant on the outcome variables.

Results: A significant time × sex interaction was identified for MMP-3 levels ($P = .021$), whereby MMP-3 levels were higher in males at return to activity (males, 2.71 ± 0.59 ng/mL; females, 1.92 ± 0.60 ng/mL; $P = .017$). Males also had higher MMP-3 levels at return to activity when compared with presurgery levels ($P = .009$). A main effect for age demonstrated that older age was associated with higher MMP-3 levels. No significant main or interaction effects were noted for C2C:CPII levels.

Conclusions: Upregulation of MMP-3 serum levels may occur after ACL reconstruction, particularly in males, which may have deleterious consequences for the cartilage matrix. Sex, BMI, and time did not influence C2C:CPII ratios, but further research with larger sample sizes is needed to confirm these findings.

Key Words: osteoarthritis, knee

Key Points

- Changes in matrix-metalloproteinase-3 (MMP-3) serum levels may be upregulated after anterior cruciate ligament (ACL) reconstruction, particularly in males, which may have deleterious consequences for the cartilage matrix.
- Accordingly, it may be necessary to account for the roles of sex, age, and time when examining MMP-3 levels in the population with ACL injury.
- Notably, body mass index did not influence MMP-3 or type II collagen turnover (C2C:CPII ratio) serum levels before or after ACL reconstruction in our small sample of patients.

Anterior cruciate ligament (ACL) rupture is a common knee injury, resulting in more than 175 000 reconstructive surgeries each year in the United States.[1] After ACL injury, and despite ACL reconstruction, individuals are at an increased risk for developing posttraumatic osteoarthritis (PTOA); 50% of individuals develop PTOA within 20 years of the initial injury.[2] Demographics such as sex and body mass index (BMI) are identified risk factors for the development of PTOA.[3] Nonetheless, how sex and BMI influence the onset and progression of PTOA after ACL injury remains unclear due to the incomplete understanding of the mechanisms contributing to PTOA and the limited sensitivity of validated measures, such as radiographs, for detecting and monitoring early osteoarthritic changes.[4]

A promising approach for assessing early PTOA changes is to measure blood-derived biomarkers, as blood is a highly dynamic tissue[5] sensitive to acute biochemical changes that may be valuable for early detection and monitoring of the cartilage changes associated with joint disease.[6] Common blood-derived biomarkers used to assess cartilage status in the population with ACL injury include matrix-metalloproteinase-3 (MMP-3), an enzyme responsible for signaling degradation of extracellular matrix substrates (eg, proteoglycans and collagens),[7] and type II collagen turnover ratio (C2C to CPII biomarker turnover ratio [C2C:CPII]), which indicates the relationship between type II collagen production and breakdown. After ACL injury, MMP-3 levels increase as soon as 1 day postinjury and can remain elevated for up to 10 years when compared

Table 1. Participants' Demographic Information Before Anterior Cruciate Ligament Reconstruction and at Return to Activity

	Time					
	Before Reconstruction			Return to Activity		
	Mean ± SD			Mean ± SD		
Characteristic	Females (n = 18)	Males (n = 14)	*P* Value	Females (n = 18)[a]	Males (n = 14)[b]	*P* Value
Age, y	16.30 ± 2.30	16.90 ± 3.25	.566	16.95 ± 2.25	17.42 ± 3.17	.641
Body mass index, kg/m^2	23.70 ± 4.62	26.20 ± 4.82	.143	24.00 ± 4.92	27.30 ± 4.79	.068
Time since surgery, mo	NA	NA	NA	9.01 ± 1.89	8.12 ± 1.34	.132
Tegner Activity Scale score[c]	9.00 ± 0.97	9.29 ± 1.07	.442	7.17 ± 1.86	7.21 ± 2.15	.948

Abbreviation: NA, not applicable.

[a] Females' anterior cruciate ligament graft types: quadriceps tendon = 2, bone-patellar tendon-bone = 14, hamstrings = 2.

[b] Males' anterior cruciate ligament graft types: quadriceps = 1, bone-patellar tendon-bone = 9, hamstrings = 4.

[c] Tegner Activity Scale scores were self-reported.

with healthy control individuals.[8–10] Type II collagen turnover ratios were lower 2 years after ACL injury compared with preinjury levels and with healthy control participants.[11] Notably, these acute and chronic biomarker changes indicate disruption to metabolic homeostasis that is characteristic of osteoarthritic cartilage.[12] Thus, given their sensitivity to the ACL injury event and their ability to reflect metabolic changes in the cartilage, MMP-3 and type II turnover may be useful biomarkers for identifying individuals at an increased risk for early-onset PTOA after ACL injury and reconstruction.

When assessing biomarkers such as MMP-3 and type II collagen turnover after ACL injury, researchers[8,13] typically accounted for the role of demographic variables such as sex or BMI. Accounting for sex and BMI is crucial, as biomarker responses may vary based on these factors and, ultimately, may influence the direction and magnitude of metabolic changes after ACL injury and reconstruction. For example, higher BMIs were moderately associated with elevated type II collagen turnover in females, but not in males, an average of 48 months after ACL reconstruction, thus suggesting a differential response in females with higher BMI versus females with lower BMI or males after ACL reconstruction.[14] Sex and BMI also influenced the risk of developing PTOA, with female and obese individuals at higher risk (odds ratios = 1.2 and 1.4, respectively) for PTOA within 5 years after ACL reconstruction.[3] Hence, it is plausible that differential metabolic responses associated with sex and BMI may contribute to the increased risk of early-onset PTOA in females and individuals with high BMI. Earlier authors[12,13] only identified an association between sex and BMI with biomarker concentrations but did not quantify how sex and BMI may alter biomarker concentrations. Therefore, it is important to directly assess the effects of sex and BMI on blood-derived biomarkers so we can better understand the role of these demographics in acute biological changes. This is an important step that may enable the development of blood-derived biomarkers as a monitoring tool after ACL reconstruction.

Age is another common demographic factor accounted for when evaluating biomarker changes, as biomarker levels tend to change with aging, independent of disease.[15] For example, healthy[16] and injured populations[17] exhibited age-related changes in MMP-3 levels: older age corresponded with higher MMP-3 levels. Studies of individuals after ACL reconstruction are limited but reflected no association of age with MMP-3 and type II turnover levels.[13] However, age remained a risk factor for the development of early-onset PTOA, with older age at the time of injury associated with earlier development of PTOA.[18] Aging-related changes in biomarkers may compound the biochemical changes associated with injury and magnify the risk for developing joint disease. Thus, examining the influence of age on MMP-3 and type II turnover levels may be informative in identifying individuals at risk for early osteoarthritic changes after ACL injury and reconstruction.

Currently, limited longitudinal research is available on MMP-3 and type II turnover changes after ACL injury and reconstruction, with no examinations to date of the influence of sex, BMI, and age on biomarkers over time. The cross-sectional nature of previous investigations provided only a small glimpse of the metabolic changes that occurred after ACL injury and reconstruction, which may have obscured valuable information, particularly when comparing individuals with large variations in BMIs, age, and time since surgery. In addition, cross-sectional research could not provide insight into the magnitude and direction of metabolic changes postsurgery or how changes in modifiable factors, such as BMI, may have further influenced biomarker concentrations over time. Although sex and BMI affect the radiographic signs of osteoarthritis (OA), incomplete knowledge of the influence of sex and BMI on biochemical markers of OA has limited the ability of clinicians and researchers to recognize individuals who may be at risk of early-onset PTOA before radiographic damage occurs. Understanding the factors influencing cartilage metabolism and health may be helpful for early identification of individuals at high risk for developing PTOA and early intervention to mitigate the risk of modifiable risk factors such as BMI. Therefore, the primary purpose of our study was to determine the relationship of sex and BMI on serum levels of MMP-3 and C2C:CPII before and after ACL reconstruction. The secondary purpose was to evaluate the relationship of sex and age with serum levels of MMP-3 and C2C:CPII before and after ACL reconstruction.

METHODS

Participants

A total of 32 individuals with a primary unilateral ACL tear participated in this study (Table 1) and were tested at 2 time points: before ACL reconstruction and when they were cleared to return to activity. All methods were approved by the Institutional Review Board at the University of

Michigan, and all recruits provided informed consent before data collection began. Participants were eligible if they (1) were between 14 and 30 years of age, (2) had a diagnosis of a primary unilateral ACL tear, and (3) were scheduled to undergo ACL reconstruction by a single orthopaedic practice. Return-to-activity criteria of the orthopaedic surgeon required (1) full range of motion; (2) appropriate joint laxity; (3) no pain, tenderness, or swelling; (4) successful completion of a leg-press test; and (5) successful completion of an agility program as described earlier.[19] Participants' demographic variables are shown in Table 1.

Body Mass Index

Participant height and weight were determined using a calibrated stadiometer before surgery and at return to activity on the days of blood sample collections. Body mass was calculated using height and weight measurements from the equation (Weight in kg)/(Height in m)^2.

Serum Collection and Immunoassays

Blood samples were obtained before surgery and at return to activity. Venous blood (approximately 4 mL) was collected from the antecubital vein, stored in anticoagulant vacuette tubes (model K2EDTA; Becton, Dickinson and Co), and centrifuged at 1000*g* for 10 minutes. The supernatant (serum) was removed for each sample, aliquoted (20 μL), and stored at –80°C for batch processing. Commercially available enzyme-linked immunosorbent assays (ELISA) kits were used to assess markers of MMP-3 (Human Total MMP-3 Quantikine ELISA Kit; R&D Systems), C2C (Collagen Type II Cleavage Assay; IBEX Technologies, Inc), and CPII (Collagen Type II Synthesis Assay; IBEX Technologies, Inc) serum concentrations.[20–22] Serum levels were expressed as ng/mL. Concentrations of C2C and CPII were determined individually and used to calculate the type II collagen turnover ratio (degradation [C2C] to synthesis [CPII]) before analysis. All assays were performed in duplicate for standards and unknowns, with intra-assay and interassay variability $\leq$10%.

Statistical Analysis

We analyzed the longitudinal data using the statistical software R (version 3.6.1; R Foundation for Statistical Computing). Normality of dependent variables was assessed using quantile-quantile plots and the Shapiro-Wilk test. Biomarker data for MMP-3 and type II turnover were nonnormally distributed and transformed using the natural log and then further assessed to remove influential points using Cook distances. We computed generalized linear mixed-effects regression models to assess the relationships of sex, BMI, time, and participant with the outcome variables (ln[MMP-3] and ln[C2C:CPII]). Sex, BMI, and time were treated as fixed effects and participant as a random effect. Time was treated as a categorical variable: T1 for baseline and T2 for return to activity. When variables included in the model were significant, post hoc analyses were performed using Tukey pairwise comparisons. In addition, a secondary analysis was conducted to assess the relationship between sex, age, time, and participant and the outcome variables (ln[MMP-3] and ln[C2C:CPII]). Sex, age, and time were treated as fixed effects and participant as a random effect.

Standardized effect sizes were calculated for all variables using Cohen f^2. Cohen f^2 is defined by Equation 1 in a regression model, where R^2 is the coefficient of determination:

$$f^2 = \frac{R^2}{1 - R^2} \quad (1)$$

Effect sizes were interpreted according to the following standard values: $f^2 \geq 0.02$, *small*; $f^2 \geq 0.15$, *medium*; and $f^2 \geq 0.35$, *large*.[23] All tests were considered significant at an α level of $P < .05$.

RESULTS

Participants

A total of 32 individuals with ACL reconstruction participated and completed the current study (females = 18, males = 14). The average time from injury to the first blood draw was 5.95 ± 5.11 days and before reconstruction. The average time from injury to the second blood draw was 282 ± 53 days and after reconstruction at the time of return to activity.

Matrix-Metalloproteinase-3

A significant time × sex interaction was found for MMP-3 levels with a medium effect size (Table 2). Males demonstrated higher levels at return to activity (females = 1.92 ± 0.60 ng/mL, males = 2.71 ± 0.59 ng/mL, $P = .017$; Figure 1). No difference was noted in MMP-3 levels between females and males before surgery (females = 1.87 ± 0.63 ng/mL, males = 2.35 ± 0.62 ng/mL, $P = .18$). The MMP-3 levels in males also increased from baseline to return to activity ($P = .009$), while no such increase was seen in females ($P = .77$). Body mass index did not influence MMP-3 levels (Table 2).

The secondary analysis showed a significant time × sex interaction for MMP-3 levels (Table 3), consistent with the primary analysis. In addition, the secondary analysis revealed a significant main effect for age (Table 3), with older ages associated with higher MMP-3 levels. No significant interactions were observed for age with sex or time (Table 3).

Type II Collagen Turnover

No significant main effects for sex, BMI, or time were observed for C2C:CPII (Table 2). Females displayed an average type II turnover concentration of –1.71 ± 0.21 before surgery and –1.71 ± 0.35 at return to activity. Males had an average type II turnover concentration of –1.80 ± 0.14 before surgery and –1.79 ± 0.18 at return to activity. (Figure 2). The secondary analysis demonstrated no significant main effect for sex, age, or time for C2C:CPII levels (Table 3).

DISCUSSION

The primary purpose of our study was to determine the effects of sex and BMI on serum levels of MMP-3 and C2C:CPII after ACL injury and at return to activity. We

Table 2. Effect Sizes and 95% CIs for Each Variable in the Statistical Model for Matrix-Metalloproteinase-3 and Type II Collagen Turnover Ratio

	Matrix-Metalloproteinase-3			Type II Collagen Turnover Ratio		
Variable	Effect Size (Cohen f^2)[a]	95% CI[b]	*P* Value[c]	Effect Size (Cohen f^2)	95% CI	*P* Value
Time	0.37	0.03, 1.04	.005	0.00	0.00, 0.11	.903
Sex	0.30	0.03, 0.86	.005	0.07	0.00, 0.40	.155
BMI	0.03	0.00, 0.32	.374	0.02	0.00, 0.29	.471
Time × sex interaction	0.23	0.00, 0.78	.021	0.00	0.00, 0.14	.859
Time × BMI interaction	0.05	0.00, 0.37	.266	0.15	0.00, 0.63	.062
Sex × BMI interaction	0.00	0.00, 0.37	.973	0.04	0.00, 0.35	.335
Time × sex × BMI interaction	0.03	0.00, 0.32	.356	0.11	0.00, 0.54	.104

Abbreviation: BMI, body mass index.

[a] According to the Cohen (1998) guidelines for effect sizes,[23] $f^2 \geq 0.02$ represents *small*; $f^2 \geq 0.15$, *medium*; and $f^2 \geq 0.35$, *large*.

[b] Because f^2 cannot be negative, CIs ≥ 0.

[c] $P < .05$ indicates a significant effect.

found that MMP-3 was related to sex but not to BMI. The collagen turnover ratio was not influenced by sex or BMI, which conflicted with our initial hypothesis. A secondary purpose was to determine the effects of age on MMP-3 and C2C:CPII levels. Older age resulted in greater serum levels of MMP-3 but not C2C:CPII.

Matrix-metalloproteinase-3 is responsible for signaling degradation of extracellular matrix substrates and theorized to be a measure of cartilage destruction,[24] with a growing body of evidence suggesting that MMP-3 levels are elevated after ACL injury.[9,10] However, whether MMP-3 levels changed over time from ACL injury to ACL reconstruction and whether MMP-3 changes depended on factors such as sex and BMI was unclear. Our study provided evidence for increased MMP-3 levels in males after ACL reconstruction compared with before reconstruction. In addition, both sex and time influenced MMP-3 levels, with higher MMP-3 levels in males than females only at return to activity. These findings suggested that MMP-3 serum levels continued to be upregulated after ACL reconstruction, particularly in males, which may have had deleterious consequences for the cartilage matrix and could have placed them at more risk for the development of PTOA. Additional research examining the ratio of MMP-3 levels relative to tissue inhibitors of metalloproteinases (TIMPs), which help regulate MMP-3 levels, would be valuable to ascertain if the increase in MMP-3 level is accompanied by a corresponding change in TIMPs activity. Furthermore, collecting biomarker levels beyond return to activity may help elucidate whether MMP-3 levels continue to be altered once patients are back to activity and in the period leading up to PTOA development. With this information, the role of MMP-3 expression in the development of PTOA can be better understood; potentially, interventions aimed at modifying the expression of MMP-3 could be used after ACL injury and reconstruction, particularly in males, to delay or prevent the development of PTOA.

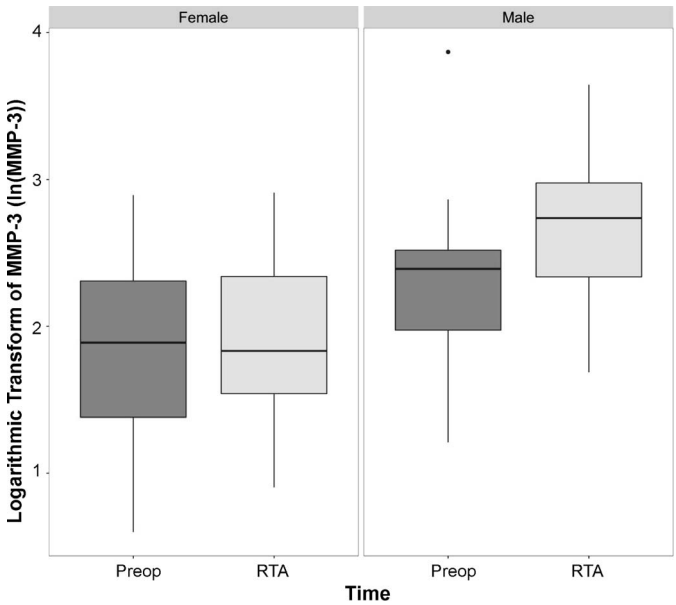

Figure 1. Boxplots depicting the data for the logarithmic transform of matrix-metalloproteinase-3 (MMP-3) separated by sex at 2 time points: (1) preoperative (preop) and (2) at return to activity (RTA). Boxes represent the interquartile range (IQR) between the 25th and 75th percentiles. The black horizontal line inside each box corresponds to the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the 25th and 75th percentiles, respectively. Black dot represents a data point that falls outside of the IQR.

We did not identify an effect of BMI on MMP-3 levels, which was consistent with a previous finding[13] that BMI was not a covariate of MMP-3 levels at 6 months after ACL reconstruction. Given that BMI did not appear to influence MMP-3 levels in other injured populations,[25,26] this outcome was not unexpected. Thus, it is possible that BMI did not influence expression of MMP-3 levels after ACL reconstruction. However, it is also possible that BMI influenced MMP-3 levels but that our sample size was too small and inadequate for detecting differences. Because of the small effect size ($f^2 = 0.03$), we contend that this was unlikely, but additional research with larger sample sizes is necessary to confirm our findings.

In addition to the effects of sex and time, our secondary analysis revealed a significant main effect of age on MMP-3 levels: older age was associated with an increase in MMP-3 serum levels. However, the current findings conflicted with another study[13] of patients with ACL reconstruction in which age was not a significant covariate of MMP-3 levels. The conflicting results were surprising, as previous investigators observed that older age was associated with higher MMP-3 levels in healthy[27] and injured populations,[16] similar to our findings. Given the relatively young participant age of the current and former

Table 3. Secondary Analysis of Effect Sizes and 95% CI for Effect Sizes of Each Variable in the Statistical Model for Matrix-Metalloproteinase-3 and Type II Collagen Turnover Ratio

	Matrix-Metalloproteinase-3			Type II Collagen Turnover Ratio		
Variable	Effect Size (Cohen f^2)[a]	95% CI[b]	*P* Value[c]	Effect Size (Cohen f^2)	95% CI	*P* Value
Time	0.35	0.03, 0.97	.004	0.00	0.00, 0.10	.908
Sex	0.38	0.04, 0.86	.003	0.07	0.00, 0.41	.176
Age	0.19	0.00, 0.67	.029	0.03	0.00, 0.30	.357
Time × sex interaction	0.24	0.01, 0.76	.016	0.00	0.00, 0.12	.869
Time × age interaction	0.04	0.00, 0.32	.315	0.00	0.00, 0.00	.989
Sex × age interaction	0.06	0.00, 0.39	.195	0.00	0.00, 0.14	.826
Time × sex × age interaction	0.00	0.00, 0.20	.664	0.04	0.00, 0.34	.305

[a] According to the Cohen (1998) guidelines for effect sizes,[23] $f^2 \geq 0.02$ represents *small*; $f^2 \geq 0.15$, *medium*; and $f^2 \geq 0.35$, *large*.
[b] Because f^2 cannot be negative, CIs ≥ 0.
[c] $P < .05$ indicates a significant effect.

assessments,[13] examining a greater age range would be valuable for confirming whether MMP-3 serum levels are age dependent in individuals with ACL reconstruction. Regardless, our results highlighted the importance of accounting for age when analyzing MMP-3 serum levels in those with ACL reconstruction.

Changes in type II turnover are a growing area of research, with evidence suggesting that collagen turnover may be disrupted after ACL reconstruction. The disruption to collagen turnover homeostasis may also be influenced by factors such as sex and BMI,[14] which are known risk factors for early-onset PTOA.[3] We found no significant effect of sex, BMI, or time on type II turnover, an outcome that was inconsistent with earlier work[14] indicating that more type II turnover was associated with higher BMIs in women after ACL reconstruction. One likely explanation for the conflicting findings was the different times that biomarkers were collected after ACL reconstruction (current study = 9.29 ± 1.71 months, previous study[14] = 48.3 ± 38.2 months). Earlier researchers[28] reported that time after ACL reconstruction was covariate for type II turnover ratios, with less type II turnover associated with greater time since reconstruction. Given that PTOA appeared to become more prevalent ≥5 years after ACL reconstruction, it was possible that sex- and BMI-related differences did not become apparent until longer after reconstruction. Another possible explanation for the different findings may have been that few of our participants were in the obese category (BMI ≥ 30.0. However, we believed this was unlikely to explain the differences between our results and those of Lane et al,[14] as both samples were in similar BMI ranges (current study = 18.08–38.63 kg/m^2, Lane et al[14] = 19.43–39.47 kg/m^2) and had similar mean BMIs (current study = 25.15 ± 4.91 kg/m^2, Lane et al[14] = 25.6 ± 4.2 kg/m^2). Thus, we concluded that BMI may not be a significant factor in type II turnover levels, which was consistent with other research in individuals after ACL reconstruction.[13] Yet BMI may not be the most appropriate measure for assessing the relationship between biomarker levels and obesity, particularly in physically active individuals in the ACL-injured population, as it can identify muscular individuals as obese[29] and is an indirect measure of adiposity. Accordingly, other factors such as body fat percentage, level of physical activity, or percentage increase in BMI may be more related to type II turnover. Further research with additional time points and validated OA measures may provide more insight into the complicated relationship among sex, BMI, and type II turnover and the effect on long-term joint health after ACL reconstruction.

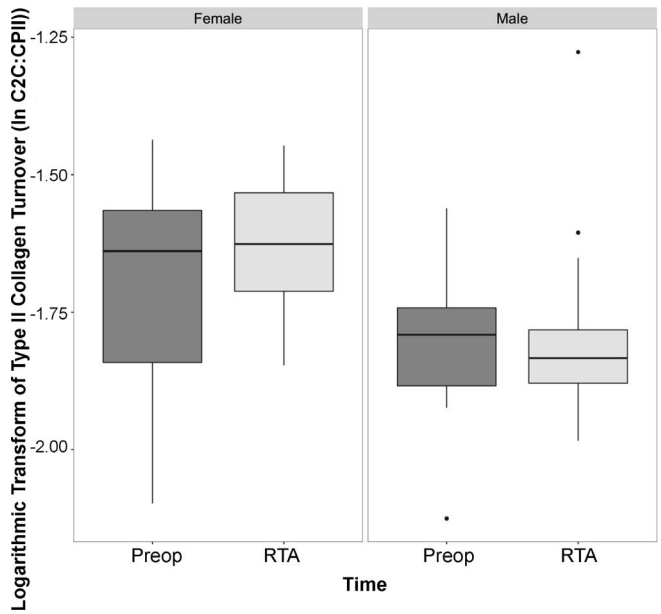

Figure 2. Boxplots depicting the data for the logarithmic transform of collagen type II turnover (C2C:CPII) serum levels separated by sexes at 2 time points: (1) preoperative (preop) and (2) at return to activity (RTA). Boxes represent the interquartile range (IQR) between the 25th and 75th percentiles. The black horizontal line inside each box corresponds to the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the 25th and 75th percentiles, respectively. Black dots represent data points that fall outside of the IQR.

Finally, the secondary analysis demonstrated no significant effect for sex, age, or time on type II turnover levels before or after ACL reconstruction. Our primary analysis indicated no effect for sex or time on type II turnover levels, so it is not unexpected that the secondary analysis confirmed our initial findings. In addition, our results regarding the effect of age were consistent with those of a previous investigation[13] of individuals 6 months after ACL reconstruction. Our results were also confirmed in earlier work with a larger age range,[30] which suggested that type II turnover levels were not age dependent and may not have required methodologic accounting for this variable. However, additional research adequately powered for age is

needed to confirm whether age influences type II turnover before and after ACL reconstruction.

This work was not without limitations. We chose to use blood-derived biomarkers for their clinical utility and cost effectiveness. Nonetheless, we acknowledge that blood biomarkers do not reflect changes occurring solely at the knee but represent systemic changes. Additional metrics that are more sensitive to smaller changes, such as synovial samples, should be used to confirm the current findings. Also, as mentioned earlier, our results may have been limited by the relatively small sample size. Further, although we denoted moderate-to-large effect sizes for MMP-3 (time and time × sex), the CIs for these values were wide and spanned a range of small-to-large effects, suggesting that the magnitude or precision (or both) of the effects of time and time × sex on MMP-3 were less clear. The wide CIs were likely attributable to our smaller sample size, as the precision of CIs is better with larger samples, but could also suggest that time and time × sex effects on MMP-3, which we interpreted as statistically significant or different based on *P* values, may not have been related to the sample size. Future investigations with larger sample sizes are necessary to provide additional insight into the influence of factors such as sex, BMI, and time on biomarker concentrations after ACL reconstruction.

CONCLUSIONS

Males demonstrated higher MMP-3 levels than females at return to activity. Their serum levels increased from before surgery to return to activity. However, MMP-3 levels did not increase over time in females. A secondary analysis confirmed these findings and revealed that older age was associated with higher MMP-3 levels. In addition, neither sex nor BMI influenced the turnover ratio in individuals with ACL reconstruction. Accordingly, sex and age should be considered when assessing MMP-3 concentrations after ACL injury and reconstruction. Further longitudinal research is needed to investigate the influence of sex, BMI, and age on blood and synovial biomarkers concurrently to gain a deeper understanding of the factors that influence biomarkers after ACL reconstruction.

REFERENCES

1. Lentz TA, Zeppieri G Jr, Tillman SM, et al. Return to preinjury sports participation following anterior cruciate ligament reconstruction: contributions of demographic, knee impairment, and self-report measures. *J Orthop Sports Phys Ther*. 2012;42(11):893–901. doi:10.2519/jospt.2012.4077
2. Roos EM. Joint injury causes knee osteoarthritis in young adults. *Curr Opin Rheumatol*. 2005;17(2):195–200. doi:10.1097/01.bor.0000151406.64393.00
3. Bodkin SG, Werner BC, Slater LV, Hart JM. Post-traumatic osteoarthritis diagnosed within 5 years following ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 2020;28(3):790–796. doi:10.1007/s00167-019-05461-y
4. Hunter DJ, Guermazi A. Imaging techniques in osteoarthritis. *PM R*. 2012;4(suppl 5):S68–S74. doi:10.1016/j.pmrj.2012.02.004
5. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood*. 1993;81(11):2844–2853.
6. Kraus VB, Burnett B, Coindreau J, et al. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. *Osteoarthritis Cartilage*. 2011;19(5):515–542. doi:10.1016/j.joca.2010.08.019
7. Sternlicht MD, Lochter A, Sympson CJ, et al. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell*. 1999;98(2):137–146. doi:10.1016/s0092-8674(00)81009-0
8. Dahlberg L, Friden T, Roos H, Lark MW, Lohmander LS. A longitudinal study of cartilage matrix metabolism in patients with cruciate ligament rupture—synovial fluid concentrations of aggrecan fragments, stromelysin-1 and tissue inhibitor of metalloproteinase-1. *Br J Rheumatol*. 1994;33(12):1107–1111. doi:10.1093/rheumatology/33.12.1107
9. Higuchi H, Shirakura K, Kimura M, et al. Changes in biochemical parameters after anterior cruciate ligament injury. *Int Orthop*. 2006;30(1):43–47. doi:10.1007/s00264-005-0023-5
10. Lohmander LS, Roos H, Dahlberg L, Hoerrner LA, Lark MW. Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. *J Orthop Res*. 1994;12(1):21–28. doi:10.1002/jor.1100120104
11. Svoboda SJ, Harvey TM, Owens BD, Brechue WF, Tarwater PM, Cameron KL. Changes in serum biomarkers of cartilage turnover after anterior cruciate ligament injury. *Am J Sports Med*. 2013;41(9):2108–2116. doi:10.1177/0363546513494180
12. Cibere J, Zhang H, Garnero P, et al. Association of biomarkers with pre-radiographically defined and radiographically defined knee osteoarthritis in a population-based study. *Arthritis Rheum*. 2009;60(5):1372–1380. doi:10.1002/art.24473
13. Davis HC, Spang JT, Loeser RF, et al. Time between anterior cruciate ligament injury and reconstruction and cartilage metabolism six months following reconstruction. *Knee*. 2018;25(2):296–305. doi:10.1016/j.knee.2018.01.009
14. Lane AR, Harkey MS, Davis HC, et al. Body mass index and type 2 collagen turnover in individuals after anterior cruciate ligament reconstruction. *J Athl Train*. 2019;54(3):270–275. doi:10.4085/1062-6050-525-17
15. Kubota K, Kadomura T, Ohta K, et al. Analyses of laboratory data and establishment of reference values and intervals for healthy elderly people. *J Nutr Health Aging*. 2012;16(4):412–416. doi:10.1007/s12603-011-0355-3
16. Manicourt DH, Fujimoto N, Obata K, Thonar EJ. Serum levels of collagenase, stromelysin-1, and TIMP-1: age- and sex-related differences in normal subjects and relationship to the extent of joint involvement and serum levels of antigenic keratan sulfate in patients with osteoarthritis. *Arthritis Rheum*. 1994;37(12):1774–1783. doi:10.1002/art.1780371211
17. Kelly D, Khan S, Cockerill G, et al. Circulating stromelysin-1 (MMP-3): a novel predictor of LV dysfunction, remodelling and all-cause mortality after acute myocardial infarction. *Eur J Heart Fail*. 2008;10(2):133–139. doi:10.1016/j.ejheart.2007.12.009
18. Roos H, Adalberth T, Dahlberg L, Lohmander LS. Osteoarthritis of the knee after injury to the anterior cruciate ligament or meniscus: the influence of time and age. *Osteoarthritis Cartilage*. 1995;3(4):261–267. doi:10.1016/s1063-4584(05)80017-2
19. Lepley LK, Palmieri-Smith RM. Pre-operative quadriceps activation is related to post-operative activation, not strength, in patients post-ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc,* 2016;24(1):236–246. doi:10.1007/s00167-014-3371-0
20. Ibex Pharmaceuticals Inc. IP. Collagen type II cleavage assay (C2C ELISA). 2017. Accessed February 15, 2022. http://ibex.ca/wp-content/uploads/2017/11/c2c_specifications_20171124-2.pdf
21. Procollagen II C-Propeptide ELISA. IBEX Pharmaceuticals Inc. Accessed February 15, 2022. http://ibex.ca/wp-content/uploads/2018/03/item-60-1003-001_cpii_assay-insert-rev_12sep2017.pdf
22. R&D Systems. Quantikine ELISA: Human Total MMP-3 Immunoassay. 2015. Accessed February 15, 2022. https://resources.rndsystems.com/pdfs/datasheets/dmp300.pdf?v=20220214
23. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. L. Erlbaum Associates; 1988.

24. Hasty KA, Reife RA, Kang AH, Stuart JM. The role of stromelysin in the cartilage destruction that accompanies inflammatory arthritis. *Arthritis Rheum*. 1990;33(3):388–397. doi:10.1002/art.1780330312
25. Grzechocińska B, Dąbrowski FA, Sierdzinski J, Cyganek A, Wielgoś M. The association between serum metalloproteinase concentration, obesity, and hormone levels in reproductive-aged women. *Endokrynol Pol*. 2019;70(1):49–56. doi:10.5603/EP.a2018.0067
26. Mulyadi, Sunnati, Azhary M, Yunus F, Nurwidya F. The correlation of age and body mass index with the level of both protease MMP3 and anti-protease TIMP-1 among Indonesian patients with chronic obstructive pulmonary disease: a preliminary findings. *BMC Res Notes*. 2018;11(1):551. doi:10.1186/s13104-018-3669-y
27. Thrailkill KM, Moreau CS, Cockrell G, et al. Physiological matrix metalloproteinase concentrations in serum during childhood and adolescence, using Luminex Multiplex technology. *Clin Chem Lab Med*. 2005;43(12):1392–1399. doi:10.1515/CCLM.2005.238
28. Pietrosimone B, Blackburn JT, Harkey MS, et al. Greater mechanical loading during walking is associated with less collagen turnover in individuals with anterior cruciate ligament reconstruction. *Am J Sports Med*. 2016;44(2):425–432. doi:10.1177/0363546515618380
29. Witt KA, Bush EA. College athletes with an elevated body mass index often have a high upper arm muscle area, but not elevated triceps and subscapular skinfolds. *J Am Diet Assoc*. 2005;105(4):599–602. doi:10.1016/j.jada.2005.01.008
30. Conrozier T, Poole AR, Ferrand F, et al. Serum concentrations of type II collagen biomarkers (C2C, C1, 2C and CPII) suggest different pathophysiologies in patients with hip osteoarthritis. *Clin Exp Rheumatol*. 2008;26(3):430–435.

Address correspondence to Riann M. Palmieri-Smith, PhD, ATC, University of Michigan, 830 N. University Avenue, Ann Arbor, MI 48109. Address email to riannp@umich.edu.