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Relationship Between Markers of Type II Collagen Metabolism and Tibiofemoral Joint Space Width Changes After ACL Injury and Reconstruction

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Abstract

Background: Those who suffer anterior cruciate ligament (ACL) disruptions are at increased risk of experiencing posttraumatic osteoarthritis (OA); however, by the time they become symptomatic, irreversible damage has likely occurred. Little is known regarding the physiological changes in articular cartilage that occur after an ACL injury and the onset of OA.

Purpose: To assess whether patient, functional, and clinical outcomes and type II collagen metabolism are associated with abnormal tibiofemoral joint space width (JSW) 4 years after injury and reconstruction.

Study Design: Cohort study; Level of evidence, 2.

Methods: A total of 35 ACL-injured patients who underwent ACL reconstruction were enrolled soon after injury, as were 32 matched controls. At baseline and 1- and 4-year follow-ups, patient-oriented subjective and objective outcomes and markers of type II collagen metabolism (considered as the ratio of cleavage to synthesis of type II collagen) were evaluated, as were radiographic measurements of JSW changes about the medial and lateral compartments of the knee. ACL-injured patients were divided into normal and abnormal JSW groups.

Results: Both ACL-injured groups (normal and abnormal JSW) had an increased ratio of collagen type I and II cleavage product (uC1,2C) to serum procollagen II C-propeptide (sCPII) compared with controls at 1- and 4-year follow-ups. Patients in the ACL group with an abnormal JSW difference had significantly increased cleavage-to-synthesis ratios of type II collagen (assessed as C-terminal cross-linked telopeptide of type II collagen [uCTX-II]/sCPII ratio) compared with controls at 4-year follow-up. ACL-injured patients with an abnormal JSW difference had significantly increased pain and decreased quality of life (Knee Injury and

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Osteoarthritis Outcome Score [KOOS]) scores than did ACL-injured patients with a normal JSW difference.

Conclusion: ACL-injured patients with an abnormal tibiofemoral JSW had diminished quality of life, increased pain, and increased type II collagen uCTX-II/sCPII ratios compared with healthy controls. These changes occurred over an interval shortly after injury in patients who were fully functional and who had normal clinical examination findings, no pivoting/giving-way episodes, and no decrease in activity level.

Keywords

posttraumatic; tibiofemoral; osteoarthritis; biomarkers; type II collagen

People who sustain significant anterior cruciate ligament (ACL) injuries of the knee are at increased risk of experiencing posttraumatic osteoarthritis (OA) within 10 to 20 years after injury.[1,19,21,25,28,30,31] After ACL injury, the clinical signs and symptoms and radiographic changes that occur with posttraumatic OA are seen in 40% to 50% of patients within 10 to 15 years after injury.[18,19,40] By the time people with these injuries become symptomatic and seek medical treatment for their condition, irreversible damage to the articular cartilage has likely occurred.[9] This poses substantial long-term quality of life consequences for the patient and greatly increased financial burden on society.[5,20,41]

The evaluation of biochemical markers indicative of type II collagen metabolism in serum and urine is thought to provide insight into the pathophysiology of the osteoarthritic joint at the time of sample acquisition and may elucidate underlying mechanisms associated with structural changes within articular cartilage.[4,16,17] Consequently, a substantial effort is underway to identify biomarkers that may serve as therapeutic targets for disease-modifying osteoarthritis drugs, markers of therapeutic efficacy, and prognostic markers capable of predicting who is at increased risk for the onset and progression of OA during its earliest stages when treatment may prove most effective at reducing future pain and loss of function secondary to irreversible articular cartilage loss.[7,16]

A recent recommendation by the joint Osteoarthritis Research Society International/Food and Drug Administration Biomarkers Working Group[7] suggested that future studies consider patients at high risk for the development of posttraumatic OA, such as those who have suffered an ACL injury, as an important research focus group. The rationale for this recommendation is that these patients are able to identify a single inciting or “index” event that places them at increased risk for the development of posttraumatic OA (unlike idiopathic OA), and researchers have the opportunity to conduct investigations that are designed to monitor the onset and earliest progression of the disease before the patients become symptomatic.

We have previously reported the approach used to quantify tibiofemoral joint space width (JSW) changes that occur after ACL injury and subsequent surgical reconstruction as well as assessment of the assumptions that must be made when utilizing this radiographic approach.[38] The purpose of this investigation was to assess whether selected patient, functional, and clinical outcomes as well as biological markers of type II collagen metabolism are

associated with the observed tibiofemoral JSW changes in the same cohort of research participants. The aim of this investigation was to elucidate relationships between pain, dysfunction, clinical assessment, and potential physiological/ biochemical pathways with the observed articular cartilage structural changes that occur in a relatively short time span after ACL injury and subsequent surgical reconstruction.

MATERIALS AND METHODS

A longitudinal cohort study was used to assess patient, functional, and clinical outcomes as they relate to markers of type II collagen metabolism (evaluated as the ratio of cleavage to synthesis of type II collagen in serum and urine) in 35 ACL-injured (18 women) patients and 32 healthy matched control participants (18 women). The ACL-injured cases were recruited from our community-based orthopaedic clinic, and controls were recruited from the surrounding community. Patients were of similar race, age, body mass index (BMI), and activity level as our control group (Table 1). Our institutional review board approved this protocol before patient enrollment, and all participants provided written informed consent before data collection.

Entry Criteria

Entry criteria for the ACL-injured participants included age at the time of enrollment between 14 and 55 years, moderate activity level (Tegner activity score ≥5), no previous injuries to any diarthrodial joint (defined as that requiring physician referral and/or >3 days of modified activities of daily living) or knee injections, no relevant knee injuries other than the index injury, no abnormal ligamentous (other than the ACL) or capsular laxity, normal radiograph results (eg, no evidence of fracture or pre-existing OA), no obvious varus or valgus malalignment (as determined by International Knee Documentation Committee [IKDC] knee examination criteria), less than 2/3 meniscectomy at the time of surgical reconstruction (only 1 patient had just over 1/3 medial meniscectomy and was consequently graded “2/3 meniscectomy” as a conservative measure per grading criteria guidelines), and articular cartilage lesions of grade IIIa or less (based on International Cartilage Repair Society grading criteria) in the tibiofemoral and patellofemoral joints.

The entry criteria used for the ACL group were also used for the control group, with the exception of a few obvious injury-related criteria that could not be controlled in the ACL group. Control participants had no subjective report of knee pain or dysfunction as determined by the Knee Injury and Osteoarthritis Outcome Score (KOOS)[34] and IKDC subjective knee evaluation form,[12] no medical history of significant trauma to any diarthrodial joint (defined as that requiring physician referral and/or >3 days of modified activities of daily living), normal clinical knee examination results (IKDC), and normal magnetic resonance imaging (MRI) findings at baseline and follow-up.

Surgical Reconstruction and Evaluation Intervals

The ACL reconstructions were performed by 1 of 2 sports medicine fellowship–trained orthopaedic surgeons. Thirty-one of 35 (88.6%) ACL reconstructions were performed with autologous bone–patellar tendon–bone (BPTB) autografts, 3 (8.6%) were BPTB allografts,

and 1 (2.8%) was reconstructed with a semitendinosus gracilis 4-strand autograft. These patients were part of a larger clinical trial and details of the surgical procedure have been described previously.[3] The load applied to the graft at the time of fixation was standardized by tensioning it until anterior-posterior (AP) laxity of the contralateral limb, as measured with a KT-1000S arthrometer (MedMetric Corp, San Diego, California), was re-created (±1 mm) using a technique that has been described.[3] The mean time between injury and for ACL-injured patients between injury and surgical reconstruction was 70.1 days (range, 18–155 days), and all patients participated in the same rehabilitation program.[3] Patients were assessed at baseline (within 3 weeks before surgery) and at 1-year and final follow-up (mean ± standard deviation, 46 ± 9.5 months after surgery). Control participants were also examined at baseline and at 1-year and final follow-up of 33 ± 6.6 months. To evaluate existing or new injuries sustained before or during the study period, control participants also underwent baseline 3-T MRI, and patients in both the control and ACL groups underwent follow-up bilateral knee MRI at final follow-up.

Clinical, Functional, and Subjective Outcome Assessments

Subjective assessments of pain, function, physical activity level, and quality of life were evaluated with the IKDC subjective score,[12] Tegner activity scale,[35] Lysholm knee scale,[22] KOOS,[34] Cincinnati functional knee score,[29] and Marx activity rating scale.[24] Functional and clinical outcomes were obtained at each visit and included the assessment of lower extremity physical function through the single-legged hop test,[2] instrumented AP knee laxity[8] (KT-1000 arthrometer), and IKDC objective knee evaluation.[11] When appropriate, comparisons were made to the uninjured limb (ACL group) or contralateral limb (controls).

Assessment of Tibiofemoral JSW

Weightbearing bilateral anteroposterior metatarsal-phalangeal view knee radiographs were obtained at baseline and all follow-ups using an approach that has been described.[10] The JSW was measured from the radiographs using a validated technique.[10] Specific details regarding the approach that was used to analyze JSW data in this cohort have been reported.[38] Patients were considered to have a significant side-to-side JSW difference, and therefore significantly abnormal tibiofemoral JSW (whether it was increased or decreased JSW), if the injured minus normal (contralateral) knee difference fell outside the 95% confidence interval (CI) of the side-to-side difference measured in controls. This was conducted separately for the medial and lateral compartments of the tibiofemoral joint.

Biomarker Assessment

Serum and midstream “clean catch” urine samples were obtained from all patients at 1-year and final follow-up evaluations. To avoid potential complications caused by normal diurnal variations of biomarkers,[15] only non–first morning void urine samples were obtained.

After completion of data collection, biomarker concentrations were evaluated with commercially available enzyme-linked immunosorbent assay (ELISA) kits, which were performed in duplicate per manufacturer recommendations by the same experienced investigators. The biological markers of type II collagen cleavage and synthesis that were chosen for this investigation were based on previous work evaluating the earliest stages

("preradiographic") of primary OA,[6] prior research focused on measuring type II collagen metabolism (or the ratio of markers of cleavage to synthesis of type II collagen),[39] as well as consultation with leading experts in the field of OA biomarker analysis (please see Acknowledgment section).

Type II collagen synthesis was evaluated by measuring concentrations of serum procollagen II C-propeptide (sCPII). The competitive ELISA (IBEX Pharmaceuticals, Montreal, Quebec, Canada) for this marker uses a monoclonal antibody that binds to type II collagen propeptide epitopes cleaved from the C-terminus of procollagen after being released into the matrix.[27] Consequently, sCPII serves as a marker of newly forming type II collagen, or collagen synthesis. The intra-assay and interassay coefficients of variation (CVs) for sCPII were 7.22% and 7.31%, respectively.

Concentrations of collagen cleavage markers were evaluated in urine via ELISA and included a collagen type II cleavage product (uC2C) (IBEX Pharmaceuticals), collagen type I and II cleavage product (uC1,2C) (IBEX Pharma-ceuticals), and C-terminal cross-linked telopeptide of type II collagen (uCTX-II) (CartiLaps, Nordic Bioscience, Herlev, Denmark). Urine biomarker concentrations were corrected for creatinine by colorimetric assay (QuantiChrom Creatinine Assay Kit, BioAssay Systems, Hayward, California). Because of known effects of age on uCTX-II concentrations in patients younger than 40 years,[26] concentrations of this marker and its ratio were adjusted by statistically controlling for age as a covariate. The intra-assay and interassay CVs for the cleavage marker uC2C were <5% and 7.23%, respectively. Intra-assay and inter-assay CVs for cleavage markers uC1,2C and uCTX-II were all <5%.

Statistical Analyses

Patient groups were established based on tibiofemoral JSW status and included (1) controls, (2) ACL-reconstructed patients with a normal JSW difference (those with medial or lateral JSW difference that fell within the 95% CI of controls), and (3) ACL-reconstructed patients with an abnormal JSW difference (those with JSW difference in either compartment that fell outside the 95% CI of controls). Relationships between patient, clinical, and functional outcomes comparing controls (baseline data) to ACL groups (those with normal and abnormal JSW difference) at final follow-up were examined statistically using Kruskal-Wallis tests.

Relationships between the markers of type II collagen metabolism (the ratio of markers of type II collagen cleavage [uC2C, uC1,2C, and uCTX-II] to type II collagen synthesis [sCPII]) and patient groupings (control, ACL reconstruction with normal JSW difference, and ACL reconstruction with abnormal JSW difference) were statistically evaluated using analysis of covariance (ANCOVA). Covariates included age, sex, BMI, and time from injury to surgical reconstruction and were retained within each model if they were found to have P values >.2. Because of the skewed distribution of biomarker concentrations, these data were transformed via square root or natural log to normalize distributions.

RESULTS

Patients and Surgical Findings

Patient-, clinical-, and functional-oriented outcomes were evaluated only in participants with complete outcome scores as well as JSW data at baseline and final follow-up, and only patients in the ACL group with complete serum and urine data sets at 1-year and final follow-up, as well as satisfactory JSW data at all visits, were retained for biomarker ratio analyses. Consequently, of the 39 patients in the ACL group who were originally enrolled in the study, 4 were excluded from statistical analyses, resulting in 35 participants (18 women) in the ACL group and 32 controls. Of the 35 participants in the ACL group, only 1 was deemed to have slightly more than 1/3 meniscectomy in the medial compartment and was consequently graded as “2/3 removed” based on the 2000 IKDC surgical documentation criteria, which was recorded at the time of surgical reconstruction. No patients had more than 1/3 meniscectomy in both medial and lateral compartments combined. Fourteen patients had no tibiofemoral articular cartilage lesions (40% of total ACL group), 8 patients had grade 1A or 1B (22.9%), 9 patients had grade 2 lesions (25.7%), and 4 patients had grade 3A lesions (11.4%) as their most significant lesion present.

Patient, Functional, and Clinical Outcomes in Control Group

Evaluation of JSW differences and patient, functional, and clinical outcomes in the control group revealed that these values did not change significantly over time (eg, between baseline and follow-up), and therefore, all outcomes for the control group were assessed at baseline exclusively (thus providing the most comprehensive data set at a single time point). These data were compared with that of patients in the ACL group at 1-year and final follow-up visits. Follow-up interval data for the ACL group are provided in Table 2.

Patient, Functional, and Clinical Outcomes in ACL Group

Highly significant differences were observed between controls and patients in the ACL group (all being more favorable to the controls) regarding IKDC subjective score; Lysholm score; KOOS pain, quality of life, activities of daily living, symptoms, and sports-related function scores; and Cincinnati knee score at 4-year follow-up (Table 3). Two of these outcome scores (KOOS pain and quality of life) were also significantly different between the ACL subgroups (normal vs abnormal difference in JSW). Patients with an abnormal JSW difference had increased pain ($P = <.03$) and decreased quality of life ($P = <.03$) scores in comparison to those with a normal JSW difference (Table 3).

Significant differences were not observed for the activity level assessments (Tegner and Marx activity scores) between groups. Similarly, there were no differences observed in the physical function measurement (single-legged hop test) between groups. The IKDC objective evaluation grade (which contains single-legged hop and instrumented AP tibiofemoral laxity measures within it) was significantly different between controls and patients in the ACL group, as was AP knee laxity (KT-1000 arthrometer), with higher IKDC scores and less side-to-side difference in AP laxity in controls compared with patients in the ACL group with either a normal or abnormal JSW difference. Neither of these values,

however, was significantly different between the ACL subgroups (normal JSW difference vs abnormal JSW difference).

Biomarker Outcomes

Comparison of the uC2C/sCPII ratio produced no significant differences between control and ACL groups at 1-and 4-year follow-up intervals, and no significant differences were observed in this ratio between ACL groups with a normal and abnormal JSW difference at the same follow-up intervals (Table 4). Urinary C1,2C/sCPII ratios, however, were significantly different between the controls and the ACL group at both 1- and 4-year follow-up visits (with patients in the ACL group having higher cleavage-to-synthesis ratios), although these ratios were not different between the ACL subgroups (normal vs abnormal JSW) at both follow-ups (Table 5). Finally, examination of uCTX-II/sCPII ratios produced statistically significant differences between the control group and ACL group at 1-year follow-up (with patients in the ACL group displaying higher cleavage-to-synthesis ratios). Although no sig-nificant differences were observed between the ACL subgroups (normal vs abnormal JSW) at 1- and 4-year follow-ups, patients in the ACL group with an abnormal JSW difference (those outside the 95% CI of controls) had significantly higher uCTX-II/sCPII ratios at 4-year follow-up than did controls. Consequently, this cleavage-to-synthesis ratio was able to distinguish between healthy patients with a normal JSW difference and patients in the ACL group with an abnormal JSW difference (Table 6).

DISCUSSION

Little is known regarding the physiological processes associated with tibiofemoral structural changes during the earliest stages of posttraumatic OA disease progression, and consequently, the choice of the outcomes and biomarkers used in this investigation was based primarily on related evidence derived from primary OA investigations that have examined the earliest stages of the disease process as well as expert opinion. To the authors' knowledge, this is the first investigation reporting significant differences between healthy control patients and ACL-injured and -reconstructed patients with an abnormal JSW difference regarding patient-oriented outcomes of pain and quality of life (KOOS) and corresponding cleavage-to-synthesis biological marker concentrations (uCTX-II/sCPII) just 4 years after injury and in advance of the clinical presentation of OA.

In this investigation, KOOS pain and quality of life scores were able to distinguish between patients in the ACL group with a normal JSW difference and those with an abnormal JSW difference: Those with an abnormal JSW difference had increased pain and decreased quality of life scores at 4-year follow-up compared to controls. It is important to point out that patients in the ACL group with an abnormal JSW difference did not report decreased function or activity level (Tegner and Marx scores) compared with preinjury baseline values or compared with patients in the ACL group with a normal JSW difference at follow-up, and although statistically significant, the amount of "pain" and decreased "quality of life" reported by this subgroup are less than values often reported with established primary knee OA (ie, Kellgren-Lawrence grade 31).[36] These KOOS pain and quality of life data are similar to those previously reported by Roos et al,[33] who studied patients at long-term

follow-up after unicompartmental partial meniscectomy and demonstrated increased KOOS pain and decreased quality of life scores compared with controls.

Type II collagen cleavage-to-synthesis ratios of uC1,2C/ sCPII and uCTX-II/sCPII were found to be significantly different in patients in the ACL group at 1- and 4-year follow-up intervals compared with healthy control values. Although ratios of uC2c/sCPII and uC1,2C/ sCPII were not able to differentiate between patients in the ACL group with a JSW difference falling within the 95% CI of controls compared with those falling outside the 95% CI, the ratio of uCTX-II/sCPII was significantly different between the control patients and those in the ACL group with an abnormal JSW difference. These results are in concurrence with those recently reported by Ishijima and colleagues.[13] In their study, the authors evaluated the relationship between the presence or absence of knee pain in patients with early OA (Kellgren-Lawrence grade 1, or pre-OA) and those with established early OA (Kellgren-Lawrence grade 2, or established early OA) with biomarkers of type II collagen metabolism (namely, serum CPII, serum C2C, and urinary CTX-II), bone resorption, and synovitis. They demonstrated that in patients with established OA (Kellgren-Lawrence grade 2), only the uCTX-II and uCTX-II/sCPII ratios were significantly increased in patients with knee pain compared to those without pain. The authors also reported increased levels of both sC2C and sCPII in patients with Kellgren-Lawrence grade 1 with knee pain compared with patients with Kellgren-Lawrence grade 1 without pain. Collagen markers were not evaluated in isolation in the present study, as cleavage-to-synthesis ratios better characterize the collagen metabolism and have been reported to be more highly associated with preradiographic OA.[6] In addition, because it is not feasible to quantify the total amount of type II collagen present in each patient, the examination of cleavage-to-synthesis ratios provides a value representative of total body type II collagen metabolism in ACL-reconstructed patients that can be compared with normative values derived from our matched healthy control patients. In addition, patients were not categorized by Kellgren-Lawrence grade for analysis in the present study, and consequently, these findings may not be directly transferable to the current study.

To the authors' knowledge, the only study evaluating associations between the markers examined in the current investigation and the earliest (preradiographic) changes in primary OA is the one reported by Cibere et al.[6] In their study, 201 patients with knee pain between the ages of 40 and 70 years were classified into "no OA," "preradiographic OA," and "radiographic OA" groups based on Kellgren-Lawrence grade combined with MRI-based cartilage scores. Serum-and urine-based biomarkers of collagen (ie, sCPII, uC2C, and uC1,2C) were then compared between the groups. The most directly comparable groups within the Cibere et al[6] investigation and the current one are the "no OA" and "preradiographic OA" groups. In their analyses, comparisons of these groups demonstrated an increased risk of preradiographic OA versus no OA with increasing uC2C levels (odds ratio [OR], 2.06; 95% CI, 1.05–4.01) and uC1,2C (OR, 2.06; 95% CI, 1.12–3.77). In addition, the authors revealed that ratios of type II collagen cleavage-to-synthesis markers were better at differentiating OA subgroups than the examination of individual markers exclusively.

Both uC2C and uC1,2C detect similar epitopes of type II collagen degradation; however, uC1,2C also detects a neoepitope produced by the cleavage of type I collagen. In our investigation, uC1,2C/sCPII ratio levels were significantly different between controls and patients in the ACL group at 1- and 4-year follow-up visits; however, uC2C/sCPII ratios were not significantly different than control values. This seems counterintuitive in that if the cause of an abnormal JSW difference in our ACL-reconstructed patients is secondary to the degradation of articular cartilage (predominantly composed of type II collagen), we would expect both C2C and C1,2C assays to detect increased levels of type II collagen cleavage products. In addition, uCTX-II values are known to be greatly influenced by the clearance of calcified cartilage after closure of the epiphyseal growth plates in patients under 40 years of age,[26] which we chose to handle statistically in our study population by adjusting for age as a covariate. It may be, however, that the antibody supplied in the uCTX-II ELISA kit is also specific (or cross-reactive) to similar neoepitopes involved with bone metabolism, and the earliest changes after ACL reconstruction as detected by these assays are related to bone (type I collagen) turnover and not type II collagen. Potential sites that may produce elevated type I collagen concentrations are the remodeling of bone tunnels after ACL reconstruction surgery and the graft harvest site and/or remodeling of subchondral bone. Caution should be used when interpreting these and other biomarker assay results, as careful validation studies (to ensure the antibody used is sensitive and exclusive to its purported epitope) using advanced cell biology and biochemistry techniques such as tandem mass spectroscopy have not been performed on the vast majority of commercially available assay kits.

Our findings from the assessment of JSW after ACL injury and subsequent reconstruction[38] indicate that the lateral compartment of the ACL-injured knee is not “normal” soon after injury (at baseline assessment, the mean time between index injury and surgery date was 70.1 days; range, 18–155 days). This suggests that significant changes in the articular cartilage structure (and not bone) seem to occur in a subset of patients soon after injury. This supports the hypothesis that the index injury may have a dramatic effect on cartilage structure immediately after the injury, whether it potentially results in an increased or decreased tibiofemoral JSW. This may be further supported by the frequent observation of femoral and tibial bone marrow edema accompanying ACL ruptures as evaluated on MRI.[14,32] This may be biomechanically driven by the large compressive and shear stresses produced at the time of ACL disruption and biologically mediated as a function of chondrocyte turnover that has yet to be well described. This concept served as the motivation for the current investigation. In addition, a recent investigation by Tochigi et al[37] reported that a “wave” of chondrocyte death occurs after impact injury over a certain threshold in tibial plafond fractures. This “wave” continues along fracture lines (and radiates outward) over the initial 48 hours after impact.[23,37] It may be that ACL-reconstructed patients with a JSW difference that starts as “abnormal” at their baseline visit experience a greater magnitude of impact energy that is produced by their specific index injury event, and consequently, they experience a greater degree of chondrocyte death or loss of aggrecan.

Limitations of this study include different follow-up intervals for our control and ACL-reconstructed patients, and while the study was originally designed to match cases and controls on a one-to-one basis, because of logistical issues, we fell short of our original case-to-control matching goal. We do not believe these limitations contribute confounding and

bias into our study, however, as the JSW, biomarker concentrations, and all other outcomes data did not change significantly over the mean 3-year follow-up interval for the controls. We have no reason to believe that the data would be different if an additional (fourth) year of data collection was included for the controls. The use of a longitudinal cohort study may be viewed by some as a limitation of this investigation; however, it is likely the strongest design that is logistically possible given the fact that a prospective approach would require many thousands of people to be screened with the outcome measures described in this study before injury and then followed over time to generate a meaningful number of ACL-injured cases for statistical analyses. Additional limitations include the lack of evaluation of biomarkers specific to bone or synovium, as the first steps of our research efforts were focused on type II collagen. Analysis based on urine and serum samples can provide information regarding systemic changes as well as metabolism of all type II collagen throughout the body; it does not, however, provide joint-specific information as would be identified through the evaluation of synovial fluid samples. This was addressed by the inclusion of an age-, sex-, BMI-, and activity level–matched healthy control group with no history of significant joint injury, and this provided normative data for comparative purposes. All patients in the ACL group in this investigation underwent surgical reconstruction of their ACL, and as such, it is not possible to differentiate between the specific effects of the index injury or the subsequent surgical reconstruction on JSW difference at follow-up. In addition, our study was not designed to perform subgroup analyses of biomarker concentrations as they related to patients who had a significantly increased JSW difference[38] of their injured knee compared with patients who had a significantly decreased JSW difference at follow-up.

Although increased AP laxity was observed overall in patients in the ACL group (evaluated bilaterally and compared with controls), none of the participants included in these analyses demonstrated a positive pivot shift, reported symptoms of pivoting or giving way, or reported decreased activities or functional limitations with athletic endeavors at the final follow-up. As was previously stated, the goal of this study was to evaluate relationships between urine- and serum-based markers of type II collagen metabolism and abnormal JSW within the first few years after ACL injury and surgical reconstruction rather than to assess individual factors associated with surgical reconstruction.

Strengths of this study include the prospective evaluation of a healthy control group that was matched with the ACL-reconstructed group by sex, age, BMI, and activity level. In addition, we studied a homogeneous group of ACL-injured patients by excluding severe concomitant injuries that were associated with each patient’s index ACL injury (ie, no patients in the ACL group had cartilage injury with exposed bone, only 1 patient received a grade of 2/3 meniscectomy as a conservative measure, and no other primary ligament injuries were included).

This investigation was the first to examine changes occurring in a relatively short time period after an acute ACL injury and reconstruction in a prospective cohort that involved the evaluation of patient, functional, and clinical outcomes as well as serum and urine type II collagen biomarkers as they relate to tibiofemoral JSW difference changes soon after injury. This is important because these findings were observed before substantial changes could be observed with conventional radiography and traditional clinical grading scales and before

patients experience a decreased capacity to participate in functional activities. Intervention at this time point (before substantial irreversible articular cartilage loss and the clinical manifestation of OA occurs) may help decrease pain and restore quality of life and potentially prevent future changes leading to progressive posttraumatic OA. It is important to note that patients with an abnormal JSW difference compared with controls in this study may not continue to progress down a pathway of degenerative joint disease. The purpose of this study was to identify some of the earliest changes that may be involved in the posttraumatic OA process, and only additional follow-up and evaluation of this cohort will be able to characterize these patients as truly having progressive posttraumatic OA that results in end-stage disease.

In conclusion, this is the first study to demonstrate significant differences in patient-oriented outcomes (KOOS pain and quality of life) as well as biomarker cleavage-to-synthesis ratios (uCTX-II/sCPII) between healthy control participants and ACL-injured patients with an abnormal JSW. These differences were observed in a relatively short period of time (4 years after injury) and in patients who were fully functional, had no pivoting/giving-way episodes, had normal clinical examination findings, and had not decreased their activity levels.

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TABLE 1

Demographic Data (Mean Values)

Groups	Baseline Age, y	Baseline Body Mass Index	Preinjury Tegner Score	Follow-up Tegner Score
ACLR[a]	28.8	24.8	7.6	6.6
Control	26.8	24.1	6.2	5.8

[a]ACLR, anterior cruciate ligament reconstruction.

TABLE 2

Follow-up Interval Data for Anterior Cruciate Ligament Reconstruction Group

	n	Female, %	Time, Mean ± SD (Range), mo
Baseline	35	51	—
1-year follow-up	23	39	17 ± 2.6 (12–25)
4-year follow-up	35	51	46 ± 8.8 (32–61)

TABLE 3

Patient, Functional, and Clinical Outcomes[a]

Outcomes Assessment by Group[b]	n	Mean ± SD	*P* Value[c]	
			Control vs ACLR Group (Combined Normal and Abnormal JSW-D)	ACLR Subgroup Comparison (Normal vs Abnormal JSW-D)
IKDC subjective score			<.0001[c]	.11
Normal JSW-D	20	91 ± 9		
Abnormal JSW-D	10	86 ± 7		
Control	32	99 ± 2		
Lysholm score			<.0001[c]	.76
Normal JSW-D	20	90 ± 10		
Abnormal JSW-D	9	91 ± 5		
Control	31	98 ± 5		
KOOS: pain			<.0001[c]	.03[c]
Normal JSW-D	21	95 ± 9		
Abnormal JSW-D	10	89 ± 10		
Control	32	99 ± 2		
KOOS: quality of life			<.0001[c]	.03[c]
Normal JSW-D	21	88 ± 13		
Abnormal JSW-D	10	76 ± 16		
Control	32	99 ± 3		
KOOS: activities of daily living			.004[c]	.23
Normal JSW-D	21	98 ± 4		
Abnormal JSW-D	10	97 ± 3		
Control	32	100 ± 1		
KOOS: symptoms			<.0001[c]	.33
Normal JSW-D	21	88 ± 13		
Abnormal JSW-D	10	84 ± 13		
Control	32	98 ± 4		

Outcomes Assessment by Group[b]	n	Mean ± SD	*P* Value[c]	
			Control vs ACLR Group (Combined Normal and Abnormal JSW-D)	ACLR Subgroup Comparison (Normal vs Abnormal JSW-D)
KOOS: sports			<.0001[c]	.06
Normal JSW-D	21	93 ± 10		
Abnormal JSW-D	10	83 ± 15		
Control	32	100 ± 1		
Cincinnati knee score			<.0001[c]	.20
Normal JSW-D	21	93 ± 8		
Abnormal JSW-D	10	90 ± 6		
Control	32	99 ± 2		
Tegner activity score			.55	.20
Normal JSW-D	20	6.8 ± 1.7		
Abnormal JSW-D	9	5.8 ± 2.2		
Control	32	6.2 ± 1.4		
Marx activity score			.83	.14
Normal JSW-D	21	10.2 ± 4.5		
Abnormal JSW-D	10	7.5 ± 5.5		
Control	32	9.6 ± 4.1		
IKDC objective evaluation			<.0001[c]	.67
Normal JSW-D	24	3.0 ± 0.5		
Abnormal JSW-D	11	2.9 ± 0.7		
Control	31	3.8 ± 0.4		
KT-1000 arthrometer difference (between knees: normal minus ACLR), mm			<.0001[c]	.84
Normal JSW-D	24	−3.7 ± 3.2		
Abnormal JSW-D	11	−3.2 ± 2.2		
Control	32	−0.6 ± 1.8		
Single-legged hop test (maximum distance by injured leg), cm			.83	.83
Normal JSW-D	23	172 ± 37		
Abnormal JSW-D	11	172 ± 31		
Control	31	171 ± 31		

[a] ACLR, anterior cruciate ligament reconstruction; JSW, joint space width; IKDC, International Knee Documentation Committee; KOOS, Knee Injury and Osteoarthritis Outcome Score.

[b] Groups: normal JSW-D = ACL-injured patients with normal JSW difference; abnormal JSW-D = ACL-injured patients with abnormal JSW difference.

[c] $p < .05$.

TABLE 4

Urinary C2C/Serum CPII Ratios[a]

	n	Mean ± SD	Median (Minimum, Maximum)
Controls (baseline)	30	0.02 ± 0.02	0.01 (0, 0.07)
ACL group, 1-year follow-up[b]			
Abnormal JSW difference	6	0.02 ± 0.01	0.02 (0.01, 0.03)
Normal JSW difference	17	0.02 ± 0.01	0.02 (0.01, 0.04)
ACL group, 4-year follow-up[c]			
Abnormal JSW difference	11	0.02 ± 0.03	0.01 (0, 0.09)
Normal JSW difference	24	0.02 ± 0.01	0.02 (0, 0.05)

[a] C2C, collagen type II cleavage product; CPII, procollagen II C-propeptide; ACL, anterior cruciate ligament; JSW, joint space width; ANCOVA, analysis of covariance.

[b] 1-year follow-up group: ANCOVA, $P = .98$ (controls vs ACL– abnormal JSW vs ACL–normal JSW).

[c] 4-year follow-up group: ANCOVA, $P = .26$ (controls vs ACL– abnormal JSW vs ACL–normal JSW)

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TABLE 5

Urinary C1,2C/Serum CPII Ratios[a]

	n	Mean ± SD	Median (Minimum, Maximum)
Controls (baseline)	31	0.01 ± 0.01	0.01 (0, 0.06)
ACL group, 1-year follow-up[b]			
Abnormal JSW difference	6	0.04 ± 0.03	0.04 (0.01, 0.08)
Normal JSW difference	17	0.03 ± 0.02	0.03 (0.01, 0.07)
ACL group, 4-year follow-up[c]			
Abnormal JSW difference	11	0.04 ± 0.03	0.03 (0, 0.10)
Normal JSW difference	24	0.03 ± 0.02	0.02 (0, 0.10)

[a] C1,2C, collagen type I and II cleavage product; CPII, procolla-gen II C-propeptide; ACL, anterior cruciate ligament; JSW, joint space width; ANCOVA, analysis of covariance.

[b] 1-year follow-up group: ANCOVA, $P = .001$ (controls < both ACL groups). No significant difference between ACL groups.

[c] 4-year follow-up group: ANCOVA, $P = .001$ (controls < both ACL groups). No significant difference between ACL groups.

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TABLE 6

Urinary CTX-II/Serum CPII Ratios[a]

	n	Mean ± SD	Median (Minimum, Maximum)
Controls (baseline)	31	0.3 ± 0.4	0.2 (0.03, 2.2)
ACL group, 1-year follow-up[b]			
Abnormal JSW difference	6	0.7 ± 0.4	0.8 (0.2, 1.2)
Normal JSW difference	17	0.5 ± 0.3	0.3 (0.1, 1.0)
ACL group, 4-year follow-up[c]			
Abnormal JSW difference	11	0.5 ± 0.6	0.4 (0.1, 2.2)
Normal JSW difference	24	0.4 ± 0.6	0.2 (0.1, 2.3)

[a] CTX-II, C-terminal cross-linked telopeptide of type II collagen; CPII, procollagen II C-propeptide; ACL, anterior cruciate ligament; JSW, joint space width; ANCOVA, analysis of covariance.

[b] 1-year follow-up group: ANCOVA, $P < .001$ (controls < both ACL groups). No significant difference between ACL groups.

[c] 4-year follow-up group: ANCOVA, $P = .001$ (controls \ ACL reconstruction abnormal JSW difference). No significant difference between ACL groups.