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Changes in Serum Biomarkers of Cartilage Turnover After Anterior Cruciate Ligament Injury

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Background: Biomarkers of cartilage turnover and joint metabolism have a potential use in detecting early degenerative changes after a traumatic knee joint injury; however, no study has analyzed biomarkers before an anterior cruciate ligament (ACL) injury and again after injury or in comparison with a similar group of uninjured controls.

Hypothesis: Changes in serum biomarker levels and the ratio of cartilage degradation to synthesis, from baseline to follow-up, would be significantly different between ACL-injured patients and uninjured controls.

Study Design: Case-control study; Level of evidence, 3.

Methods: This case-control study was conducted to examine changes in serum biomarkers of cartilage turnover following ACL injury in a young athletic population. Specifically, 2 markers for type II collagen and aggrecan synthesis (CPII and CS846, respectively) and 2 markers of types I and II degradation and type II degradation only (C1,2C and C2C, respectively) were studied. Preinjury baseline serum samples and postinjury follow-up samples were obtained for 45 ACL-injured cases and 45 uninjured controls matched for sex, age, height, and weight.

Results: Results revealed significant decreases in C1,2C ($P = .042$) and C2C ($P = .006$) over time in the ACL-injured group when compared with the controls. The change in serum concentrations of CS846 from baseline to follow-up was also significantly different between the ACL-injured patients and uninjured controls ($P = .002$), as was the change between groups in the ratio of C2C:CPII over time ($P = .013$). No preinjury differences in the ratio of C1,2C:CPII or C2C:CPII were observed between groups; however, postinjury differences were observed for both ratios.

Conclusion: Changes in biomarker concentrations after an ACL injury suggest an alteration in cartilage turnover and joint metabolism in those sustaining ACL injuries compared with uninjured matched controls.

Keywords: cartilage turnover; cartilage metabolism; ACL injury; biomarkers; posttraumatic osteoarthritis

Acute traumatic knee joint injury is common in athletes[32] and physically active populations,[22,36] and traumatic knee joint injury has been identified as an unequivocal risk factor for subsequent osteoarthritis.[8,9,13,14,19,20,28] Cross-sectional and longitudinal studies have demonstrated a strong association between traumatic knee joint injuries and posttraumatic osteoarthritis (PTOA), and the annual health care costs associated with PTOA are estimated to be nearly $12 billion.[7] Participants in the Clearwater Osteoarthritis Study with a history of severe knee joint injury at baseline were 7.4 times more likely to have incident radiographic osteoarthritis at follow-up when compared with those with no history of injury.[46] Other studies have reported that subjects with a history of knee injury were more than 5 times more likely to have osteoarthritis when compared with those with no history of injury.[16,44] In a study of 67 young female soccer players, 51% had radiographic evidence of knee osteoarthritis only 12 years after anterior cruciate ligament (ACL) injury.[30] The

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mean age at injury was 19 years in this population, and radiographic osteoarthritis was present in half of this group soon after they turned 30 years old. Despite the strong association between joint trauma and the development of PTOA, early biological changes in cartilage turnover and metabolism after acute knee joint injuries remain poorly understood.[11]

Recently, the osteoarthritis disease process has been characterized as a continuum beginning with an inciting event, such as a traumatic knee joint injury, and progressing through molecular, preradiographic, and radiographic stages, resulting in end-stage disease.[24,25] Emerging evidence suggests that biochemical biomarkers of cartilage turnover and metabolism offer potential utility in assessing early molecular changes in cartilage turnover and metabolism after traumatic knee joint injury.[11,27,31] It has also been suggested that emerging biomarkers may be useful in formulating early diagnoses, assessing disease prognosis, monitoring disease progression and burden, and assessing the efficacy of interventions.[3,35] Regardless of recent advances in the development of biomarkers of cartilage turnover and metabolism, most biomarkers are still at the exploration and demonstration phases of clinical qualification.[26] One of the critical needs in the further development of biomarkers is studying the effects of joint-specific contributions such as traumatic injuries to systemic concentrations in existing biomarkers.[26] In fact, the Arthritis Foundation[2] has recently made it a funding priority to assess the cartilage matrix of knees with acute ACL injury using biochemical measures.

Current practice relies mainly on standard radiographs to diagnose PTOA after acute traumatic knee joint injury; however, radiographs are not sensitive to early degenerative joint changes, and treatment options are often limited by the time advanced PTOA is evident on traditional radiological studies. As a result, our ability to prevent and treat PTOA after knee joint injury is severely limited by our capacity to detect this debilitating condition earlier in its clinical course. Magnetic resonance imaging (MRI) is more sensitive than radiographs,[12] but this is expensive and often unavailable. The advantages of most emerging biomarkers of cartilage turnover and metabolism are that they can be measured in urine and/or serum, they may be less costly than other methods (eg, MRI), and they show potential as being sensitive to early changes in disease.[26] Biomarkers provide the only current potential means of identifying the early molecular stages of osteoarthritis after an inciting event that precedes structural changes.[26] While several recent review articles discuss the potential role of biomarkers in osteoarthritis[5,35,45] and assess changes in cartilage turnover and metabolism after joint injury,[27,31] a recent report provides evidence-based guidelines for the study of emerging biomarkers related to cartilage turnover, joint metabolism, and osteoarthritis in vivo.[26] Despite the potential importance of these emerging biomarkers, little is known about how these markers are affected by acute traumatic knee joint injury, particularly in comparison with the preinjury baseline state. Furthermore, no study has analyzed biochemical biomarkers before ACL injury and again after injury and reconstruction in the same population or a similar group of uninjured controls.

The objective of the current study was to address this existing gap in the research literature related to the effect of ACL injury and reconstruction with respect to specific serum biomarkers of cartilage turnover and metabolism. We looked for possible changes in serum biomarker levels by comparing baseline samples taken before injury with samples taken after ACL injury and reconstruction. This study also examined changes in serum biomarker concentration levels in an uninjured control group matched for sex, age, height, and weight. Our a priori research hypothesis was that the change in serum biomarker levels, and the ratio of cartilage degradation to synthesis, from baseline to follow-up would be significantly different between the ACL-injured cases and the uninjured control subjects.

MATERIALS AND METHODS

Design and Setting

A case-control study was conducted to examine changes in serum biomarkers of cartilage turnover after an ACL injury. The independent variables of interest were time and group. Time was a within-subjects variable consisting of 2 levels: baseline before ACL injury and follow-up after ACL injury. Group was a between-subjects variable and also included 2 levels: those who sustained an ACL injury during follow-up and an uninjured control group matched for sex, age, height, and weight. The dependent variables for this study included the serum concentration of 4 commercially available biomarkers of cartilage turnover. Specifically, 2 markers for type II collagen and aggrecan synthesis (procollagen II carboxy propeptide [CPII] and aggrecan chondroitin sulfate 846 epitope [CS846], respectively) and 2 markers of type I and II degradation and type II degradation only (Col2 3/4 short assay and collagen type II cleavage [C2C], respectively) were studied. All subjects were cadets or active-duty military personnel at the United States Military Academy (USMA) at West Point, New York, and all had similar physical activity requirements during the study period.[32] Based on our a priori sample size calculations, assuming an α level of $P < .05$, power of .80, and a 12% difference in the change in C2C concentration between groups, a minimum of 30 subjects per group was required for the current study. This study was reviewed and approved by the institutional review board at Keller Army Hospital (West Point, New York) with a secondary review by the United States Army Clinical Investigation Regulatory Office (Fort Sam Houston, Houston, Texas).

Case Subjects

The ACL-injured cases for this study were 45 of 71 subjects from the randomized and nonrandomized arms of an existing clinical trial comparing outcomes for ACL reconstructions performed with 2 different autograft techniques (patellar tendon vs hamstring tendon).[43] All subjects had confirmed preinjury and postinjury serum samples stored in the Department of Defense Serum Repository (DODSR). Subjects were eligible for inclusion in the clinical trial if

they were between the ages of 17 and 45 years and had (1) a history of traumatic knee joint injuries, (2) symptoms and physical examination findings consistent with the diagnosis of an ACL rupture, and (3) MRI findings indicating an ACL rupture.[43] In all subjects, the knee was evaluated arthroscopically to verify the diagnosis of an ACL rupture and any concomitant intra-articular injuries to determine eligibility based on the inclusion and exclusion criteria.[43] Potential subjects were excluded if they (1) had previously undergone ACL reconstruction in either knee, (2) sustained multiple knee ligament injuries that required concomitant surgical repair, (3) had a full-thickness chondral lesion, or (4) had a history of major joint injuries or surgical interventions before arrival at our institution or other major joint injuries during their 4 years at the USMA.[43]

Control Subjects

A matched control group of 45 healthy subjects with serum samples drawn upon entry to (baseline) and graduation (follow-up) from the USMA, following the same timeline as the ACL-injured patients with whom they were paired, was also studied. The 45 subjects in the matched control group (1) had no history of major joint injuries or surgical interventions before arrival at our institution or during their 4 years at the USMA; (2) met matching criteria for age, sex, height, and weight with a subject in the ACL-injured group; and (3) had serum samples in the DODSR for entry and graduation from the USMA. The control subjects were drawn from a control pool of 710 potential subjects consisting of 10 possible subjects who matched each ACL-injured study subject. The criteria used to match the cases with controls were as follows: same sex, age ± 2 years, height ± 2 inches, and weight ± 15 lb. Identification of the 10 potential control subjects for each of the 45 ACL-injured subjects was performed by the Office of Institutional Research at the USMA.

After the identification of potential control subjects by the Office of Institutional Research, each potential control subject was screened for a history of major joint injuries or surgeries during their 4 years at the USMA. This was possible because all of the potential control subjects received their health care through a closed health care system during the 4 years between the collection of baseline and follow-up serum samples. We reviewed injury surveillance records using the Cadet Injury and Illness Tracking System (CIITS), electronic medical records using the Armed Forces Health Longitudinal Technology Application (AHLTA), and surgical records using the Surgery Scheduling System (S3) for each potential control subject. Potential control subjects were excluded if they had a history of major joint injury or surgery during the 4 years between baseline and follow-up serum samples. After screening for a history of major joint injury or surgery, the list of remaining eligible control subjects was forwarded to the DODSR. The control subject chosen for each ACL-injured study subject was chosen by the DODSR from among the potential controls who met the inclusion criteria based on the availability of entry and graduation serum samples in the DODSR. The research team was blinded to the identity of the control subjects ultimately selected by the DODSR.

Outcome Measures

Sera were analyzed using 4 specific enzyme-linked immunosorbent assay (ELISA) tests of cartilage turnover: 2 measuring degradation (C2C and C1,2C) and 2 measuring synthesis (CS846 and CPII). The assays for CPII, CS846, C1,2C, and C2C are commercially available in 96-well ELISA kits using precoated plates from IBEX (Montreal, Quebec, Canada). These biomarkers of type II collagen and aggrecan metabolism were selected for this initial study because type II collagen and aggrecan are the most abundant proteins in articular cartilage. Furthermore, the biomarkers selected were identified as having sufficient evidence to justify further study in vivo based on the recommendations of a recent study commissioned by the Food and Drug Administration.[26] These biomarkers have been qualified for various osteoarthritis outcomes based on the BIPED classification of osteoarthritis biomarkers.[3] Levels of CPII have been found to directly correlate with the synthesis of type II collagen.[33] Also, CS846 is most concentrated in human fetal cartilage and is almost absent in adult articular cartilage.[17] It reappears in osteoarthritic cartilage, being present on the largest, most intact molecules.[40] Furthermore, CS846 increases markedly in content in joints after an injury.[29] With regard to collagen degradation, the cleavage of type II collagen by collagenases produces a specific neoepitope at the carboxy-terminus of the three quarters–length type II collagen cleavage product. A specific assay for C2C has been developed and can be used in both serum and synovial fluid.[38] Cleavage of type I and II collagen by collagenases can also be detected in a similar fashion using the C1,2C or Col2 3/4 short assay.[4]

Specimen Acquisition and Testing

Preinjury and postinjury serum samples for all cases and controls were obtained from the DODSR.[1,41] This repository is a secure facility jointly maintained by the Armed Forces Health Surveillance Center and the United States Army Public Health Command. The DODSR maintains sera drawn from service members for routine administrative testing and currently stores over 50 million specimens. The repository stores sera from all cadets entering the USMA at 2 time points: (1) entry into the academy and (2) graduation from the academy (~4 years later). The interval for testing is typically every 2 years once in the regular military as well as upon redeployment from overseas assignments. Serum, if available, is released in 0.5-mL volumes except when only the last 0.5 mL remains in the DODSR.

Serum samples for cases and controls were routinely drawn first thing in the morning, allowed to clot at room temperature for 30 to 60 minutes, centrifuged at approximately 1300*g*, aliquoted into separate microcryotubes, and stored at $-80°C$ until subsequent analyses. All subjects were healthy, were free of any history of major joint injuries, and met the medical and physical induction standards for military service at the time that the preinjury baseline sample was obtained. At the time of the follow-up sample, all control subjects had remained free of any major joint injuries, and the ACL-injured cases

TABLE 1
Within-Group Comparisons (Preinjury Baseline to Postinjury Follow-up) for Change in Serum Biomarker Concentrations of Cartilage Turnover and Metabolism Over Time[a]

	ACL-Injured Cases					Uninjured Matched Controls[b]				
	Preinjury (Baseline)		Postinjury (Follow-up)			Preinjury (Baseline)		Postinjury (Follow-up)		
	Median	IQR	Median	IQR	*P* Value[c]	Median	IQR	Median	IQR	*P* Value[c]
Measures of degradation										
C1,2C	10.97	1.32	9.96	1.40	<.001	9.78	1.47	9.52	1.10	.019
C2C	10.31	1.89	8.98	1.89	<.001	9.34	1.29	8.81	0.90	.059
Measures of synthesis										
CPII	11.09	1.25	10.41	1.44	.002	9.74	1.51	8.94	1.39	.005
CS846	24.53	2.20	24.76	1.91	.509	25.16	1.28	24.68	1.64	.001

[a]IQR, interquartile range.
[b]Matched for age, sex, height, and weight.
[c]Matched pairs (n = 45).

had remained free of other major joint injuries with the exception of the incident ACL injury sustained during the follow-up period. All assays for each individual subject were performed at the same time to minimize the potential effect of multiple freeze/thaw cycles. Each sample was assayed in triplicate for the 4 biomarkers of cartilage turnover using commercially available precoated ELISA kits (IBEX), according to manufacturer guidelines. A Wallac-Victor 1420 (PerkinElmer Life Science, Boston, Massachusetts) multilabel plate reader was used to detect serum absorbances for each cartilage turnover marker at an optical density of 450 nm. Serum concentrations were determined against the known standard curve provided by the manufacturer by utilizing Workout 2.5 software (Dazdaq Solutions Ltd, Brighton, United Kingdom). This process was performed by the same member of the research team, who was blinded to the case-control status of each subject. The ELISA kits for each biomarker were from the same respective lot numbers to further minimize interassay coefficients of variation (CVs). In the current study, intra-assay CVs ranged from 2% to 4%; interassay CVs ranged from 3% to 9%.

Data Analysis

Descriptive statistics were presented using median and interquartile range for each group because not all biomarkers were found to be approximately normally distributed. The Wilcoxon matched-pairs signed-rank test, a nonparametric method analogous to the paired *t* test for normally distributed data, was used to evaluate within-group differences from preinjury baseline to postinjury follow-up. Because controls were matched to each ACL-injured case, statistical comparisons between groups (ACL injury vs control) were also calculated using the Wilcoxon matched-pairs signed-rank test. For comparison of biomarker changes from preinjury baseline to postinjury follow-up between the 2 groups, the individual differences were first taken (postinjury − preinjury concentration) within each subject, and then these differences were compared between the 2 groups using the same method discussed above. All statistical analyses were completed using STATA/SE software version 10.1 (StataCorp, College Station, Texas), and comparisons used an α level of $P < .05$.

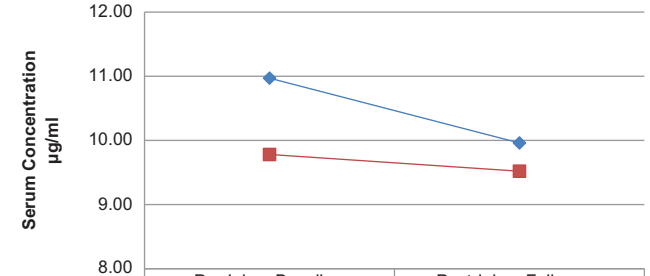
RESULTS

Sample Characteristics

Of the 45 ACL-injured cases, 39 (86.7%) were male, and the remaining 6 (13.3%) were female. Among the male ACL-injured cases, the mean age was 20.26 ± 1.29 years, the mean height was 179.76 ± 7.37 cm, and the mean weight was 83.99 ± 12.09 kg. The ACL-injured female patients had a mean age, height, and weight of 19.33 ± 1.03 years, 172.72 ± 2.78 cm, and 68.33 ± 6.61 kg, respectively. The mean time from the baseline preinjury serum sample until the ACL injury was 621 ± 377 days or approximately 21 months, and the mean time from the ACL injury until the follow-up sample was 713 ± 393 days or approximately 24 months. The mean time from the baseline sample until the follow-up sample in the ACL-injured cases was 1334 ± 104 days or approximately 44 months. Based on the matching criteria, the ACL-injured cases and uninjured controls were matched for the same sex, age ±2 years, height ±2 inches, and weight ±15 lb. Baseline and follow-up samples were also obtained for the uninjured control group, and the mean time from the baseline sample until the follow-up sample in the control group was 1421 ± 100 days or approximately 47 months.

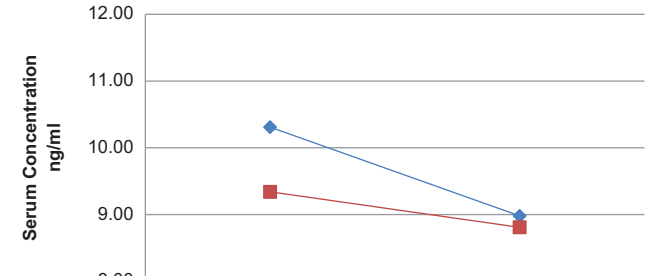
Change in Biomarker Concentrations Over Time

Within Groups. Significant within-group differences were observed in both the ACL-injured cases and the uninjured matched controls when preinjury baseline serum biomarker concentrations were compared with postinjury follow-up concentrations (Table 1). Serum concentrations were significantly lower at the time of postinjury follow-



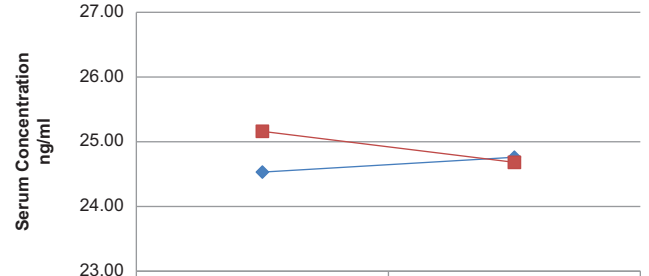
	Pre-Injury Baseline	Post-Injury Follow-up
ACL Injured	10.97	9.96
Control	9.78	9.52

Figure 1. Change in median serum concentration of C1,2C over time for ACL-injured cases and uninjured controls matched for sex, age, height, and weight.



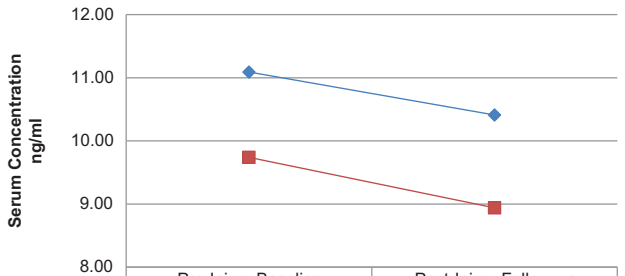
	Pre-Injury Baseline	Post-Injury Follow-up
ACL Injured	10.31	8.98
Control	9.34	8.81

Figure 2. Change in median serum concentration of C2C over time for ACL-injured cases and uninjured controls matched for sex, age, height, and weight.



	Pre-Injury Baseline	Post-Injury Follow-up
ACL Injured	24.53	24.76
Control	25.16	24.68

Figure 3. Change in median serum concentration of CS846 over time for ACL-injured cases and uninjured controls matched for sex, age, height, and weight.



	Pre-Injury Baseline	Post-Injury Follow-up
ACL Injured	11.09	10.41
Control	9.74	8.94

Figure 4. Change in median serum concentration of CPII over time for ACL-injured cases and uninjured controls matched for sex, age, height, and weight.

up in the ACL-injured cases for both biomarkers of degradation (C1,2C and C2C) and for CPII when compared with preinjury baseline concentrations. There was a slight increase in the concentration of CS846 at the time of postinjury follow-up in the ACL-injured cases, but the difference in comparison with preinjury baseline concentrations was not statistically significant. Postinjury follow-up concentrations were lower for all biomarkers studied in the uninjured matched controls when compared with baseline preinjury levels, and all differences were statistically significant with the exception of C2C, which approached significance.

Between Groups. Both serum biomarkers of type II collagen degradation decreased at a significantly greater rate from baseline to follow-up in the ACL-injured cases when compared with the uninjured control subjects (Table 2). The rate of decline in serum concentrations of C1,2C, from the preinjury baseline sample to the postinjury follow-up sample, was significantly greater in the ACL-injured cases when compared with the uninjured controls (Figure 1). Similar and more significant changes were observed between cases and controls for serum concentrations of C2C (Figure 2) probably because of the greater specificity of the C2C assay for type II collagen degradation. The change in serum concentrations of cartilage proteoglycan-specific CS846 from baseline to follow-up was also significantly different between the ACL-injured cases and uninjured controls. After ACL injuries, the serum concentrations of CS846 increased; however, in the uninjured controls, this marker of aggrecan synthesis decreased from baseline to follow-up (Figure 3). In contrast, the change in CPII (a marker of type II collagen synthesis) from preinjury baseline to postinjury follow-up was similar between ACL-injured cases and uninjured controls (Figure 4).

The change in the ratio of serum biomarker concentrations of type II collagen degradation to synthesis was also significantly different over time between the ACL-injured cases and uninjured controls (Table 3). Specifically, the change in the C2C:CPII ratio was significantly different from the preinjury baseline sample to the postinjury follow-up sample between the ACL-injured cases and the uninjured controls. Similar results were observed for the C1,2C:CPII ratio, which approached significance (P = .077). We observed no significant differences in the C1,2C:CPII ratio or the C2C:CPII ratio at baseline before the ACL injury between the ACL-injured cases and the matched controls; however, there were significant differences between the ACL-injured cases and uninjured controls

TABLE 2
Between-Group Comparisons (Preinjury Baseline to Postinjury Follow-up) for Change in Serum Biomarker Concentrations of Cartilage Turnover Over Time (Postinjury – Preinjury)[a]

	ACL Injury (n = 45)	Control (n = 45)	Matched-Pairs Difference: *P* Value
Measures of degradation			
C1,2C	–0.91 (2.45)	–0.38 (1.21)	.042
C2C	–1.02 (1.37)	–0.54 (1.53)	.006
Measures of synthesis			
CPII	–0.64 (1.34)	–0.79 (2.01)	.765
CS846	0.10 (2.05)	–0.52 (1.41)	.002

[a]Values are expressed as median (interquartile range).

TABLE 3
Between-Group Comparisons for Serum Biomarker Concentration Ratios in Preinjury Baseline Levels (Pre), Postinjury Follow-up Levels (Post), and Change in Marker Levels Over Time (Post – Pre)[a]

	ACL Injury (n = 45)	Control (n = 45)	Matched-Pairs Difference: *P* Value
C1,C2:CPII[b]			
Pre	1.00 (0.13)	1.07 (0.29)	.126
Post	0.96 (0.16)	1.07 (0.12)	<.001
Post – pre	–0.04 (0.20)	–0.01 (0.31)	.077
C2C:CPII[b]			
Pre	0.92 (0.10)	0.94 (0.15)	.153
Post	0.86 (0.12)	0.98 (0.12)	<.001
Post – pre	–0.06 (0.14)	0.04 (0.20)	.013

[a]Values are expressed as median (interquartile range).
[b]Ratio of degradation to synthesis in serum biomarkers of cartilage turnover.

for both ratios when the postinjury follow-up samples were examined (Table 3). Both ratios decreased in the ACL-injured cases but either remained unchanged (C1,2C:CPII) or slightly increased (C2C:CPII) in the controls at the time of postinjury follow-up.

DISCUSSION

We utilized existing preinjury baseline and postinjury follow-up serum samples stored in the DODSR to examine changes in biomarker concentrations of cartilage turnover and metabolism over time between ACL-injured cases and uninjured controls matched for sex, age, height, and weight. This is the first study, to our knowledge, to examine changes in serum biomarkers of cartilage turnover and metabolism from the preinjury baseline state to postinjury follow-up in a young and active population at high risk for traumatic knee joint injuries.[32] We observed that the change in individual biomarker concentrations over time was significantly different between the ACL-injured cases and uninjured controls for all of the biomarkers studied, with the exception of CPII, which is a marker of type II collagen synthesis. We also observed that the change in the ratio of type II collagen degradation to synthesis over time was significantly different between the ACL-injured cases and uninjured controls. These findings suggest that a traumatic ACL injury in the knee leads to changes in cartilage turnover and metabolism that can be detected by serum biomarkers. Furthermore, the change in serum biomarker levels observed in the present study after an ACL injury appears to indicate an alteration in joint metabolism at the molecular level.[24,25]

An imbalance in type II collagen degradation and synthesis is believed to be a critical component in the development of osteoarthritis.[18] As a result, the simultaneous assessment of cartilage degradation and synthesis in assessing cartilage turnover and joint metabolism has been advocated as a promising approach.[5,10,12,15,21] In the current study, we observed no significant differences in the preinjury baseline C2C:CPII ratio or the C1,2C:CPII ratio between ACL-injured cases and uninjured controls, despite observed baseline differences in the individual markers. However, these ratios were significantly different between cases and controls at postinjury follow-up. Furthermore, the change in the C2C:CPII ratio over time was significantly different between the ACL-injured cases and uninjured controls and approached significance for the C1,2C:CPII ratio. This is likely because the C2C assay has greater specificity for type II collagen found in articular cartilage.[38] These findings suggest that there was a balance in cartilage degradation and synthesis at baseline before the ACL injury in both groups; however, after ACL injury, an apparent loss of cartilage homeostasis emerged. Cahue et al[10] reported similar findings for the C2C:CPII ratio and the C1,2C:CPII ratio. Specifically, they noted that the C2C:CPII ratio and the C1,2C:CPII ratio were associated with the odds of joint space grade progression for osteoarthritis in the knee, and they also reported similar findings for worsening Kellgren-Lawrence grades.[10] However, in contrast with the current study, the patient population studied was much older (mean age, 69 years) and already had significant signs of knee osteoarthritis at baseline.[10]

In contrast to previous studies related to osteoarthritis,[5] the imbalance in the ratio of cartilage degradation to synthesis in the current study appears to be caused by initial decreases in serum concentrations of type II collagen degradation relative to synthesis after acute traumatic joint injuries. Consistent with the findings for the ratio of cartilage degradation to synthesis reported above, we observed that serum biomarkers of type I and type II collagen degradation (C2C and C1,2C) decreased at a significantly greater rate over time in the ACL-injured cases when compared with controls; however, the decline in type II collagen synthesis (CPII) was comparable between the groups. In a recent study, Catterall et al[11] reported decreases in serum concentrations of CPII, C2C, and C1,2C from the

time of an acute incident ACL injury to follow-up approximately 50 days later; however, these decreases were not statistically significant, which may be because of the small sample size or short duration of follow-up compared with the current study. More importantly, they reported a moderate and statistically significant inverse relationship between serum and synovial fluid concentrations for C2C, suggesting that higher concentrations of C2C in the target joint were moderately associated with lower concentrations of C2C in serum.[11] While not statistically significant, similar observations were noted for C1,2C and CPII in which concentrations increased from the time of ACL injury to follow-up in synovial fluid but decreased from the time of injury to follow-up in serum. The decreases in serum concentrations of C1,2C and C2C after ACL injuries relative to uninjured controls observed in the current study may be caused by increased concentrations in the synovial fluid of the target joint; however, further study is needed to confirm the relationship between serum and synovial fluid concentrations for these biomarkers relative to ACL injury.

Previous studies have suggested that imbalances in cartilage degradation and synthesis in osteoarthritis progression are likely because of suppressed collagen synthesis rather than increases in degradation.[10] Furthermore, studies in humans and in bovine cartilage suggest that type II procollagen synthesis decreases significantly from childhood into adulthood,[33,34] and significant reductions in CPII have been observed in patients with osteoarthritis when compared with healthy controls.[33] While we observed baseline and follow-up differences between the ACL-injured cases and controls in the current study, the change in type II collagen synthesis (CPII) over time did not differentiate those who were injured during the follow-up period from those who were not, whereas type I and type II collagen degradation (C1,2C and C2C, respectively) did. Further study is needed to elucidate how traumatic joint injuries affect cartilage turnover and metabolism and how these changes are similar and distinct from observations in older patients with advanced osteoarthritis.

The change in serum levels of CS846 over time was significantly different between ACL-injured cases and uninjured controls in the current study, with levels increasing in the ACL-injured cases and decreasing in uninjured controls. Available data suggest that the proteoglycan-specific CS846 is likely a marker of aggrecan synthesis.[37,39] Previous studies have reported that CS846 is almost absent from mature adult cartilage; however, this epitope is generally present in increased content in cases of osteoarthritis[17,39,40] and after joint injuries.[29] Poole et al[39] previously reported that serum levels of CS846 were slightly elevated in patients with osteoarthritis when compared with healthy control subjects; however, their analysis was cross-sectional in nature and did not evaluate changes in CS846 over time. Nevertheless, the postinjury change in CS846 between the ACL-injured cases and uninjured controls in the present study is comparable with that observed between patients with osteoarthritis and healthy controls by Poole et al.[39] Furthermore, the apparent group-by-time interaction for CS846 provides preliminary evidence to suggest that CS846 may be specific to posttraumatic alterations in cartilage turnover and joint metabolism. Prior studies suggest that the level of CS846 in adult serum is normally very low but increases and remains elevated with osteoarthritis, making CS846 specific to degenerative joint changes.[39]

Although we did not observe any preinjury baseline differences in the ratio of collagen degradation to synthesis between the ACL-injured cases and the uninjured matched controls in the current study, we unexpectedly observed preinjury baseline differences between the ACL-injured cases and uninjured controls for all biomarkers of cartilage turnover and metabolism studied. Between-group differences in the concentration of individual biomarkers before injury may be less important in measuring posttraumatic changes in cartilage turnover and joint metabolism than changes over time or the ratio between markers of degradation to synthesis.[10-12,15,21,37] However, these observations raise questions about the association between preinjury baseline concentrations for serum biomarkers of cartilage turnover and the subsequent risk of ACL injuries. Preinjury differences may be caused by different biomechanical risk profiles between those who go on to subsequently tear their ACL and those who do not[6]; however, this hypothesis requires further examination, and further study is needed to expand and confirm these preliminary observations.

Strengths and Limitations

As with any study, the current investigation has limitations that should be considered when interpreting the findings. While samples were available before ACL injury and after ACL injury and reconstruction within a fairly large cohort of patients and matched controls, these samples were not all drawn at standardized intervals in relation to the incident injury. Serum samples within the cohort were drawn at standardized intervals upon entry to the USMA or active-duty military service and immediately before graduation. Therefore, the time between each sample and ACL injury was variable. Serum samples were drawn shortly after rising during morning sick call, which should have mitigated the effect of diurnal variation; however, it is still possible that diurnal variation may have affected the results of the current study. Kong et al[23] reported that no diurnal variation was observed in serum levels of C1,2C, C2C, or CS846; however, serum levels of CPII were elevated 4 hours after rising in older patients (mean age, 70 years; range, 59-91 years) with radiographic osteoarthritis. Serum levels of CPII were not elevated 1 hour after rising or between 6 PM and 8 PM relative to concentrations taken at 8 AM.[23] As a result, the effect of any diurnal variation in the current study was likely limited because samples were taken shortly after rising. Although the ACL-injured cases and uninjured controls were matched on age, sex, height, and weight, an important limitation of this study is that information on activity level before collection of the preinjury baseline samples was not available; however, between preinjury baseline and postinjury follow-up, the activity levels were comparable

for ACL-injured cases and controls, as all subjects had the same physical requirements while at our institution. Furthermore, while we used relatively narrow matching criteria for age, height, and weight, the potential for residual confounding due to the range of values within the matching categories exists.[42] Similarly, although we matched on important confounding variables,[26] because of the case-control study design, the potential for selection bias remains. While serum biomarkers are the least invasive and most risk-free method of evaluating cartilage turnover and metabolism, the specific levels of these biomarkers for various pathological processes have not been defined for all types of diseases, particularly in young and active patient populations. Finally, a primary disadvantage of serum biomarkers of cartilage turnover and metabolism is their lack of specificity for a particular joint. Therefore, although we controlled for prior injuries, it is possible that differences in general cartilage metabolism, from structures unrelated to the knee joint, may have been responsible for the observed differences in the current study.

Despite the noted limitations with the current study, the DODSR provided a unique opportunity to evaluate changes in serum biomarkers of cartilage turnover and metabolism from the preinjury baseline state to postinjury follow-up in a relatively large sample of ACL-injured cases and uninjured controls matched for potential confounding variables including sex, age, height, weight, body mass index, and activity level.[26] Controlling for the total body burden of disease has been a limitation of most previous biomarker studies[26] that we were able to overcome in the current study, which is particularly important when assessing serum biomarker levels. Because all subjects were medically screened to meet entry level standards for military service and subsequently received their medical care through a closed health care system, we were able to be relatively certain that the ACL-injured cases and matched controls were free from a history of ACL injuries and other significant joint injuries at baseline and that the control group remained free of major joint injuries during their 4 years at the USMA between serum samples. As a result, we were able to account for the total body burden of disease at baseline and during the follow-up period. Because all subjects had the same physical activity requirements during the 4 years between the baseline preinjury sample and postinjury follow-up sample, we are also relatively certain that differences in activity level did not influence our results. Finally, regardless of the case-control study design, the temporal relationship between the preinjury serum samples contained within the DODSR, subsequent ACL injury, and postinjury follow-up samples was maintained for both ACL-injured cases and control subjects. Therefore, the results of the current study provide insight into the temporal relationship between acute traumatic ACL injuries in the knee and serum biomarker concentrations of cartilage turnover and metabolism.

CONCLUSION

We observed no preinjury baseline differences but significant postinjury follow-up differences between ACL-injured cases and matched controls for the ratio of type II collagen degradation to synthesis in the current study. These findings suggest that there was a balance in cartilage degradation and synthesis at baseline before ACL injury in both groups; however, after ACL injury, an apparent loss of cartilage homeostasis emerged, which may be an early indication of posttraumatic changes in cartilage turnover and metabolism and possibly the initiation of PTOA. Significant changes in individual markers of type I/II and type II collagen degradation (C1,2C and C2C, respectively) and aggrecan synthesis (CS846) between the ACL-injured cases and matched controls further suggest that an ACL injury leads to changes in cartilage turnover and metabolism that can be detected in serum. Further study is needed to prospectively confirm these results based on serum samples taken at the time of the incident ACL injury and at systematic follow-up points after the injury in cases and controls. Prospective clinical studies correlating serum biomarker concentrations with clinical outcomes will also be needed to determine the clinical relevance of the observed differences between the ACL-injured cases and uninjured controls.

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