Osteoarthritis and Cartilage 27 (2019) 286–293

Osteoarthritis and Cartilage

Serum cartilage oligomeric matrix protein (COMP) expression in individuals who sustained a youth sport-related intra-articular knee injury 3–10 years previously and uninjured matched controls

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ARTICLE INFO

Article history:
Received 16 March 2018
Accepted 26 September 2018

Keywords:
Cartilage oligomeric matrix protein
Youth knee injury
Fragmentation

SUMMARY

Objective: This study investigates the relationship between a youth sport-related intra-articular knee injury and cartilage oligomeric matrix protein (COMP), a biomarker of cartilage turnover.
Design: Participants included a sub-sample (n = 170) of the Alberta Youth Prevention of Early Osteoarthritis (PrE-OA) study group. Specifically, 85 individuals with a 3–10 year history of sport-related intra-articular knee injury and 85 age, sex and sport-matched controls. COMP levels were investigated in serum. Between group differences in COMP levels, COMP fragmentation patterns and, the relationship between serum COMP and clinical outcomes (i.e., Magnetic Resonance Imaging (MRI) Osteoarthritis Knee Score; MOAKS, Knee Osteoarthritis Outcome Score; KOOS, Fat mass index; FMI) were examined.
Results: Participant median age was 22.3 years (range 16–26) and 63% were female. Although there was no difference in COMP levels between previously injured and uninjured females, previously injured males demonstrated an ~15% greater (171.5 ng/ml, 95% CI 11.0–428.0, $P = 0.04$) serum COMP level than uninjured males. However after controlling for FMI, this difference was absent. Within the injured participants, COMP levels were associated with $MOAKS_{SYNOVITIS}$ and FMI. Furthermore, COMP fragmentation patterns were distinct between injured and uninjured individuals.
Conclusions: In this study group, serum COMP levels were greater in injured males, but not females, compared to matched controls. However, after controlling for FMI, no differences in COMP were observed. A unique COMP fragmentation pattern was observed in injured vs uninjured participants. These results further the hypothesis that COMP levels and/or degradation of the protein may be a marker of cartilage injury which could predispose to later OA.

Introduction

Osteoarthritis (OA) is a pathological degenerative joint condition defined by the presence of pain, reduced mobility, and radiographic changes including cartilage loss, joint space narrowing, and osteophytes[1]. While OA typically develops in older populations, the incidence of post-traumatic osteoarthritis (PTOA) is significant in

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https://doi.org/10.1016/j.joca.2018.09.011
1063-4584/

young to middle-aged adults 12–20 years post injury[2]. Knee injury is a common sport-related injuries in youth and PTOA is most commonly described in the knee joint[3]. In Canada, sport participation is the leading cause of injury in youth, with knee and ankle injuries accounting for over 40% of the burden[4]. Furthermore, studies indicate a 10-fold increased risk of knee OA in the 12–20 years post injury[4]. Overall, it is estimated that over 50 percent of individuals with an anterior cruciate ligament (ACL) tear or meniscal injury will develop knee OA[5]; making these high risk individuals a key demographic to focus on for both research endeavors and novel secondary prevention approaches to reduce the risk, or slow the progression of OA.

While biomarkers have been used to identify and assess presence and progression of a variety of diseases; to date, no single biomarker has been found that predicts the presence of OA. Furthermore, no biomarker with clinically relevant (e.g., approved diagnostic test) prognostic value for the diagnosis of OA prior to disease onset has been identified. There is evidence that suggests that cartilage oligomeric matrix protein (COMP) may have prognostic value within the progression of osteoarthritis[6–9]. COMP is a structural protein integral to proper articular cartilage function and increasing levels of COMP have been correlated to subsequent radiographic degradation of articular surfaces in the knee[6,7,9,10]. Many have suggested that COMP, specifically that found in the serum (sCOMP) and/or urine, may prove to be of prognostic value in regards to early OA diagnosis[9], and some have demonstrated the prognostic potential of COMP in patients that go onto develop OA[11,12].

To date, only a very few studies have investigated COMP in healthy young adults (16–26 years)[13] or specifically to defined/characterized PTOA. Given the potential utility of COMP as a diagnostic and prognostic indicator of OA development, there is a notable age gap in published literature concerning COMP and early OA development. Studies tend to focus on pediatric (under 18 years) and older (45+ years) populations.

The association of serum COMP (sCOMP) values in relation to a history of intra-articular knee injury in a youth cohort is an area that has not been investigated to date. It is known that these intra-articular injuries increase the likelihood of OA later in life within the injured joint, but the potential relationship to sCOMP is unclear. The potential relationship between sCOMP and OA (risk and/or progression) are of significant research interest given the prevalence of this disease in the general population and the impact on the health care system. Knowledge surrounding the potential associations between OA and sCOMP levels could provide the basis for identifying participants at an increased risk of developing PTOA before they reach a potential 'point of no return' in the disease.

Furthermore, sCOMP has been shown to fragment in uniquely different patterns depending on the type of disease afflicting the joint. Categorical differences between rheumatoid arthritis (RA) and OA have been shown in the literature[14] with smaller fragments of COMP observed within the serum of OA patients vs RA patients. While the role of these fragments in OA and/or RA is not completely understood, it has been found that specific neo-epitopes of COMP in OA are enriched in patients with chronic joint pain[15]. It has been hypothesized that the difference in COMP fragmentation patterns in OA vs RA may be in part due to different disease processes driving expression of different proteases that cleave COMP. It is therefore possible that these fragments/neo-epitopes may be present in the serum prior to clinical OA diagnosis.

Presently, there is some research examining sCOMP outcomes immediately following intra-articular knee injury or after PTOA onset[16–20]. However, there is a paucity of research examining outcomes during the interval between joint injury and disease onset (<10 years post-injury). Therefore, the purpose of this research is to determine if serum COMP levels change following intra-articular knee injury compared to matched controls in a youth cohort; and if the levels of COMP are associated with clinical outcome measures (i.e., Magnetic Resonance Imaging (MRI) Osteoarthritis Knee Score; MOAKS, Knee Osteoarthritis Outcome Score; KOOS, Fat mass index; FMI). Our hypothesis was that participants that had suffered a previous intra-articular knee injury would present with elevated levels of serum COMP compared to matched controls and this would be associated with changes in MOAKS and KOOS sub-scale scores, and FMI.

Methods

Ethics approval

Ethics approval was granted from the conjoint health research ethics board at the University of Calgary, Canada (CHREB, ETHICS ID # E-25075) and consent was obtained for all participants prior to testing. All testing was performed in accordance with the Declaration of Helsinki.

Participant recruitment

This study includes youth/young adults recruited into the PrE-OA study group (n = 200) between June 2013 and April 2015. One hundred participants who had sustained a sport-related intra-articular knee injury 3–10 years previously and 100 age (within ±12 months), sex and sport (at time of injury) uninjured matched controls; all active youth in Calgary. Recruitment sources (Sport Injury Prevention Research Centre, and Sport Medicine Centre, both at the University of Calgary), injury diagnoses procedures and inclusion/exclusion criteria have previously been reported[21,22]. In summary, injured participants had sustained an intra-articular knee injury (i.e., a clinical diagnosis of knee ligament, meniscal or other intra-articular tibiofemoral or patellofemoral injury within the past 3–10 years that required both medical consultation and resulted in disruption of regular sport participation) during a previous study or, presented to a Sport Medicine Centre with a sport-related knee injury 3–10 years previously when they were ≤18 years of age. Injury diagnoses were based upon diagnostic codes recorded on injury report forms (physiotherapist clinical examination) or medical records (physician clinical examination) and confirmed by participants. The 3–10 year window post-injury was chosen to examine chronic changes in participant physiology and knee condition, with the assumption that all acute responses to the injury had subsided. Uninjured participants included individuals with no previous time-loss knee injury. Exclusion criteria included pregnancy, non-steroidal anti-inflammatory use or cortisone injection within 3 months prior to testing, a musculoskeletal injury within the previous 3 months prior to testing that resulted in time loss (work, school or sport), diagnosis of other arthritides, or any current medical problem that prevented participation in the functional testing aspect of the study (e.g., neurological conditions). The sample size of 85 was estimated based on the ability to detect a meaningful clinical difference between study groups for serum COMP levels ($1 - \beta = 0.8$, $\alpha = 0.05$) based on previously published serum COMP levels in ACL injury patients[23–25].

Procedures

After completing a study questionnaire that gathered demographic, knee injury/surgery and medical history information and KOOS[2], individual participants rotated through testing stations that collected blood serum samples, height (cm), weight (kg), and body composition (dual X-ray absorptiometry, DXA). In addition

MRI studies were conducted at an off-site diagnostic imaging facility.

Knee injury and osteoarthritis outcome score

The KOOS is a self-report measure designed to evaluate knee related symptoms and function in young active patients with knee injury and OA. It has been validated in different populations varying in age, disease duration and activity levels and it has been shown to have high test–retest reliability[2]. The KOOS consists of 42 items in five subscales: pain, other symptoms, function in daily-living, function in sport and recreation, and knee-related quality of life. Each item was scored on a five-point Likert scale. Subscale scores were then summed, and the total sub-scale score transformed to a 0–100 scale (higher scores indicating better outcome).

Fat mass index

A Hologic Discovery A (Hologic, Bedford, MA) dual-energy X-ray absorptiometry (DXA) scanner with Discovery QDR^{TM} software was used to capture whole body composition scans. Daily calibration of the scanner employed a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned in supine on the scanner bed according to the manufacturer's recommendations and instructed to remain as still as possible for the duration of the scan[16]. All procedures were consistent with the official positions of the International Society for Clinical Densitometry[26]. FMI was calculated as fat mass, relative to stature squared (kg/m^2).

Blood collection, serum COMP (sCOMP) expression and Western Blot analysis

Blood samples (4–5 ml) were collected from each participant by a certified phlebotomist using standard venipuncture in untreated 5-mL vacutainers. Collections were performed at the very beginning of the test protocol. The WIESLAB hCOMP assay[27] (human COMP quantitative ELISA) was used to quantify the concentration of sCOMP proteins. This assay did not distinguish between intact and fragmented COMP, and binds both with equal affinity. The assay was conducted according to the manufactures instructions. Serum was diluted with a Tris–HCl/SDS based lysis/sample buffer, and separated on a 10% poly-acrylamide gel. The gels were transferred to nitrocellulose membranes and probed with primary antibodies specific to COMP (monoclonal – MA37C94 and polyclonal – PA5-72491, all Thermofisher). The monoclonal antibody (ref # AB_2083692) was used at a concentration of 1:500, while the polyclonal antibody (ref # AB_2718345) was used a concentration of 1:2000. An infra-red secondary was utilized for detection of the signal with the Odyssey imaging system (LICOR).

Magnetic resonance imaging (MRI)

Participants underwent bilateral knee MRI using typical clinical sequences (i.e., sagittal proton density, sagittal and coronal proton density fat saturated and 3D gradient echo FIESTA; 1.5 T). All studies were rated by a musculoskeletal fellowship trained radiologist (JLJ) with 13-years of imaging experience, blinded to injury history or surgical intervention (other than those that required fixation that is visible on MRI) using the semi-qualitative MOAKS[28]. MOAKS is a semi-quantitative scoring system for providing a comprehensive inventory of possible abnormalities related to knee OA, including sub-scales for osteophytes, synovitis/effusion and bone marrow lesions, articular cartilage, meniscal and ligamentous lesions. MOAKS scoring has very good to excellent reliability in this study group as previously reported[29].

Statistical analyses

STATA (v11.1, Collage Station, Texas, USA) was be used for all statistical analyses. 'Data was tested for normality (Shapiro–Wilk Test, $\alpha = 0.05$) to ensure appropriateness of paired *t*-test analyses'. There was no need to assess the homogeneity of variance. Covariates (i.e., age, sex, weight, height, FMI, and time since knee injury) at historical study group recruitment were summarized. To account for the matched design, paired *t*-tests (mean difference; 95% CI) or two-sample *t*-test (i.e., age comparison between male and female participants) were calculated for all primary outcomes. Primary analyses compared sCOMP levels between matched pairs. Analyses were stratified by sex. Multivariable linear regression (95% CI), taking into account matching via identification of cluster (pair), was used to assess the association between COMP and history of injury adjusting for FMI. The value entered for the time-since-injury variable for control (non-injured) participants was the same as that of their matched-case and reflected an equivalent time period. The correlations between COMP and clinical measurements were access using Pearson correlation (for continuous measurements, e.g., FMI) and Spearman's coefficient (for ordinal measurements, e.g., MOAKS and KOOS sub-scale scores).

Results

In total, 200 study participants completed baseline data collection for aspects of the study. Of the 200 participants, 192 (96 matched pairs) participants had blood samples collected successfully of which 170 (85 matched pairs) attended MRI testing. Missing blood samples were related to difficulties in collecting the samples, (e.g., phlebotomist error, withdrawn consent, non-steroidal anti-inflammatory use and/or inability to complete the entire protocol) and the primary reasons for not attending MRI studies was accessibility and time-restraints.

Participant characteristics

Table I summarizes participant characteristics by study group and sex. There was no difference in the age between study groups (males, $P = 0.32$; females, $P = 0.36$) or males and females ($P = 0.13$). Across the 85 previously injured participants, the mean time of injury was 82.7 months (just under 7 years since time of injury). Of the 85 injured participants, 13 (three female and 10 male) had patellofemoral subluxations, 13 (six female and seven male) had grade I-II medial collateral ligament (MCL)/lateral collateral ligament (LCL) sprain, or grade I-II ACL sprain, 14 (six female and eight male) had isolated meniscal injuries, 44 (30 female and 14 male) experienced grade III ACL sprains and one male had suffered a fracture. All grade III ACL sprains were surgically reconstructed. Although there was no difference in FMI between previously injured and uninjured females, previously injured males demonstrated significantly higher FMI in comparison with uninjured males. In both sexes, previously injured participants scored lower on the $KOOS_{SYMP}$ subscale indicating greater presence of knee symptoms compared to the uninjured controls.

sCOMP levels & matched pair comparisons

Differences between study groups were assessed by a paired *t*-test and are summarized in Table II. Differences in sCOMP levels existed by injury history and sex. In male participants sCOMP values were substantially higher than female participants in both injured and uninjured study groups. Males with a previous injury demonstrated a significantly higher sCOMP level than uninjured

Table I
Participant characteristics and outcome measures

Characteristic	Male		Female	
	Injured n = 40	Uninjured n = 40	Injured n = 45	Uninjured n = 45
Age; (years), median, range	21.5 (17–26)	22 (18–26)	23 (16–26)	23 (15–26)
Height; (m) mean, 95% CI	1.80 (1.78, 1.82)	1.80 (1.77, 1.82)	1.67 (1.65, 1.69)	1.67 (1.65, 1.69)
Time since injury (months) median, range	79.8 (35.1–118.5)	NA	85.5 (44.3–124.2)	NA
Weight; (Kg) mean, 95% CI	83.1 (79.9, 86.3)	77.4 (3.5, 80.2)	68.0 (64.6, 71.5)	65.1 (62.0, 68.2)
$KOOS_{SYMP}$ (normalized) mean, 95% CI	**84.0 (80.3, 87.7)***	**91.9 (89.1, 94.7)***	**83.7 (79.1, 88.3)***	**92.1 (89.4, 94.9)***
Fat mass index (FMI) mean, 95% CI	**4.5 (4.0, 5.1)***	**3.7 (3.3, 4.1)***	6.5 (5.8, 7.2)	6.0 (5.4, 6.6)

*Significant at $P < 0.05$, $KOOS_{SYMP}$; knee osteoarthritis outcome symptoms sub-scale score (0–100 scale with higher scores indicating better outcome), Fat Mass Index was calculated as fat mass, relative to stature squared (kg/m^2).
Bold values: Just highlighting values that reached significance (in addition to *).

Table II
Serum COMP (sCOMP) Levels & Matched Pair Comparisons. *Significant findings ($P < 0.05$) are bolded within the table

	Male (n = 40 matched pairs)			Female (n = 45 matched pairs)		
	Injured (mean, SD)	Uninjured (mean, SD)	*P*-value (paired *t* test)	Injured (mean, SD)	Uninjured (mean, SD)	*P*-value (paired *t* test)
COMP (ng/ml)	1666.3, 672.7	1494.8, 617.6	**0.04***	1210.2, 568.9	1147.6, 536.8	0.928

males (1666.3 ± 672.7 ng/ml vs 1494.8 ± 568.9 ng/ml, $P = 0.040$). Since FMI was significantly different between injured and uninjured males, it was decided to examine if FMI was confounding the sCOMP results between these groups. After adjusting for FMI, sCOMP levels between injured and uninjured males were no longer significant (Table III). In female participants, there was no evidence of a significant difference in sCOMP values (1210 2 ± 568.9 ng/ml vs 1147.6 ± 536.8 ng/ml, $P = 0.928$) between groups with and without a history of intra-articular knee injury (Table II) regardless covariates adjustment (Table III). There was also no significant correlation observed between sCOMP levels and time since injury for injured males (r = .028, $P = .782$) or females (r = .160, $P = .144$) (Fig. 1).

FMI vs sCOMP levels

Exploratory assessments were made to investigate the possibility of a relationship between FMI and sCOMP levels. Pearson correlation analysis demonstrated a negative relationship between sCOMP levels in both injured and matched control study groups, however, this correlation only reached significance in the injured study group (Table IV).

KOOS score vs sCOMP levels

Due to the non-continuous nature of the KOOS sub-scale scores, Spearman's correlation analysis was used to examine any correlation between the KOOS sub-scales and sCOMP levels. No significant association were identified between any of the KOOS sub-scale scores and the sCOMP levels in the injured or matched control study groups (Table V).

Table III
Linear regression conditioned for matched design. When Fat Mass Index (FMI) was accounted for in the model, COMP levels were no longer significant in males with a previous injured vs matched controls

	Coefficient (95% CI)	
	Male	Female
Injury	242.85 (−1.97, 487.67)	11.07 (−201.82, 223.97)
FMI	−28.68 (−134.52, 77.15)	−2.40 (−58.35, 53.55)

MRI MOAKS scoring vs sCOMP levels

Spearman's correlation analysis was also used to examine any correlation between MOAKS sub-scale scores and sCOMP levels. Correlation analysis demonstrated a relationship between sCOMP levels and $MOAKS_{SYNOTIVIS}$ score within the injured study group but not within the uninjured controls (Table VI). No other MOAKS sub-scale scores were found to be significant between the injured study group and the uninjured controls.

sCOMP fragment analysis

Western blot analysis of serum from males and females with or without a previous joint injury (n = 5 each group) was undertaken. Using both a monoclonal and polyclonal COMP antibody, a unique sCOMP fragmentation pattern was observed in previous injured participants regardless of sex (Fig. 1). Specifically, the polyclonal COMP antibody recognized ~37 and ~20 kilo-Dalton (kD) bands in males and females with or without a previous joint injury (Fig. 2 arrows), however, it recognized higher weight fragments (~50–100 kD) only in female participants with a previous joint injury (Fig. 2 arrows). The monoclonal antibody recognized a ~37 kD in male and female participants with or without a previous joint injury (Fig. 1 arrows), but recognized a ~23 kD band only in male and female participants with a previous joint injury (Fig. 2 arrows).

Discussion

The current study was designed to determine whether sCOMP levels differed between individuals that had suffered a youth sport-related knee injury 3–10 years previously and uninjured age, sex and sport matched controls. Primary analyses demonstrated a significant elevation in sCOMP values amongst previously injured male participants compared with uninjured matched controls, while, in female participants, no significant difference sCOMP values was observed. However, after accounting for FMI, the difference in COMP levels observed with univariate analysis was no longer observed. While no evidence of a relationship was observed between COMP and any KOOS sub-scale score, a negative correlation was observed between COMP and FMI (previously injured participants only). Although sCOMP is often recognized as

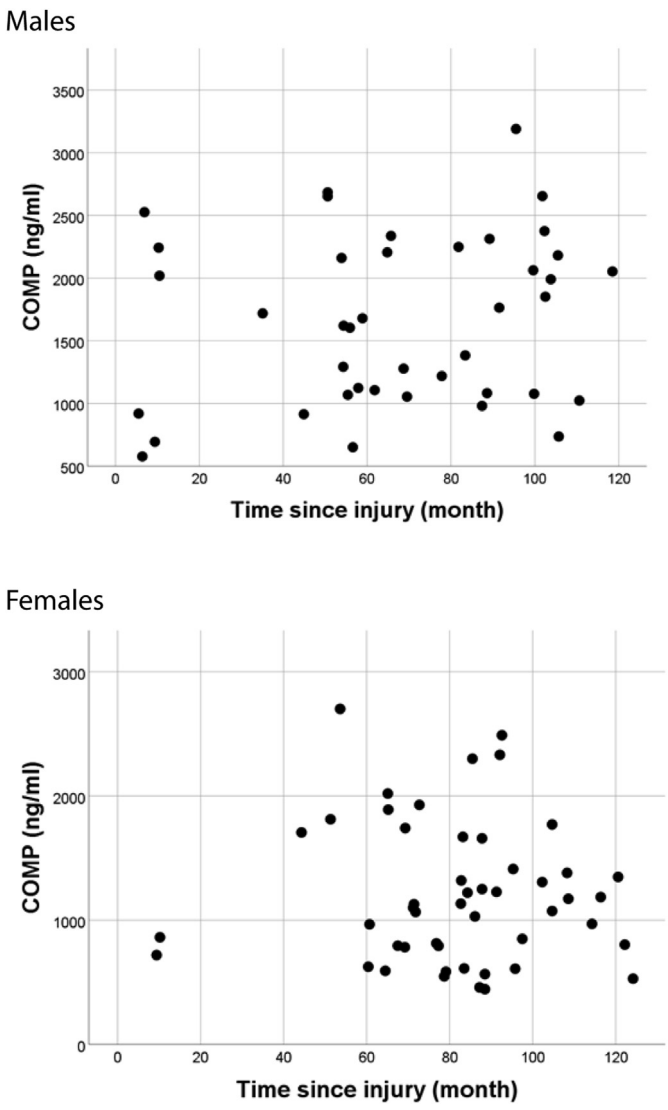

Fig. 1. Association between Serum COMP (sCOMP) levels and time since injury in males and females. There was no significant correlation observed between sCOMP levels (ng/ml) and time since injury in males (r = .028, $P = .782$) or females (r = .160, $P = .144$).

Table IV
sCOMP levels & FMI correlation

sCOMP levels		FMI
Uninjured	Pearson correlation	−0.19
n = 85	Sig. (2-tailed)	0.065
Injured	Pearson correlation	**−0.218***
n = 85	Sig. (2-tailed)	**0.036***

*Significant findings ($P < 0.05$) are bolded within the table.

a marker of cartilage turnover, no association with $MOAKS_{CARTILAGE}$ was observed. Further, there was no evidence of a relationship between MOAKS bone marrow lesion; meniscus; or MOAKS total score, however, a significant correlation was observed between $MOAKS_{SYNOVITIS}$ score and sCOMP levels (previously injured participants only). Western blot analysis of serum demonstrated unique banding patterns in males and females with a previous joint injury, and also a distinct banding pattern within previously injured females.

Since the seminal studies demonstrating release of COMP into the joint environment after injury[8,9]; a number of studies have found that synovial fluid and/or serum COMP is increased with joint injury and/or the progression of OA (in both cross-sectional and longitudinal studies)[16,17,30]. It is also important to note that not all studies have demonstrated that COMP increase after joint injury and/or correlates with structural changes within the joint[31]. This discrepancy may contribute to why no clinically relevant deviations from baseline sCOMP values have been established in regards to prognosis/diagnosis of OA.

Previous studies have correlated OA progression [Kellgren-Lawrence (K-L) grading] with an increase in sCOMP values over one and 3 year follow up cohorts (40 years of age and older)[7,32]. While our present study had only a single measurement point to evaluate; injured male participants did demonstrate an increase in baseline sCOMP values vs matched controls. While it would be of interest to follow up this study group to determine if individuals went on to develop OA, it is important to note that no participant in the current study met the clinical definition of OA. This result is important to evaluate in the context of a previous longitudinal study that demonstrated increased baseline sCOMP levels are a risk factor for developing incident knee OA[33]. The concept that increased sCOMP may have prognostic utility to determine which patients will develop radiographic OA, may allow for identification and therefore earlier treatment of this sub-group of individuals.

In regards to the differences observed between COMP levels in males vs females, there are many potential explanations for these sex-specific differences. A likely contributor is the disparity in injury types between sexes. Females experienced a much higher proportion of ACL injuries than male participants, which could be a contributing factor. Over 66% of females in the study had complete ACL tears, while only 35% of males presented with complete ACL tears. The difference in physiological response to the type of injury likely has an effect on sCOMP values. Alternately, it is possible that the physiological response to an intra-articular knee injury is fundamentally different in regards to sCOMP levels between sexes. Sex differences have been reported in other literature[7]; however these sex-specific differences were identified in participants with a minimum age of forty five, substantially higher than the ages in this study. In that study, the authors hypothesized that anatomical differences between the sexes (e.g., skeletal size and a difference in total cartilage mass) could be potential explanation behind their observation. The design of the current study cannot provide further insight on this hypothesis and further study will need to be

Table V
sCOMP levels & KOOS correlation

sCOMP levels		Normalized KOOS Score				
		koos_symp	koos_pain	koos_adl	koos_sp	koos_qol
Uninjured	Spearman's correlation	0.007	0.038	−0.084	0.016	−0.41
n = 85	Sig. (2-tailed)	0.945	0.713	0.417	0.879	0.694
Injured	Spearman's correlation	−0.061	−0.006	−0.118	−0.134	−0.008
n = 85	Sig. (2-tailed)	0.569	0.957	0.268	0.207	0.938

koos_symp (KOOS Symptom Score, normalized), koos_pain (KOOS Pain Score, normalized) koos_adl (KOOS Total Daily Living Score, normalized), koos_sp (KOOS Total Sport and Recreation Score, normalized), koos_qol (KOOS Total Quality of Life Score, normalized).

Table VI
sCOMP levels & MRI OA Knee Score (MOAKS) Magnetic Resonance Imaging (MRI) scoring correlation

sCOMP levels		MOAKS score (index knee)				
		BML	Cartilage	Synovitis	Meniscal	Total
Uninjured	Pearson correlation	0.181	−0.156	0.008	0.208	0.197
n = 85	Sig. (2-tailed)	0.139	0.204	0.949	0.089	0.108
Injured	Pearson correlation	0.208	0.137	**0.404***	−0.024	0.098
n = 85	Sig. (2-tailed)	0.084	0.257	**0.001***	0.841	0.421

BML (bone marrow lesion), Cartilage (cartilage), Synovitis (synovitis), Meniscal (meniscal), Total (combined score). *Significant findings ($P < 0.05$) are bolded within the table.

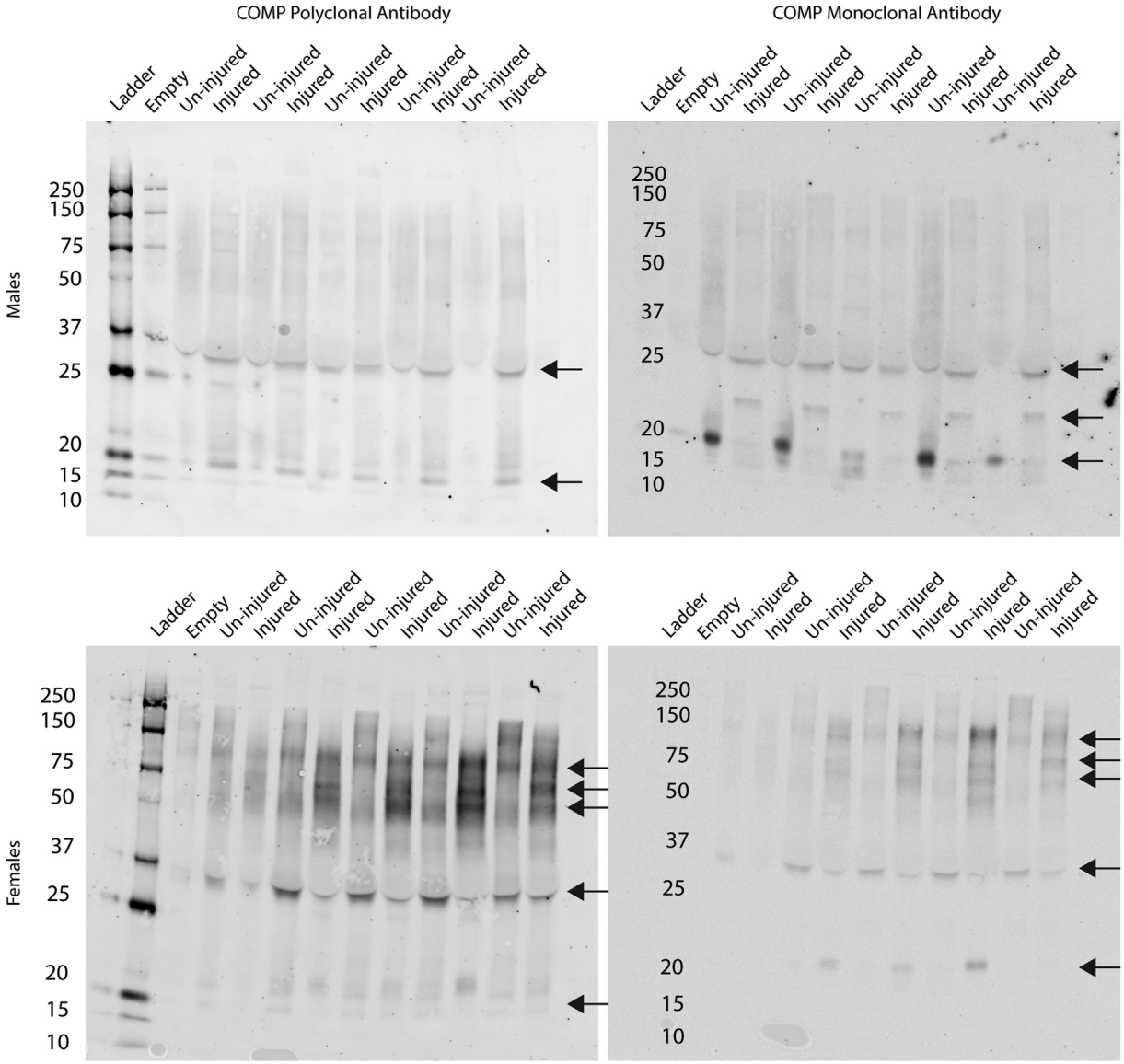

Fig. 2. cartilage oligomeric matrix protein (COMP) Fragmentation Analysis. Serum samples from males and females with or without joint injury were analyzed by western blot. A COMP polyclonal and monoclonal antibody were both utilized and present different results. A ~37 kD band was observed in all samples with both antibodies. Higher molecular weight fragments were only observed in previous injured females. A ~23 kD fragment was observed with the serum of previous injured males and females only, but only with the monoclonal antibody.

conducted. Age-related differences in sCOMP values have also been noted in both sexes[20]. One study of 82 healthy controls aged 22 and under found that controls under 16 years had significantly higher baseline sCOMP values than participants aged 16 and older[13]. This clear change in sCOMP expression with age suggests that skeletal maturity and turnover/homeostasis is most likely a significant contributor to sCOMP levels in younger and older populations respectively. It appears that sCOMP values are relatively high during skeletal immaturity, and decline after maturity completes. Decades later, the trend reverses, and age-related degradation within the body serve to increase sCOMP levels regardless of the presence or absence of OA.

sCOMP levels were also examined in the context of FMI, assessed as subgroups of injured and uninjured participants, a significant association between sCOMP and FMI was found in the injured study group alone. However, the association was negative, such that injured individuals with higher FMI has lower sCOMP levels. This finding is at odds with the findings of the Johnston County OA cohort, which found a positive association between body mass index (BMI) and baseline ln sCOMP values[6,20]. These differences could be due to a combination of substantially lower n-values ($n = 769$ vs $n = 45$), statistical method employed, and a different range of participant ages. Furthermore, we observed that correction for FMI in males abolished the previous significance observed in injured males vs uninjured male controls. This result suggests there is a relationship between COMP and FMI, however, the direction of the relationship (e.g., COMP acting on FMI or vice versa), or even if this is a direct or indirect relationship remains unknown. To our knowledge there is no evidence of a direct and functional relationship between adiposity and the expression of COMP in the serum and/or synovial fluid. We therefore suggest this phenomenon deserves further study.

The absence of correlation between COMP and $MOAKS_{CARTILAGE}$ degeneration scoring is not particularly surprising given the age range and time from injury in the current injured study group. However, the correlation between synovitis and COMP levels may potentially be a surrogate measure[34] demonstrating an increased level of cartilage breakdown is occurring, yet has not progressed to the level required to be observed using the MRI techniques employed in the current study. We did not perform dedicated cartilage imaging such as T2* or DGEMRIC, which are more sensitive to cartilage signal changes. As with differences in sex, this study was not powered on MRI MOAKS scoring and therefore it may be possible that with increased study participants a relationship between COMP and cartilage degeneration may have been observed. Another limitation in the current study was although the radiologist was blinded to the medical history of the patients in the study, in cases of ACL reconstruction, the fixation would have been visible on the MRI. This may have created a measurement bias and potentially influenced how the rater performed the rating, (i.e., was more likely to identify lesions in those they knew where cases). However, we suggest that this potential bias was minimal since there were few individuals categorized as MRI-defined OA in both groups included in the current study.

While the level of COMP in serum/urine/synovial fluid may be of potential use as a prognostic/diagnostic for OA and/or cartilage turnover, the fragmentation pattern of the protein has also been investigated. Previous studies have demonstrated that COMP may have distinct fragmentation patterns depending on the health status of the participant. In one study, fragmentation patterns in OA were not distinguishable from healthy controls, yet, a categorical difference in fragmentation was noted between participants with RA and all other study participants[14]. In the current study, while no differences in sCOMP levels were observed in females with a previous joint injury, a unique sCOMP fragmentation pattern was observed in previously injured (males and females) vs non-injured controls. A recent study examining COMP fragments in synovial fluid identified a neo-epitope (Ser^{77}) that was present in patients present with acute knee trauma, and also in patients with OA and RA[15]. Interestingly, Ser^{77} is approx. a 40 kD fragment and we observed a similar sized fragment in all participants (injured and non-injured) in the current study group. This difference could be due to the difference of serum vs synovial fluid, but could also be due to differences in participant age between our study group and the published study. Interestingly, this previous study also identified a ~15 kD band in a sub-set of patients with acute knee trauma and in the current study we found a ~23 kD fragment present in the previously injured participants. It would be of interest to determine if an antibody against the 23 kD fragment could discriminate between previously injured vs matched control and/or patients with OA.

In conclusion, this study has demonstrated sex-specific differences in sCOMP levels between participants with and without a traumatic intra-articular knee injury. Injured male participants showed a ~15% increase in sCOMP values compared to their uninjured controls, but this difference was lost when FMI was taken into account. While COMP is typically seen as a marker of cartilage turnover, sCOMP was not correlated with MRI cartilage degeneration scores. However, COMP levels were correlated with increased levels of synovitis potentially suggesting that breakdown of the cartilage is occurring, yet is in a very early stage not detectable by MRI. This suggests that COMP may be a marker of very early cartilage breakdown, however, further study would be required to test this hypothesis.

Author contributions

Conception and design: JL, JLW, CAE, RJK. Analysis and interpretation of the data: JL, JLW, GR, CAE, RJK. Drafting of the article: JL, JLW, CAE, RJK. Critical revision of the article for important intellectual content: JL, JLW, GR, JLJ, CAE, RJK. Final approval of the article: JL, JLW, GR, JLJ, CAE, RJK. Provision of study materials or patients: JL, JLW, CAE, RJK. Statistical expertise: JL, JLW, GM. Obtaining of funding: JLW, CAE, RJK. Collection and assembly of data: JL, JLW, GR, JLJ, CAE, RJK.

Conflict of interest statement

The authors declare no conflict of interests.

Role of the funding source

Grants through the Alberta Innovates Health Solution (AIHS) Osteoarthritis Team Grant to CAE and RJK, Canada Foundation for Innovation (CFI) to RJK, Grace Glaum Professorship to RJK, Canadian Institutes for Health Research To JLW, CAE and RJK. GR was supported by an Arthritis Society PhD Studentship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The authors would like to acknowledge the assistance of research coordinators Gabriella Nasuti, Jamie Rishaug and Lisa Loos, the numerous Sport Injury Prevention Research Centre (SIPRC) research assistants/students and the participants. We would also like to thank Drs. Linda Woodhouse and David Hart for their support of the project and their feedback.

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