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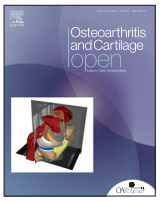

A novel mechanosensitive stress test in individuals following anterior cruciate ligament reconstruction: A pilot study

Prakash Jayabalan [a,b,*], Rose Darcy [a,b], Vikram Darbhe [a], Zander Neuville [b], Vehniah Tjong [b], Levi Hargrove [a,b], Leah Welty [b], Thomas Schnitzer [b]

[a] Shirley Ryan AbilityLab, USA
[b] Northwestern University Feinberg School of Medicine, Chicago, IL, USA

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ABSTRACT

Objectives: 1. Assess serum biomarker responses of the anterior cruciate ligament reconstructed (ACLR) knee compared to the non-injured contralateral knee in a lateral tilt paradigm. 2. Determine the relationship between kinematics at the ACLR knee and serum biomarker responses.
Method: Cross-sequential study. 16 participants 2–7 years post-ACLR, undertook two 30 min walking sessions on a 10° angular tilted treadmill towards the ACLR knee and/or contralateral non-injured knee. Serum collected at baseline and after 30 min of walking was tested for biomarkers associated with osteoarthritis (Cartilage oligomeric matrix protein [COMP]), C-terminal cross-linked telopeptides of type II collagen, matrix metalloproteinase-3,-13, ADAM a disintegrin and metalloproteinase with thrombospondin motifs 4-,-5 (ADAMTS-4,-5), interleukin-1β,-6,-8 and RANTES (regulated on activation, normal T-cell expressed and secreted). Kinematic measurements were also taken.
Results: When tilted towards the ACLR knee, there was a significant increase in COMP compared to baseline (mean 41.1 ng/mL (95%CI:13.8,68.4), not observed when tilted towards the contralateral knee. There was a significant correlation between change in COMP concentration and change in knee adduction/abduction angle ($r = -0.58$,$p = 0.02$). There were no relationships to kinematics and the other biomarkers.
Conclusions: The tilt paradigm identified a differential response of a serum mechanosensitive marker (COMP) when testing an ACLR and contralateral knee. There was also a significant relationship between COMP response to tilt walking and the change in knee adduction angle of the ACLR knee. This paradigm could be an aid to provide individual knee biomarker response to a mechanical stress in asymptomatic individuals at risk of developing post-traumatic osteoarthritis at least 2 years post-ACLR.

Following anterior cruciate ligament (ACL) injury, there are an estimated 250,000 ACL injuries annually in the United States, often in relatively young (less than 35 years of age) and physically active individuals [1]. ACL reconstruction (ACLR) is commonly undertaken to improve the stability and potentially allow for an earlier return to sport. However even if surgery is initially considered successful, approximately 50 %–90 % of patients develop post-traumatic knee osteoarthritis (PTOA) within 5–10 years post-reconstruction [2]. While PTOA is diagnosed with clinical symptoms and imaging, the disease process starts long before it can be detected [3].

The development of PTOA is known to be driven by both mechanical and biologic factors [3–5], yet there are a paucity of studies that have investigated the underlying mechanistic effects of these factors. An understanding of this relationship could help to identify those at most risk for PTOA development and be of significant clinical benefit. One approach to studying this relationship is to employ a stimulus response framework, a methodology in which a known mechanical stimulus (typically walking exercise) is utilized and the response assessed based on specific disease-related markers. These could include serum biomarkers of cartilage metabolism or imaging studies [6–14]. Most prior studies utilizing serum in this realm have focused on change in a articular cartilage turnover marker, specifically cartilage oligomeric matrix protein (COMP) [10,15]. COMP is a structural glycoprotein that binds and stabilizes type-II collagen fibers [16]. Prior studies have shown that this biomarker is mechanosensitive to joint loading [17] with serum concentrations of COMP increasing in as quickly as 30 min of walking and

* Corresponding author. Shirley Ryan AbilityLab, USA.
E-mail address: pjayabalan@sralab.org (P. Jayabalan).

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can remain raised up to 5.5 h post walking exercise [17]. In terms of kinematic evaluation, a recent meta analysis found that significant gait alterations persist following ACL reconstruction [18]. Specifically there are reductions in peak knee flexion angle and external knee-flexion moment that persist up to 64 months. However peak knee-adduction angle is greater in the ACLR knee for the first 20 months but smaller compared to healthy knees at 64 months. Studies have also shown that there are relationships between gait kinematics during walking in individuals with knee OA [19] or with cartilage thinning [20] that associations with changes in systemic biomarkers of OA and inflammatory cytokines. Minimal studies have further related biomechanics to serum biomarker changes in individuals with knee OA.

Another major limitation of stimulus-response frameworks in knee OA is that studies typically utilize ground level walking. Utilizing this methodology, it is challenging to apportion biomarker responses to a specific knee joint, since both sides (ACLR and contralateral knees) receive the same biomechanical input [10,12,21]. A methodology that preferentially alters loading profile on each knee during walking is an angular tilted treadmill (Fig. 1) which can be tilted in either a medial or lateral direction and as such, each knee has a different biomechanical loading pattern.

The goal of this study was to assess serum biomarker responses (COMP as the primary marker as well as other mechanosensitive, inflammatory and metabolic biomarkers for exploratory analyses) of the ACLR knee compared to the non-injured contralateral knee in a lateral tilt paradigm. We also wanted to determine the relationship between kinematic changes at the ACLR knee and serum biomarker responses. Our primary hypothesis was that when the treadmill was tilted towards the ACLR knee and the individual walked for 30 min, there would be a significantly larger serum COMP response compared to baseline that would not be observed when the contralateral knee was tested. In addition, we hypothesized that the change in serum COMP concentration from baseline would correlate to changes in knee adduction angle of the ACLR knee.

1. Methods

The study was approved by our local institutional review board (IRB). All participants provided written informed consent prior to entering the study.

1.1. Study cohort

All participants were recruited from local research registries and through community advertisements. Inclusion criteria included participants who were aged 20–45 years with a history of unilateral ACL-reconstruction and had 'no pain' on the numeric pain rating scale (NPRS) within the month prior to study initiation both at rest or during walking. An individual's contralateral knee also must not have had a history of injury or surgery. Individuals were also screened to ensure they could walk for 30 min on a standard flat treadmill. Exclusion criteria included, ACLR more than 7 years prior to testing, on-going ankle, hip or spine pain, history of rheumatoid arthritis, ankylosing spondylitis, other inflammatory arthropathies, lower extremity total joint arthroplasty, or unstable cardiovascular disease which precluded walking exercise for 30 min. Individuals who had more than one ACL injury or surgery on their ACLR knee were excluded from the study.

1.2. Walking sessions (Fig. 2)

A sequential crossover design was used for this study, with all participants performing two walking trials (ACLR tilt versus contralateral tilt) on two separate days more than 1 week apart. Following the 30-min pre-walking rest period, participants walked on a custom-fabricated treadmill (Treadmetrix, Park City, UT) that allows a sideways tilt in either the left or right directions to 10° angulation. The order in which individuals performed the two walking sessions was randomized, using a random number generator. For example, following randomization an individual with a right ACLR was tilted to the right (so the right knee was

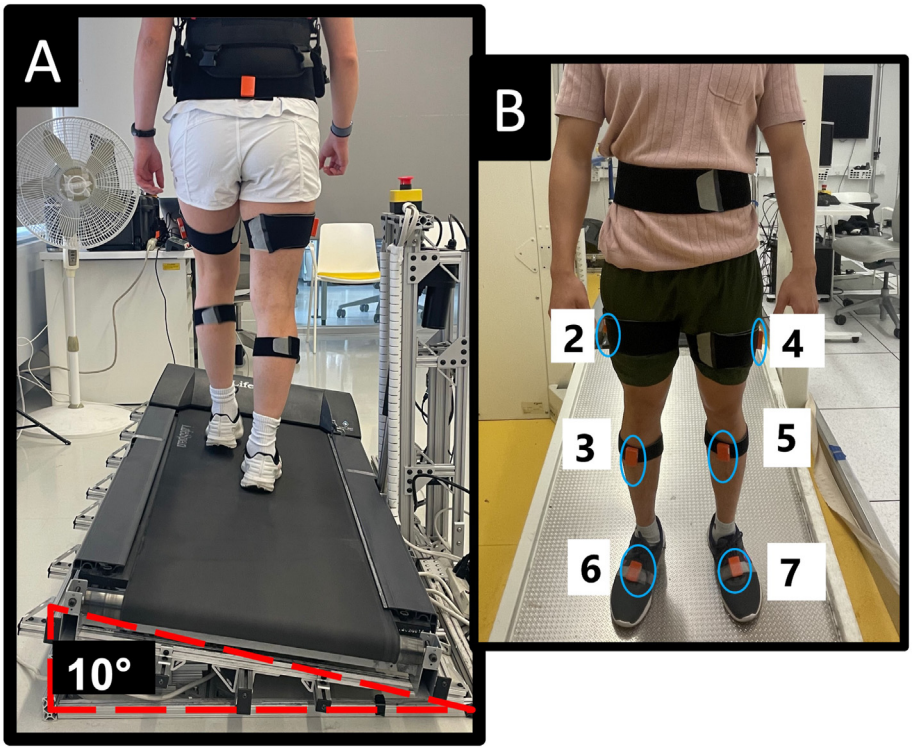

Fig. 1. Tilted treadmill at 10° angulation (A). Anterior XSENS marker placements are also shown (B). Tilting towards the ACLR knee, (in the image shown, right knee which is lower) was considered to be testing the ACLR knee whereas tilting towards the contralateral knee (left) was testing the opposite knee.

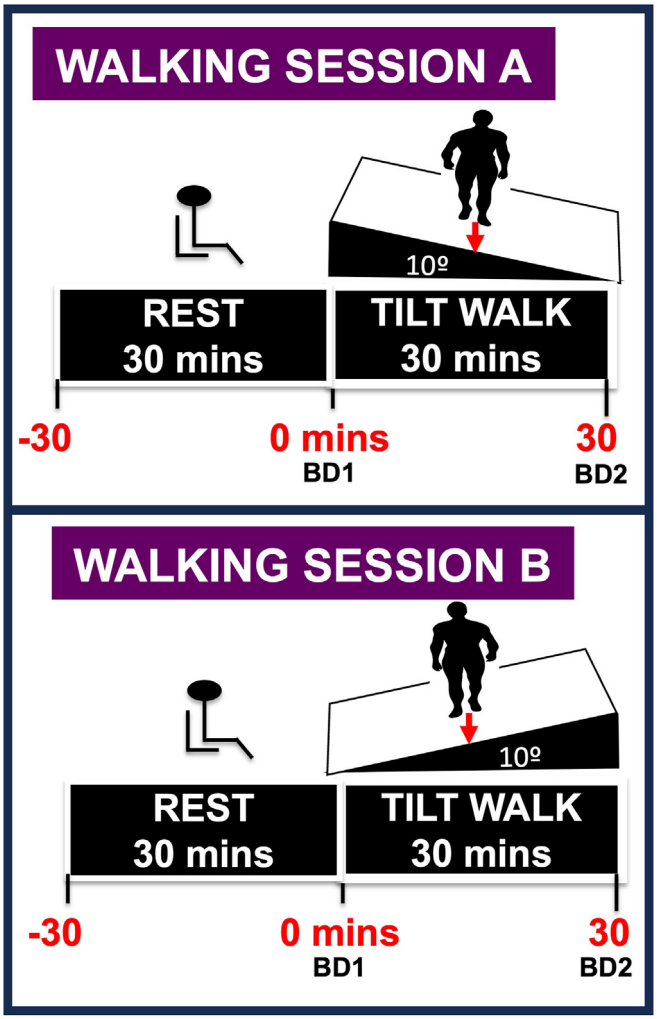

Fig. 2. Crossover sequential study design. Participants would attend the lab and be seated for 30 min. After 30 min of rest, participants will then walk with the treadmill tilted towards or away the ACLR knee (session A). In the other session, the treadmill would be tilted towards the alternate knee (session B). The second session was done 1 week later. The order of sessions (A vs. B) was randomized). Blood draws were performed at baseline (Blood Draw 1) and after 30 min of walking (BD2).

lower than the left during walking), and this was considered testing of the ACLR knee. The angle at which individuals walked was kept the same for both sessions.

For both walking sessions the same methodology was used. Participants did not exercise strenuously 24 h prior to the day of testing. On the day of testing, participants arrived at the laboratory and then had the inertial measurement unit (IMU) sensors placed and calibrated as outlined below. They were then seated in a chair, resting for 30 min. At the completion of 30 min, a research team member inserted an intravenous (IV) cannula into the antecubital vein and a 5 mL vial of blood was drawn via BD vacutainer® tubes (baseline sample). The individuals then walked 30 min with the respective tilt. At the completion of 30 min of walking, a further blood sample was taken.

1.3. Serum samples and testing

Blood samples were allowed to clot for 30 min on ice, centrifuged at 3500rpm, serum separated and transferred for storage at −80 °C until assayed. Serum levels of biomarkers associated with OA were measured in three domains:

(1) Tissue Turnover – COMP and C-terminal telopeptides of type II collagen (CTX-II). CTX-II is a biomarker associated with type II collagen degradation. Recently, CTX-II has previously been found to originate from the interface between subchondral bone and articular cartilage, which is a site of potential remodeling [22,23]. The inter- and intra-assay variability for COMP was 5.2 % and 7.7 % respectively in our study. For CTX-II the inter-and intra-assay variability from our study was 6.7 % and 8.3 % respectively.

(2) Cartilage degradative enzymes - Matrix metalloproteinase-3 (MMP-3) MMP-13, ADAM a disentegrin and metalloproteinase with thrombospondin motifs 4-,-5 (ADAMTS-4,-5) ADAMTS-4 and ADAMTS-5. MMPs are major contributors to the degenerative process occurring during OA pathogenesis, MMP-3 is a stromelysin and MMP-13 is a collagenase with substrates that include collagen II present in articular cartilage [24]. ADAMTS-4 and -5 are aggrecanases involved in aggrecan degradation [25]. The inter- and intra-assay variability for MMP-3 was 6.3 % and 9.7 % and for MMP-13 the inter-and intra-assay variability wshows biomarker as 7.7 % and 9.3 %. The inter- and intra-assay variability for ADAMTS-4 was 5.3 % and 8.7 % and for ADAMTS-5 the inter-and intra-assay variability from our study 9.2 % and 9.3 %.

(3) Inflammatory cytokines – Interleukin-1β (IL-1β), IL-6, IL-8, RANTES (regulated on activation, normal T-cell expressed and secreted). These cytokines are commonly cited as being involved in the pathophysiology and progression of knee OA [26]. The inter- and intra-assay variability from our study for IL-1β was 7.3 % and 9.5 %, for Il-6 it was 8.7 % and 10.5 %, for IL-8 it was 6.5 % and 8.3 % and RANTES it was 7.2 % and 8.5 %.

COMP (catalog #DCMP0), CTX-II (Catalog #EEL037), ADAMTS-4 (Catalog #DY4307-05) and ADAMTS-5 (Catalog #DY2198-05) were determined in duplicate per sample using commercially available solid-phase enzyme-linked immunosorbent assays (R and D Systems, Minneapolis, MN, USA) per the manufacturer's instructions. For inflammatory cytokine and MMP analysis, a custom multiplex immunoassay (Luminex, R&D Systems, Minneapolis, MN) was used, also in duplicate based on the xMAP platform and concentrations tested in duplicate.

1.4. Kinematic analysis

Kinematic data were collected using an inertial measurement unit (IMU) based motion capture system- MTw Awinda (Xsens Inc., Enschede, The Netherlands) [27]. The system employs real-time data collection at a sampling frequency of 100 Hz and processes the data through advanced filtering algorithms, including an Extended Kalman Filter (EKF), low-pass filtering, and biomechanical model constraints, to ensure high accuracy and reliability in estimating joint angles and motion trajectories [27–29]. In the experiments, the IMUs were placed at the numbered locations shown in Fig. 2 namely the right foot, right thigh, right calf, left foot, left thigh, left calf, and lumbar region.

Xsens MVN software that estimates orientation of segments by with a biomechanical model of the human body were used [27,30]. The raw data was analyzed using custom-written Matlab code that identified gait patterns using foot contact data and normalized the kinematic data across the 100 % gait cycle. Kinematic parameters (flexion/extension and abduction/adduction) during the midstance of the gait cycle were measured in keeping with prior studies [31]. In addition, participant cadence and stride length were averaged across the gait cycle.

1.5. Statistical analysis

Data analysis was conducted using Stata 17 (StataCorp. 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC.), for regression models, R 4.0.2 (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical

Computing, Vienna, Austria.) for figures and StatTag for reproducibility [32]. We estimated differentces in serum biomarker concentrations between time points and knees using linear mixed effects models with interactions for time and knee and a participant specific random intercept to account for repeated measurements. Absolute changes in serum biomarker concentration was utilized in this study as has been commonly utilized in prior stimulus-response studies [8,10,33]. We used $p < 0.05$ to determine statistical significance of individual fixed effects terms, equivalent to the associated 95 % confidence interval not crossing zero. For the ACLR knee, we used linear regression to estimate associations (beta coefficients, 95 % confidence intervals, correlations and associated p-values) between changes in biomarker concentrations from 0 to 30 min and changes in stride characteristics. We examined normality of all biomarker outcomes prior to regression analyses. Because both ADAMTS4 and ADAMTS5 were skewed, we log transformed values for analyses.

We also used linear regression to confirm that changes in biomarker concentrations in the ACLR knee were not significantly associated with months since surgery. In addition, we estimated linear mixed effects models for our primary outcome, COMP, that included sex, age, months since surgery, and interactions between sex and time, age and time and months and time. Because results were substantially similar, and there was no evidence with significance set at $p < 0.05$ that sex, age or months explained changes in COMP over time, or that changes in COMP in the ACLR knee over time were associated with sex, age or months since surgery, we report estimates from our original, unadjusted models.

2. Results

2.1. Study participant characteristics (Table 1)

All participants completed all study visits. The mean age of participants was 27.9 ± 5.6 years. Participants (n = 16) consisted of 11 females (69 %) and 5 male (31 %) individuals who had a history of unilateral ACLR. At the time of testing, participants had an average of 54.9 months (± 31.5) since their ACLR. The mean speed of participants walking was 2.4mph (± 0.5).

2.2. Numeric Pain Rating Scale

None of the participants had any knee joint pain (0 out of 10 pain) at baseline during study visits or during tilted walking trials (either when tilted towards the ACLR or contralateral knee).

2.3. Changes in serum biomarker concentrations in response to tilt walking

Supplemental Table 1 shows average serum biomarker concentrations at baseline and 30 min, by knee. Table 2 shows differences in serum biomarker concentrations from baseline to 30 min for the contralateral and ACLR knees. We discuss findings for COMP followed by those for degradative markers and inflammatory cytokines. Fig. 3 illustrates levels of COMP at 0 and 30 min, by knee, for each participant and overall. The boxplot on the right shows differences in changes in COMP for the ACLR and contralateral knees. There was a statistically significant increase in COMP over 30 min for the ACLR knee (41.1 ng/mL, 95 % CI: 13.8, 68.4) (Table 2). In contrast, the change in COMP for the contralateral knee was not statistically significant (22.9 ng/mL, 95 % CI -15.8, 61.5) (Table 2). Baseline levels of COMP were not significantly different between knees (−7.0 ng/mL, 95 % CI -34.4, 20.3) (Supplmental Table 1).

Table 1
Participant characteristic's, including age, gender, time since ACLR and treadmill speed (n = 16).

Characteristic (N = 16)		
Gender		
Male, N (%)	5	(31 %)
Female, N (%)	11	(69 %)
Age, years, mean (SD)	27.9	(5.6)
Time since ACLR, mean (SD)	54.9	(31.5)
Treadmill speed, mph, mean (Standard Deviation)	2.4	(0.5)
Stride length - contralateral, cm, mean (SD)	122.5	(21.4)
Stride length - ACL, cm, mean (SD)	124.1	(21.6)

2.3.1. Cartilage degradative markers and inflammatory cytokines (Figs. 4 and 5)

Figs. 4 and 5 illustrate levels of degradative markers and inflammatory cytokines, respectively, by knee, for each particiant and overall. When tilting towards the ACLR knee, there was no increase in degradative markers compared to baseline, with the exception of log(ADAMTS5), which increased modestly (0.37 units, 95 % CI 0.01, 0.74). Similarly, there was no significant increase in any of the inflammatory cytokines (Fig. 5, Table 2). When the contralateral knee was tested in a similar manner, there was also no significant increase in concentration compared to baseline.

2.3.2. Knee kinematics

The mean stride length was 125.8 cm (± 20.9) and 123.8 (± 20.4) cm in the contralateral knees and ACLR knees, respectively when tilted towards that particular side. Mean cadence was 102.1 steps/min when tilted towards the ACLR knee and 102.9 steps/min when tilted to the contralateral side. Compared to baseline, when tilting towards the ACLR knee, the knee adduction/abduction angle of the ACLR knee changed by −0.33° (± 0.266, i.e more adduction) and the knee flexion angle changed by −2.18° (± 1.92 i.e more flexion). In the contralerateeral knee the adduction angle changed by −0.11° (± 0.18) and the knee flexion angle changed by −2.18° (± 2.08).

2.4. Biomarker changes and knee kinematics

Supplemental Figs. 1 and 2 illustrate the association between changes in cartilage degradative markers and inflammatory cytokines, respectively, and gait parameters. We discuss findings for COMP followed by those for degradative markers and inflammatory cytokines. Fig. 6 illustrates the relationship between change in COMP concentration and knee adduction/abduction angle at 30 min. There was a significant negative association: the more the knee progressed toward adduction, the larger the increase in COMP concentration ($r = -0.58$, $R^2 = 0.34$, $p = 0.02$, Beta = −100.6, 95 % CI −180.9, −20.2). There was no significant correlation in other kinematic and gait parameters measured — flexion/extension angle, stride length and cadence (Supplemental Fig. 1). There were no statistically significant associations between any of the other degradative markers and inflammatory cytokines and knee kinematics.

3. Discussion

The aim of our pilot study was to assess the impact of a selective loading paradigm on serum biomarker responses in individuals with ACLR knees and their contralateral non-injured knee. In keeping with our primary hypothesis, we found a significantly higher serum COMP response compared to baseline when tilting towards the ACLR knee after 30 min of walking. This was not observed when tilting towards the non-surgical knee.

COMP was the primary biomarker chosen for this study since it is known to be mechanosensitive to joint loading [8,10,12,17,34]. COMP diffuses from the synovial fluid (SF) into the lymphatics and then bloodstream with correlations between its concentration in the SF and serum [35–37]. Serum COMP concentration is related to an increase in cartilage metabolism [38] and independently associated with a decrease in articular cartilage volume in individuals in OA [11]. A recent study utilizing both imaging and serum biomarker evaluations in individuals with ACL injury and bone marrow edema lesions (BMELs) found higher pre-surgical COMP concentrations in ACL-injured patients compared

Table 2
Differences in serum biomarker concentrations at baseline and at 30 minutes of tilt walking.

Biomarker ng/mL	Baseline Differences (Contralateral – ACL)		Change in Contralateral Knee (30 Min – Baseline)		Change in ACL Knee (30 Min – Baseline)		Differences in Changes (ACL – Contralateral)	
	Mean	(95 % CI)	Mean	(95 % CI)	Mean	(95 % CI)	Mean	(95 % CI)
COMP	−7.0	(-34.4, 20.3)	18.2	(-9.1, 45.6)	**41.1**	**(13.8, 68.4)**[a]	22.9	(-15.8, 61.5)
CTX-II	−1.1	(-1.9, −0.3)	0.8	(0.0, 1.6)	0.7	(-0.1, 1.5)	−0.1	(-1.2, 1.0)
MMP-3	3186.2	(-3173.2, 9545.6)	2697.6	(-3661.8, 9057.0)	6064.3	(-295.1, 12423.7)	3366.7	(-5626.9, 12360.3)
MMP-13	56.7	(-500.9, 614.3)	489.6	(-68.0, 1047.2)	374.0	(-183.6, 931.6)	−115.5	(-904.1, 673.0)
ADAMTS4	−0.0	(-0.5, 0.5)	−0.0	(-0.6, 0.5)	−0.4	(-0.9, 0.2)	−0.3	(-1.1, 0.4)
ADAMTS5	−0.0	(-0.2, 0.1)	0.0	(-0.1, 0.1)	0.0	(-0.1, 0.1)	0.0	(-0.1, 0.2)
Log(ADAMTS4)	−0.2	(-0.7, 0.3)	0.1	(-0.4, 0.6)	−0.1	(-0.6, 0.4)	−0.2	(-0.9, 0.5)
Log(ADAMTS5)	0.2	(-0.2, 0.5)	0.2	(-0.2, 0.6)	0.4	(0.0, 0.7)	0.2	(-0.3, 0.7)
IL-1Beta	−15.7	(-39.8, 8.5)	16.0	(-8.5, 40.5)	−7.4	(-31.1, 16.2)	−23.4	(-57.5, 10.6)
IL-6	−11.1	(-55.2, 33.0)	16.2	(-27.0, 59.4)	−4.4	(-49.0, 40.2)	−20.6	(-82.7, 41.5)
IL-8	2.5	(-33.5, 38.6)	11.5	(-24.9, 48.0)	20.9	(-14.4, 56.3)	9.4	(-41.3, 60.2)
RANTES	284.0	(-2615.3, 3183.3)	1448.6	(-1450.7, 4347.9)	635.7	(-2263.6, 3535.0)	−812.9	(-4913.1, 3287.3)

Estimates are derived from a linear mixed effects model with an interaction for time and knee with a subject specific random intercept, and are based on n = 16 participants, except for CTX-II (n = 14), MMP-3 (n = 13), and MMP-13 (n = 10).
[a] Significant difference in biomarker concentration at 30 min compared to baseline.

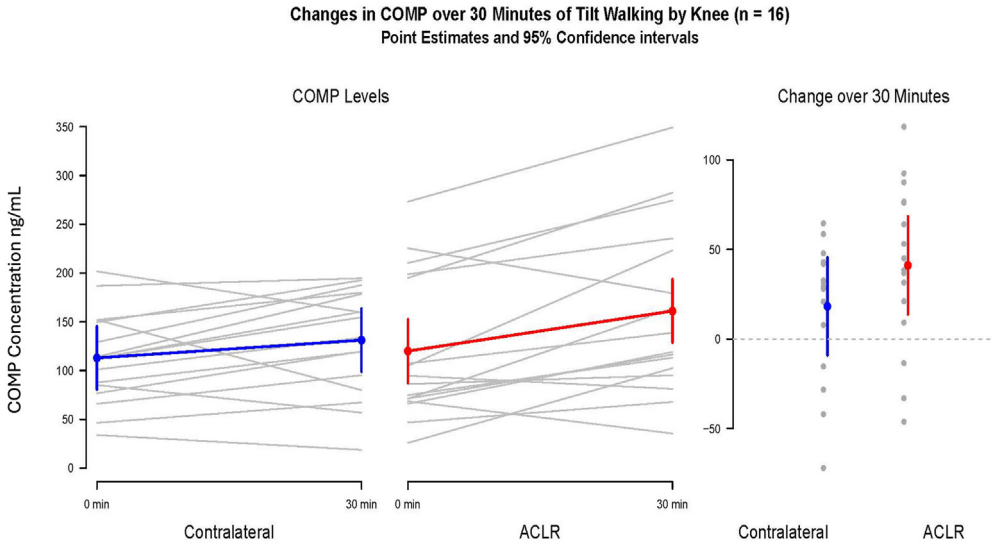

Fig. 3. Point estimates and 95 % confidence intervals for serum COMP concentration at baseline and after 30 min of tilt walking, as well as changes in COMP concentration, in 16 participants, separately for ACLR and contralateral knees.

with controls. Similarly Nishida et al. [39] found that. in individuals with known ACL deficient knees, serum concentration of COMP was higher in individuals with early OA changes on arthroscopy compared to those without. Prior studies have measured biomarker responses in response to loading in an ACLR cohort, typically usually flat walking as the stress, with challenges in differentiating biomarker changes to a specific knee [40–43]. Our study utilizing a novel mechanosensitive stress, showed that in a cohort of ACLR individuals without joint pain or clinical evidence of OA, at least 2 years post-surgery, there were differing biochemical responses when testing the ACLR knee to the contralateral knee at 30 min. However our study also suggested that the other biomarkers tested did not have the same level of mechanosensitivity as COMP. This is similar to Fischer et al. [33] who found that there were no changes compared to baseline in the serum pro-inflammatory cytokine (TNF-α and IL-1β) concentration even at 3.5 h following 30 min of ground level treadmill walking.

Few studies have related biomarker changes to kinematic parameters in post-ACLR individuals. In our study, in keeping with our secondary hypothesis, the increase in serum COMP noted also had a significant association with the change in the knee adduction/adduction of the ACLR knee when the treadmill was tilted towards this knee. In one of the few studies that have investigated the relationship change in biomarkers and biomechanics in response to walking in ACLR individuals, Fischer et al., showed there was a significant correlation of change in TNF-α SF concentration to knee extension moment and IL-1β SF concentration to knee adduction moment at 3.5 h following 30 min of ground level walking in individuals 2 years post-ACLR. This was not shown in our study and may have been due to our biomarker concentrations only being measured immediately after 30 min of walking (rather than 3.5 h). In addition, the type of walking exercise chosen (tilted walking) may have led to differential findings compared to flat walking. Of note, Dewig et al. [44] showed that aberrant gait biomechanics were only shown in individuals post-ACLR when walking on graded surfaces (uphill or downhill) which was not shown with flat walking. The tilted treadmill utilized in our study provides a methodology by which an individual knee receives a differing loading profile and therefore comparison can be made of each individual joint. A prior study showed that walking on side-sloped surfaces similar to our treadmill was associated with greater external rotation of the lower knee at heel strike [45]. Our pilot study does show that this methodology is able to differentiate ACLR knees without pain from contralateral non-injured knees based on their biochemical response to walking and this biochemical response was associated with the change in kinematics which may play a role in OA development. Prior studies have shown potential mechanosensitivity of

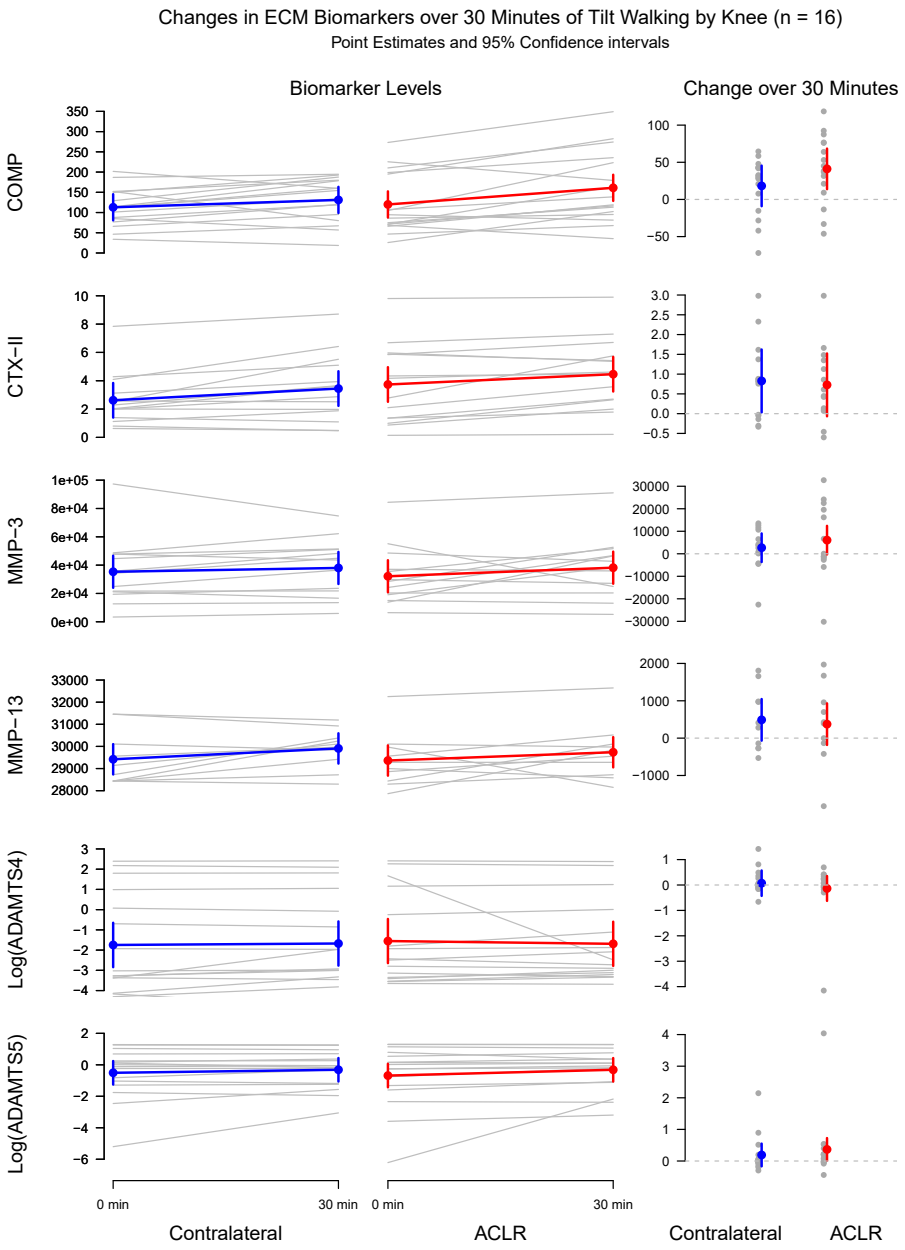

Fig. 4. Point estimates and 95 % confidence intervals for serum concentrations of degrative markers (CTX-II, MMP-3, MMP-13, and log transformed ADAMTS4 and ADAMTS5) at baseline and after 30 min of tilt walking, as well as changes in these biomarkers, separately for ACLR and contralateral knees. Estimates are based on 16 participants, except for CTX-II (n = 14), MMP-3 (n = 13), and MMP-13 (n = 10). Biomarker concentrations are in ng/mL for all.

some of the biomarkers tested in this study (MMP-3 [46], ADAMTS-4 [46], IL-1β [38,46,47], and IL-6 [38,46]). However the majority of these studies were performed in individuals without OA in which potential biomarker responses are likely different, or following long-duration and/or high impact exercise such as marathon running.

Cartilage oligomeric matrix protein (COMP), C-terminal cross-linked telopeptides of type II collagen, matrix metalloproteinase-3,-13, ADAM metallopeptidase with thrombospondin type 1 motif 4-,-5 (ADAMTS-4,-5), interleukin-1β,-6,-8 and RANTES (regulated on activation, normal T-cell expressed and secreted.

The ultimate goal of this type of methodology is to identify individuals who will have an accelerated development of PTOA following ACLR. The majority of longitudinal studies of OA progression have attempted to assess the relationship between pre-surgical variables of joint disease and progression of cartilage pathology post-ACLR. For example, the age of an individual at time of injury, BMI [48], presence of bone marrow lesion on MRI [49] and concentration of pre-surgical inflammatory mediators [50] in the SF have all been shown to have some relationship to the development of PTOA. Importantly, in our study, there were no significant association between participant age or months since ACLR surgery (known risk factors for the development of PTOA) and change in serum COMP concentration. A recent study [51] showed changes in serum levels of inflammation and matrix degradative markers (COMP and monocyte chemoattractant protein-1 (MCP-1)) pre-operatively to 6-months post-surgery had relationships to MRI changes of lateral tibiofemoral cartilage proteoglycan density at 12 months [51]. However, beyond 1–2 years there still remains a lack of methodologies to identify early developers of OA. By stressing the knee joint post-ACLR using the tilted treadmill in our study, we were able to elucidate a biomarker response that has relationships to the biomechanics of the knee joint and gait parameters associated with the presence and progression of knee OA, specifically the knee adduction angulation [52–55].

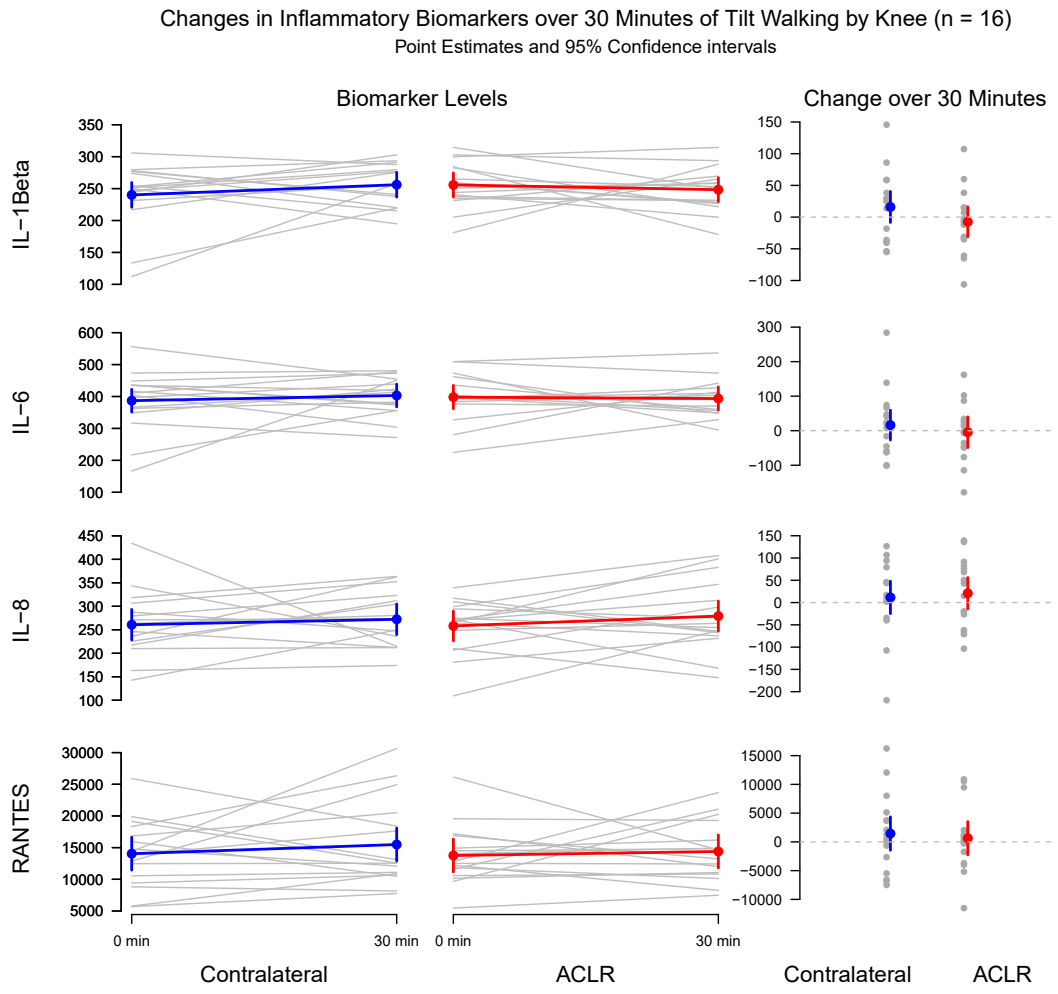

Fig. 5. Point estimates and 95 % confidence intervals for serum concentrations of inflammatory cytokines (IL-1β,-6,-8 and RANTES) at baseline and after 30 min of tilt walking, as well as changes in these cytokines, in 16 participants, separately for ACLR and contralateral knees. Inflammatory cytokine concentrations are in ng/mL for all.

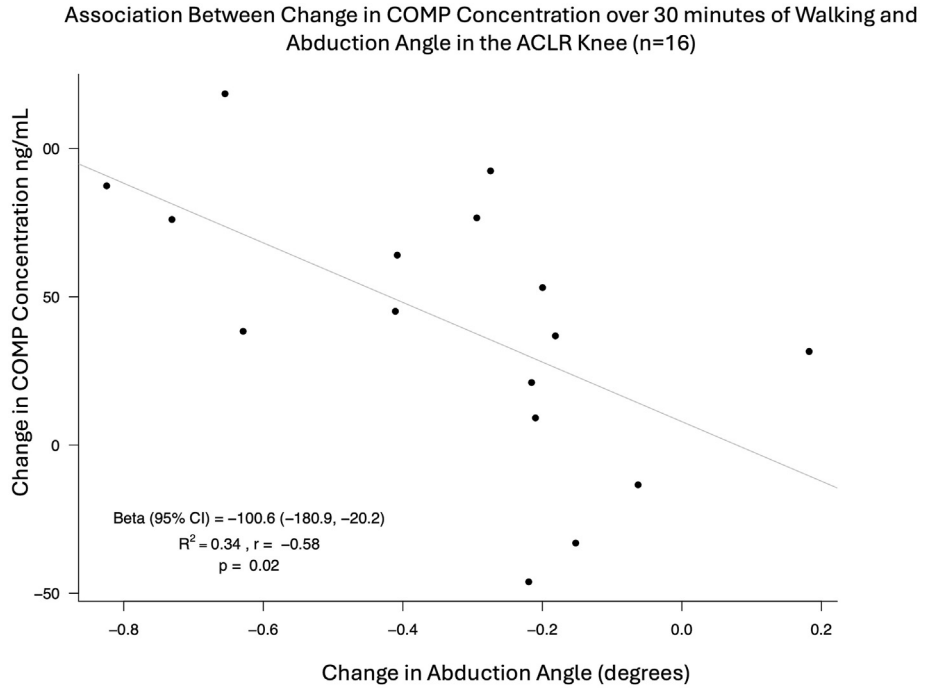

Fig. 6. Association between change in serum COMP concentration and change in knee adduction/abduction angulation in the ACLR knee for n = 16 participants.

3.1. Limitations

Due to the small sample size and pilot nature of our study, we were not statistically powered to look at differences across conditions (ACLR tilt vs. contralateral tilt). Although participants varied in time post-ACLR surgery, we found no evidence that this was associated with biomarker changes in the ACLR knee. Unfortunately surgical findings from the original reconstruction were unavailable and hence we did not have information on ACL graft source and/or concomitant meniscal injury.

Serum COMP is present in a number of tissues in the body; however, the concentration of COMP in non-articular tissues is several magnitudes lower than the articular cartilage. It is possible that the serum COMP and other biomarker responses observed may be from other tissues containing articular cartilage such as the hip or ankle since their biomechanical parameters also change during tilt walking. To limit this issue, participants were excluded if they had a history of prior spine, hip or ankle surgery or injury. However, it is important to note that baseline biomarker concentrations could be contributed from both knees rather than a specific knee (ACL or contralateral). Future studies would need to aspirate SF to provide a more accurate measurement of COMP contribution of each knee. Kuhne et al. [56], did find persistent significant correlations between serum and SF COMP concentrations in indviduals at two years following traumatic knee injury. Finally, our study measured biomarker responses immediately after 30 min of walking; however, future studies could measure the longer post-walking biomarker changes and in turn may observe a mechanosensitive response in inflammatory and cartilage degradative markers.

4. Conclusion

This study provides evidence of the ability of the tilted treadmill paradigm to identify a differential response of a serum mechanosensitive marker (COMP) when testing an ACLR knee compared to non-injured knees. In addition, our study also found a significant relationship between COMP response to tilt walking and the change in the knee adduction angle of the ACLR knee. It is important to note that COMP concentrations could also be raised due to biomechanical changes in other joints. However, this paradigm can serve as an aid to provide individual knee biomarker response to a mechanical stress in asymptomatic individuals at risk for the development of PTOA at least 2 years post ACLR. This pilot study is the first step to creating a database of biomarker responses that could serve as the basis for the development of a longitudinal study that relates these responses to the development of PTOA or cartilage loss on imaging studies. Identifying those at most risk for PTOA development could lead to earlier intervention strategies in these individuals.

Author contributions

Prakash Jayabalan – Study design, recruitment, analysis, write-up.

Rose Darcy – Study recruitment, experimental setup, biomarker analysis.

Vikram Darbhe – Study experimental setup, biomechanical testing and analyses.

Alexander Neuville – Study recruitment, experimental setup, biomarker analysis, Vehniah Tjong – Study recruitment, experimental setup.

Levi Hargrove – Experimental setup for biomechanical analysis.

Leah Welty – Statistical analytical plan, Data analysis, Thomas Schnitzer - Study design, recruitment, analysis, write-up.

Declaration of competing interest

All authors declare that they have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocarto.2025.100619.

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