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Title: **Body Mass Index influences tibiofemoral cartilage composition and biochemistry in individuals with anterior cruciate ligament injury**

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Declaration

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Body Mass Index Influences Tibiofemoral Cartilage Composition and Biochemistry in Individuals With Anterior Cruciate Ligament Injury

Context: A history of anterior cruciate ligament (ACL) injury and high body mass index (BMI) are strong risk factors for incident knee osteoarthritis (KOA). Limited research has evaluated the interaction between ACL injury and high BMI on early deleterious changes in cartilage health.

Objectives: The purpose of this study was to determine differences in T1ρ relaxation time and serum cartilage oligomeric matrix protein (sCOMP) concentration between individuals with high and normal BMI following an ACL injury.

Design: A cross-sectional study

Setting: A controlled laboratory setting

Patients: Forty-two participants with primary ACL injuries were assigned to either the high-BMI (>25kg/m^2; n=20; age: 22.9±5.1; time between injury and visit: 3.6 ± 2.2 weeks) or normal-BMI (≤25kg/m^2; n=22; age: 21.1±4.2; time between injury and visit: 3.3 ± 1.7 weeks) group based on their BMI.

Main Outcome Measure(s): T1ρ relaxation time for the medial and lateral tibia (MTC and LTC) and femur for each participant, sCOMP concentrations, Knee Injury and Osteoarthritis Outcome Score (KOOS).

Results: The high-BMI group, regardless of limbs, demonstrated greater T1ρ relaxation times in LTC (mean difference: 2.1±0.1ms; *p*=0.004; *d*=0.77) and MTC (mean difference: 1.7±0.4ms; *p*=0.04; *d*=0.44) knees compared to the normal-BMI group. The high-BMI group showed greater concentrations in sCOMP compared to the normal-BMI group (mean difference: 26.4±15.8 ng/mL; p=0.02; d=0.72). There were no differences in KOOS scores between group.

Conclusions: Overweight individuals experiencing primary ACL injuries exhibit higher T1ρ relaxation times in both their injured and uninjured limbs and sCOMP concentrations compared to normal-weight individuals with primary ACL injuries. Our findings indicate that BMI significantly impacts cartilage composition and biochemical changes associated with KOA development in individuals with ACL injuries within 3.5 weeks of injury.

Keywords: knee, proteoglycan density, biomarkers, osteoarthritis

Key Points

- Overweight individuals after ACL injuries show higher T1ρ relaxation times both injured and uninjured limbs.
- Overweight individuals with ACL injuries exhibit higher sCOMP concentrations compared to normal-weight individuals.
- BMI significantly impacts cartilage composition and biochemical changes associated with KOA development.

INTRODUCTION

History of a knee injury, such as anterior cruciate ligament (ACL) injury,[1] and high body mass index (BMI)[2] are primary risk factors for knee osteoarthritis (KOA) development. Between 30-50% of individuals with ACL injury develop radiographic KOA within two decades of ACL injury regardless of whether they undergo ACL reconstruction (ACLR).[3] People with knee injuries increase their BMI percentile by up to 5 units more than someone of the same age without an injury.[4] An increase in body weight has been linked to the development of aberrant movement biomechanics[5] and excessive limb and joint-level forces during daily activities, which may adversely load joint tissues and hasten knee cartilage breakdown.[6] Further, high BMI is often associated with greater body adiposity which is linked to systemic biological changes (i.e., greater inflammation) that may also contribute to accelerated joint tissue breakdown following ACL injury and ACLR.[7]

Most studies exploring the development of altered biomechanical and biological factors that increase the risk for joint tissue breakdown include patients many months to years following ACL injury and ACLR.[7,8] While previous research has shown that high BMI negatively affects cartilage composition and mechanical properties in otherwise healthy,[9] often sedentary individuals, it remains unclear whether similar degenerative changes are present in more active individuals with high BMI at the time of ACL injury. Furthermore, preoperative (i.e., post ACL injury but prior to ACLR) measures of cartilage health are commonly used as a baseline assessment for studies seeking to track KOA-related outcomes longitudinally;[10] however, it remains unknown whether individuals with high BMI exhibit different cartilage outcomes compared to those with normal BMI following ACLR. In the current study, we measured T1ρ relaxation times and serum cartilage oligomeric matrix protein (sCOMP) concentration that have

been identified as indicators of knee cartilage degeneration.[11] Identifying such early associations between preoperative BMI and outcomes of cartilage breakdown could inform timely, BMI-targeted interventions that begin preoperatively and during the acute recovery phase post-ACLR. Further, identifying patients at highest risk of cartilage breakdown following ACL injury should inform the development of future clinical trials seeking to apply targeted treatment strategies to patients with the greatest need for intervention.

T1ρ relaxation times acquired from quantitative compositional magnetic resonance imaging (MRI) have been utilized to estimate proteoglycan density in the tibiofemoral articular cartilage and assess early compositional changes related to pre-structural KOA development following ACL injury and ACLR.[12] Reduced proteoglycan density is an early marker of articular cartilage degeneration and a sign of KOA development.[13] Altered tibiofemoral T1ρ relaxation times, indicating worse proteoglycan density, have been reported in the ACLR limb as early as 6 to 12 months, compared to contralateral limb and limbs of uninjured control.[10,14] Obesity is also linked with higher T1ρ MRI relaxation times in uninjured tibiofemoral cartilage, observed in individuals with high BMI compared to those with normal BMI.[9]

Cartilage oligomeric matrix protein (COMP) is a glycoprotein essential for organizing and stabilizing the extracellular matrix of articular cartilage to withstand mechanical stress.[15] Serum COMP (sCOMP) is associated with KOA onset and progression.[11] Additionally, higher sCOMP levels can predict future cartilage breakdown after an ACL injury, as seen by arthroscopy and MRI.[16,17] Greater sCOMP is also associated with higher BMI ($r=0.63$, $p<0.001$) in individuals with KOA.[16] Overall, T1ρ relaxation times and sCOMP have been identified as indicators of knee cartilage degeneration for individuals with high BMI and ACLR. However, it remains unclear how T1ρ relaxation times and sCOMP differ after ACL injury in people with

high and normal BMI. Understanding the role of preoperative BMI on outcomes of cartilage health in the weeks following ACL injury is critical for: 1) determining how to best clinically manage BMI preoperatively and 2) manage BMI in future longitudinal ACLR clinical trials that use cartilage measurements as main outcome measures.

Therefore, the purpose of the current study was to determine differences in T1ρ relaxation time between individuals with high ($\geq 25 kg \cdot m^{-2}$) and normal BMI ($<25\ kg \cdot m^{-2}$) following an ACL injury and between limbs in individuals with high BMI. We also aimed to determine differences in sCOMP levels between individuals with high and normal BMI following an ACL injury. We hypothesized that ACL patients with high BMI will demonstrate greater tibiofemoral articular cartilage T1ρ relaxation times in their injured knees compared to their uninjured knees and the injured knees of ACL patients with normal BMI. Furthermore, we hypothesized that ACL patients with high BMI would have higher sCOMP levels compared to other patients with normal BMI.

METHODS

Study Design

MRI data were collected at a preoperative timepoint as part of a larger longitudinal cohort study evaluating biomechanical and biological outcomes in ACL injured patients scheduled for ACLR. Our current study was cross-sectional in nature and incorporated outcomes of tibiofemoral cartilage composition and sCOMP concentration in individuals (aged 16-35 years old) with ACL injuries categorized as exhibiting high (BMI $\geq 25 kg/m^2$; n=20) and normal (BMI $< 25 kg/m^2$; n=22) BMI based on World Health Organization guidelines.[18] Each participant completed a compositional MRI session and a venous blood collection session on the same day 25±13 days following ACL injury but prior to ACLR. For purposes of characterizing our cohort,

all participants completed the Knee Injury Osteoarthritis Outcomes (KOOS) survey, assessing self-reported knee function through five scales, including symptoms, pain, activities of daily living, sport and recreation, and quality of life. The study methods and recruitment procedures received approval from the Institutional Review Board at the XXX, and all participants provided written informed consent before participation. For participants under the age of 18, informed assent was obtained directly from the minors, and written parental permission was secured from their legal guardians.

Participants

Participants with ACL injuries were recruited from orthopaedic practices within the university health system. Participants with concomitant meniscal injuries at the time of ACL injury were included in the study if no more than 1/3 of their meniscus was removed at the time of ACLR. Participants were excluded if they had a history of multiple ACL injuries, planned multi-ligament reconstruction at the time of ACLR, lower extremity fracture at the time of ACL injury, any type of arthritis, BMI $\geq$ 36 kg/m^2, or an immunodeficiency disorder. Pregnant females were also excluded. We calculated that 18 participants in each group would be required to identify statistically significant differences (p<0.05) in this cross sectional study with an alpha level of 0.05 and a power (1-β) of 0.8. Due to the lack of directly applicable previous data for our specific research question, we used a medium effect size (Cohen's d = 0.69) as recommended by Cohen for studies where prior data is unavailable or insufficient to determine an effect size.[19]

Magnetic Resonance Image Acquisition

Prior to acquiring MR images, participants remained seated with their legs extended for 30 minutes to unload the knee cartilage. T1ρ MRI images were acquired with a Siemens Magnetom Prisma 3T PowerPack scanner with an XR 80/200 gradient knee coil (60 cm × 213

cm, Siemens, Munich, Germany). We used a 3D magnetization-prepared angle-modulated partitioned-k-space spoiled gradient echo snapshots sequence within one acquisition, a spin-lock power at 500Hz, four different spin lock times (TSL: 70, 30, 10, 0ms) and a voxel size of 0.44mm x 0.884mm x 4mm (field of view=140mm x 140mm x 96mm, slice thickness=4.0mm, TR = 6.37ms, 320 x 160 x 24 matrix, phase encode direction of anterior/posterior).[20] Double echo steady state (DESS) MR images were also taken using the same scanner and gradient coil for use in training an automatic image segmentation network (TR/TE = 17/6.3ms, FA = 25˚, field of view = 140mm x 140mm x 96mm, matrix = 512 x 512 x 96, voxel size = 0.3125mm x 0.3125mm x 1mm).

Segmentation of the Articular Cartilage and T1ρ Calculation

To speed up the image segmentation process, a 55-layer U-net image segmentation network trained in MatLab (MatLab R2023b [23.2.0] MathWorks, Natick, MA, USA) on 34 investigator-labeled DESS knee images was used to semi-automatically segment the medial/lateral tibial (MTC and LTC) and femoral (MFC and LFC) cartilage in T1ρ MR images. Subjects used for the training of the image segmentation network were not used in the study, but the subjects' respective DESS and T1ρ MR images were taken using the same sequence parameters outlined previously. Before being used to train the segmentation network, DESS images were cut down from 512 x 512 x 96 voxel matrices to 480 x 480 x 96 voxel matrices and, if necessary, flipped to a right leg orientation. The network was trained with a randomly selected train/validate/test split of 88/10/2 to output binary images identifying three classes of voxels (background, femoral cartilage and tibial cartilage) over 65 epochs with a mini batch size of 2 and a reshuffling of the training data every epoch. Validation loss was calculated every 20 iterations of the segmentation network and the network with the lowest calculated validation loss

was the final network output by the training process. Once a network was acquired from the training process, the test set was semantically segmented with the trained network and compared with the respective subject's manual segmentation of the cartilage via DICE and IoU scores. This trained network could then be adapted to semi-automatically segment cartilage in T1ρ MR images with different resolution and voxel sizes. The process involved automatically segmenting a subject's DESS knee image and then moving the acquired cartilage label map to the T1ρ image coordinate system from the DESS image coordinate system via an affine transform. The DESS resolution cartilage label map could then be resampled to T1ρ image resolution and the investigator could then modify any non-anatomically accurate data points output by the network.

The three regions of interest that were sub-sectioned represent load-bearing regions and included: 1) the cartilage that corresponds with the anterior horn of the meniscus (Anterior-MFC/LFC & Anterior MTC/LTC); 2) the central portion of the cartilage that lies between the anterior and posterior meniscus (Central MFC/LFC & Central MTC/LTC; and 3) the cartilage corresponding with the posterior horn of the meniscus (Posterior-MFC/LFC & Central-MTC/LTC). The analyses for this study utilized global weightbearing T1ρ relaxation time values made up of the anterior, central, and posterior regions of interest (ROI) for each condyle.

Blood Collection

Blood was drawn from the participant's antecubital fossa using 21-gauge needles and collected into 5mL serum separator tube vacutainers. The samples were then refrigerated for a minimum of 30 minutes to allow clotting. Next, blood samples were centrifuged at 3000 rpm at 4º C for 10 minutes.[21] Serum samples were aliquoted equally into 1mL or 1.8 mL internally and externally threaded cryovials and stored in a freezer at -80º C.

sCOMP Processing

Upon completion of the study, stored serum samples were thawed, and batch processed using a commercially available enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, Minnesota, United States) to determine the concentrations of sCOMP in compliance with the manufacturer's protocol. All standards and unknown samples were performed in duplicates and unknown samples were prepared using 100 µL assay diluent for analysis.[21] A microplate reader was used to determine optical density within 30 min of assay processing. The COMP assay detection sensitivity was <10 $pg \cdot mL^{-1}$, and the intra-assay variability was 2.35%. Samples for a single individual were analyzed on a single plate to control for interassay variation within participants. All unknown sample concentrations were greater than the mean minimal detectable amount (>0.01 ng/mL).

Statistical Analysis

We enrolled 57 participants with ACL injuries who were between the ages of 16 and 35 years. Data normality was assessed using the Shapiro-Wilk test, and Q-Q plots were utilized to visually inspect potential outliers for all outcomes. Among 57 participants, we excluded 7 participants whose T1ρ relaxation times exceeded z score greater than ±3 for at least one of the ROIs. Also, we excluded 8 participants who did not complete either the MRI session and/or blood draw following ACL injury. Thus, the analysis included 42 ACL participants assigned either the high-BMI group (n=20) or the normal-BMI group (n=22) based on their BMI on the testing day (Figure 1).

We assessed differences in group demographics using independent t-tests. Mixed-model ANOVAs were used to determine differences between high-BMI and the normal-BMI groups and between the ACL injured and uninjured limbs for 4 ROIs (LFC, LTC, MFC, and MTC). We chose to analyze T1ρ relaxation times as absolute values rather than using an injured-to-

uninjured limb ratio to preserve the ability to examine systemic influences such as BMI. Normalizing the contralateral limb could obscure the effect of BMI, which may similarly impact cartilage composition in both limbs. We planned to run Tukey HSD post-test when there were significant interactions ($p<0.05$) from the ANOVAs. An independent t-test was performed to compare sCOMP concentrations between the high-BMI and the normal-BMI groups. Two-tailed alpha levels ($p<0.05$) were used to separately assess the differences. Cohen's *d* effect sizes and 95% confident intervals were calculated to estimate the magnitude of differences in T1ρ relaxation times and sCOMP concentrations between groups and limb. (0.21 to 0.50, 0.51 to 0.80, and > 0.80 represented small, moderate and large effect sizes).[19]

RESULTS

The high-BMI group exhibited statistically higher body mass (kg) and BMI than the normal-BMI group ($p<0.0001$, both; Table 1). There were no differences in age, height, time between injury and study visit, or any of the five scales of KOOS scores ($p>0.05$).

Mixed model ANOVAs (Group × Limb) were conducted to examine the effects of BMI and limb on T1ρ relaxation times across cartilage regions. The high-BMI group demonstrated greater T1ρ relaxation times in LTC (mean difference: 2.1±0.1ms; group main effect: $F_{(1, 42)}=9.16$; $p=0.004$; $d=0.77$) and MTC (mean difference: 1.7±0.4ms; group main effect: $F_{(1, 42)}=4.04$; $p=0.04$; $d=0.44$) in both limbs compared to the normal-BMI group (Table 2). There were statistically significant group main effects for MFC and LFC ($p>0.05$). ($p>0.05$). No statistically significant differences were found between limbs for any ROI (limb main effect: $F_{(1, 42)}=0.04$-1.32; $p=0.26$-0.83) and there were no group by limb interactions for any ROIs (interaction: $F_{(1, 42)}=0.003$-0.78; $p=0.38$-0.96).

The high-BMI group demonstrated greater concentrations in sCOMP compared to the normal-BMI group (mean difference: 26.4±15.8 ng/mL; p=0.02; d=0.72) (Table 2).

DISCUSSION

The findings of the current study support our hypotheses that the high-BMI group demonstrates worse articular cartilage composition and more deleterious biochemical outcomes in the several weeks following ACL injury prior to ACLR compared to the normal-BMI group. Further, both the ACL injured limb and uninjured limb demonstrated greater tibial T1ρ relaxation times in the high-BMI group suggesting that high-BMI has deleterious effects on articular cartilage bilaterally prior to unilateral ACLR. It is well established that individuals with ACL injuries experience rapid deleterious changes in cartilage due to biomechanical and biological alterations in joint tissue.3 Our findings suggest that high BMI may exacerbate these early changes even before surgery, potentially independent of the ACL injury itself. These data highlight a particular clinical concern for cartilage health in individuals with BMI ≥ 25 kg/m² and suggest that high BMI may be an important therapeutic target as early as the initial preoperative timepoint. Additionally, future clinical trials aiming to prevent KOA development following ACL injury may benefit from considering preoperative BMI as a modifiable factor influencing early cartilage composition.

Greater T1ρ relaxation times were found in the LTC and MTC of the high-BMI group compared to the normal-BMI group. Partially consistent with our findings, a previous study reported that greater tibial T1ρ relaxation times were found in individuals with high-BMI (average BMI: 32.8kg/m^2) than normal-BMI (average BMI: 22.2kg/m^2) who did not have a history of lower limb injury, surgery, or symptoms related to KOA.[9] Another study also reported that people who reduced their body weight by 9.3% showed a relationship between greater

weight loss and increased proteoglycan content, as well as reduced cartilage thickness loss.[22] Based on the current and previous findings, increased body mass may negatively influence articular cartilage health. Importantly, chondrocyte metabolism is closely linked to its mechanical environment,[23] and previous studies have demonstrated that excessive cartilage loading induced by high BMI can result in reduced synthesis of extracellular matrix components, increased production of pro-inflammatory cytokines, and possibly cell death.[24] Thus, changes in biochemical composition can alter the mechanical properties of cartilage, which in turn can modify the mechanical environment experienced by chondrocytes. This can affect their metabolic activity and potentially contribute to a continuous cycle of degeneration, which can be seen as depleted proteoglycan density.

Interestingly, our results showed greater T1ρ relaxation times of LTC and MTC in the high-BMI group than the normal-BMI group in both limbs of people with unilateral ACL injury. Notably, no statistically significant differences in T1ρ relaxation times were observed between limbs in either BMI group. The absence of interlimb differences early after ACL injury may suggest that BMI may influence cartilage composition systemically, rather than through injury-specific effects. This supports the idea that high BMI contributes to cartilage degeneration both before and after ACL injury. Unfortunately, we were unable to collect a history of BMI, which prevents us from determining the length of time knee cartilage was exposed to excessive load induced by high BMI. We also cannot confirm whether their BMI increased from the point when they had ACL injuries as their activity levels decreased, which could influence cartilage composition. Another potential cause for our results is that unilateral ACL injuries may lead to aberrant loading in both limbs that could affect cartilage changes within approximately 3.5 weeks after ACL injuries. Previous studies found that under and/or overloading can impact T1ρ

relaxation times in individuals with ACLR.[14] However, our data cannot confirm whether the participants exhibited abnormal loading patterns during activities that might lead to harmful changes in cartilage composition. Future studies are warranted to determine how early the cartilage adaptations occur as a result of altered biomechanics during activities in individuals with ACL injuries.

The current study also found that the high-BMI group demonstrated greater sCOMP concentrations than the normal-BMI group. In other words, elevated sCOMP concentrations may reflect the influence of ACL injury and/or high BMI, as both factors have been independently associated with altered cartilage metabolism and joint tissue changes linked to the development of KOA.[25] Consistent with the findings, previous studies separately reported increased sCOMP concentrations in individuals with ACL injuries and high BMI.[17] While our study design does not allow us to determine whether BMI and ACL injury have a synergistic effect, our results suggest that among individuals with ACL injuries, those with high BMI exhibit higher sCOMP concentrations than those with normal BMI. This may indicate that BMI plays a substantial role in influencing sCOMP concentrations in ACL injured patients. A high BMI, resulting from increased body mass, could elevate sCOMP concentrations through more intensive cartilage metabolism due to overloading and/or increased expression of COMP in adipose tissue.[26] However, it is important to note that we did not assess body composition, so we were unable to confirm whether our high-BMI participants have more adipose tissue than the normal-BMI group. Our results suggest that individuals with both ACL injury and high BMI may experience more significant early detrimental changes in cartilage associated with KOA progression and development compared to individuals with ACL injuries and normal BMI. However, further

research—including appropriate control groups—is needed to determine whether BMI and ACL injury interact to influence early cartilage degeneration.

To mitigate detrimental changes in cartilage among individuals with high BMI, dietary weight loss interventions may be primarily recommended.[27]. Weight loss caused by high intensity exercise and diet decreased knee pain, knee joint loading,[28] and level of pro-inflammatory cytokines (i.e., Interleukin-6)[29] associated with cartilage degradation in overweight and obese adults with KOA. Moreover, high BMI is recognized as a primary predictor of initial ACL injuries[3,30] which is a critical factor in the development of KOA. Therefore, it is crucial to manage body mass to prevent people from accelerating cartilage degradation leading to onset of KOA before and after ACL injuries. However, given that high-intensity exercise may not be appropriate during the early stages of ACL injury recovery, such exercise-based interventions may be more feasible and beneficial during later rehabilitation phases. Also, weight loss might not be appropriate for individuals who reach a level of high BMI due to their high muscular volume and/or short height. Still, given that high BMI negatively affects cartilage composition, alternative therapeutic interventions should be applied to prevent cartilage degradation. For instance, previous studies have investigated effects of therapeutic interventions that manipulate vertical ground reaction force during walking on sCOMP concentration and T1ρ relaxation time by providing real time biofeedback for individuals with ACLR. They found that the walking with biofeedback to elicit bouts of high loading increased dynamic nature of vertical ground reaction force during walking, which was related to less sCOMP concentration and T1ρ relaxation time. This intervention has not been yet applied to high BMI individuals with ACL injuries prior to ACLR. Future studies are needed to see whether applying the intervention early could positively affect articular cartilage health.

Limitation

While the current study provides valuable insights into the effects of ACL injuries and high BMI on detrimental changes in cartilage health, several limitations should be considered for future research. First, this study is retrospective, so it is unknown whether participants already had altered proteoglycan density or sCOMP concentration before ACL injuries. Second, some participants reported medial and/or lateral meniscal tears at the time of their ACL injuries, which could affect T1ρ relaxation times and sCOMP concentrations. Third, we did not include an uninjured control cohort with T1ρ relaxation time or sCOMP concentration, which limits us to directly compare cartilage outcomes between injured and uninjured individuals across BMI groups. Our data answer an initial important clinical question about how high BMI impacts cartilage in a patient population (i.e., ACL injured individuals); however, we acknowledge without an uninjured control group we are unable to fully disentangle the underlying mechanisms linking high BMI to more deleterious T1ρ MRI and sCOMP outcomes. Future studies should determine if high BMI or a combination of high BMI and ACL injury impact these outcomes prior to ACLR. Fourth, individuals with a BMI greater than 35 kg/m² were excluded in the parent study, which limits the generalizability of our findings to those with Class II obesity (BMI 35–39.9). Therefore, our results may not fully represent cartilage health or biomechanical adaptations in individuals with higher levels of obesity following ACL injury. Fifth, although individuals with high BMI and ACL injury showed worse baseline cartilage and COMP outcomes, it remains unclear whether this leads to faster PTOA progression after ACLR. Longitudinal studies are needed to clarify these trajectories. Lastly, although BMI is commonly used to assess its associations with ACL injury risk, cartilage degradation, and KOA, it does not capture body composition comprehensively. Therefore, future studies could benefit from

incorporating body composition measures to better understand the relationship between adiposity and early changes in knee articular cartilage associated with KOA development.

CONCLUSION

Individuals with BMI over 25kg/m^2 experiencing primary ACL injuries exhibit higher T1ρ relaxation times in both their injured and uninjured limbs compared to the injured limbs of normal-weight individuals with primary unilateral ACL injury. Additionally, the high BMI group demonstrated greater sCOMP concentration than the normal BMI group. Our findings indicate that BMI impacts cartilage composition and biochemical changes associated with KOA development in individuals an average of 3.5 weeks post-ACL injury. Therefore, future studies are needed to identify effective management of body weight to reduce the risk of developing KOA following ACL injuries.

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445 **Figure 1. Enrollment. A flow chart.**

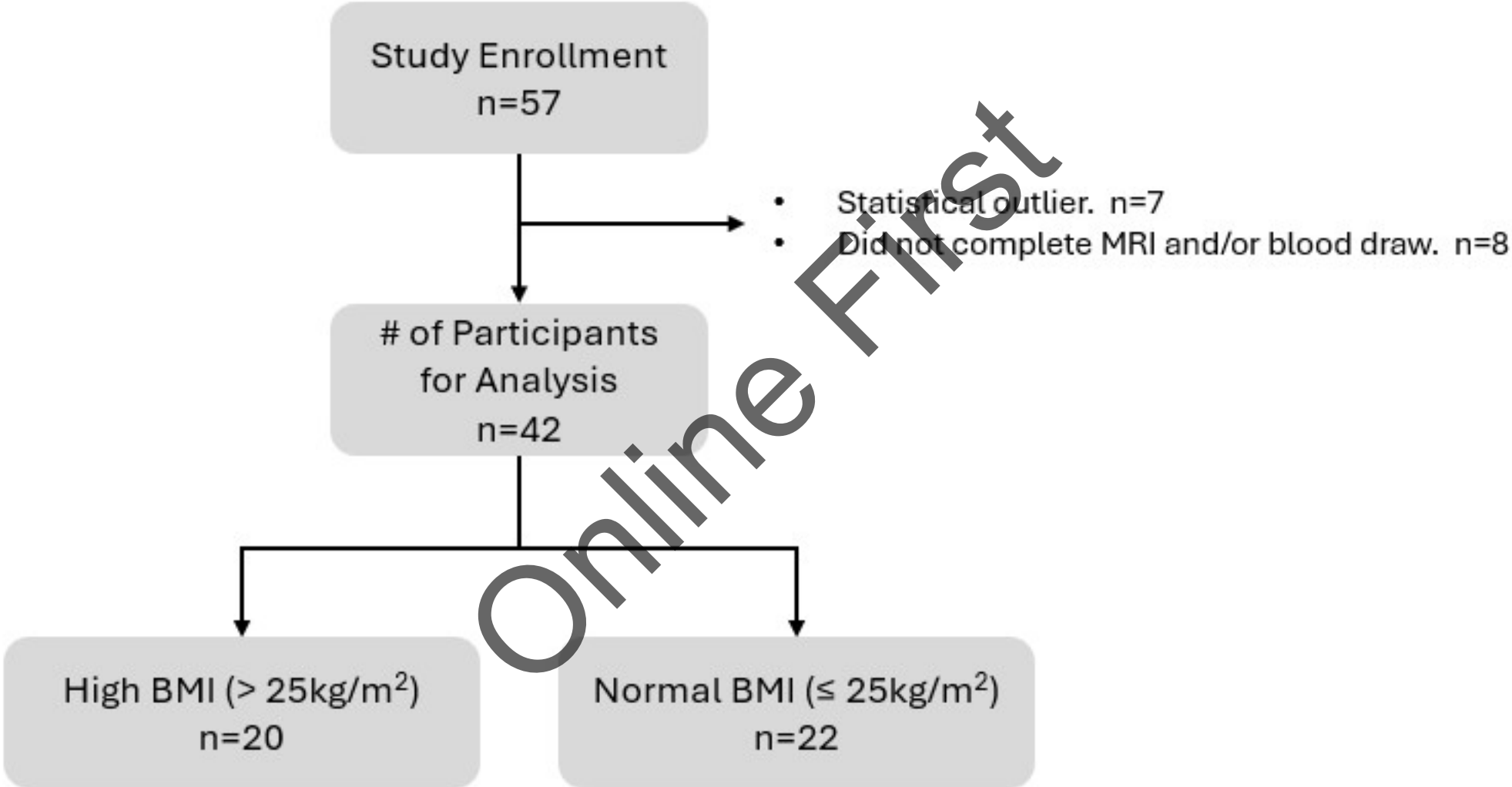

Table 1. Participants' Demographics

	High-BMI (n=20)	Normal-BMI (n=22)	Independent T-test (*p*-value)
Gender	10F / 10 M	14F / 8M	
Age	22.9±5.1	21.1±4.2	0.21
BMI, kg/m^2†	27.9±3.4	22.0 ± 1.8	**< 0.001**
Height, m	1.71 ± 0.08	1.75 ± 0.10	0.13
Mass, kg†	81.5 ± 11.6	67.9 ± 9.3	**< 0.001**
Time btn injury and visit, days	25.2 ± 15.4	23.1 ± 11.9	0.74
KOOS, Pain	68.9 ± 13.7	74.3 ± 11.9	0.11
KOOS, Symptom	64.0 ± 16.5	67.8 ± 13.8	0.35
KOOS, ADL	78.3 ± 16.3	82.7 ± 16.6	0.27
KOOS, Sport	39.0 ± 29.0	40.8 ±20.6	0.79
KOOS, QOL	32.8 ± 20.1	40.7 ± 16.3	0.11
sCOMP, ng/mL†	148.8 ± 44.4	122.4 ±28.6	**0.02**

Abbreviations: BMI, body mass index; btn, between, KOOS, knee injury and osteoarthritis outcome score; ADL, active daily living; QOL, quality of life; sCOMP; serum cartilage oligomeric matrix protein

†: Statistically significant difference (p<0.05) between groups

Table 2. A mixed model ANOVA to investigate differences in T1σ relaxation Time between groups and limbs

	T1ρ relaxation Time (ms)				Mixed-model ANOVA				
	High BMI		Low BMI		Group Main Effect		Limb Main Effect		Group x Limb Interaction
	Injured	Uninjured	Injured	Uninjured	F-ratio (p value)	ES (95% CI)	F-ratio (p value)	ES (95% CI)	F-ratio (p value)
LFC	52.0 (3.4)	51.7 (3.2)	50.6 (4.2)	50.3 (4.5)	2.22 (0.14)	0.38 (-0.05, 0.81)	0.15 (0.70)	0.30 (-0.31, 0.90)	0.003 (0.96)
MFC	51.8 (3.5)	52.9 (3.3)	51.0 (3.0)	51.8 (5.3)	0.85 (0.36)	0.22 (-0.21, 0.65)	1.32 (0.26)	0.61 (-0.03, 1.23)	0.003 (0.96)
LTC†	50.1 (2.6)	49.6 (2.6)	48.0 (2.4)	47.8 (3.1)	**9.16 (0.004)**	**0.77 (0.32, 1.22)**	0.23 (0.63)	0.64 (-0.00, 1.26)	0.03 (0.87)
MTC†	51.8 (5.2)	51.3 (2.0)	49.4 (3.4)	50.3 (3.7)	**4.04 (0.04)**	**0.44 (0.01, 0.88)**	0.04 (0.83)	0.67 (0.03,1.29)	0.78 (0.38)

†: statistically significant difference (p<0.05) in group main effect.

Abbreviations: BMI, body mass index; LFC, lateral femoral condyle; MFC, medial femoral condyle; LTC, lateral tibial condyle; MTC, medial tibial condyle; ES, effect size