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Effect of unloading time, leg, and sex on distal femoral cartilage thickness

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Abstract

Numerous studies that have investigated distal femoral cartilage thickness before and after a prescribed loading activity have included a rest period prior to the loading activity to remove the effects of previous activities that may have compressed the cartilage. The purpose of this study was to systematically determine how unloading time affects cartilage thickness, as well as how sex and leg preference influence the thickness in young, active adults with no knee injuries. Thirty-two healthy college students (16 female, 16 male) who regularly participated in moderate or vigorous physical activity were recruited and instructed to refrain from any physical activity at least 12 h prior to data collection. Ultrasound images were initially obtained for each leg and then every 15 min for an hour (T0, T15, T30, T45, T60) using a Mindray 7 machine. Between collections, they had their legs extended in front of them. Their leg preference was determined using the Waterloo Footedness Questionnaire. Cartilage thickness was measured at the intercondylar notch (IC), medial condyle (MC), and lateral condyle (LC). The results suggest that the cartilage at the LC experiences less compression than the other regions. Among individuals who have reduced their physical activity for at least 12 h, resting with legs extended for 30 min appears to allow the IC and MC cartilage to recover and create equivalent conditions for all participants. While female values were lower than those of males across all regions, there was no difference in their response to unloading.

Keywords: Ultrasound; Leg preference; Medial condyle; Lateral condyle

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1. Introduction

Articular cartilage is an avascular connective tissue that covers bone surfaces and acts to decrease friction within synovial joints. Due to the lack of blood vessels, lymphatics, and nerve input, this tissue has a limited ability to heal.[1] Along with creating a low-friction environment, articular cartilage helps distribute compressive and shear forces while protecting the subchondral bone.[2] At the knee, articular cartilage is especially important due to the large forces acting there in everyday life. Level walking involves forces at the knee that are typically 2.5 times body weight, whereas ascending stairs creates forces nearly six times body weight.[3,4] These repetitive, often large forces make the articular cartilage at the knee particularly vulnerable to injury. Individuals with osteoarthritis have been reported to have thinner femoral cartilage than those without osteoarthritis.[5] Articular cartilage thickness can be easily measured with good reliability

with ultrasound, which has been noted to be an accurate and more affordable option to magnetic resonance imaging (MRI).[6-8]

Numerous studies have investigated the effect of loading on cartilage thickness. Several examples of activities considered include walking,[9-11] running,[9,12-15] drop landings,[10,12] and squats.[16] These studies have focused on young, healthy individuals with no knee injuries, current or past. For the most part, researchers have determined that these weight-bearing activities lead to decreases in cartilage thickness. Additionally, diurnal activities have been shown to decrease cartilage thickness, with cartilage being thicker at 8:00 am than at 4:00 pm.[17]

Many of the studies that have investigated cartilage thickness before and after a prescribed loading activity have included a rest period prior to the loading activity. The purpose of this rest period is to remove the effects of previous activities that may have compressed the cartilage and potentially mask the effects of the experimental loading movement. This rest period typically involves the person lying supine or sitting upright with their legs extended in front of them. The duration of this rest has ranged from 0 to 60 min, with the most common durations being 30 min,[9,11,14] 45 min,[18] and 60 min.[10,19] None of these studies measured cartilage thickness before and after rest to determine the effect of rest on cartilage thickness. There have been studies that have measured cartilage thickness before and after rest, using a control group to compare with their loading intervention groups.[9,12] However, their participants rested for 30 min prior to the initial scan, comparing 30 min rest to 60 min rest. The results of these studies are inconsistent, with one reporting a difference and the other reporting no difference. The amount of time required to be certain that cartilage deformation from prior activities is not known. It would be helpful to know how long a rest period is needed to be confident that all participants have a common starting point regarding their cartilage status from prior activities. To be respectful of participants' time, it would be particularly helpful to know the minimum time required.

Several studies that used ultrasound to investigate distal femoral articular cartilage thickness measured one leg, whereas others measured both legs. Those who have compared legs have compared right-to-left knees, without regard to leg preference or dominance.[5,20] The results of these studies have been mixed, with one study reporting no differences[20] while the other reported differences between the right and left medial condyle (MC) cartilage thickness, but not other regions.[5] Since loading influences cartilage thickness and not all loading is symmetrical, it is likely that cartilage thickness may differ between legs based on leg preference. If prior activities do create a difference, this may alter the rest time required for cartilage to rebound from prior deformation. How leg preference may alter cartilage thickness and its behavior with unloading has not been investigated.

Most research comparing cartilage thickness between females and males has determined that females have thinner cartilage in some or all regions of the distal femoral articular cartilage.[17,20-25] There has been no focus on whether there is a difference in recovery from cartilage compression between females and males. The purpose of this study was to determine the effect of unloading time on distal femoral cartilage thickness in young, healthy adults with healthy knees and to investigate if leg preference or sex has an influence on how the cartilage is affected by unloading time. In particular, the thickness at the intercondylar notch (IC) and at the medial and lateral condyles (LCs) was investigated over 1 h, with scans every 15 min.

2. Materials and methods

2.1. Participants

Thirty-two healthy college students (16 female, 16 male) were recruited for this study. To participate in the study, individuals had to be between the ages of 18 and 25 years old and regularly participate in at least 30 min of moderate or vigorous activity at least three times a week for the past three months or longer. They had to be injury-free and had not sustained a back or lower-body injury in the past six months. Additionally, they must not have had a back or lower-body surgery or a serious injury. Finally, they had to be free of any neuromuscular disease that might alter their movement patterns (e.g., muscular dystrophy, multiple sclerosis, peripheral neuropathy). Upon verifying that a person met the inclusion criteria, an appointment was scheduled for testing. They were asked to refrain from any physical activity for at least 12 h prior to the testing appointment, which was verbally verified. If a person had participated in physical activity within 12 h of testing, they were rescheduled to another day. Testing appointments were scheduled primarily prior to noon. Exceptions to this timing due to participant availability occurred for two males and two females who arrived at the same time in the early afternoon, so the scheduling was consistent across the two groups to achieve equivalent diurnal effects.[17]

2.2. Testing protocol

Upon arrival at the laboratory, each participant was given a consent form approved by the university's Institutional Review Board. All elements of the project were performed in accordance with the Declaration of Helsinki. After reading the form and asking any questions, they signed

the form indicating their desire to participate in the study. They also completed a health and activity questionnaire to confirm their eligibility. Their height was measured to the nearest mm with a wall stadiometer, and their weight was measured to the nearest 0.1 N using a force plate (Kistler 9260AA, Kistler Instrument Corporation, USA). Body mass values were obtained from weight data, and body mass index (BMI) was calculated from body mass and height.

For ultrasound image data collection, the participant was supine on a padded plinth with their legs extended in front of them. The leg being scanned was positioned with the person's knee flexed to 140° (confirmed with a goniometer) and their foot flat on the end of the plinth (Figure 1A).[9] All ultrasound images were collected by the same investigator with an ultrasound machine (Mindray M7, Mindray North America, USA) set at a frequency of 8–12 MHz. The machine settings were consistent for all scans taken for each participant. A Mindray L9-3E linear probe was positioned transversely between the medial and LCs, perpendicular to the femoral cartilage surface, above the superior patella to obtain the crispest image.[6] The probe was rotated, forward or backward as needed, to obtain a clear image of the distal femoral cartilage. For the initial scan of each leg, a transparency sheet was placed over the ultrasound machine screen.[9] After the initial scan, a clear image for each leg was selected, and the top edge of the cartilage-bone interface was traced on the transparency sheet with a liquid chalk marker. For subsequent imaging, the sheet was placed over the ultrasound machine display, and the top edge of the cartilage-bone interface was aligned with the marker curve on the sheet to ensure consistent images were obtained over time. Two or three (depending on image quality) ultrasound images were collected for each knee at five time points, separated by fifteen min: T0, T15, T30, T45, and T60. The order of leg scans (right, then left or left, then right) was consistent across the time periods for each participant, and the initial leg was randomized across participants.

2.3. Data collection

A single image was selected for each participant for each leg and time. The IC was measured at the low point of the supracondylar ridge. MC and LC measurements were taken at the point of inflection, and three adjacent measures were taken and averaged (Figure 1B).[26] Two investigators made all measures and the average of their values was used for analysis. The Waterloo Footedness Questionnaire was completed during the final rest time to determine leg preference.[27] Data was organized to compare the preferred leg to the non-preferred leg.

2.4. Statistical analysis

Characteristics of the two groups (females and males) were compared using independent *t*-tests. Levene's test for equal variance was performed, and if variances were unequal, the Welch–Satterthwaite correction was used. For the cartilage thickness in each region (LC, IC, MC), a three-way factorial analysis of variance (ANOVA) (leg, time, sex) was conducted using Statistical Package for Social Sciences (SPSS 30.0, IBM, United States). Mauchly's test of sphericity was performed; if violated, a Greenhouse–Geisser correction was applied. If a significant main effect for time was identified, Bonferroni post hoc testing was performed. Significance was determined with $p < 0.05$.



Figure 1. Example of ultrasound data collection set up (left) and ultrasound image (right) showing the three regions where cartilage thickness was measured (lateral condyle, intercondylar groove, medial condyle)

3. Results

3.1. Subject characteristics

A total of 32 participants were recruited and completed the testing. Participant age, height, body mass, BMI, and leg preference are shown in Table 1. Levene's test indicated equal variances between the two groups for all values except body mass. The Welch–Satterthwaite correction was used to compare body mass between the two groups. The female participants were significantly younger, shorter, and lighter. There was no significant difference between the two groups' BMI or leg preference, as determined using the Waterloo Footedness Questionnaire.

3.2. Time

Figure 2 shows the cartilage thickness data for all three regions over the five time points. Results of the factorial ANOVA run for each region determined some significant changes in cartilage thickness over time. There was a main effect for time for the IC cartilage thickness ($F(2.980,120) = 2.76$, $p = 0.049$, $\eta^2 = 0.083$). Post hoc tests indicated a difference in thickness between T0 and T15 (2.327 ± 0.441 mm vs. 2.398 ± 0.487 mm; $p = 0.004$), T0 and T30 (2.327 ± 0.441 mm vs. 2.406 ± 0.533 mm; $p = 0.015$) and T0 and T60 (2.327 ± 0.441 mm vs. 2.418 ± 0.464 mm; $p < 0.001$). There was also a trend towards a difference between T0 and T45 (2.327 ± 0.441 mm vs. 2.385 ± 0.492 mm; $p = 0.077$). In all cases, the IC cartilage thickness was smaller at T0. There were no other pairwise differences. There was also a main effect for time for MC cartilage thickness ($F(4,120) = 4.456$, $p = 0.002$, $\eta^2 = 0.129$). Post hoc tests indicated a difference in MC thickness between T0 and T30 (2.289 ± 0.393 mm vs. 2.375 ± 0.421 mm; $p = 0.002$) and T0 and T60 (2.289 ± 0.393 mm vs. 2.378 ± 0.416 mm; $p = 0.002$). There was also a trend towards a difference between T0 and T45 (2.289 ± 0.393 mm vs. 2.345 ± 0.387 mm; $p = 0.076$). In all cases, the MC cartilage thickness was smaller at T0. There were no other pairwise differences. There was no main effect of time for the LC cartilage thickness.

3.3. Leg

Table 2 shows the cartilage thickness values for the preferred leg and the non-preferred leg, averaged over all times, for each of the three regions, and Figure 3 displays the cartilage thickness data for all three regions over time for each leg. There was a main effect for leg for the MC cartilage thickness ($F(1,30) = 11.292$, $p = 0.002$, $\eta^2 = 0.273$) with the preferred leg values being 2.408 ± 0.419 mm compared to 2.280 ± 0.369 mm for the non-preferred leg. The MC cartilage thickness for the preferred leg was greater than that of the non-preferred leg at all times. The IC cartilage thickness for the preferred leg was greater than the non-preferred leg at all times, but there was no main effect for leg for the IC cartilage thickness. The LC cartilage thickness had no main effect for leg, and the leg with the thicker cartilage was not consistent over time.

3.4. Sex

Table 2 provides the cartilage thickness values for females and males, averaged over all times, for each of the three regions, and Figure 4 displays the cartilage thickness data for all three regions over time for each sex. The LC cartilage thickness was significantly smaller for females than males (2.096 ± 0.285 mm vs. 2.575 ± 0.362 mm; $F(1,30) = 20.641$, $p < 0.001$, $\eta^2 = 0.408$). There was also a main effect for sex for the IC cartilage thickness ($F(1,30) = 14.559$, $p < 0.001$, $\eta^2 = 0.327$). The IC thickness was 2.130 ± 0.377 mm for Females and 2.644 ± 0.438 mm for males. There was also a main effect for sex for the MC thickness ($F(1,30) = 5.396$, $p = 0.027$, $\eta^2 = 0.152$). The medial cartilage thickness was 2.201 ± 0.392 mm for females and 2.486 ± 0.369 mm for males. Across all regions and times, female cartilage thickness was smaller than male values.

3.5. Interactions between time, sex, and leg

There were no statistically significant interactions among the independent variables (time, leg, sex) across the three regions. However, there was a trend towards a difference for the interaction between the leg and time for the LC cartilage thickness ($F(4,120) = 2.164$, $p = 0.077$, $\eta^2 = 0.067$). There was also a trend towards a difference in the values at the initial time between the two legs (2.268 ± 0.389 mm for preferred leg vs. 2.347 ± 0.408 mm for non-preferred leg, $p = 0.083$).

4. Discussion

The aim of this study was to determine the effect of several factors (time, sex, leg preference) on the distal femoral articular cartilage thickness in three regions (MC, IC, LC) in healthy, young adults with no knee injuries. A main effect was found for each factor, but not for all regions. The primary question of this study was how the duration of leg unloading affected cartilage thickness. There was a main effect for time for the IC and MC, but not the LC. This may imply that the LC cartilage was initially less compressed due to the activities participants engaged in prior to the initial scan. This reasoning is supported by previous studies reporting that maximum joint stress is placed on the MC and that the medial compartment bears more load than the lateral compartment.[28,29] The results for the IC and MC suggest that rest periods of 15 and 30 min, respectively, were adequate to significantly increase cartilage thickness from its initial state and to prevent further significant change over time. It is noteworthy that in both regions, the

Table 1. Participant characteristics (mean ± standard deviation) for the entire group, female, and male participants

Characteristics	Group (n = 32)	Females (n = 16)	Males (n = 16)	Significance
Age (y)	20.4 ± 1.6	19.8 ± 1.6	21.0 ± 1.4	$p = 0.034$
Height (cm)	174.7 ± 8.9	168.6 ± 5.1	180.9 ± 7.7	$p < 0.001$
Mass (kg)	72.3 ± 9.2	65.6 ± 7.7	79.0 ± 4.5	$p < 0.001$
BMI (kg/m^2)	23.6 ± 2.1	23.1 ± 2.3	24.2 ± 1.7	NS
Leg preference	28 right, 4 left	14 right, 2 left	14 right, 2 left	NS

Abbreviations: BMI: Body mass index; NS: Not significant.

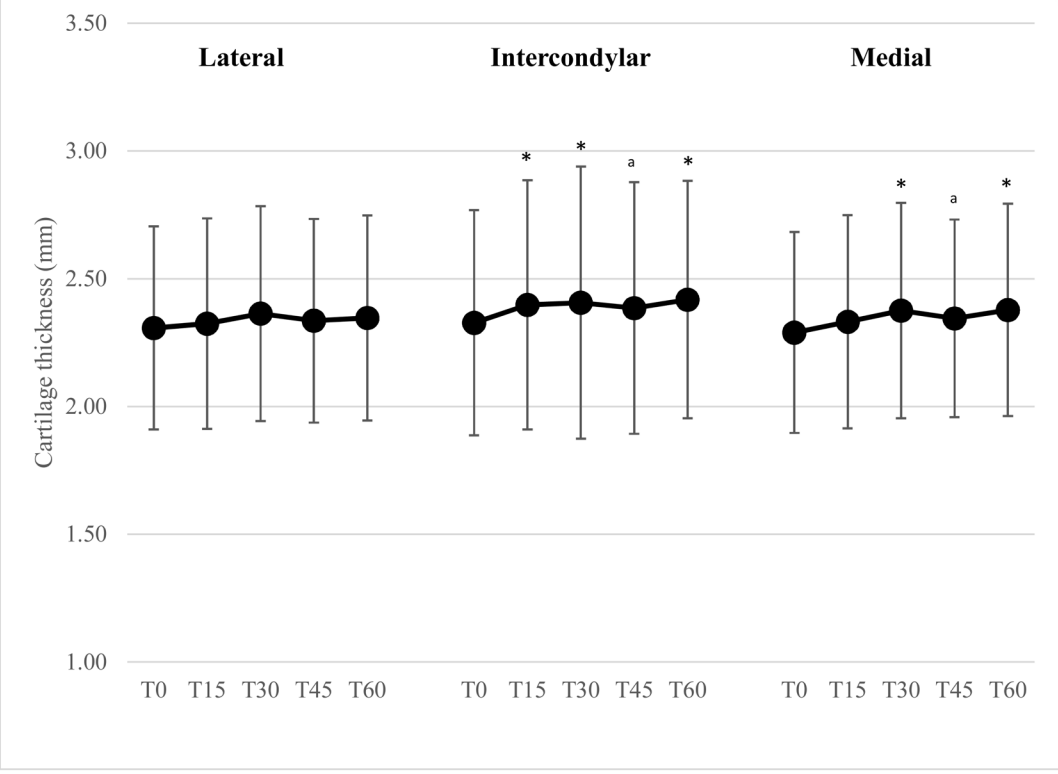


Figure 2. Mean cartilage thickness over time for each region. Error bars indicate standard deviation values.
Notes: *$p < 0.05$ (significant difference from T0); [a]trend towards a difference from T0.

difference between the initial thickness and the thickness after 45 min was not statistically significant, but a trend towards significance. There is a small, non-significant decrease in mean thickness between 30 and 45 min, which also occurred for the LC values. The mechanism underlying this small decrease in cartilage thickness after 30 min is unclear and warrants further investigation. This slight dip, then slight increase, could support the use of 60 min of rest, as suggested in a previous study, which found a difference in cartilage thickness after 30 min of rest.[9] However, to minimize participant burden and optimize testing efficiency, a rest period of 30 min may be sufficient, as cartilage thickness appeared to have increased by this time and did not change significantly thereafter.

While no previous research has investigated the effect of unloading time on cartilage thickness, several studies

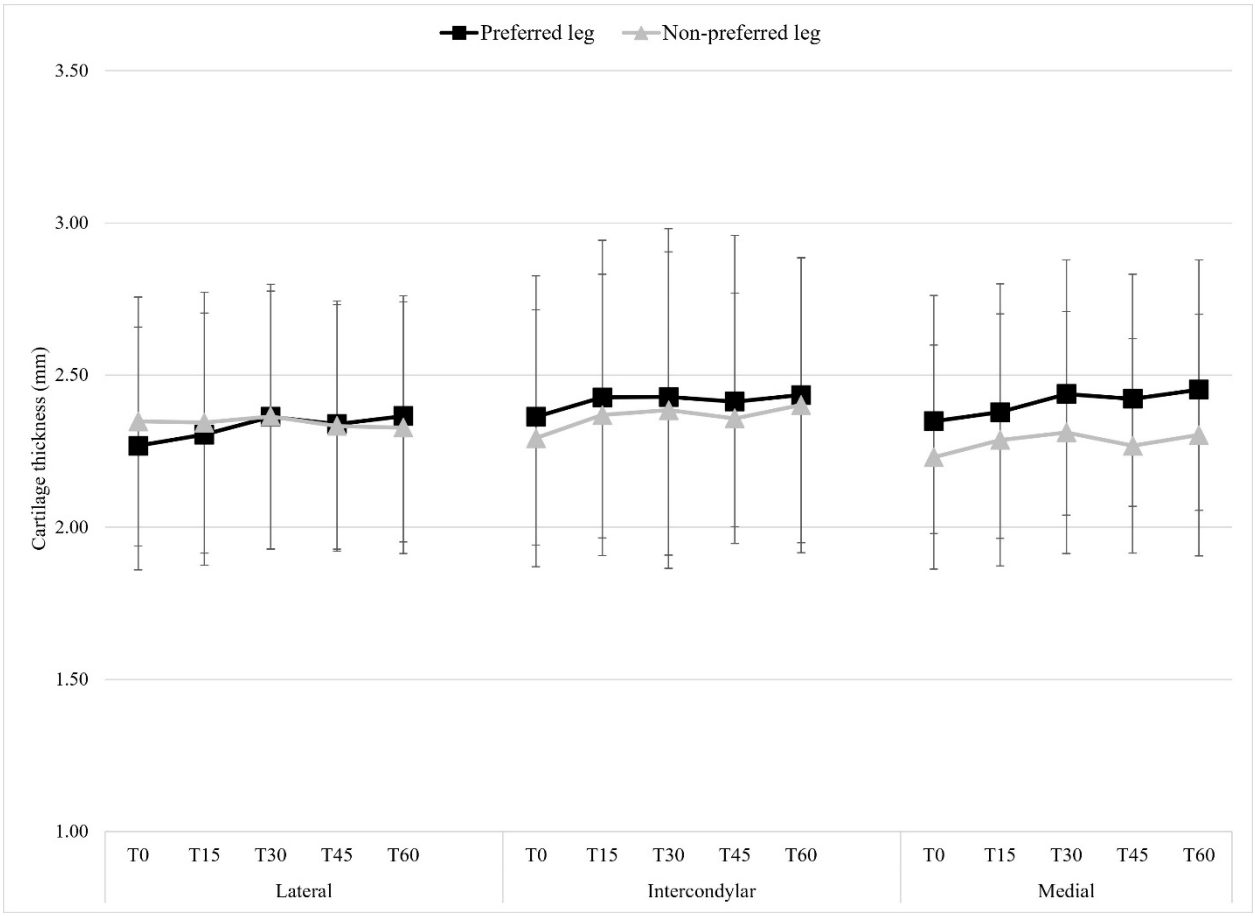


Figure 3. Mean cartilage thickness for each region over the five time points for the preferred leg (black squares) and non-preferred leg (grey triangles)

Table 2. Cartilage thickness (mean ± standard deviation) for each leg and for each sex averaged over all times

Parameters	Lateral (mm)	Intercondylar (mm)	Medial (mm)
Preferred leg	2.328 ± 0.395	2.413 ± 0.502	2.408 ± 0.419
Non-preferred leg	2.343 ± 0.362	2.361 ± 0.438	2.280 ± 0.369*
Females	2.096 ± 0.285	2.130 ± 0.377	2.201 ± 0.392
Males	2.575 ± 0.362*	2.644 ± 0.438*	2.486 ± 0.369*

Note:*$p < 0.05$ (significant difference from the preferred leg or females).

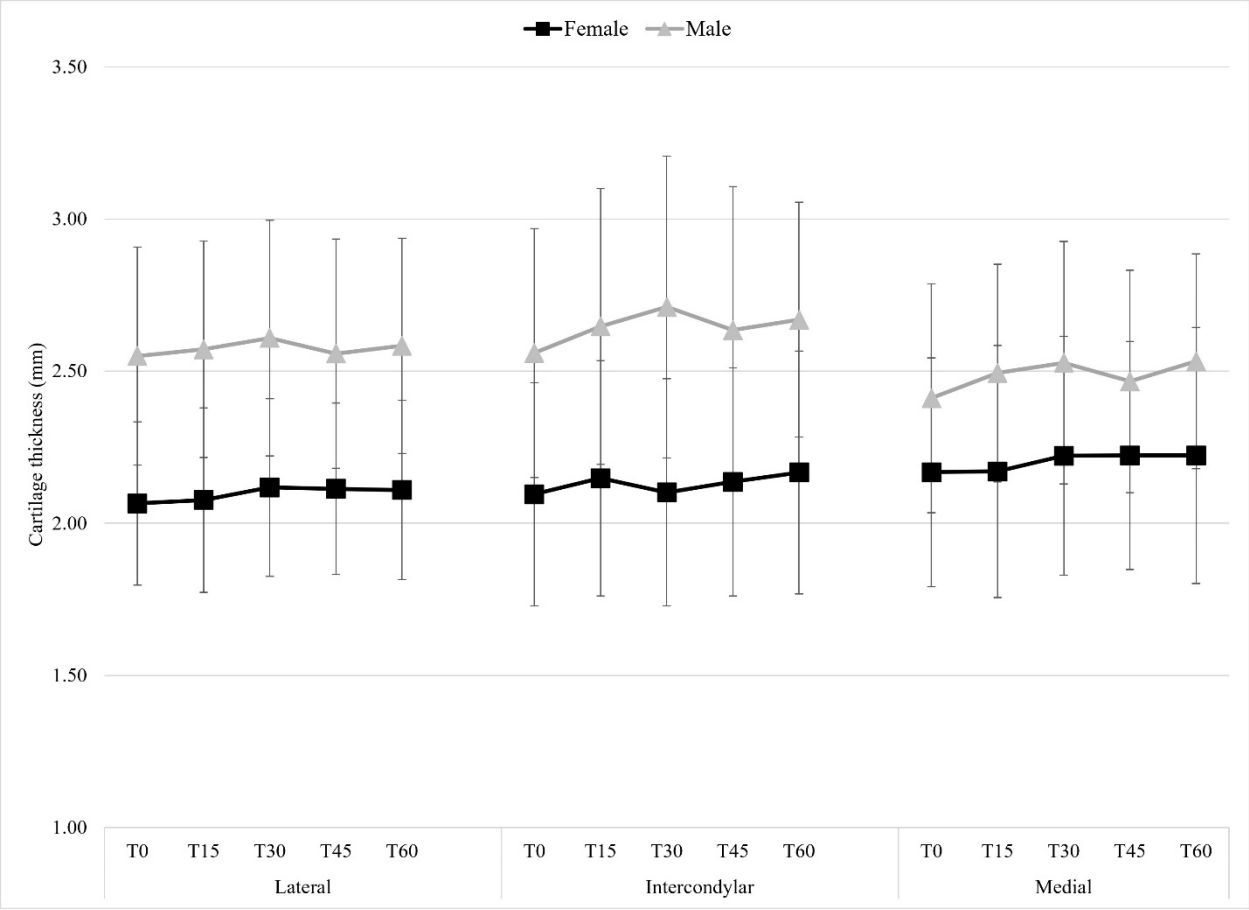


Figure 4. Mean cartilage thickness for each region over the five time points for females (black squares) and males (grey triangles)

have examined the effect of a specific loading condition, including a 30-min rest control.[9,11,12,30] However, these studies already had their participants rest (unload with legs extended) for 30 min prior to the initial ultrasound scan, so they were comparing scans after 30 min to 60 min of unloading. For the studies that used similar resting protocols (legs fully extended while sitting on a table), the results were mixed. One study, like the current study, reported no significant differences across the three regions at 30–60 min of rest.[30] In contrast, two other studies only investigated the MC cartilage thickness and reported thickness increases of 3.4% and 6.3%, respectively.[9,11] Neither of these studies mentioned asking participants to restrict activities prior to the test. Additionally, the time of day the testing took place was not specified, and it is known that diurnal effects affect cartilage thickness.[17] If their participants were tested late in the day and/or engaged in significant physical activity prior to testing, their MC cartilage thickness could have been compressed more significantly than that of the current study participants. This sheds light on the importance of clear criteria for testing conditions, especially prior activities and the time of day during testing, when determining the effect of loading or unloading on cartilage thickness.

When comparing the preferred leg to the non-preferred leg, there was only a main effect for the MC cartilage thickness, with the preferred leg having greater thickness at all times. The IC cartilage thickness was also greater for the preferred leg, but not significantly. When articular cartilage is exposed to compressive and shear forces in a cyclical fashion, there is an increase in the cartilage thickness, volume, and deformation capacity.[31] The preferred leg, particularly the medial aspect of the joint, is likely exposed to more of these facilitatory forces, and the cartilage has seemingly responded with greater thickness. The behavior over time between the two legs was consistent for all regions. While several studies investigating distal femoral articular cartilage thickness have measured both legs, comparisons between legs are limited. Two previous

studies compared the cartilage thickness between the right and left leg (with no regard for preference) and reported either no difference between the two legs for the LC, IC, or MC cartilage thickness[20] or a difference in thickness only at the LC.[23] These findings are not necessarily in disagreement with the current study results, as their participants may have had a mix of leg preferences, and so comparing right to left legs would mask any differences that leg preference may exhibit.

The effect of sex on cartilage thickness was significant across all three regions, with females having thinner cartilage than males, and this difference was consistent over time. The smaller thickness in females was consistent with other ultrasound research, which has also reported significant differences in some or all regions for a similar population.[20,22,23,25] However, a study focused on cartilage thickness in a diabetic population reported no difference in cartilage thickness between female and male subjects in either leg or across the three regions in their control subjects who were older than those in the current study (mean age = 33 years).[32]

There were no significant interactions between the three independent variables (time, sex, leg). This absence of significant interactions may be attributed to the sample size being too small and the study lacking the power to reliably detect interaction effects. Any future studies that focus on interaction effects should ensure they have a larger number of participants.

While the current study focused on an active, young, healthy population, its results may not apply to a less active, older, or injured population. If the knowledge of the effect of unloading was desired for a different population, a similar study would need to be conducted. Previous research has reported greater cartilage thickness in high-intensity elite athletes than in age-matched sedentary adults.[33] Additionally, studies have reported age-related differences in cartilage thickness.[25] These studies highlight the current study's limitation in generalizing its findings to other populations. The time of data collection was controlled in this study to avoid diurnal effects on cartilage, with most participants tested in the morning. It is possible that if data collection occurred later in the day, when there was a greater opportunity for the cartilage to be previously compressed from activities of daily living, the time needed to fully decompress the cartilage would be longer. Additionally, all participants in the current study were asked to refrain from physical activity for the previous 12 h, and this was confirmed prior to data collection. If there were no restrictions, this might also alter the time needed to completely decompress the cartilage.

While ultrasound has been determined to be a valid technique to collect distal femoral articular cartilage thickness, it is limited to only viewing the anterior aspect of the distal femur. Other imaging techniques (e.g., MRI) could be used to obtain information from additional regions. In addition to determining cartilage thickness, measuring serum cartilage oligomeric matrix protein (sCOMP) levels could provide insight into cartilage recovery. Previous research has concluded that these levels increase with several physical activities (running, jumping, drop landings, walking).[12,34,35] Another previous study reported that sCOMP values did not change over 30–60 min of rest.[12] It would be relevant to the current study to determine changes between 0 and 30 min of rest.

5. Conclusion

The distal articular cartilage thickness response to the removal of loading depended on the region, with the greatest change happening at the MC. For the LC, thickness was not affected by time, while the IC and MC required 15 min and 30 min, respectively, to increase significantly from initial thickness values. In both regions, the thickness did not significantly increase with additional time of unloading. The preferred leg has significantly greater MC thickness, and females have thinner cartilage thickness in all regions. Future studies investigating the effects of a loading intervention should direct participants to avoid physical activity for at least 12 h, then have them rest with their legs extended for 30 min to ensure equivalent initial conditions.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: All authors
Formal analysis: Michele LeBlanc
Funding acquisition: All authors
Investigation: All authors
Methodology: All authors
Writing–original draft: Michele LeBlanc
Writing–review & editing: All authors

Ethics approval and consent to participate

The Institutional Review Board (IRB) of California

Lutheran University reviewed the study and granted approval (IRB-FY2025-48). Participants in the study signed an IRB-approved consent form in person before any data were collected from them.

Consent for publication

All research subjects provided written consent in person. They signed that they understood that only group data would be included in any publications or presentations and that any photos taken would mask their faces so their identities would not be revealed.

Availability of data

Data are available upon reasonable request from the corresponding author.

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