



The reliability of supra-patellar transverse sonographic assessment of femoral trochlear cartilage thickness in healthy adults

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1 **The reliability of supra-patellar transverse sonographic assessment of femoral trochlear**
2 **cartilage thickness in healthy adults**

3

4 Running title: Femoral cartilage thickness evaluated by sonography

5

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26

27 **Abstract**

28

29 **Objectives**

30 To determine the intra-session reliability of femoral cartilage thickness measurements using ultrasonography
31 and extend the pool of normative data for cartilage thickness measurements assessed by ultrasonography.

32 **Methods**

33 77 healthy participants (55 male and 22 female), with an average age of 43 ± 18 (mean \pm SD) years, volunteered.
34 Resting supra-patellar ultrasound was used to image trochlear cartilage thickness on two separate occasions a
35 maximum of 7 days apart. Reliability was evaluated with intraclass correlation coefficients (ICC), Bland &
36 Altman analysis, standard error of measurement (SEM and SEM%), and the smallest real difference (SRD and
37 SRD%). Normative data was assessed using linear, multiple regression models and independent group t-tests.

38 **Results**

39 The test-retest level of agreement at all locations was high (ICC 0.779-0.843), which increased to high-very
40 high in young adults (ICC 0.884-0.920). The SEM% was 8.2-8.3% at all locations and reduced further to 5.4-
41 6.3% in younger adults. The SRD% was between 22.8-23.1% for the full sample and 14.9-17.5% in young
42 adults only. Multiple regression analyses demonstrated that age, weight, female gender and a high physical
43 activity frequency could significantly predict cartilage thickness at all locations ($P<0.05$); however, female
44 gender was the only significant independent predictor in all models (all $P<0.01$). Females also had thinner
45 cartilage at all locations ($P<0.01$).

46 **Conclusion**

47 Supra-patellar ultrasonography demonstrates high intra-tester reliability and measurement precision and is a
48 promising method to assess trochlear cartilage thickness. Being female may impact femoral cartilage thickness
49 more than other potential risk factors for knee osteoarthritis such as age, weight, and high physical activity
50 frequency.

51

52 **Keywords:** Ultrasonography, femoral trochlear cartilage thickness, reproducibility of results, normative data

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54

55

56

57 Introduction

58 In recent years, ultrasound (US) has been increasingly used to assess cartilage thickness. A commonly adopted
59 technique is axial supra-patellar US imaging (1–6), although longitudinal US scanning of the knee has also been
60 used (7,8). Despite its emergence as a method to assess trochlear cartilage thickness, to date, only a few studies
61 report the validity (through the comparison of US measurements with cadaver specimens or MRI imaging) and
62 reliability of sonographic evaluation of cartilage (7–9). Previous studies using US to measure trochlear cartilage
63 thickness have utilized either a young adult sample (4,5), or have been confined to clinical populations (2,3,10).
64 Therefore, the value of sonographic measurement of trochlear cartilage thickness in a healthy adult sample is
65 restricted by limited normative data. Further examination of the accuracy and repeatability of this technique is
66 required to establish whether US can be used as an effective tool to measure trochlear cartilage thickness.
67 Overall, the ability to reliably measure trochlear cartilage thickness may offer an important tool to for the
68 assessment of patellofemoral disorders, such as patellofemoral pain syndrome, chondromalacia patella and
69 patellofemoral knee arthritis, in both a clinical and research setting.

70

71 The primary aim of this study was to assess the intra-session reliability of cartilage thickness measurements
72 using sonography. Measurement precision was also assessed to identify the smallest change that can be
73 considered actual change and not just a result of a test-re-test error. A secondary aim of this study was to also
74 extend the pool of normative healthy adult data for cartilage thickness measurements assessed by
75 ultrasonography.

76

77 Materials and methods**78 Study participants**

79 Seventy-seven healthy volunteers (55 male and 22 female), with an average age of 43 ± 18 years, and with an
80 average body mass index (BMI) of 24.9 ± 3.2 , were enrolled. Participants were targeted through word of mouth,
81 poster advertisement, generic emails, and social media from the Bangor University community and the
82 surrounding North Wales area. The inclusion criteria of entry to the study included being: (i) healthy, (ii) male
83 or female, (iii) aged between 18-80 years. Exclusion criteria included: (i) diagnosed osteoarthritis (OA),
84 rheumatoid arthritis, or other inflammatory diseases, (ii) history of knee malalignment (varus / valgus) greater
85 than 15° , (iii) previous knee injury (including meniscus tear or ligament damage or tear), (iv) recent fracture of
86 lower extremity (within last 6 months), (v) current or prior use of lipid-lowering therapy, corticosteroid

87 injections, or high dose oral steroids (vi) current or past (within last four weeks) glucosamine and/or chondroitin
88 supplementation use, (vii) pregnancy. This study was approved by the local Research Ethics Committee (School
89 of Sport Health and Exercise Sciences (SSHES), Bangor University) and conducted in accordance with the
90 Helsinki Declaration (2013). Written informed consent was obtained from all participants.

91

92 Experimental protocol

93 Participants were required to visit the School of Sport, Health and Exercise Science, Bangor University on two
94 occasions with each session lasting approximately 60 minutes. During the initial visit, participants completed a
95 medical and basic physical activity questionnaire. Anthropometric measurements (body mass and height) were
96 also assessed using a calibrated balance beam scale (SECA, California, USA) and wall-mounted tape measure
97 (SECA, California, USA), respectively. Ultrasonography was subsequently used to obtain images of the femoral
98 articular cartilage as outlined below. All participants completed their first and second visit at the same time of
99 day and within a 7-day period. Participants were also asked to refrain from any strenuous physical activity for
100 48 hours prior to each visit.

101

102 Ultrasonography

103 The ultrasound (US) assessment was performed using a 12 MHz linear-array probe (Esaote S.P.A. MyLab50
104 ultrasound, Firenze, Italy) and acoustic coupling gel (Aquasonic 100, Parker Laboratories, Inc, Fairfield, NJ,
105 USA) following a period of between 15-30 minutes of seated rest. With participants lying in a supine position,
106 and with the knee maximally flexed, the superior margin of the patellar was located and a line was marked on
107 the skin using a washable marker at the point immediately above the superior margin of the patellar and at 1 cm
108 intervals in a superior direction. The transducer was placed in a supra-patella transverse position, perpendicular
109 to the bone surface and orientated to optimize the US image (5,9). The location at which the cartilage thickness
110 of the intercondylar notch appeared greatest was marked on the skin and recorded to enable the examiner to
111 return the transducer to the exact location for all subsequent scans. The same researcher performed all
112 ultrasonography scans following training by a consultant rheumatologist with expertise using this technique.

113

114 US images were analyzed by 'Image J' software (Image J, National Institute of Health, Bethesda, MD, USA) to
115 determine the minimal cartilage thickness. The distance from the thin hyperechoic line formed at the synovial
116 space-cartilage border to the line formed at the cartilage-bone border was used to measure minimal cartilage

117 thickness at the lateral facet, medial facet and intercondylar notch. Anatomic reference points used in the present
118 study corresponded to the midpoint of the intercondylar notch and 1 cm apart in the medial and lateral directions
119 were used as an estimate of the cartilage thickness at the medial and lateral facet, respectively (11). Naredo and
120 colleagues previously demonstrated good reproducibility in femoral cartilage thickness measurement when
121 using comparable anatomical reference points (5). Prior to analysis, all images were de-identified by a second
122 researcher for blinded analysis. Based on the pixel resolution (15.8 pixels/mm) of the images captured by
123 ultrasonography, the ImageJ software allowed images to be measured to an accuracy of greater than one-tenth
124 off a mm, or more specifically, one pixel was equal to 0.06 mm. The cartilage thickness of each image was
125 measured in triplicate and an average of the three measurements was used for all data analysis. As required, the
126 image contrast was adjusted to assist in appropriately identifying the hyperechoic line formed at the synovial
127 space-cartilage border to the line formed at the cartilage-bone border.

128

129 **Statistical analysis**

130 *Reliability analysis*

131 Agreement between measurements was evaluated using a one-way mixed, absolute agreement type, intraclass
132 correlation coefficient (ICC) (12). ICC values can be classified as low: 0.20–0.49; moderate: 0.50–0.69; high:
133 0.70–0.89; or very high: 0.90–1.00 (13). Paired t-tests, together with Bland-Altman plots, were used to provide
134 an indication of the systematic error (14). The standard error of the measurement (SEM) and the SEM% were
135 calculated as previously described (15,16). SEM and the SEM% were used to establish the measurement
136 precision between visit 1 and 2 and to provide a measure of the smallest value that represents a real change in a
137 group of individuals. Furthermore, to calculate the smallest error in a single individual score, the smallest real
138 difference (SRD) and SRD% were also calculated as previously described (17). All analyses were initially
139 completed using the full dataset. Finally, a split-group analysis was performed for each of the following groups:
140 young adults (18-25 years of age), middle-aged adults (26-50 years of age), older adults (\geq 51 years of age),
141 male only, and female only groups. This analysis provided an opportunity to determine whether age or sex of
142 the participant influenced the level of intra-tester reliability and measurement precision.

143

144 *Analysis of normative cartilage thickness data*

145 Independent t-tests were used to determine differences between measurements made on the right and
146 measurements made on the left knee. If data was not available for either the right or left side (i.e. a measurement

147 could not be made for one of the locations), both visit 1 and visit 2 data points were removed to ensure an equal
148 sample size. Simple linear regression analyses were performed to determine the relationship between mean
149 cartilage thickness of the right knee (at each location) and participant characteristics (age, body mass, height,
150 and BMI). Multiple linear regression models were subsequently used to explore the relationship between
151 cartilage thickness (at each location) and potential risk factors; including age, BMI, and female gender and high
152 frequency of weekly physical activity. Physical activity was considered 'high' when participants completed
153 structured exercise training on a minimum of 5 days per week. In addition to multiple regression, mean cartilage
154 thickness between sexes was also assessed by creating an equal sized ($n = 17$) sample matched for age and BMI.
155 Independent t-tests were used to determine whether cartilage thickness differences existed between males and
156 females at each measurement location (intercondylar notch, lateral facet, medial facet). For the multiple
157 comparisons of cartilage thickness between the three locations, Bonferroni corrections were used with $P < 0.016$
158 ($0.05/3$) for statistical significance. Finally, a one-way analysis of variance (ANOVA) was used to assess the
159 differences in cartilage thickness measurements at each measurement location. The left side was not used within
160 the analysis of normative data as the side to side differences were found to be small and within measurement
161 error in the present study. Moreover, others have reported limited side-to-side differences in femoral cartilage
162 thickness measurement (18) and have advocated the use of unilateral OA models in research (19).

163

164 **Results**

165 The results from the US measurement of cartilage thickness and participant characteristics are displayed in
166 Table 1. A total of 308 knees were scanned (right and left knee of 77 participants on two occasions). This
167 produced a total of 1168 blinded images (77 participants were imaged three to four times per side on two
168 occasions, i.e. visit 1 and visit 2). Some individual images could not be measured as the hyperechoic line
169 formed at the synovial-cartilage border and/or cartilage-bone border could not be clearly delineated. Thus, it
170 was not possible to confidently measure cartilage thickness for 129 (11%), 180 (15%), and 221 (19%) of the
171 available images for the intercondylar notch, medial and lateral facet, respectively. For the cartilage thickness
172 reliability to be assessed a minimum of one image per location was required. Overall, cartilage thickness could
173 be measured in 306 knees (99.4%) at the medial facet, 304 knees (98.7%) at the intercondylar notch, and 296
174 knees (96.1%) at the lateral facet.

175

176 **Reliability analysis**

177 The ICC and 95% confidence intervals (95% CI) for the data are shown in Table 2. The ICC's indicate that the
178 level of agreement at all locations was high (ICC between 0.779 – 0.843), with the highest at the intercondylar
179 notch, followed by the medial facet and then the lateral facet. Subsequent analyses revealed that the level of
180 agreement between measurements was considerably improved when considering younger participants (≤ 25
181 years of age) only (Table 2). In addition, the image quality and clarity were typically better in younger
182 individuals as highlighted in Figure 1. Results also demonstrated that the intra-tester reliability of cartilage
183 thickness measurements was generally similar when male and female groups were analyzed separately (Table
184 2).

185 Systemic variation in cartilage thickness measurements at the notch, medial facet, and lateral facet are shown by
186 Bland-Altman plots (Figure 2). The plots suggest that slightly higher variation (i.e. heteroscedasticity) may exist
187 for higher cartilage thickness measurements, particularly at the notch and medial facet. Moreover, results of the
188 paired t-tests showed no significant difference in cartilage thickness between visit 1 and visit 2 for the medial
189 (1.83 vs 1.82 mm; $P = 0.760$) and lateral facet locations (1.81 vs 1.81 mm; $P = 0.860$). However, at the
190 intercondylar notch, a small but significantly greater cartilage thickness measurement was obtained at visit 2,
191 thus indicating that measurements made during the second visit may be systematically higher compared to
192 measurements made at visit 1 (2.03 vs 2.08 mm; $P = 0.016$). When data was split based on the age of the
193 individuals, paired t-tests (visit 1 *versus* visit 2) did not reveal any systematic differences in measurements made
194 in young participants. Furthermore, although mean differences in cartilage thickness measurements tended to be
195 slightly higher in middle-aged and older participants at most measurement locations compared to the young
196 group, a significant difference between visit 1 and visit 2 was only present at the intercondylar notch in the
197 middle-aged participants.

198 Measurement precision

199 The SEM is provided for each location in Table 2. The value ranged from 0.15 - 0.17 mm for all locations. In
200 agreement with the intra-class correlation analysis, the SEM was lowest in the split group analysis of young
201 participants only (Table 2). Moreover, the SEM%, which provides a measure independent of units, indicates that
202 differences in groups of individuals above 8.2 - 8.3% can be considered a real change and not the difference
203 associated with measurement error. Overall, the SEM% values for all analyses (Table 2) provide evidence of a
204 relatively low range (5.4 – 9.6%). Moreover, the smallest real change is the measurement error in a single
205 individual cartilage thickness. Table 2 demonstrate that the SRD is between 0.42 and 0.47 mm for all locations.

206 In relative terms, this equals 22.8 – 23.1%. An improvement in the smallest real change was shown in young
207 participants (0.28 – 0.32 mm) and when analyzing females only (0.27 – 0.34 mm).

208

209 Sonographic assessment of cartilage thickness

210 Femoral cartilage thickness did not differ between the right and left intercondylar notch, or between the right
211 and left medial facet. Although differences were observed at the lateral facet between the left and right knee
212 (1.78 vs 1.88 mm, $P = 0.04$), the difference was small (5.6%) and within the SEM. For this reason (and as
213 previously stated in the methodology), normative data analyses were based on the right side only.

214

215 Age was found to have a negative relationship with lateral cartilage thickness in men (Figure 3A). Participant
216 weight was found to have a positive relationship with cartilage thickness at the intercondylar notch (Figure 3C).
217 When females and men were assessed separately, this positive correlation was only found in men (Figure 4C).
218 Participant height was also found to have a positive relationship with cartilage thickness at all locations (Figure
219 3B); however, when the analysis was separated for males and females, a positive relationship remained between
220 height and lateral facet cartilage thickness in males only. Moreover, a negative relationship between height and
221 intercondylar notch thickness was found in females. In addition, BMI was found to have a negative relationship
222 with lateral and medial facet thickness in men, but not women (Figure 4D). The correlation coefficient, levels of
223 significance and regression equation are presented in Figure 3 for the full dataset, and in Figure 4 for the
224 comparison between males and females.

225

226 Age, weight, female gender and high physical activity frequency (> 5 sessions per week) were the independent
227 variables included in the multiple regression model. This four-predictor model was able to account for 28.8% of
228 the variance in femoral cartilage thickness at the intercondylar notch [$F(4, 59) = 5.953, P < 0.001, R^2 = 0.288$].
229 However, gender was the only independent variable to significantly contribute to the model. The beta
230 coefficient ($\beta = -0.367$) indicates that in this sample the cartilage thickness in females was 0.38 mm lower than
231 males ($P < 0.01$). At the lateral facet, the regression model could predict 16% of the variance in cartilage
232 thickness [$F(4, 60) = 2.857, P < 0.05, R^2 = 0.160$]. As per the previous regression model, gender was the only
233 independent variable to significantly contribute to the model ($\beta = -0.253, P = 0.008$). Finally, age, weight,
234 female gender and a high physical activity frequency at the medial facet could significantly predict 15.1% of the

235 variance in cartilage thickness at the medial facet $F(4, 60) = 2.659, P < 0.05, R^2 = 0.151$]. However, again,
236 gender was the only independent variable that contributed to the model ($\beta = -0.355, P = 0.03$).

237

238 Analysis of the stable model, i.e. with sex (a dummy variable) as the only predictor variable, the following
239 models were produced: For the femoral intercondylar notch, femoral cartilage thickness in females could be
240 calculated as $2.204 + (-0.450 \times 1) = 1.754$ mm, and in males it could be calculated as $2.204 + (-0.450 \times 0) =$
241 2.204 mm. The model was significant ($P < 0.001$). For the femoral lateral facet, femoral cartilage thickness in
242 females could be calculated as $1.933 + (-0.219 \times 1) = 1.714$ mm, and in males it could be calculated as $1.933 +$
243 $(-0.219 \times 0) = 1.933$ mm. This model was also significant ($P < 0.01$). Finally, for the femoral medial facet,
244 femoral cartilage thickness in females could be calculated as $1.946 + (-0.295 \times 1) = 1.651$ mm, and in males it
245 could be calculated as $1.946 + (-0.295 \times 0) = 1.946$ mm. This model was also significant ($P < 0.01$).

246

247 Results demonstrated that cartilage thickness was thicker at the intercondylar notch compared to the medial and
248 lateral facet (Figure 5). However, there was no difference in cartilage thickness between the medial and lateral
249 facet ($P > 0.05$). To further assess for differences in mean cartilage thickness between sexes an equal sized ($n =$
250 17) sample matched for age and BMI was created. Results demonstrated that mean cartilage thickness was
251 lower in females at the intercondylar notch, lateral facet and medial facet than that of the matched male group
252 (Figure 6). The biggest difference in mean cartilage thickness between males and females was at the medial
253 facet (2.00 mm *versus* 1.60 mm, respectively).

254

255 **Discussion**

256 The use of supra-patellar transverse sonography to assess trochlear cartilage thickness is a novel technique,
257 which required the further study into its reliability and accuracy. The purpose of the present study was to
258 ascertain the intra-tester reliability of supra-patellar transverse US of trochlear cartilage thickness in a group of
259 healthy males and females across a wide range of ages. Notably, the present study demonstrates high intra-tester
260 reliability for trochlear cartilage thickness at the intercondylar notch, medial facet, and lateral facet, as well as a
261 reasonably small measurement error. Additional analysis revealed that both intra-tester reliability and
262 measurement precision reliability was better in young healthy individuals when compared with older
263 counterparts.

264

265 In healthy individuals, supra-patellar transverse ultrasonography allowed a quick and straightforward
266 assessment of trochlear cartilage. The high ICCs found in the present study [intercondylar notch 0.843 (0.790 -
267 0.883), medial facet 0.834 (0.778 - 0.835) and lateral facet 0.779 (0.707 - 0.876)], are comparable to previously
268 reported ICCs using a very similar standardized protocol in a small sample of flexed cadaver knee (age of death
269 was 76-89 years) (9). Interestingly, in both studies, the level of agreement at the lateral facet was lower
270 compared to the intercondylar notch. One possibility is that the lateral and medial facets are prone to an
271 increased level error related to the inclination and positioning of the US transducer (9). This is supported by
272 previous evidence using MRI, which reported that central weight regions often provide greater accuracy than
273 boundary areas (20). Results in the present study also revealed that intra-tester reliability was considerably
274 greater in younger individuals compared to middle-aged and older individuals. Given that a limited degenerative
275 change would be expected in young healthy adults, the increased reliability in young individuals might be due to
276 the trochlear cartilage appearing considerably clearer in young participants. In contrast, image quality in older
277 individuals was often lower, thus reducing the ability of the investigator to delineate images and offer such
278 precise measurements.

279

280 Images obtained from this study provided a clear hyperechoic line formed at the synovial-cartilage border
281 and/or cartilage-bone border that allowed femoral cartilage thickness to be assessed in most but not all cases.
282 Compared to the study by Yoon and colleagues, cartilage thickness could be measured in a greater proportion of
283 knees at the medial facet (98.7 vs 70.6%) and lateral facet (96.1 vs 90.1%) in the current study (8). Differences
284 in the ability to measure cartilage thickness between the two studies are likely to relate to the participants (i.e.
285 OA vs healthy individuals in the current study). Several degenerative changes, including, roughened and
286 fibrillated articular cartilage, cartilage loss, asymmetrical narrow, as well as abnormalities at the subchondral
287 bone have previously been associated with poorly defined hyperechoic cartilage borders (5,8). Moreover,
288 despite great care being used to standardize the US assessment of the knee and to replicate the positioning of
289 both the participant and transducer between sessions, other factors such as poor transducer positioning or
290 movement artefact, may also contribute to poor image quality (9). In the current study, of the 7 knees which
291 could not be measured, individuals were all male, mostly older and had a higher BMI. These factors and the
292 relationship with the ability to measure cartilage thickness using US warrant further investigation.

293

294 Femoral trochlear cartilage thickness measurements in the present study were comparable to several previous
295 studies using the same US methodology in similarly aged healthy individuals (3,21). In contrast, others have
296 reported slightly greater femoral trochlear cartilage thicknesses in young (25–40 years) healthy individuals
297 compared to the present study (5). The present study also found a significantly thicker cartilage thickness at the
298 intercondylar notch compared to the lateral and medial facets. This difference has not been observed to the same
299 extent in several other studies (3,5,21) and may be related to differences in biomechanical loading. In addition,
300 femoral trochlear cartilage thickness did not differ between the right and left intercondylar notch, or the right
301 and left medial facet. Although differences were observed at the lateral facet between the left and right knee
302 (1.78 vs 1.88 mm, $P = 0.04$), the difference was small (5.6%) and within the SEM. Side to side differences in
303 thickness have previously been reported; however, differences in cartilage thickness tend to be small (total knee
304 joint: $3.8 \pm 3.1\%$) with no significant differences for limb dominance (22). A previous report indicates good
305 correlations between morphological dimensions of the left and right side and advocates the use of unilateral OA
306 models in research (19).

307

308 The current study also found females had lower cartilage thickness at all locations compared to males, which is
309 consistent with previous studies using both MRI and US (5,23,24). Furthermore, regression analyses in the
310 present study found that female gender was the only variable that could explain the variation in cartilage
311 thickness. The lower trochlear cartilage thicknesses observed in the present study may relate to differences in
312 body size between men and women. This is supported by the current finding that women have thinner trochlear
313 cartilage thickness compared to men after the adjustment for age and BMI (Figure 6), and previously, after
314 adjustment for body height and weight (24). Differences between males and females may also relate to
315 differences in the sex hormone estrogen (25), which is understood to act upon estrogen receptors found in
316 articular cartilage (26), and/or to differences in the dynamic loading across the knee joint between men and
317 women (27).

318

319 Further analyses demonstrated that age was negatively associated with cartilage thickness at the lateral facet, but
320 only in males. Similarly, several studies have previously found ageing to be negatively associated with femoral
321 cartilage thickness assessed by both US (5) and MRI (28). The results of the current study suggest that the
322 lateral femoral facet might be the most prominent site for age-related change. Furthermore, although age has
323 previously been found to be negatively associated with femoral cartilage thickness in both men and women (5),

324 the present study suggests that men are more at risk of age-related change in cartilage thickness at the lateral
325 facet. This finding is surprising given that older women are at increased risk of OA (29) and may relate to the
326 small sample of females in the present study.

327

328 Anthropometric variables such as body height and weight may also influence trochlear cartilage thickness. In
329 the current study, a positive relationship was found between body height and cartilage thickness for all three
330 locations. Several previous studies have also found body height to be positively associated, albeit weakly, with
331 cartilage thickness (24,28). In contrast, a positive relationship between weight and cartilage thickness was
332 observed only at the intercondylar notch. Moreover, when the relationship was explored separately for males
333 and females, weight and body height demonstrated a different relationship with femoral cartilage thickness.
334 Both body height and weight were shown to have a positive relationship with cartilage thickness at various
335 locations in men, while in females, weight was unrelated to femoral cartilage thickness and body height was
336 negatively related at certain locations. Previous research also demonstrated that neither weight nor height was
337 correlated with femoral cartilage thickness in women, and only body height was positively correlated with
338 femoral cartilage thickness in men (30). Reasons for the difference between men and women are unknown.
339 However, it appears that the higher joint loads that are related to body size may have a more favorable impact
340 on cartilage thickness of healthy men compared to women. Whether the relationship extends to a group of men
341 with a greater variation in body size remains unclear. The present study also found that BMI had a negative
342 relationship with both lateral and medial facet thickness in men. This supports previous research indicating
343 having a high BMI may increase the risk of reduced cartilage thickness and knee OA (31). The results of the
344 current study would suggest that while being either heavier or taller may be positive for cartilage thickness in
345 men, an unfavorable body composition may reduce cartilage thickness. This may also suggest that muscle
346 function and physical fitness may have a key role in cartilage thickness morphology. Although exercise
347 frequency as a measure of physical activity level was not associated with cartilage thickness in the present
348 study, future research, together with more refined measures of physical activity is required to explore the
349 potential relationship and determine whether a moderation effect exists.

350

351 A primary limitation of this study was the inability to determine the validity of femoral cartilage thickness
352 measurements made using US with a gold standard such as MRI. Nonetheless, US may be regarded as a
353 promising measurement technique that has demonstrated a good agreement in both cartilage thickness

354 measurements made using US and MRI (7) as well as US and anatomical specimens (9). Nevertheless, this level
355 of agreement is not a universal finding, particularly when using supra-patellar axial US to assess medial facet
356 thickness (8) and when severely damaged knees are included in the analysis (9). Importantly, caution is
357 warranted when considering the validity and reliability of sonographic measures of cartilage thickness when the
358 sample includes older individuals, and individuals with significant knee OA. In addition, unlike the analysis of
359 MRI, US cartilage thickness measurements are largely limited to the femoral plate and do not offer the ability to
360 assess other morphological measurements such as cartilage volume. Unlike MRI, the use of US to detect
361 changes in cartilage thickness following acute loading is unclear. Although acute changes in femoral cartilage
362 thickness following walking and running have recently been reported (32), our recent work demonstrated that
363 such change was not a universal finding (11). A further limitation of the present study relates to the fact that
364 inter-tester reliability was not assessed. This is particularly important given the usefulness of sonographic
365 cartilage thickness measurements as a clinical and research tool relies on the ability to make direct comparisons
366 between studies.

367

368 This cross-sectional study of the healthy adults, with a wide age range, demonstrates high intra-tester reliability
369 for all femoral cartilage locations (ICC's between 0.779-0.843) and measurement precision (SEM% between
370 8.2-8.3%), which is better in younger adults (ICC's between 0.884-0.920 and SEM% 5.4-6.3%). Thus, in
371 younger adults, differences between groups or because of an intervention, that is greater than 6.3% would
372 represent real difference and not just measurement error. Finally, this study also provides normative data for
373 knee cartilage thickness measured by sonography. Considerable variability exists in the femoral cartilage
374 thicknesses of healthy individuals. However, cartilage thickness appears greatest at the intercondylar notch
375 compared to the medial and lateral facets. Furthermore, the data suggest that females have reduced cartilage
376 thickness compared with males and that both ageing and anthropometric measures affect cartilage thickness
377 differently in males and females. This research offers interesting data for the study of the patello-femoral joint.

378

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477 **Table** Error! No text of specified style in document. Physical characteristics of participants and knee cartilage thickness

	Men (n = 55)		Women (n = 22)		Total (n = 77)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Age (years)	45 (18)	18 - 70	38 (20)	20 - 79	43 (18)	18- 79
Body height (m)	1.77 (0.06)	1.64 - 1.95	1.67 (0.06)	1.51 - 1.78	1.74 (0.08)	1.5 – 1.95
Body mass (kg)	79.7 (11.1)	63.3 - 120.7	66.3 (11.6)	40.5 – 89.4	75.8 (12.7)	40.5 - 120.7
BMI	25.4 (3.0)	21.0-35.7	23.7 (3.5)	17.7 - 30.2	24.9 (3.2)	17.7 - 35.7
Knee cartilage thickness (mm)						
Right						
Lateral	1.93 (0.29)	1.43 - 2.73	1.71 (0.29)	1.23 - 2.37	1.87 (0.30)	1.23 - 2.73
Notch	2.20 (0.40)	1.28 – 3.22	1.75 (0.23)	1.27- 2.36	2.07 (0.43)	1.27 – 3.22
Medial	1.95 (0.38)	1.15 - 2.97	1.65 (0.28)	1.06 - 2.30	1.86 (0.38)	1.06 - 2.97
Left						
Lateral	1.79 (0.30)	1.02 - 2.37	1.68 (0.34)	0.93 – 2.44	1.76 (0.32)	0.93 - 2.44
Notch	2.17 (0.40)	1.44 - 3.12	1.84 (0.32)	1.18 - 2.46	2.08 (0.41)	1.18 - 3.12
Medial	1.84 (0.34)	1.01 - 2.60	1.63 (0.31)	1.08 – 2.28	1.78 (0.34)	1.01 - 2.60

478 **Table 2** Reliability of cartilage thickness measurements made at visit 1 and visit 2 for all locations with
 479 comparisons between age and gender

Location	ICC (95% CI)	SEM	SEM%	SRD	SRD%
Overall (n=77)					
Notch	0.843 (0.790 - 0.883)	0.17	8.2	0.47	22.9
Medial	0.834 (0.778 - 0.876)	0.15	8.2	0.42	22.8
Lateral	0.779 (0.707 - 0.835)	0.15	8.3	0.42	23.1
Young adults (n=20)					
Notch	0.920 (0.854 - 0.957)	0.12	5.7	0.32	15.8
Medial	0.884 (0.792 - 0.937)	0.11	6.3	0.32	17.5
Lateral	0.906 (0.830 - 0.949)	0.10	5.4	0.28	14.9
Middle-aged adults (n=29)					
Notch	0.843 (0.747 - 0.905)	0.18	8.4	0.49	23.3
Medial	0.800 (0.684 - 0.877)	0.13	6.8	0.35	18.9
Lateral	0.639 (0.453 - 0.772)	0.17	9.2	0.47	25.4
Older adults (n=28)					
Notch	0.779 (0.651 - 0.864)	0.19	9.2	0.51	25.4
Medial	0.832 (0.731 - 0.898)	0.17	9.6	0.48	26.5
Lateral	0.788 (0.661 - 0.872)	0.15	8.7	0.42	24.2
Male only (n=55)					
Notch	0.804 (0.725 - 0.862)	0.18	8.3	0.50	23.3
Medial	0.803 (0.725 - 0.861)	0.16	8.7	0.45	24
Lateral	0.744 (0.645 - 0.819)	0.15	8.2	0.42	22.8
Female only (n=22)					
Notch	0.838 (0.723 - 0.908)	0.12	6.9	0.34	19.0
Medial	0.870(0.775 - 0.927)	0.10	5.8	0.27	16.2
Lateral	0.828 (0.708 - 0.902)	0.12	6.8	0.32	19.0

ICC = intra-class correlation; CI = confidence intervals; SEM = standard error of measurement; SRD = smallest real difference); young (≤ 25 years of age), middle-aged (26-50 years of age), and old age groups (≥ 51 years of age)

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482 **Figure 1** US transverse image of the femoral articular cartilage demonstrating the difference in image quality
483 and clarity between young, middle-aged, and old groups. Image A) represents the ‘young’ group (23-year-old
484 male), image B) represents the ‘middle-aged’ group (44-year-old male), and image C) represent the ‘old’ group
485 (69-year-old male). M = the location of medial facet; N = the intercondylar notch; L = the lateral facet

486

487 **Figure 2** The Bland-Altman plots demonstrate the mean difference between the cartilage thickness
488 measurements at visit 2 and visit 1 (i.e. visit 2 minus visit 1) plotted against the mean of the two visits (i.e. visit
489 1 plus visit 2, divided by 2). Plot A) represents intercondylar notch, B), lateral facet and C) medial facet (solid
490 line represents mean difference and dashed lines represent upper and lower limits of agreement)

491

492 **Figure 3** Variation of mean femoral cartilage thickness at the intercondylar notch, lateral facet and medial facet
493 with physical characteristics of the participants. A) Age, B) Height, C) Weight, and D) BMI. R-value,
494 significance value, and regression equation are also presented above with significant findings highlighted in
495 bold. Solid, dashed and round dot trendline = intercondylar notch, lateral facet, and medial facet, respectively

496

497 **Figure 4** Presents variation of mean femoral cartilage thickness at the intercondylar notch, lateral facet and
498 medial facet with physical characteristics of the participants for both males and females A) age, B) height, C)
499 Weight, and D) BMI. R-value, significance value, and regression equation are also presented above with
500 significance highlighted in bold. Black trendline = male; grey trendline = female; Solid line = notch; dashed
501 trendline = lateral; round dot trendline = medial

502

503 **Figure 5** Mean cartilage thickness measurements at the medial facet, intercondylar notch and the lateral facet: *
504 = significant difference between groups at $P < 0.01$ level. Data are means \pm SD

505

506 **Figure 6** Mean cartilage thickness measurements at the medial facet, intercondylar notch and the lateral facet
507 for both male ($n = 17$) and female ($n = 17$) participants, matched for age and BMI. * = significant difference
508 between groups at $P < 0.01$ level. Data are means \pm SD

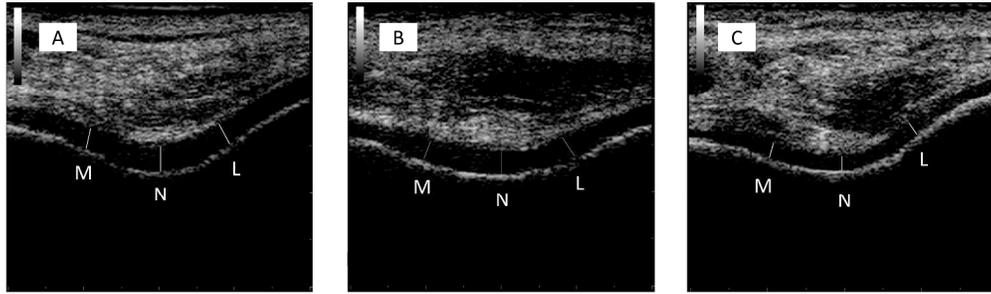


Figure 1. US transverse image of the femoral articular cartilage demonstrating the difference in image quality and clarity between young, middle-aged, and old groups. Image A) represents the 'young' group (23-year-old male), image B) represents the 'middle-aged' group (44-year-old male), and image C) represent the 'old' group (69-year-old male). M = the location of medial facet; N = the intercondylar notch; L = the lateral facet

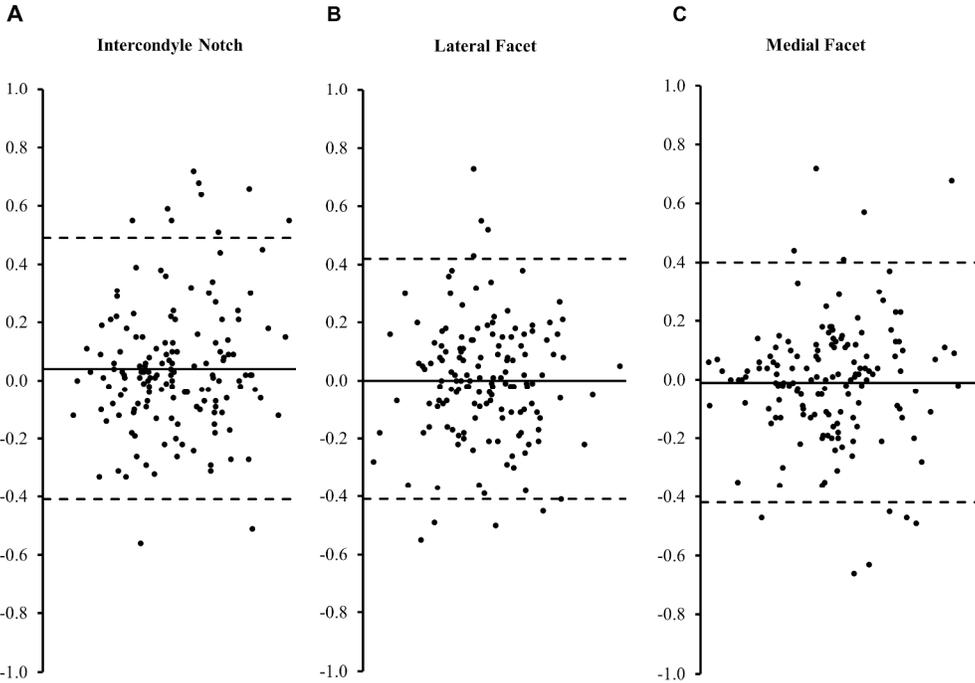


Figure 2. The Bland-Altman plots demonstrate the mean difference between the cartilage thickness measurements at visit 2 and visit 1 (i.e. visit 2 minus visit 1) plotted against the mean of the two visits (i.e. visit 1 plus visit 2, divided by 2). Plot A) represents intercondylar notch, B), lateral facet and C) medial facet (solid line represents mean difference and dashed lines represent upper and lower limits of agreement)

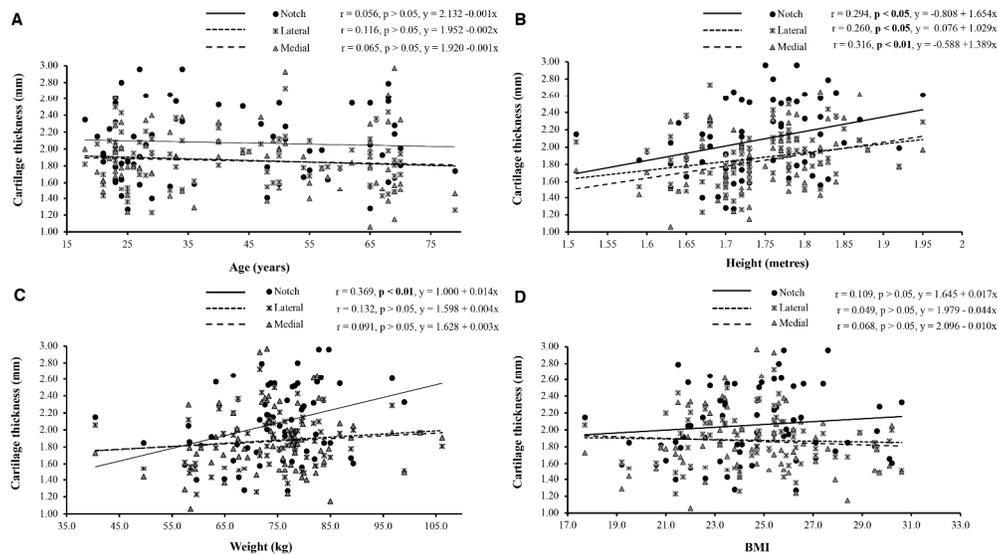


Figure 3. Variation of mean femoral cartilage thickness at the intercondylar notch, lateral facet and medial facet with physical characteristics of the participants. A) Age, B) Height, C) Weight, and D) BMI. R-value, significance value, and regression equation are also presented above with significant findings highlighted in bold. Solid, dashed and round dot trendline = intercondylar notch, lateral facet, and medial facet, respectively

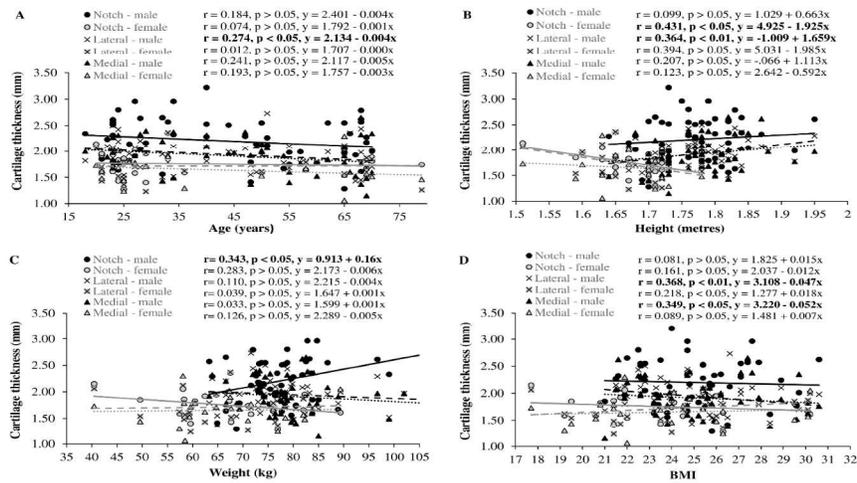


Figure 4. Presents variation of mean femoral cartilage thickness at the intercondylar notch, lateral facet and medial facet with physical characteristics of the participants for both males and females A) age, B) height, C) Weight, and D) BMI. R-value, significance value, and regression equation are also presented above with significance highlighted in bold. Black trendline = male; grey trendline = female; Solid line = notch; dashed trendline = lateral; round dot trendline = medial

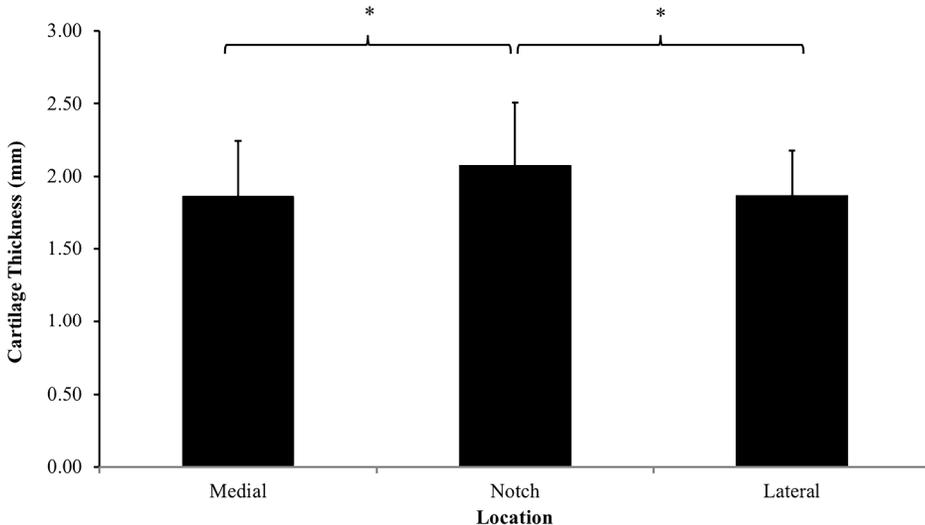


Figure 5. Mean cartilage thickness measurements at the medial facet, intercondylar notch and the lateral facet: * = significant difference between groups at P < 0.01 level. Data are means ± SD

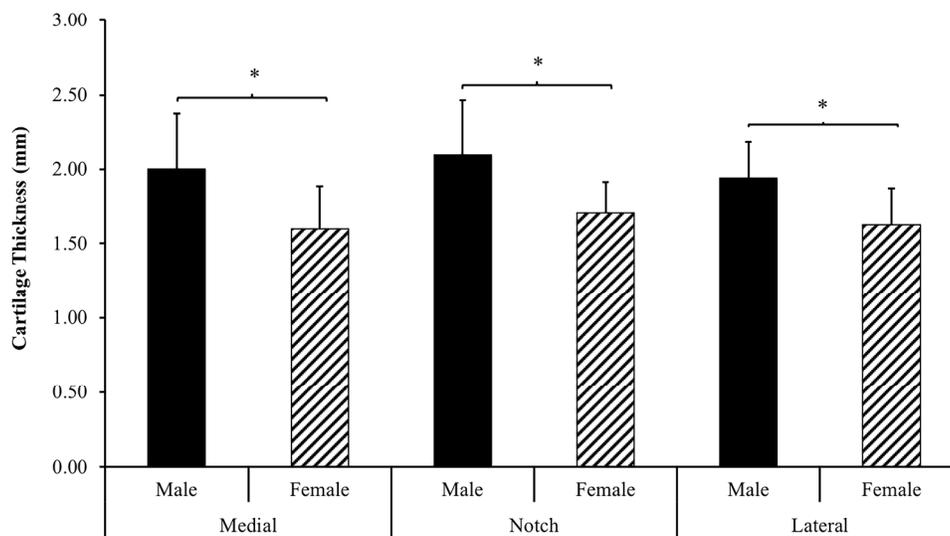


Figure 6. Mean cartilage thickness measurements at the medial facet, intercondylar notch and the lateral facet for both male (n = 17) and female (n = 17) participants, matched for age and BMI. * = significant difference between groups at P < 0.01 level. Data are means \pm SD