The effect of limb position on measured values of vastus lateralis muscle morphology using B Mode ultrasound

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Abstract

It is not known whether different leg positions influence measurements of muscle thickness, pennation angle and fascicle length. The primary aim of this study was to determine whether extension or flexion of the leg affected measurements of muscle morphology in the vastus lateralis. Thirty-two male professional football players participated in the study. B mode ultrasound (LOGIQ e, GE Healthcare, United States) was used to capture images of the vastus lateralis from the dominant leg of each participant when their leg was (i) extended at the knee and (ii) flexed at the knee by 90 degrees. Data was analysed using paired t-tests. Muscle thickness and pennation angle were was greater when the leg was extended (2.43 ± 0.18cm vs. 2.36 ± 0.17cm; $t(31) = 2.76$, $p < 0.010$; 18.47 ± 1.18° vs. 16.87 ± 1.14°; $t(31) = 7.59$, $p < 0.001$, respectively). Fascicle length was greater when the leg was in flexion (9.87 ± 0.53cm, flexion vs. 9.04 ± 0.92cm; $t(31) = -7.652$, $p < 0.001$). The intra rater reliability of the investigator was assessed using a 2-way mixed-effects model. In conclusion, leg position affects measurements of muscle morphology in vastus lateralis, this should be considered when comparing findings between studies.

Key words: muscle thickness, pennation angle, fascicle length, ultrasound, knee flexion, knee extension.

Introduction

Real time brightness mode (B mode) ultrasound is an imaging technique that allows for the visualisation of internal anatomy by producing cross-sectional greyscale two dimensional images made up of echoes returning from the tissues (English et al., 2012). B mode ultrasound is used in clinical practice, for example to screen and diagnose sarcopenia via measurements of muscle thickness, cross-sectional area, fascicle length, pennation angle and tissue composition (Perkisas et al., 2016 and 2021; Ticinesi et al., 2018; Cruz-Jentoft et al., 2019, and within research to investigate the effects of loading and unloading (Narici and Cerretelli, 1998), exercise training (Reeves et al., 2004 and 2009; Narici et al., 2005; Franchi et al., 2014) and ageing (Narici et al., 2003; Abe et al., 2011, 2014a and 2014b) on muscle morphology. Advantages of B mode ultrasound compared to Magnetic Resonance Imaging and Computed Tomography, include portability, lower cost, shorter test duration, and fewer hazards (Reimers et al., 1993; Sipilä and Suominen, 1993; Harris-Love et al., 2016; Stringer and Wilson, 2018). Variation in techniques and procedures in research studies using ultrasound has been reported (English et al., 2012). Moreover, it has been suggested that ultrasound technique depends on the experience and expertise of the operator posing a risk to the consistency, accuracy and precision of measurements (Wagner, 2013).

Various factors influence measurements taken using B mode ultrasound including equipment, operational settings, anatomical landmarks (to standardise the measurement site), probe orientation, patient positioning (Ticinesi et al., 2018; Perkisas et al., 2021) and probe pressure (Vahlgren et al., 2019). In clinical practice, measurements of muscle thickness, pennation angle and fascicle length of the leg are usually undertaken with the patient supine, knee fully extended and the muscle 'relaxed' (Ticinesi et al., 2018; Perkisas et al., 2021).

Within research settings, the position of the leg for the measurement of muscle thickness, pennation angle and fascicle length varies considerably (Perkisas et al., 2021). For example, measurements of muscle thickness have been taken from a leg in extension when participants were supine (Melo et al., 2016) and whilst standing (Sanada et al., 2006; Abe et al., 2011, 2014a and 2014b). Muscle thickness has also been measured in flexion at 50° (Kleinburg et al., 2016), and 45° (Blazevich et al., 2006). Muscle morphology differs according to leg position. Significantly larger measurements of muscle thickness, pennation angle and cross-sectional area have been reported for the vastus lateralis when standing vs. supine (Wagle et al., 2017). Furthermore, measurements of pennation angle are larger (Fukutani et al., 2015) and fascicle length shorter (Fukunaga and Kurihara, 1997) in knee extension vs. knee flexion.

The position of the leg has also been shown to result in changes to fluid levels present in the anterior thigh near the lateral epicondyle (Jesling et al, 2013). Specifically, measurements of fluid using ultrasound were larger when the participant was standing vs. supine with the knees extended (Jesling et al, 2013). The lowest levels of fluid measured were when the participants were supine with the knees flexed at 30° (Jesling et al, 2013). Collectively the evidence suggests that positioning of the leg is an important procedure to consider when measuring muscle morphology of the anterior thigh region.

There is a paucity of studies that have measured muscle thickness, pennation angle and fascicle length of the vastus lateralis in full knee extension vs. knee flexion of 90° whilst the participant is supine, and the muscles are relaxed. Studies which have investigated vastus lateralis muscle morphology have participants seated with the hip flexed (Fukutani et al., 2015), or participants supine but with muscles actively contracted to move the leg from flexion to extension (Fukunaga and Kurihara, 1997). The primary aim of our study was to compare the effect of knee extension and knee flexion (at 90°) on measurements of muscle thickness, pennation angle and fascicle length in vastus lateralis when the participant is supine and the leg relaxed. A secondary aim of the study was to determine the intra rater reliability of the investigator when taking measurements of muscle morphology in the vastus lateralis.

Materials and methods

Study design

This study used a cross-over design to measure muscle thickness, pennation angle and fascicle length in 2 leg positions; (i) knee extended, and (ii) knee flexed at 90^{o}. Ethical approval was obtained from the Leeds Beckett University Research Ethics Committee, and the study was conducted in accordance with the Helsinki Declaration for human research.

Participant recruitment, screening and enrolment

Participants provided written informed consent before participating in the study. A convenience sample of thirty-two full time male professional football players with no current lower leg injury took part in the study (mean ± SD age = 18.3 ± 0.5 years, height = 179 ± 1.7cm, mass = 77.3 ± 3.5kg). A sample of this nature was chosen to provide homogeneity of physical characteristics.

Data collection

Participants were told to refrain from exercise for 24 hours prior to a single study visit which lasted no more than 30 minutes. During the study visit participants were familiarised with equipment and measurement procedures. Images of vastus lateralis of the dominant leg were taken with the participant supine on a plinth with knee extended and then with knee joint off the plinth and flexed at 90 degrees (Fig. 1). The order of the measurements and leg positions were the same for each participant. The investigator moved the leg passively from the knee extension position to the flexion position to avoid a muscle contraction.

Fig.1

Ultrasonography: Muscle thickness and architecture

Images of the vastus lateralis were captured using B mode ultrasound (LOGIQ e, GE Healthcare, Illinois, United States) with a 5cm linear array probe (5Hz) using the following sequence: participant preparation, image capture and measurement of muscle morphology.

Participant preparation

The measurement site was determined by measuring 50% of the distance between the greater trochanter and lateral femoral condyle. This was marked using a skin friendly pen. To ensure the measurement site was on the belly of the muscle, participants were asked to contract the quadriceps whilst the investigator applied appropriate resistance and palpated the muscle to determine the location of the bulk of the muscle belly. If the original mark was not on the muscle belly, a new mark was made whilst ensuring to keep in line with the original 50% mark.

Image capture

Images were taken from each participant for each position. The head of the probe was coated with water soluble transmission gel to provide acoustic contact and reduce the pressure applied to the dermal surface. The probe was orientated longitudinally and parallel to the fascicular path in the sagittal plane. The subcutaneous fat and the superficial and deep aponeuroses of the muscle were identified onscreen. The probe was positioned on skin of the measurement site ensuring minimal pressure was applied and manipulated until the superficial and deep aponeurosis were parallel and three clear fascicles originating from the deep aponeuroses were visible. Two images were captured in each leg position and downloaded to imaging software (Image J, v.1.51k; National Institute of Health; Bethesda; USA).

Measurements of variables were made from images after the participant had completed the study visit.

Measurement of muscle morphology

Muscle thickness was measured as the perpendicular distance between the superficial and deep aponeuroses, with care being taken to not include them in the measurement. Pennation angle was measured as the angle at which the fascicle inserted into the deep aponeurosis. Fascicle length was measured as the length of the fascicular path from the superficial to the deep aponeurosis. The entire length of the fascicles was not visible due to the fascicles extending from the images, so fascicle length was measured using the extrapolation method, whereby a straight line was drawn extending off the edge of the superficial aponeurosis and another line was drawn from the end of the visible fascicle until it intercepted the superficial aponeurosis. Fascicle length was measured as the length of the visible fascicle plus the estimated fascicle - a technique which has been used by others and for which only a small error has been reported, as outlined by Blazevich et al. (2006), see Fig. 2.

Fig.2.

Data analysis

Data analysis was conducted using SPSS v25. Descriptive statistics were calculated for each condition. Measurements of muscle thickness, pennation angle and fascicle length were taken from two separate images for each leg position, for each participant. The mean of the repeat measurements of muscle thickness, pennation angle and fascicle length was calculated from the two images taken in both extension and flexion. Data was assessed for significant outliers via a box plot and normal distribution via the Shapiro Wilkes. Paired samples t-test was used to determine whether there was a statistically significant mean difference between measurements of muscle thickness, pennation angle and fascicle length in knee extension and knee flexion. No assumptions for a paired t test were violated. An Alpha of $P< 0.05$ was classed as significant.

To determine the intra rater reliability of the investigator for taking measurements of muscle morphology in the vastus lateralis, the intra class correlation coefficient (ICC) and associated 95% confidence interval (95% CI) were calculated using a 2-way mixed-effects model (absolute agreement) (Koo and Li, 2016). The standard error of measurement (SEM) was calculated using the following formula: SEM = $SD\ x \sqrt{1 - ICC}$ (Atkinson and Nevill, 1998)

Bland Altman plots were created to determine the agreement between the repeated measurements of muscle thickness, pennation angle and fascicle angle taken. To construct the plots, the mean difference between the measurements were calculated (A – B) and plotted on the Y axis and the mean of the two measures ((A+B/2) were plotted on the X axis. To determine whether 95% of the data points lay within ± 1.96 SD of the mean difference, the 95% limits of agreement (LoA) were calculated using the following formula:

LoA (95%) = $mean\ difference\ \pm (1.96\ x\ SD\ difference)$ (Atkinson and Nevill, 1998).

Results

Muscle thickness and pennation angle were larger when the knee was extended compared to flexed ($p < 0.01$ and $p < 0.001$, respectively) whereas fascicle length was larger in the knee flexed position ($p < 0.001$, Table 1).

Table 1

To show the spread of muscle thickness, pennation angle and fascicle length measurements, including the median of the measurements, the minimum and maximum measurements, in the two positions, box plots with whiskers were constructed, see Fig. 3A, B and C. Figure 3A box plot illustrates a larger dispersion of data around the median in the knee extended position, but the data is skewed to the left of the median in the knee flexed position indicating the larger means of muscle thickness measurements taken are closer together than the smaller means of muscle thickness measurements in the knee flexed position. Figure 3B illustrates the dispersion of fascicle length data is similar in the two leg positions, yet the median of the knee flexion box plot sits outside the knee extension box plot, highlighting the difference in fascicle length measurements in the two leg positions. Figure 3C shows a larger dispersion of data around the median and a wider distribution of data in the knee extended position compared to the knee flexed position.

Fig. 3A.

Fig. 3B.

Fig .3C.

Excellent levels of intra rater reliability was achieved for measurements of muscle thickness (ICC= 0.95, 95% CI 0.9-0.98, $p<0.01$), good levels of reliability were shown for measurements of pennation angle (ICC= 0.88, 95% CI 0.75-0.94, $p<0.01$) and moderate levels of reliability were attained for measurements of fascicle length (ICC= 0.83, 95% 0.65-0.92, $p<0.01$) (Koo and Li, 2016). The smallest SEM was observed for measurements of muscle thickness (SEM = 0.04cm, mean = 2.4cm, 95% CI = 2.32 – 2.47cm), compared to the SEM for pennation angle (SEM = 0.5°, mean = 17.67°, 95% CI = 16.69 – 18.65°) and fascicle length (SEM = 0.29cm, mean = 9.46cm, 95% CI = 8.89 – 10.03cm).

The Bland Altman plots to examine the differences between the repeated measures of muscle thickness, pennation angle and fascicle length are displayed in figures 4A, 4B and 4C, respectively. Visual inspection of the graphs illustrates that most of the data points fall within the 95% LoA for muscle thickness, pennation angle and fascicle length. The mean difference between the repeated measurements were minimal: muscle thickness = 0.00cm (95% CI = - 0.01, 0.02cm), pennation angle = 0.01° (95% CI = - 0.18, 0.20°) and fascicle length = 0.03cm (95% CI = - 0.09, 0.15cm).

Fig. 4A.

Fig. 4B.

Fig .4C.

Discussion

Vastus lateralis muscle thickness, pennation angle and fascicle length are different when measured in supine knee extension and supine knee flexion while the muscle is relaxed. Specifically, differences in muscle thickness and pennation angle were larger in the supine position with the knee in full extension (2.43 ± 0.18cm *vs.* 2.36 ± 0.17cm and 18.47 ± 1.18˚ *vs.* 16.87± 1.14˚) and differences in fascicle length were larger in the supine position with the knee flexed at 90 degrees (9.87 ± 0.53 *vs.* 9.04 ± 0.52cm). These findings are supported by Reeves et al. (2004) who reported the longest vastus lateralis fascicles when the knee was angled at 90° and the highest pennation angle measurements of the vastus lateralis when the knee was angled at 10° (near to full extension).

Previous studies which have compared measurements of muscle morphology in various angles of knee extension and flexion have measured pennation angle and fascicle length, but not thickness (Fukunaga and Kurihara, 1997; Reeves et al., 2004; Fukutani et al., 2015). Measurements of muscle thickness are larger in knee extension compared to knee flexion. This study has both clinical and research implications. In clinical practice it is suggested that the muscles should be relaxed during examination (Ticinesi et al., 2018; Perkisas et al., 2021). The clinical implications of these findings, particularly relating to muscle thickness, are that measurements of muscle thickness when assessing and diagnosing sarcopenia across the community should be taken in the same position. It is important to consider that the sample was made up of younger adults for this study and it could be possible that the younger adults were more sensitive to changes in the knee joint angle compared to older adults who have less muscle quantity (Janssen et al., 2000). Conversely, the muscles of the quadriceps insert into the same location and functionally these muscles will shorten when the knee extends and lengthen when the knee flexes regardless of age. Thus, it could be argued that the findings of this study are not affected by the samples age. That said, it may be prudent that another study is conducted which includes a sample of healthy adults with a mixed age -range to warrant these study findings. Regardless of the age of the sample, it is important that any normative data which is reported should state the position of the participant and leg that measurements were taken in and any comparisons of muscle thickness measurements to the normative data should be taken in the same leg position as the position used to calculate the normative data. In research settings the validity of comparisons between measurements of muscle morphology taken can now be improved.

More importantly, the consequence of the findings from this study are that previous and/or future studies which have measured muscle thickness and pennation angle in a supine knee extended position will be larger than studies which have/may measure(d) muscle thickness and pennation angle in a supine knee flexed position. On the other hand, previous and/or future studies which have measured fascicle length in a supine knee extended position will be smaller than studies which have/may measure(d) fascicle length in a supine knee flexed position. Meaningful comparisons between studies with similar aims are not valid when different leg positions have been reported. For example, Ema et al. (2013). Aagaard et al. (2001) and Seynnes et al. (2007) measured pennation angle and fascicle length after a resistance training intervention. However, Ema et al. (2013) positioned the knee in full extension, whereas Aagaard et al. (2001) and Seynnes et al. (2007) positioned the knee into flexion at 90° and 80°, respectively. Therefore, comparisons between the findings from Ema et al. (2013) to the other studies (Aagard et al., 2001; Seynnes et al., 2007) should be made with caution due to the different knee positions used. Future studies should report the position of the participant and leg in the measurement protocol as this will enable researchers to make an informed decisions on whether comparisons between the studies are valid. Furthermore, a standard measurement protocol for measuring vastus lateralis muscle morphology in research would help to reduce the variability of techniques used and improve the consistency of measurements taken both within and between studies.

Measurements of fascicle length are smaller in knee extension compared to knee flexion. Possible reasons for these differences may be the joint position and muscle activation altering both the shape and structure of the muscle (Thoirs et al., 2009). Although no active contraction of the muscle occurred during the imaging, it could be argued that when the knee was extended the bulk of the muscle would be larger than if the knee was flexed as this would stretch and elongate the muscle. The shorter fascicles in the knee extended position may have been due to the fascicles taking up the elongation of the muscle tendon when the muscle is shortened (Reeves and Narici, 2003; Fukunaga et al., 2015) and the larger pennation angle measurements may be a result of the contractile elements shortening (sarcomeres, actin and myosin) and producing tension resulting in a greater angle at which the fascicles pull on the tendon (Nordin and Frankel, 2001).

Prediction equations for estimating skeletal muscle mass via measurements of muscle thickness have been developed (Sanada et al., 2006). However, these equations were based upon measurements of muscle thickness taken in the standing position. A study by Thoirs et al. (2009) reported larger measurements of muscle thickness in the recumbent position compared to standing and concluded that new prediction equations for estimating skeletal muscle mass based on measurements of muscle thickness taken in the recumbent position are required. Our study suggests that new prediction equations for estimating skeletal muscle mass is required for measurements of muscle thickness taken in a supine knee extended and supine knee flexed position.

The ICC results reveal that measurements of fascicle length are less reliable than measurements of muscle thickness and pennation angle. Lower ICC scores for measurements of fascicle length (ICC = 0.8, moderate) compared to muscle thickness (ICC = 0.96, excellent) or pennation angle (ICC = 0.87, good) within this study provide evidence of this. Further supporting this point is the larger SEM value observed for fascicle length (0.29cm) compared to muscle thickness (0.04cm). The larger variability in the fascicle length measurements is important to consider as this may result is a misdiagnosis in a clinical setting (depending on the cut -off value) or a mis interpretation of a finding in research. That said, the Bland Altman plots revealed that 95% of the data points fell within the 95% LoA for all the variables investigated. Additionally, the mean differences observed were close to zero and regression analysis revealed beta values close to zero and non- significant relationships between the absolute difference (A-B) and the mean of the repeated measurements ((A+B/2), indicating no systematic bias in the measurements of muscle thickness, pennation angle and fascicle length. Taking all the results into account, it can be concluded that measurements of fascicle length using B mode ultrasound are reliable, however there are larger variances of measurement error when measuring fascicle length compared to muscle thickness and pennation angle.

The consequence of the lower reliability scores and larger variance of measurement error for fascicle length measures is that conclusions made regarding alterations to fascicle length may be due to the reliability of the measurements of fascicle length taken within the study rather than the dependent variable being assessed. Although small error rates have been reported for the extrapolation technique to measured fascicle length used in this study (Blazevich et al., 2006), architectural differences along the entire length of the muscle have been reported, including the vastus lateralis (Blazevich et al., 2009). The extended field of view technique is more advantageous as it allows for a panoramic view of the entire fascicle and does not rely on any extrapolated estimation of the length of the fascicle (Franchi et al., 2018). If researchers do not have access to the extended field of view technique, interpretation of data may be limited. Future studies should evaluate the reliability of investigators and this should be stated in study reports as this will help to identify whether alterations in fascicle length, if observed, may be due to measurement error or the variable (s) being investigated variable.

Limitations

As a single investigator took the measurements of vastus lateralis muscle morphology it could be argued that this may have resulted in subconscious investigator bias towards a specific result when imaging the muscle, for example bias towards larger pennation angle measurements in knee extension compared to knee flexion. To reduce the effect of investigator bias, measurements of morphology were not taken on the same day as the images were taken and the images were coded randomly, therefore when the investigator came to take the measurements, they did not know which position the image was taken in. Another limitation of this study is that only one knee joint angle of flexion was selected in this study (90° of knee flexion) which limits the comparability of the findings from this study to other studies which have also positioned the knee in 90° of flexion.

Perspectives

Leg position (knee extension *vs.* knee flexion) has an effect on vastus lateralis muscle morphology. The leg position used to measure vastus lateralis morphology in studies may be a possible reason for some of the differences in study findings between studies with similar aims. Future studies should state the position of the participant and leg in the measurement protocol. Comparisons between studies which report different participant and limb positions should be made with caution. Ultrasound measures of fascicle length are less reliable and have larger errors of measurement than measurements of muscle thickness and pennation angle. Future studies should assess the reliability of investigators when measuring fascicle length in the vastus lateralis and where possible the extended field of view technique should be utilised.

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Table 1: Vastus lateralis mean muscle thickness, pennation angle and fascicle length in supine with knee extended and supine with knee flexed and paired t test analysis

	Knee ext	Knee flex	Δ means	t	95% CI (lower-upper)
Muscle thickness (cm)	2.43 ± 0.18	2.36 ± 0.17	0.07	2.76*	.16 - .11
Pennation angle (°)	18.47 ± 1.18	16.87 ± 1.14	1.6	7.59**	1.20 - 1.17
Fascicle length (cm)	9.04 ± 0.52	9.87 ± 0.53	0.83	-7.65**	.62, -1.06

Values are displayed as mean ± standard deviation

Δ means = difference between the mean of the repeat measurements of MT, PA & FL measured in supine knee extended position and supine knee flexed position; T = the t-test statistic; * = p < 0.01; ** = p < 0.001; 95% CI = lower and upper 95% confidence intervals

Figure legends

Fig.1. Image A represents the leg positioned in knee extension and Image B shows the position of the leg in knee flexion

Fig.2. Longitudinal image of the vastus lateralis demonstrating the measurement of muscle thickness, pennation angle and fascicle length via linear extrapolation**.**

Fig. 3A. The box plot illustrates the mean muscle thickness in the two positions: supine with the knee extended and supine with the knee flexed. The whiskers on the box plot represent the spread (the minimum to the maximum measurement) of the mean muscle thickness measurements.

Fig. 3B. The box plot illustrates the mean fascicle length in the two positions: supine with the knee extended and supine with the knee flexed. The whiskers on the box plot represent the spread (the minimum to the maximum measurement) of the mean fascicle length measurements.

Fig .3C. The box plot illustrates the mean pennation angle in the two positions: supine with the knee extended and supine with the knee flexed. The whiskers on the box plot represent the spread (the minimum to the maximum measurement) of the mean pennation angle measurements.

Fig. 4A. Bland Altman plot illustrating the test- retest reliability of muscle thickness measures based upon the mean of two repeated trials.

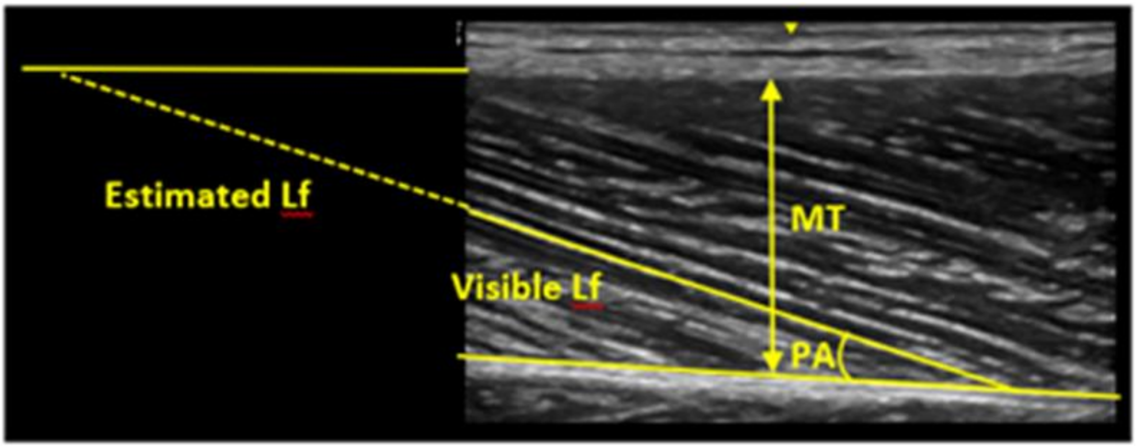
Fig. 4B. Bland Altman plot illustrating the test- retest reliability of pennation angle measures based upon the mean of two repeated trials.

Fig. 4C. Bland Altman plot illustrating the test- retest reliability of fascicle length measures based upon the mean of two repeated trials.

1A

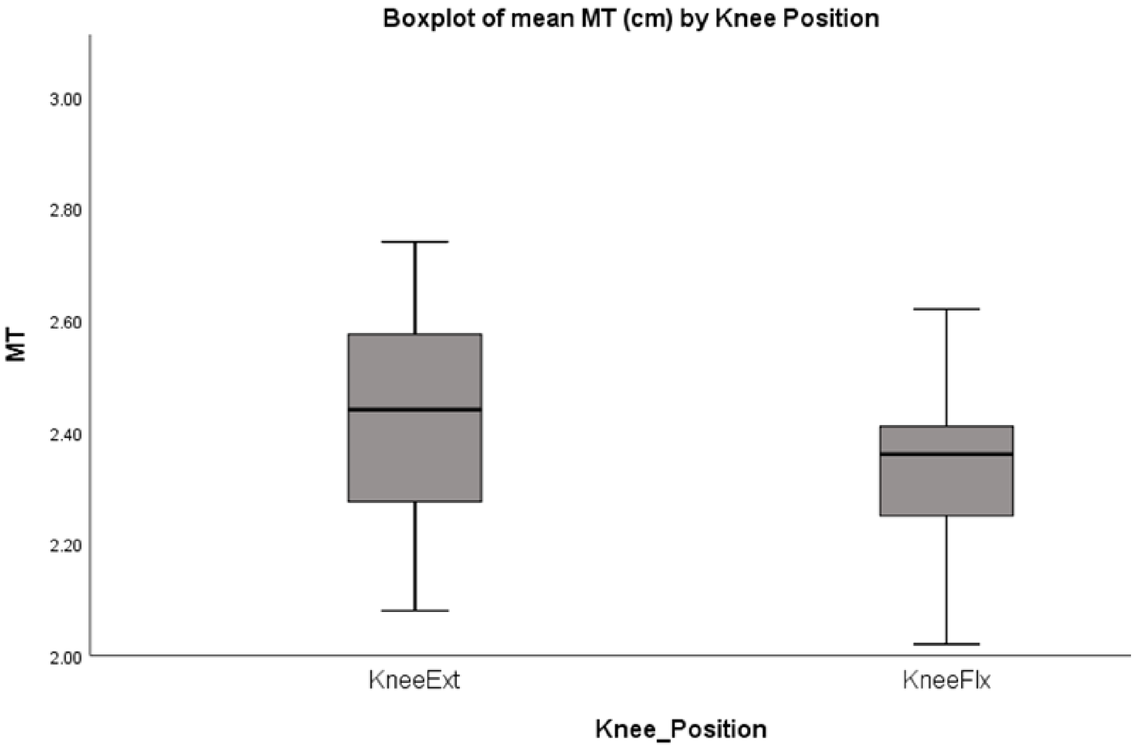
1B

2



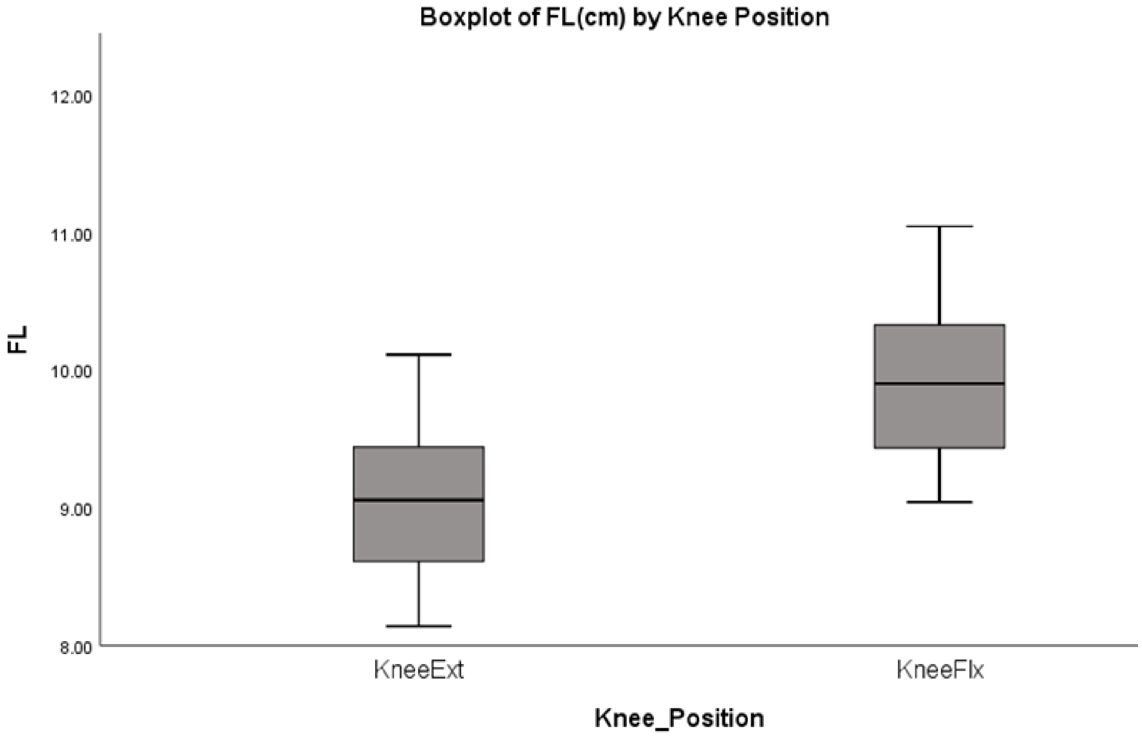
Estimated Lf
MT
Visible Lf
PA

3A



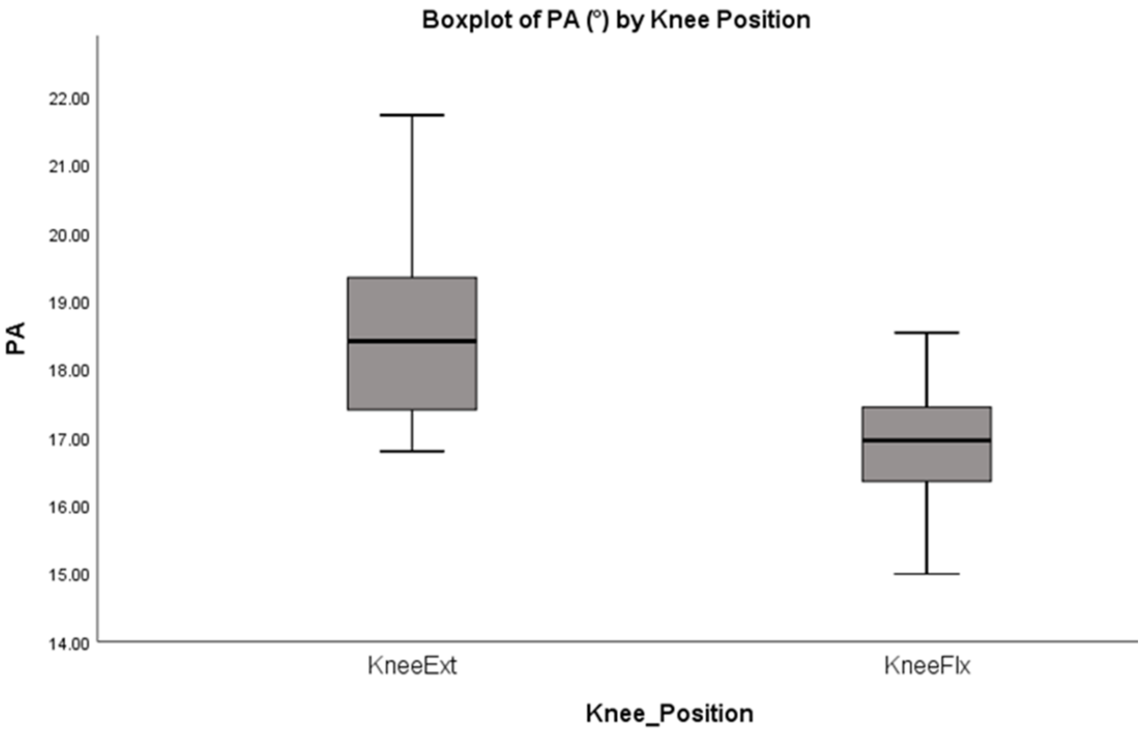
Boxplot of mean MT (cm) by Knee Position
MT
3.00
2.80
2.60
2.40
2.20
2.00
KneeExt
KneeFlx
Knee_Position

3B



Boxplot of FL(cm) by Knee Position
12.00
11.00
10.00
9.00
8.00
FL
KneeExt
KneeFlx
Knee_Position

3C



Boxplot of PA (°) by Knee Position
22.00
21.00
20.00
19.00
18.00
17.00
16.00
15.00
14.00
PA
KneeExt
KneeFlx
Knee_Position