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The validity and reliability of the Achilles tendon moment arm assessed with Dual-energy X-ray absorptiometry, relative to MRI and ultrasound assessments

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Abstract

Dual-energy X-ray absorptiometry (DXA) in single energy mode has been shown to permit the visualisation of bone and soft tissue, such as the patellar tendon through two-dimensional sagittal imaging. However, there is no validated DXA-based measurement of the Achilles tendon moment arm (d_{AT}). The aims of this study were: 1) to compare *in vivo* DXA derived measurements of the d_{AT} at rest against two previously validated methods: tendon excursion (TE) and magnetic resonance imaging (MRI) at three ankle angles (-5°, 0° and +10°). 2) analyse the intra-day reliability of the DXA method at all ankle angles and compare between methods. Twelve healthy adults (mean±SD: 31.4±9.5 years; 174.0±9.5 cm; 76.2±16.6 kg) participated in this study, involving test-retest DXA scans, ultrasound scans and one MRI scan. The d_{AT} was defined as the distance from the centre of the calcaneal-tibial joint axis to the Achilles tendon (AT) muscle-tendon line of action. DXA derived d_{AT} measures were significantly greater than MRI measurements (19.7-24.9%) and were 45.2% significantly larger than the TE method. The test-retest reliability of the DXA technique at 0° was high [CV=1.38%; ICC=0.96] and despite the consistently larger d_{AT} lengths obtained using DXA, MRI and DEXA data were strongly correlated (r=0.878, p<0.001). In conclusion, the DXA technique allowed for highly reproducible *in vivo* d_{AT} measurement at rest, which has implications for the calculation of AT forces *in vivo* and the ability to predict the measurement from one tool to the other, thereby providing a novel basis to contrast existing and future studies.

Introduction

The Achilles tendon moment arm (d_{AT}) is a measure widely used within biomechanics and important when converting individual muscle forces from the *gastrocnemius* (GM) and *soleus* into a moment about the ankle or vice versa (Clarke et al., 2015; Maganaris et al., 1998; Rasske et al., 2017). d_{AT} length is defined as a perpendicular distance from the ankle joints axis of rotation to the Achilles tendon (AT) muscle-tendon line of action (Fletcher and MacIntosh, 2018; Maganaris, 2003; Sheehan, 2012). Its length has implications both clinically and experimentally as it can relate joint torques and muscle forces, with the d_{AT} vital in musculoskeletal modelling (Clarke et al., 2015; Maganaris, 2004; Maganaris et al., 1998; Sheehan, 2012). Accurate measurement of the d_{AT} length is important when discussing the muscle-tendon at the ankle joint as larger d_{AT} lengths allow for greater muscle-tendon displacement, velocities and larger joint moments (Maganaris, 2004).

Reliable and valid methods are required when obtaining parameters such as d_{AT} which proves difficult due to the complex nature of defining the axis of rotation (Alexander et al., 2017) and determining the line of action. Numerous techniques have been implemented in the measurement of d_{AT} *in vivo* such as magnetic resonance imaging (MRI) and ultrasound imaging as the accuracy and repeatability associated with medical imaging techniques surpass the simplicity of surface measurements and cadaver tissue (Alexander et al., 2017; Wilson et al., 1999). The ultrasound and MRI techniques have become common practice in the measurement of the d_{AT} (Fath et al., 2010). Whereas, to our knowledge, dual-energy X-ray absorptiometry (DXA), has yet to be routinely utilised in the measurement of d_{AT}. However, DXA under single energy mode has been shown to produce two-dimensional (2D) sagittal images that permit the visualisation of bone and soft tissue with high water content, formerly validated against MRI in the patellar tendon moment arm (d_{PT}) (Erskine et al., 2014). With a ‘high definition instant

vertebral assessment' (IVA-HD) DXA protocol, high-quality sagittal images of the knee joint allowed the d_{PT} to be measured when rested at full extension (Erskine et al., 2014).

MRI provides high visibility of the anatomical structure capturing bony configurations of the calcaneal-tibial joint, depicting soft tissue and allowing the line of action to be easily identified (Fath et al., 2010; Fletcher and MacIntosh, 2018; Hashizume et al., 2012; Rugg et al., 1990). This permits d_{AT} to be measured directly without erroneous assumptions regarding the actual path of the tendon (Rugg et al., 1990). With MRI, d_{AT} is estimated as a distance using the centre of rotation (COR) technique where the distance from the joint axis to the muscle-tendon line of action is measured (Rugg et al., 1990). The ultrasound technique termed tendon excursion (TE) operates without clear identification of the joint COR or muscle-tendon action line, providing an estimation of d_{AT} length (Fath et al., 2010). TE calculates d_{AT} during passive rotation of the ankle as the ratio of tendon displacement at the musculotendinous junction (MTJ) to joint rotation (Fletcher and MacIntosh, 2018).

Since no previous study has measured d_{AT} utilising DXA, the opportunity to directly compare the three techniques discussed above *in vivo* has not been available. The ability to utilise DXA in the measurement of d_{AT} is advantageous, as the novel method proposed, would be quicker, cheaper and more accessible compared to an MRI and allows the calculation of d_{AT} length whereas TE provides an estimation of d_{AT} length. Therefore, by directly comparing DXA, MRI and ultrasound a comparison can be provided for any measurement differences, which is essential when looking to reliably compare results between studies (Erskine et al., 2014). Furthermore, a relatively novel application of DXA d_{AT} requires validation, preferably against a recognised or the current gold standard technique such as MRI (Erskine et al., 2010; Onambele-Pearson and Pearson, 2012). The aims of the current study were twofold: 1) to investigate the *in vivo* assessment of d_{AT} at rest using: DXA, MRI and ultrasound to observe whether differences exist between the imaging protocols. 2) to determine the reliability of d_{AT}

measurements using DXA. It was hypothesised that: 1) the intraday reliability of the DXA protocol would be high, and 2) ultrasonography would underestimate measurements made using the MRI technique and that DXA measurements would overestimate those of the MRI and ultrasound at all ankle joint angles. We hypothesised that DXA would overestimate MRI measurements of d_{AT} length as a previous study which investigated this novel DXA method found DXA to overestimate MRI when comparing measurements of the d_{PT} (Erskine et al., 2014). Similarly, we hypothesised ultrasonography or the TE method to underestimate MRI d_{AT} measurements due to previous research highlighting this (Hashizume et al., 2016).

Methods

Participants

Twelve healthy adults (eight men and four women) were recruited to participate in this study in line with previous moment arm validation studies (Erskine et al., 2014; Hashizume et al., 2016). Age, stature and body mass (mean±SD) were as follows: 31.4±9.5 years, 174.0±9.5 cm, and 76.2±16.6 kg, respectively. Participants self-reported as physically active and free of lower limb musculoskeletal injuries, ankle joint/AT disorders with no history of ankle surgery and not pregnant (relating to the radiation exposure during the DXA scan). Participants provided written informed consent before participating in this study, which conformed with the Declaration of Helsinki (World Medical, 2013). This study was approved by the local ethics committee of Manchester Metropolitan University.

Experimental Protocol

Over two laboratory visits the participants d_{AT} of their right ankle was quantified using three techniques. On the first laboratory visit, the participants d_{AT} was assessed twice using a

Discovery W DXA scanner [Hologic Inc., Bedford, USA] to allow the reliability of the DXA protocol to be investigated, and assessed once using ultrasonography [Esaote Biomedica, Genoa, Italy]. On the second laboratory visit, 24 hours later, the participants d_{AT} was measured once using a 0.25-T G-Scan MRI scanner [Esaote Biomedica, Genoa, Italy].

Scanning Protocols

For the MRI protocol, participants laid on their left side and were instructed to remain still and relaxed inside a 0.25-T G-Scan MRI scanner [Esaote Biomedica, Genoa, Italy] with their right knee extended (Figure 1a). The sole of the right foot was positioned against a custom-made wooden device, with the ankle secured to the device using two non-elastic Velcro straps. The custom-made device allowed manipulation of the ankle and fixed the angle of the ankle joint for the subsequent scans (-5°, 0° and +10°). Sagittal ankle scans were acquired using a Turbo 3D T1-weighted sequence with the following scanning parameters: time of repetition=40 ms; time to echo=16 ms; matrix=256 x 256; field of view=180 mm x 180 mm; slice thickness=3.4 mm; interslice gap=0 mm.

[Insert Figure 1]

For the DXA session, six single energy IVA-HD scans [Hologic Inc., Bedford, USA] with the participant lying on their right side (Figure 1b) were taken at the three pre-determined ankle joint angles, with two scans taken per joint angle (-5°, 0° and +10°). The right ankle was manipulated and fixed at these angles using the same custom-made wooden device utilised in the MRI protocol (Figure 1b). The IVA-HD parameters were as follows: scan length=20.3 cm; scan width=13.7 cm; line spacing=0.0241 cm; point resolution=0.1086 cm; scanning time=11s. The acquired sagittal images of the participant's right ankle were attained by placing the ankle

151 joint with the lateral aspect of the limb within the imaging zone. To enable the reliability of
152 IVA-HD DXA scans to be analysed, the procedure was repeated, which required participants
153 to be removed and then repositioned back on the scanner in between DXA scans.
154 For the TE protocol, participants were seated on an isokinetic dynamometer [Cybex Norm,
155 Cybex International, New York, USA] with their right ankle securely fixed with inextensible
156 straps. The right knee was fully extended with the right thigh strapped to the dynamometer
157 chair (Figure 1c). The participants hip angle was set to 85° and the participants were secured
158 to the dynamometer at the shoulders and hip with inextensible straps. The centre of the lateral
159 malleolus was aligned visually to the axis of rotation of the dynamometer. Participants
160 preconditioned the muscle-tendon complex using five ramped isometric plantarflexions at 50%
161 of self-perceived maximum (Maganaris and Paul, 1999), and then performed three maximum
162 voluntary plantar-flexion contractions. Thereafter, the maximum range of motion (ROM) of
163 the dynamometer was set to the participant's voluntary maximum plantarflexion and maximum
164 dorsiflexion. To familiarise the participants, the ankle was passively rotated through the ROM
165 limit at a constant velocity of $1°\cdot s^{-1}$ prior to assessing d_{AT}. To measure d_{AT} the ankle was
166 passively rotated through the ROM, during which the displacement of the GM MTJ was
167 recorded using B-mode ultrasound with a 7.5 MHz, 50 mm linear probe [Esaote AU5,
168 Biomedica, Genoa, Italy]. The probe was fixed in position using a custom-built foam cast. To
169 ensure accurate measurements of displacement, a 2 mm echo-absorptive tape was placed over
170 the MTJ at 0° ankle position to check for movement of the probe. If any movement was detected
171 the test was repeated. This procedure was repeated to measure the reliability of the TE protocol,
172 the participant was removed from the dynamometer between assessments, with all pen
173 markings and tape removed before the measurement process was repeated.
174
175 **Image Analysis**

All DICOM images from the MRI and DXA scans were imported to a DICOM viewer [Osirix 2.7.5, Osirix Foundation, Geneva, Switzerland]. For MRI scans, the midsagittal slice was identified (typically between slice 12-15) and d_{AT} was then calculated from the joint COR identified using the Reuleaux' method (see Figure 2a). The d_{AT} for the single 2D DXA DICOM image was measured as reported for the MRI analysis (see Figure 2b).

When analysing d_{AT} with the TE method at 0° ankle angle, the maximal plantarflexion and dorsiflexion of one ROM test was screen grabbed and analysed using ImageJ [ImageJ 1.45s; National Institutes of Health] software. The displacement of the MTJ away from the echo absorptive marker was measured at maximal plantarflexion (See Figure 2c). The total displacement was then divided by the change in the ankle angle through a full ROM (Tomlinson et al., 2014).

[Insert Figures 2]

Statistical Analyses

Statistical analysis was performed using SPSS (Version 25, SPSS Inc., Chicago, IL, USA). Data reduction reliability was confirmed using two datasets for the TE, DXA and MRI. Coefficients of variance (CVs) for the novel DXA technique were calculated between datasets. The intra class correlation coefficients (ICC, model: 2-way mixed; types: absolute agreement and consistency) were calculated for the DXA method to determine the test-retest reliability of the novel DXA method (See Table 2). The mean value of the first dataset measurements for TE, DEXA and MRI was used in the statistical analysis. Parametricity of the d_{AT} data utilised the Shapiro-Wilk test (sample normally distribution) and the Levene's test (homogeneity of variance). Thus, a repeated-measures ANOVA (rANOVA) was used to examine between-methods differences of DXA, MRI and TE with a Bonferroni correction for post hoc pairwise

comparisons at 0° ankle angle. An rANOVA was used to examine between-methods differences of DXA and MRI with a Bonferroni correction for post hoc pairwise comparison at -5° and 10° ankle angle. A Pearson's product-moment correlation was used to examine the associations between the DXA and MRI d_{AT} data at -5°, 0° and 10° and between TE and DXA and TE and MRI at 0°. Bland-Altman plots were developed using SPSS to illustrate the mean difference between DXA and MRI derived d_{AT} measurements; the limits of agreement were set at 1.96×SD of the method difference. Statistical significance was accepted with $p<0.05$ and all data are presented as means±SD unless otherwise stated.

Results

DXA-originated d_{AT} values were consistently higher at all ankle angles measured (Table 1) than those determined from the MRI and TE techniques, especially at 0° ankle angle (Figure 3). Thus at 0° ankle angle, there was a main effect of measurement method on attained d_{AT} value ($F(1.144, 12.588)=133.571$, $p<0.001$). Post hoc tests revealed that DXA d_{AT} measurement resulted in a 19.7% greater d_{AT} measurement compared to MRI ($p<0.001$). MRI d_{AT} measurement resulted in a 40.8% greater d_{AT} measurement compared to TE ($p<0.001$) and when comparing TE and DXA, DXA d_{AT} measurements were 45.2% greater than TE d_{AT} measurements ($p<0.001$).

[Insert Figure 3]

There was an interaction between the DXA and MRI techniques and ankle angles ($F(1,11)=10.808$, $p=0.007$). Post hoc pairwise comparison revealed that at -5° ankle angle DXA d_{AT} measurements were greater (22.6%) than those derived from MRI technique ($p<0.001$) and at 10° ankle angle DXA d_{AT} measurements were larger (24.9%) than MRI ($p<0.001$).

There was a strong positive correlation between DXA and MRI (r=0.878, n=12, p<0.001) (See Figure 4).

[Insert Figure 4]

No relationship was observed between TE and DXA (r=0.383, n=11, p=0.219) or TE and MRI (r=0.293, n=11, p=0.356).

The test-retest reliability of the DXA method was excellent, demonstrated by low CVs and ICC values >0.75 at all ankle angles measured (See Table 2).

Heteroscedasticity was observed in the residuals (i.e. [DXA-MRI]^2] between DXA and MRI d_{AT} measurements) at -5° (p=0.010) and 10° (p=0.046) ankle angle. At 0° ankle angle, the distribution of the residuals was homoscedastic, suggesting that the between method difference was not dependent on d_{AT} when measured at this ankle angle.

This study observed a systematic over-approximation of d_{AT} length when measured using the DXA technique compared to the MRI technique. If DXA was utilised in place of MRI, DXA would overestimate MRI d_{AT} length by 9 to 11 mm (See Figure 5a, 5b and 5c).

[Insert Figure 5]

Discussion

In this study, the reliability of a novel DXA technique, for the measurement of individuals d_{AT} length *in vivo*, was assessed in a healthy young adult population. The main findings from this study have shown that the novel 2D DXA method produced highly reproducible d_{AT}

251 measurements given its low CVs and high ICCs. Thus, to our knowledge, this shows for the
252 first time that the DXA imaging technique enables a reliable measure of d_{AT} length. The
253 hypothesis was accepted as the novel DXA technique gave significantly larger d_{AT}
254 measurements at all ankle angles measured when compared to the established 2D MRI method
255 and the TE technique. Despite the consistently larger DXA d_{AT} measurements, the DXA and
256 MRI techniques were in strong agreement irrespective of inter-individual differences in ankle
257 joint dimensions. The agreement was shown through Bland-Altman plots and the significant
258 positive correlation between the DXA and MRI techniques when assessing d_{AT} length, *in vivo*.
259 A lack of association between TE vs. MRI and DXA was observed, suggesting its suitability
260 in measuring the d_{AT} needs further analysis.
261 The novel DXA technique permitted the visualisation of the AT, allowing the measurement of
262 individuals d_{AT}, through a single high-quality 2D sagittal image. The single energy IVA-HD
263 scan protocol discerns soft tissue with high water content such as the AT and enables the resting
264 ankle joint and more specifically the AT to be visualised at the investigated -5°, 0° and 10°
265 ankle angles. The sagittal images attained could depict the tibia, fibula, medial malleolus,
266 calcaneus, talus and navicular bone clearly (Figure 2b). Hence it was possible to measure the
267 d_{AT}, i.e. the perpendicular distance from the estimated ankle joints axis of rotation to the AT
268 muscle-tendon line of action (Maganaris et al., 2000). The visualisation of the AT through the
269 DXA derived image compared to the MRI derived image is less clear and appears as a shadow
270 (though distinct) image compared to the clear anatomical image provided by the MRI technique
271 (See Figure 3a and 3b). While this may cause a small potential error in discerning the AT from
272 the DXA sagittal images compared to MRI, we propose that a suitably trained researcher with
273 knowledge of the AT and the anatomical image of the ankle joint will be able to discern the
274 AT from the DXA derived sagittal image.

The 29.5±6.2 mm (TE) *in vivo*, d_{AT} values reported within this study were comparable to previous findings using TE. These previous studies have reported similar d_{AT} lengths compared with our data at 32 mm (29 to 36 mm 95% confidence intervals (CI)) (Baxter and Piazza, 2018) and 31±3.7 mm in sprinters when measuring d_{AT} with the TE technique (Lee and Piazza, 2009). However, even larger d_{AT} measurements have been reported when using the TE technique at 38 mm (35 to 42 mm 95% CI) (Fath et al., 2010) and 41.6±5.5 mm (Lee and Piazza, 2009) that would be comparable to our MRI derived d_{AT} values (43.3±3.7 mm (MRI)). The measurements reported using the novel 2D DXA technique, 53.9±5.2 mm, are closely comparable to previous d_{AT} values where researchers measured d_{AT} with the MRI method. Previously, d_{AT} values of 53 mm (51 to 56 mm 95% CI) (Baxter and Piazza, 2018) and 54 mm (51 to 57 mm 95% (CI) (Fath et al., 2010) have been reported when measured using the MRI technique. When rationalising the larger estimations of d_{AT} length through the DXA technique, it was previously addressed that the DXA method produces a single sagittal image which represents an 'average' view of the calcaneal-tibial joint (Erskine et al., 2014). Whereas the MRI technique allows for analysis at a multitude of 'slices' in the sagittal plane, possibly accounting for some of the 19.7-24.9% difference in d_{AT} lengths between DXA and MRI at the different ankle angles as the COR point will not be identical.

Previous research has demonstrated that the use of dissimilar techniques such as TE and MRI as well as dissimilar methodologies when using the same technique in measuring d_{AT} has led to variances in d_{AT} estimations (Sheehan, 2012). A weak relationship was reported, in our results, when comparing the TE method to the 3D MRI protocol. Previous literature has suggested that individuals have varying AT stiffness and slack length which makes evaluating individuals d_{AT} difficult when utilising the TE technique (Baxter and Piazza, 2018; Hashizume et al., 2016). Variances in AT stiffness and slack length dictate the potential tendon elongation

during passive ankle movements (TE) and illustrates variability within the healthy adult cohort (Fletcher and MacIntosh, 2018; Muraoka et al., 2002).

Hashizume et al. (2016) reported a significant correlation between TE and 3D MRI, however reporting a correlation value of (p=0.05, r^2=0.352) which constitutes a 'weak' relationship. This finding was similarly reported by Baxter and Piazza (2018) who revealed only a positive trend (p=0.052, r^2=0.21) but not either a significant or a 'strong' relationship. This led those researchers to suggest that the TE method may not be suitable to evaluate the singular variability of individuals d_{AT} (Hashizume et al., 2016). The lack of correlation observed within our results between TE and MRI (p=0.356, r^2=0.085) is thus comparable to previous research (Baxter and Piazza, 2018).

Our results observed larger d_{AT} lengths at greater plantarflexion ankle angles, with -5° ankle angle exhibiting the shortest d_{AT} length (Table 1). The novel DXA technique was shown to be highly reliable at 0° ankle angle, with low CV's and high ICC's. Both -5° and +10° were also shown to provide reliable measurements (Table 2).

The difference between methods in absolute d_{AT} lengths described here would have significant implications for the calculation of AT force. For example, the AT force in a healthy adult population with an ankle plantarflexion moment of 20 N m and a d_{AT} of 43 mm (measured via MRI) would be ~ 465 N. However, if the d_{AT} value measured via DXA replaced the MRI measure in the equation (e.g. 53 mm) the AT force would be calculated as ~ 377 N, a difference of ~ 88 N. It is acknowledged though, that investigations in different populations and additional work would be required before a universal correction factor may be applied (Erskine et al., 2014). A post hoc power analysis (1–β err prob) of this study was <0.99, thus confirming that the results of this study can be generalised to young healthy adult populations.

The findings of this study could allow for the use of DXA-derived d_{AT} measurements to be used in the calculation of the GM and AT properties. The results may also mean that future

research should be cautious when selecting the TE method to determine d_{AT} (Hashizume et al., 2016). With popularity of DXA rising in recent years to measure muscle mass and body composition, it would be beneficial to utilise DXA based d_{AT} measurements. The ability to utilise the DXA technique to measure d_{AT} would allow for researchers to determine muscle-tendon forces, which would be helpful in illustrating group differences and/or intervention induced variations in calf 'muscle strength'. The DXA technique, however, delivers radiation to participants, with the effective dose estimated to be 56 μSv with the IVA-HD protocol. Fortunately, this effective dose is 'extremely low' and is well below the maximum recommended annual dose regarded as safe, i.e. 1,000 μSv (Njeh et al., 1999). Despite the 'extremely low' radiation, researchers may wish to use DXA in future studies examining d_{AT} length as this novel protocol is quicker than the MRI technique and DXA is cheaper and more easily accessible. Studies examining muscle mass refer to DXA as the gold standard measurement technique and therefore if already being utilised in the assessment of muscle mass or body composition, researchers will be able to accurately determine d_{AT} length and therefore muscle-tendon forces in one laboratory visit.

This study utilised 2D imaging in all methods investigated, which needs to be addressed when comparing its findings to previous work. Hashizume et al. (2012) found that when employing 2D methods to measure d_{AT} length as opposed to 3D imaging techniques that the d_{AT} length will be overestimated. However, as all techniques investigated were 2D, the reliability and agreement between techniques can be accepted at the 2D level. Further investigation would be required to examine the agreement between the novel DXA technique and 3D MRI analysis. Following on from the results of this study, the use of DXA in the assessment of individuals d_{AT} has been shown to be reliable at rest. Further investigation should explore the measurement of d_{AT} during differing isometric muscle contraction intensity at a range of ankle joint angles.

Conclusion

This study has illustrated that reliable measurements of d_{AT} length at rest can be estimated from the IVA-HD DXA scan protocol. The novel DXA method gave consistently longer d_{AT} lengths (19.7-24.9%) at all ankle angles measured (-5°, 0°, +10°) compared to the reference MRI method. However, we have shown the novel DXA and MRI techniques were in strong agreement irrespective of inter-individual differences in ankle joint dimensions. This finding provides a novel method in the calculation of AT forces *in vivo* and allows comparisons to be drawn between existing and future studies.

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Conflict of interests

AJ Buffey - no conflict of interests

GL Onambélé-Pearson – no conflict of interests

RM Erskine - no conflict of interests

DJ Tomlinson - no conflict of interests

Data Reference

The datasets generated and/or analysed during the current study will be available in the Manchester Metropolitan University repository (link will provided upon publication).

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Tables

Table 1. The Achilles tendon moment arm (d_{AT}) length measured using dual energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) at the three predetermined ankle angles and tendon excursion (TE) at 0°.

	DXA	TE	MRI	*p-Value*
d_{AT} (-5°) (mm)	50.9±4.5	-	41.5±3.4	<0.001
d_{AT} (0°) (mm) (*)	53.8±5.0	29.5±6.2	43.3±3.6	<0.001
d_{AT} (10°) (mm)	56.7±5.8	-	45.4±3.8	<0.001

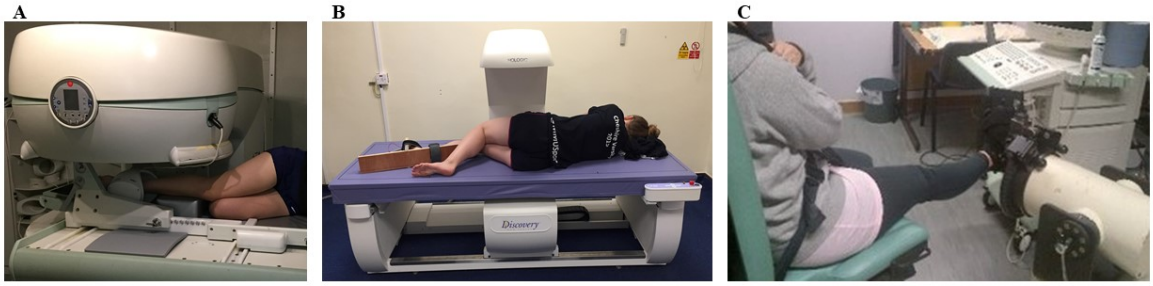
Data are reported mean±SD. (* denotes that the *p*-value reported was from the Bonferroni corrected post hoc).

Table 2. Achilles tendon moment arm (d_{AT}) measurement along with the CV (%) between the two datasets (test-retest) and ICC for the dual energy x-ray absorptiometry (DXA) method.

	DXA (-5°)	DXA (0°)	DXA (10°)
d_{AT} (mm) Test 1	50.9±4.5	53.8±5.0	56.7±5.8
d_{AT} (mm) Test 2	51.4±4.8	53.4±4.3	58.2±6.7
CV (%)	2.15	1.38	3.01
ICC (Lower CL - Upper CL) *	0.89 (0.68-0.97)	0.96 (0.86-0.99)	0.76 (0.38-0.92)
ICC (Lower CL - Upper CL) **	0.89 (0.66-0.97)	0.96 (0.86-0.99)	0.77 (0.37-0.93)

(* indicates absolute agreement and ** indicates consistency).

503 **Figure**



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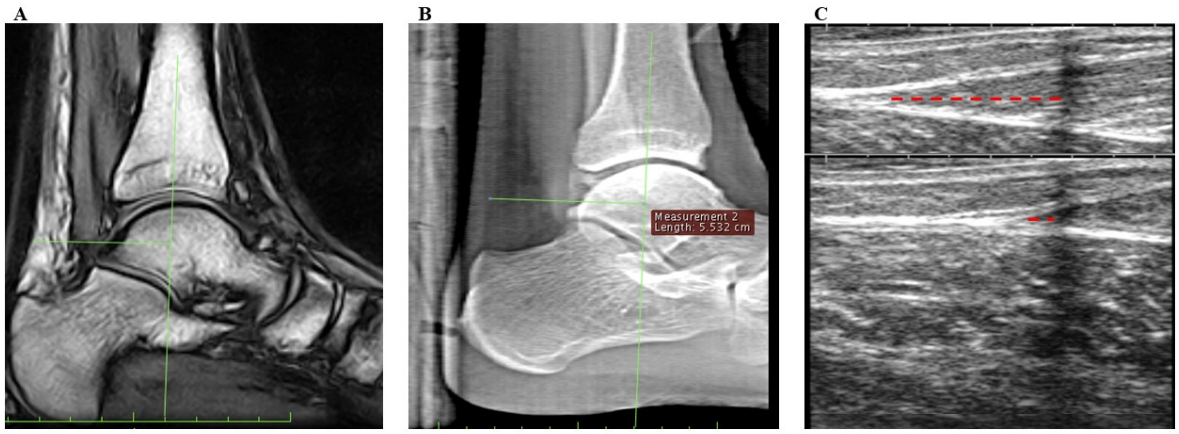
505 **Figure 1.** Representative images illustrating (A) the lower limb positioning within the (A)
506 magnetic resonance imaging, (B) dual energy x-ray absorptiometry (DXA) and (C) tendon
507 excursion protocol.



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509 **Figure 2.** Representative images demonstrating: (A) the anatomical landmarks used to measure
510 Achilles tendon (AT) moment arm *(d_{AT})* in the MRI COR method. The near horizontal green

line represents the measurement of the individuals d_{AT} from the AT line of action (straight white line) to the estimated centre of the calcaneal joint. (B) The measurement process of a single 2D dual energy x-ray absorptiometry (DXA) scan for the analysis of d_{AT}. The near horizontal green line represents the measurement of the individuals d_{AT} from the AT line of action to the estimated centre of the calcaneal joint and (C) illustrating tendon elongation measured during the tendon excursion technique. The dashed red lines represent the movement of the gastrocnemius medialis muscle-tendon junction during passive rotation of the ankle. The echo absorptive is shown as the faint black line through the ultrasound image.

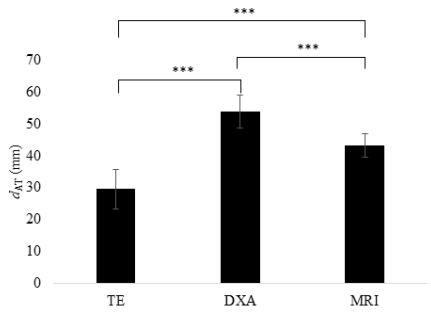

Figure 3. Displaying Achilles tendon moment arm (d_{AT}) length at 0° ankle angle measured by tendon excursion (TE), dual energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI). *** denotes a significant difference between tendon excursion (TE) and dual energy x-ray absorptiometry (DXA) measurements (p<0.001); between DXA and MRI (p<0.001); *** and between TE and MRI (p<0.001).

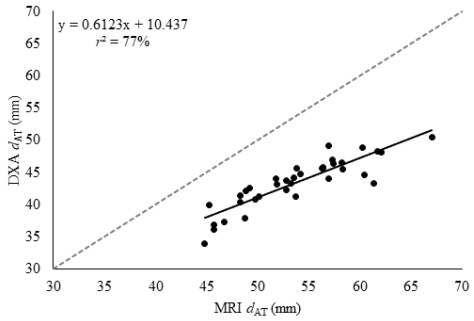

Figure 4. Displaying the Pearson correlation between measurements attained using the dual energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) techniques comparing measurements taken at all ankle angles (solid black line=linear correlation (r=0.878, p<0.001); dashed line=line of identity; n=12).

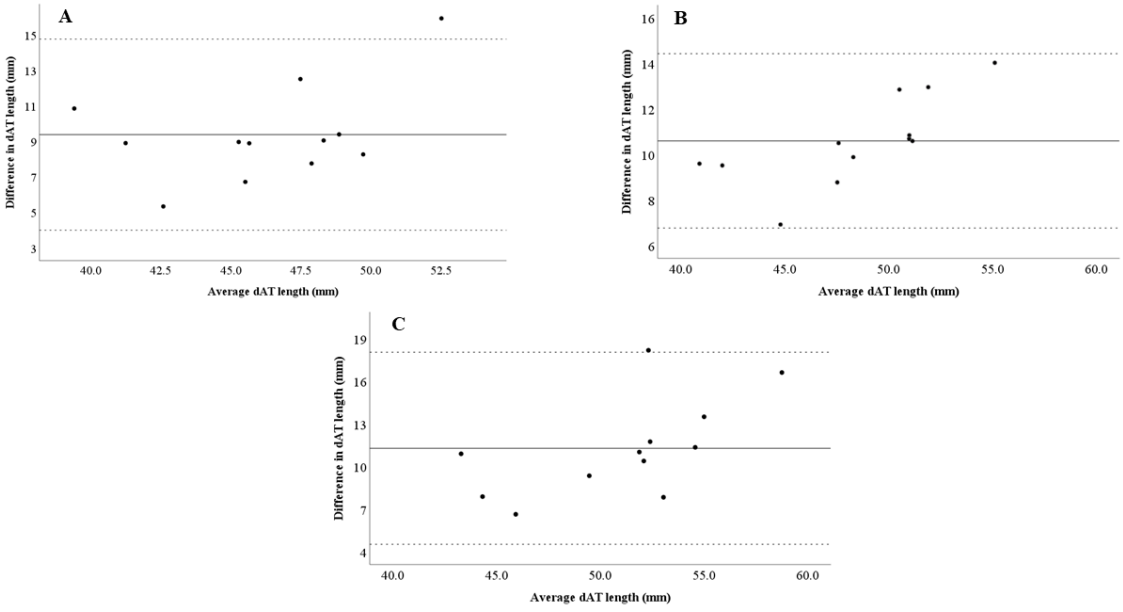

Figure 5 Bland-Altman plots demonstrating: (A) the systematic bias (+9.4 mm) when measuring d_{AT} at -5° ankle angle when measured by the novel dual energy x-ray absorptiometry (DXA) technique compared to the magnetic resonance imaging (MRI) protocol. (B) The systematic bias (+10.4 mm) when measuring d_{AT} at 0° ankle angle when measured by the novel DXA technique compared to the MRI protocol. (C) The systematic bias (+11.3 mm) when measuring d_{AT} at 10° ankle angle when measured by the novel DXA technique compared to the MRI protocol. Solid line=mean differences; dashed lines=limits of agreement.