Title: Reliability of a two-probe ultrasound imaging procedure to measure strain in the Achilles tendon

Authors:

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Author’s response to reviews:

Reviewer 1

Comment 1

Please consider adding a clear statement about to whom your paper is targeted; e.g. is this a technical paper for researchers or are you suggesting that this is a method that could be developed for clinical use - if the latter please expand on this, including discussion of current limitations, within your discussion section.

Authors response

Thank you for your comment our conclusion has been amended to reflect this comment. Specifically, ‘However, to improve the accuracy of this tool in the research environment further reliability trials are warranted to determine which variables contributed to poor between-session reliability.’

Comment 2

Background:

Paragraph 1, line 73: suggest inclusion of a linking sentence here to explain the relationship between your initial mention of strain and then statement about Achilles injury prevalence - this link is inferred but as this is your central hypothesis it may be useful to state this explicitly here.
You clearly explain the previously reported association between strain and injury of the Achilles. However, this was demonstrated using different methodological approaches - does this mean that you are questioning the validity of this association? Please could you clarify.

Authors response

Thank you for your comment. We are seeking clarity as we feel this comment is addressed by the following wording ‘Alteration in the strain properties of the Achilles tendon, induced by mechanical loading, is associated with risk of injury and long-term tendon adaptation such as pathological stiffening (3, 8, 9). Stiffer tendons have less adaptive ability, which may predispose to the development of tendinopathy at relatively lower tendon loads (10).’ Located on lines 78 to 82

Comment 3

Methods:

Please provide justification for your sample size.

Authors response

Thank you for this comment. We purposively targeted 30 participants as we have conducted numerous trials related to reliability and image where we routinely recruit 25-50 participants. No power calculation guided the calculation of the sample size.

Comment 4

Please provide brief overview of other participant demographic details if available (e.g. ethnicity or smoking status and how these may relate to your primary outcome). If not available please provide brief mention within your discussion section.

Authors response

Ethnicity data has been added to methods line 114

Comment 5

Inclusion/exclusion criteria are included; Please provide brief justification for the upper age restriction to recruitment.

Authors response
The age of 65 was chosen in alignment with previous studies examining tendon properties. It was also felt that if an age group older than 65 years of age was included we would see pathological tendon change. We were also the first reliability trial using this technique to include participants aged in their fourth and fifth decades.

Comment 6

Ultrasound image acquisition: Overall this is a well presented section. However, it would add further clarity if you could outline how you ensured that the probe was placed accurately within medial/lateral movement away from the central tendon region if possible.

Authors response

Thank you for your comment. For the proximal location, medial and lateral movement was minimised by the use of a custom probe holder located at the musculoskeletal tendinous junction, this is detailed in the methodology and pictured in Figure 4. The probe holder held the transducer in a fixed location. Medial and lateral probe movement for the probe at the calcaneal insertion may have occurred and we have accounted for this in the limitations. However, the sonographer carefully observed the transducer during the data collection in order to mitigate this. This has been added to the manuscript line 381-382. Furthermore, MAT-lab takes into account any potential movement of the skin relative to the ultrasound probe. Pixel shift measurements within the background ultrasound field, from stationary structures such as subcutaneous tissue (skin), was subtracted from the pixel shift measurements from the tendon. Removing potential error which may arise from any relative movement of the ultrasound probe. This is explained in lines 195-199 of the manuscript.

Comment 7

Image acquisition: use of randomisation of participants to achieve the inter-session sample is mentioned - please provide detail about how these participants were randomised or selected.

Authors response

The following wording has been added to lines 217-218 ‘10 participants randomly-selected by a random number generator application’

Comment 8

Please justify your reduced sample size for inter-session analysis.
Authors response

The intra-session sample size was a pragmatic decision based upon the ability to recruit return participants and resourcing. We have also authored previous reliability studies where a reduced sample size for subsequent measures has been acceptable.

Comment 9

Please provide a supporting reference for your strain calculation or make explicit that this is provided for the first time by yourselves.

Authors response

The following sentence has been added to lines 204-205 ‘Strain (%) was calculated using the following equation based on the methodology described by (19)’.


Comment 10

Analysis - line 205: please review this sentence for grammatical accuracy.

Authors response

Sentence corrected

Comment 11

Analysis - line 210: this is the first mention of a reduced sample for inter-rater analysis -please review all sections were sample size is mentioned and consider consolidation of information into one clear section.

Authors response

The data analysis section has been amended to ensure consistency. Reference to sample size has been removed from the image acquisition section.
Comment 12

Results:

Line 220 - spelling error - amend 'trail' to 'trial'

Authors response

Spelling corrected

Comment 13

Inter-rater within session reliability - line 258: The way the data is presented is a little confusing - this line suggesting that there was a significant difference between raters and yet the previous lines reporting the agreement between raters was excellent - please consider revising to aid clarity.

Authors response

The following commentary under the section titled Inter-rater within-session reliability has been amended to add clarity.

‘Data revealed a statistically significant difference between the mean strain values for Rater 1 and 2 (P = 0.003). However, the inter-rater within-session reliability was excellent (ICC = 0.88) with low measurement error (Table 5).’ (line 267)

Comment 14

Discussion:

Overall this is a well-written section. However, it would be of use if you could include outline discussion about to whom this paper would be use - e.g. are you proposing that this is a viable method for future researchers? Is this an approach that should be further developed for use in clinical practice?

Authors response

Thank you for the comment. We have added the following sentence to the conclusion (line 403-405):

‘However, to improve the accuracy of this tool in the research environment further reliability trials are warranted to determine which variables contributed to poor between-session reliability.’
Reviewer #2:

Comment 1

The methods are appropriate, and in most part very detailed with support from the literature, but would benefit from further refinement/clarity around methods of ultrasound image acquisition. This includes details of the sonographer/researcher undertaking the scans, standardised B-mode settings between scans and further clarity around the probe positioning and measurements for Achilles tendon elongation between ankle joint positions.

Authors response

The following sentences have been added to the ultrasound image acquisition section:

‘Two Terason ultrasound machines were set up identically. In all participants, the right leg was imaged, using B-mode imaging, with both ultrasound probes positioned in the longitudinal plane by one examiner (PM).’ (lines 140-142)

Comment 2

Does the manuscript adhere to the relevant standards for reporting and data deposition?

Authors response

Thank you for the comment. The following detail has been added to the ultrasound image acquisition section:

‘The machine was set with one focal zone, depth was of 2cm and gain was adjusted to ensure a clear image was obtained. Depth was adjusted to 3cm for two participants who had greater subcutaneous fat in their calf region, requiring deeper penetration of the ultrasound to capture the Achilles tendon.’ (line 142-145)

Comment 3

Yes, the title and abstract both convey the nature of the study and findings. The only comment I would make is that from the title and discussions of tendon stiffness in the abstract, I wondered if the study might have included strain elastography in measuring tendon stiffness & elasticity. I wondered if this approach had been considered as not mentioned in the introduction or discussion, although I understand not of direct relevance to measurements of tendon excursion.

Authors response
Thank you for the comment. Yes, elastography was included in the literature review prior to this study. However, we did not have access to a sonoelastography machine. In addition, sonoelastography grades strain by colour opposed to elongation of the structure. Therefore, employing a quite different method. We therefore did not include sonoelastography as a component of this study.

Comment 4

I think that this is an interesting study with valuable information for the field in testing the reliability of this technical technique. However, I think that this paper would benefit from some further detail and clarity around the significance of the probe positioning in terms of the measurements taken for measuring differences in tendon length and what this means for the regions of interest captured and overall measurement of tendon excursion. I understand that the software motion analysis captures the tendon excursion using the ROIs, but I am not too sure of the significance of the measurements of the probe on the skin or the regions of interest shown in Figure 5. The methods section (ultrasound image acquisition) may benefit from some more explanation of the importance of probe positioning in relation to the different regions of interest on ultrasound (figure 5) and measurements of length taken between ankle positions to measure tendon elongation.

Authors response

Thank you for your comment.

1. Choice of probe locations: The locations for the placement of the probes were chosen for two reasons. Firstly, it was necessary to have two clearly defined and consistent anatomical locations with which to measure from and ensure standardisation. Visualisation of and placement at the MTJ and tendon insertion allowed this standardisation to occur. Secondly, the two locations allowed a full consideration of the tendon to occur, along its length from the insertion to the MTJ. Reference to this has been added to the manuscript in lines 152-158.

2. Ramification of measuring from two defined ROI’s across the length of the tendon: although for the reasons mentioned above, we chose probe locations that would capture a greater length of the tendon, we acknowledge that the two ROIs did not examine this entire length. Inferences have been made about tendon length and strain changes for the whole length from the two ROI’s which are representative of the entire length, but not necessarily absolute measures for its entire length. We acknowledge this is a potential limitation, and as such we have added this point to the study limitations (lines 384-386).

3. Potential for probe movement on the skin: MAT-lab takes into account any potential movement of the skin relative to the ultrasound probe. Pixel shift measurements within the
background ultrasound field, from stationary structures such as subcutaneous tissue (skin), was subtracted from the pixel shift measurements from the tendon. Removing potential error which may arise from any relative movement of the ultrasound probe. This is explained in lines 194-199 of the manuscript.

Comment 5

Methods (ultrasound image acquisition): In terms of the probe positioning overall, I apologise if I am misunderstanding the methodologies discussed in the paper, but I am unclear how the same level of calcaneal bone in the right side of the screen at the Achilles tendon insertion could be standardised between scans (in each ankle joint position). Could this have perhaps been measured in terms of tendon distance from calcaneal bone? Was the probe held consistently on the line that was drawn at the distal aspect of the probe, or was this able to move as per tendon elongation and a second line was drawn? I am unclear if the probes were held in situ as the ankle moved through range of motion in the biodex and motion was captured for use in motion analysis software? I wonder if some further detail could be included to clarify, following on from information presented at line 153?

Authors response

Thank you for your comment. You are correct, the same level of calcaneal bone in the right side of the ultrasound screen cannot be standardised between scans. The justification for having the calcaneus just visible on the right side of the ultrasound screen, is that during tendon excursion the insertion of the Achilles tendon is still visible within the predetermined range. The hand held probe remained in a fixed position over the Achilles tendon insertion. The only movement would be as a result of skin displacement relative to the ultrasound probe. MATLAB software takes into account pixel shift movement within the background ultrasound field, from stationary structures such as subcutaneous tissue. Pixel shift movement within the background was subtracted from pixel shift measurements from the tendon. This method provides a more specific calculation of tendon elongation by removing potential error. The second probe was positioned in a customised probe holder which reduced the amount of skin displacement was subtracted from the tendon. We have added further detail regarding our justification for the placement of the two probes in comment 4. This is explained in lines 152-158 of the manuscript.

Comment 6

Methods (ultrasound image acquisition) (144-147): Clarity was provided for placement of the first probe at the medial aspect of the myotendinosus junction, but was the Achilles insertion scanned in the most central portion of the tendon (rather than more medial/lateral?).
Authors response

The following sentence has been added for clarity:

‘The second probe was used to capture the central region of the Achilles tendon insertion’ (lines 148-149)

Comment 7

Methods (line 167): Details are provided in the expertise of the second rater (RE) in terms of expertise with the motion analysis software, but no details are provided for the first rater or expertise of the researcher placing this probe on the Achilles tendon. Does this rater have a qualification/training in ultrasound? Does the second rater have this training also?

Authors response

The following sentence has been added into the manuscript in the image acquisition section:

‘All images were acquired by (PM) who attended specialised imaging training workshops and underwent supervised education regarding ultrasound imaging and using a two-probe procedure, image motion analysis and calculation software by an experienced musculoskeletal ultrasonographer (RE).’ (lines 172-175)

Comment 8

Methods: Sufficient detail is provided for the ultrasound machine and transducer utilised between scans, as well as images and timing of cineloops, but there could be more detail provided in the standardisation of B-mode settings/pre-settings between scans, including depth, focal zones, gain, frequency between scans.

Authors response

Thank you for the comment. The following detail has been added to the ultrasound image acquisition section:

‘The machine was set with one focal zone, depth was of 2cm and gain was adjusted to ensure a clear image was obtained. Depth was adjusted to 3cm for two participants who had greater subcutaneous fat in their calf region, requiring deeper penetration of the ultrasound to capture the Achilles tendon.’ (lines 142-145)
Results. Table 1: To be clear, are these the measurements between trials taken from a mean of both raters or first rater?

Authors response

The data presented in Table 1 is the mean of Rater 1. Rater 2 was not involved in the Trial 1 Trial 2 data. The following section has been added to the data analysis section for clarity:

‘Ultrasound images for Trial 1 and Trial 2 were obtained in a single session by a single assessor (PM) for…….’ (lines 210-211)

Discussion (lines 323-324): There is a statement that Achilles tendon measurements were undertaken in longitudinal planes rather than in transverse, contradictory to another paper. It is stated that this is because the transverse planes may influence Achilles tendon strain measurement but there is no explanation of why this might be the case, which could be included.

Authors response

Thank you for your comment. Following consideration, we have deleted the sentence.

The longitudinal plane is the common method used. Whether studies included transverse and/or longitudinal strain was dependent on the location of measurement, as the aponeurosis is tensioned transversely as well as longitudinally, due to muscle bulging. The transverse plane was more commonly used following an exercise protocol compared to the current studies activation procedure of passive elongation. Furthermore, strain was calculated based on the methodology described by (Dilley, 2001), where measurements of nerve excursion were undertaken in the longitudinal plane.

Discretionary Revisions

1. Out of interest, in the exclusion criteria, were there any ultrasound features in this Achilles tendon that caused participants to be excluded (i.e. subclinical inflammatory changes/tendinopathy, calcifications)?

2. Discussion (limitations) (lines 370-371) - should these sentences follow on from one another?

Authors response
1. In response to point one the participants did not undergo any pre-study entry screening related to study inclusion

2. The sentence has been amended to read:

‘Second, the ultrasound probe positioned over the calcaneal insertion of the Achilles tendon, was manually held. The structure of the posterior calcaneus meant it was not possible to manufacture a custom-made brace to stabilise the probe as was the case for the probe located at the MTJ. Subsequently, at the calcaneal insertion measurement point there may have been some probe movement across the surface of the skin.’ (lines 376-381)