Author’s response to reviews

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

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Author’s response to reviews:

Revision 2

Reviewer #1:

Thank you for addressing the previous review comments. I have no further comments or recommendations. This is an interesting article that will add new evidence to the scientific field.

Reviewer #2:

Comment 1

In the abstract, it is stated that repeat measurements are undertaken at the initial study visit and then again in a second measurement session four weeks later. Could more details be added in please to indicate to readers that:

The inter-rater reliability was undertaken with a smaller sample of patients, not all 29

The between day measurement was undertaken with a smaller sample of patients, not all 29

Thank you for your comment. Repeat measurements were taken in all participants at the initial study visit and ten participants in a second measurement session four weeks later. This has been clarified in lines 33-34.
Comment 2

Could there please be more detail added in to the methods section discussing 'ultrasound image acquisition' (lines 140-163) to explain the probe positioning at the MTJ of the Achilles tendon. I am still a little unclear about the following:

The initial placement of the probe at the MTJ is achieved through placement at the medial gastroc, then moved down distally to meet the MTJ. There is then a line at the proximal point of the probe. To then capture tendon elongation at the MTJ, the probe is placed half way between this line and the proximal line made from the probe at the Achilles insertion. Could you please provide a rationale for this approach to still capture the MTJ or a reference to paper documenting this technique as I am not too sure of how this method still captures the MTJ shown in Figure 2b (as observed from previous submission as not included in revised submission, my apologies if this has been amended).

Thank you for your comment. The rationale for this approach was to account for tendon excursion. If the probe was left at the MTJ and not repositioned the MTJ would have moved out of the field of view. Repositioning of the probe meant that the MTJ remained in the field of view during tendon excursion. This has been clarified at lines 159-162.

Comment 3

Could you please explain in the image acquisition section (lines 169-171), where/when in the video sequences obtained, the four quality images were captured from (two from each probe), perhaps with accompanying images? Could I clarify please, if these four images are static images (or videos), taken from certain points of ankle dorsiflexion/plantarflexion? Does figure 5 show an example of this static image. To further clarify, do these two images captured relate to trial 1 and 2 or was this full process repeated twice in 2 separate trials?

Thank you for your comment, the following has been added to the text (Lines 168-172):

A video sequence of tendon excursion in the longitudinal plane was recorded and four quality sequences from each probe were obtained. The two best video sequences were selected for analysis and named Trial 1 and Trial 2. The selected sequences had to be the matched sequence for both MTJ and insertion.

Comment 4

In image acquisition (lines 172-175), there may be a grammatical error in the wording of this sentence: '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)'

Could this possibly have the initials PM out of brackets, or perhaps the professional background of this researcher could be added in here, for example 'All images were acquired by a podiatrist (PM)…

Could this sentence perhaps be written in 2 separate sentences, with the information about the image motion analysis and calculation software being captured by an experienced ultrasonographer written in separately? It might be interesting in the discussion to note any impact that any differences in professional background/training had on the inter-observer agreement, in addition to any impact on inter-observer reliability findings that one researcher scoring another's images may have had (as within session reliability may have been different if included full repeated measurements including image acquisition, as well as image analysis).

Thank you for your comment the paragraph has been reformatted to below (Lines 173-176):

All videos were acquired by a podiatrist (PM), who had attended specialised imaging training workshops and received supervised education regarding ultrasound imaging and image motion analysis software by an experienced musculoskeletal ultrasonographer (RE).

Comment 5

In 'Image motion analysis and calculation software' more detail could be added to explain how the 3 contiguous ROI and varied dimensions were selected (shown in figure 5) (lines189 -190) as variation between these could possibly impact intra/inter observer reliability? Were these selected across the MTJ, mid-portion and insertions for example?

Thank you for the comment the paragraph has been amended for clarity (Lines 187-192):

To analyse the movement of the tendon, three contiguous rectangular regions of interest (ROI) of varied dimensions within the Achilles tendon were selected within a predetermined range (Figure 5). The predetermined range was standardised through division lines on the ultrasound screen that enabled the image to be split into quarters. The ROI was standardised in the middle half of the divisions for all images at the MTJ and insertion.

Comment 6

Could I also clarify please if figure 5 shows an example of a saved image from the sequence captured, as explained in the image acquisition section (line 170) and is this image an example of data collected from the 2 separate probes or probe positioned at the calcaneal insertion?
Yes, this is static image of the video sequence from the ultrasound probe capturing the Achilles tendon insertion.

Comment 7

In the 'data analysis' section, in line 210, it is explained that ultrasound images for Trial 1 and 2 were obtained in a single session. Could I confirm please that this involved two repeated measures in the same day by one researcher and if so, that detail could be added in please that all measurements (including probe placement and image acquisition) were undertaken as well as image analysis for both trials. Any potential for researcher recall bias caused by this approach could be acknowledged in the limitations - with details in how the lines drawn on the skin were removed between trials and if this did not occur (which I think may apply to this study, looking at details provided in lines 398-400 in the conclusion?), this could lead to better within session reliability and poor between session reliability which could be acknowledged in the discussion to a greater extent?

Thank you for your comment, we feel the current discussion adequately details the points you raise. Specifically:

1. Line (279)…The excellent within-session reliability with small measurement error may be attributable to the standardised methodology used in the within-session trials.

2. Line (283)…and the use of a custom-built ultrasound probe holder, removing the need for manual probe repositioning

Comment 8

Further in data analysis, it is stated in line 223 that inter-rater reliability was assessed in a further 10 participants. Were these 10 participants randomly selected from measurements taken at the initial study day (from trial 1 or both trials?). This could be amended with detail as may read as a further 10 participants recruited. It might also be useful to know if all patients measured at trial 1 only.

Thank you for the comment. The paragraph has been reformatted (lines 224-227):

For the total sample (29 participants), 10 pre-captured images were randomly selected to determine the within-session inter-rater reliability of the motion analysis software. Tendon excursions were compared between the first rater (PM) and a second rater (RE), who had expertise in the use of the motion analysis software and was blinded to the results.
Comment 9

In the discussion, 2 ROI are discussed here (lines 383-384), could I clarify please that this relates to the 2 ROI areas captured by each probe and not the 3 ROI discussed during image analysis and shown in figure 5?

Thank you for your comment. The text has been corrected to read (Lines 377-381):

Third, we chose probe locations that would capture a greater length of the tendon; however, the two probe locations (MTJ and insertion) did not examine the entire length of the tendon. Inferences were made about tendon length and strain changes for the whole length from the MTJ and insertion, which are representative of the entire length but not necessarily absolute measures for its entire length.

Comment 10

Minor discretionary comment: Line 336, two fullstops included at end of the sentence.

Text corrected