Reviewer’s report

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

Version: 1 Date: 17 Jul 2019

Reviewer: Lisa Newcombe

Reviewer's report:

Thank you to the authors for considering, reviewing and addressing all comments. Overall, I really appreciate the time and consideration by the authors in addressing all points and I can confirm that the majority of these have been addressed in full. Thank you for the fuller explanations provided around the methods and limitations sections in relation to image acquisition in particular, these were really helpful and assisted in my understanding of this technical protocol. I do still have a few comments and enquiries related to some of the methodologies utilised and handling of these methods in the limitations section, which I feel might be useful to further improve reader understanding, ahead of publication. I have included these for review point by point below. Again, overall, I found this to be an interesting paper and adds value to the field and thank you to the authors and editorial time for the opportunity to review this paper. Please see comments as below:

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

* Perhaps in the main body methods section, it could be clearer that the repeated measures within day at initial study did not include full re-positioning of probes (as was undertaken between days - which may account for better reliability within day than between day)

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).

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?

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).

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?

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?

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?

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.

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?

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

Many thanks for considering these points and happy to provide any further clarity for all comments as required.

**Level of interest**
Please indicate how interesting you found the manuscript:

An article whose findings are important to those with closely related research interests
Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal