Author’s response to reviews

Title: Reliability and methodology of quantitative assessment of harvested and unharvested patellar tendons of ACL injured athletes using ultrasound tissue characterization

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Version: 2 Date: 10 Mar 2019

Author’s response to reviews:

REPLY TO EDITOR

Technical Comments

(1) Please include the email addresses of all authors on the title page. Please ensure that it is identical to the one entered on the submission system.

Reply: Noted
Action: Included in page 1

(2) Please change the heading 'Introduction' to 'Background'.

Reply: Noted
Action: Modified in page 3

(3) Please provide a 'Conclusions' section heading for the concluding paragraph of your manuscript.

Action: Included in pages 12
(4) Thank you for including your figures as an additional file; the copy embedded within the manuscript can be removed.

In addition, please provide figure titles/legends under a separate heading of 'Figure Legends' after the References. Figure files should contain only the image/graphic, as well as any associated keys/annotations. If titles/legends are present within the figure files, please remove them.

Reply: We understood from the formatting guidelines that tables that fit into the A4 size page should be embedded into the manuscript. And due to the fact, they fit in landscape mode we included at the end.

However, now after the request of one of the reviewers we will add as a separate file.

“Tables less than one A4 or Letter page in length can be placed in the appropriate location within the manuscript. •Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.

•Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files. Please see [below] for more information”

Action: We removed all the embedded figures and tables from the manuscript and uploaded them as separate files.

We included the separate headings of Figure titles in page 21 Table titles in page 21 and Table legends in page 22.

We also removed any additional text rather than the image/ table in the separate files.

General comments

(1) Please explain how a 12cm track works on a ~ 4.8 cm patella tendon. (Line 88 – 93).
Reply: The UTC tracker allows acquisitions with 12 cm length. It doesn’t mean that all the 598 images resulted of one-time acquisition will be taken into consideration. The number of images to be used depend on the length of the tendon, as well, the height of the participant. For Achilles tendons, most of the images are used since the examiner may want to visualize the calcaneus and myo-tendon junction. For patellar tendons, less transversal images are necessary to cover the whole tendon length. For instance, in this sample, the longest tendon was 6.32 cm, and the shortest 3.38 cm. However, the data acquisition is not interrupted because the length of the tendon was covered. All 598 images are acquired, and only the images representing the length of interest are included in the analysis. This selection is done by drawing the contours on the desired area, as demonstrated in figures 3 and 4.

Action: We included two new figures to clarify the methodology. (Fig 1) (Fig 2) page 5

(2) Line 94  Lay, not laid
Reply: Noted
Action: Modified in line 101 page 5.

(3) Line 97 – 98 – can you rephrase to make this flow? Line 110 “resulting in 598 sequential transverse images…” but you have said to both reviewers 1 and 2 you only used ~250 – 300 images. Please make it clearer how many images were used.

Reply: As explained above, the UTC tracker allows the capture of 598 sequential transversal images, however, only the area of interest that it is selected during the analysis is taken into consideration by the UTC software to calculate the proportions of each echo-type.

Action: In Methods:

Inclusion of Fig 2.

Lines 131 – 150 explain the process of drawing contours to determine the patellar tendon length, i.e. length of interest.

About the number of selected images in lines 149-150 in page 7.

We have also modified the order of the text, bringing the section “UTC Data Analysis and Processing” to page 6, before the section “Repeated Measures - intra-rater and inter-rater reliabilities” now in page 8.
(4) Line 137 on different days (not in different days).
Reply: Noted
Action: Modified line 129 Page 6.

(5) Line 155 Please review this sentence
Reply: Reviewed and modified
Action: Modified text now in lines 151-152 in page 7. Also add the thickness measurement in Fig 5.

(6) Line 181 How can most of the data be normally distributed for all parameters? Either the data was or was not. Please clarify.
Reply: Normality of the difference between 2 acquisitions (due to test-retest objectives) was calculated again for each one of the variables studied.
Majority of them presented normal distribution, some of them, didn’t. However, such results did not interfere in which test to perform to access the test-retest reliability.
Therefore, we decided to use the test-retest reliability to calculate the SEM and MDC.
Additionally, other papers using UTC imaging had similar findings and methodology (Docking, Rosengarten, Daffy, & Cook, 2015; Rosengarten et al., 2015; Wezenbeek et al., 2017), despite non-parametric data the test-retest reliability was used to calculate SEM and MDC.
Action: We included which variables were not normally distributed in page 8 lines 187-191 in Statistical Analysis section.

(7) Line 183 Please review this sentence…In case of
Line 195-196 Please review this sentence …Confidence interval (CI)….Its values will be presented
Line 197 which version of SPSS?
Reply: The sentence that was in Line 183 of the previous version was confusing thus removed.
Line 195-196 we modified the sentence.

Line 197- SPSS v.21 was used.

Action: Removed the sentence in previous line 183 and included a new table with similar information (Table 1).

We modified the sentence that was in line 195-196, but now is in line 202-203 in page 9.

We modified to SPSS version 21 in line 203, page 9.

(8) Line 200 – 204 much of this information could go in a table about the participants and which imaging they had (see below)

Reply: We agree. A new table was included

Action: Table 1 contain now the information mentioned, graft information and which participant was included in each moment of their rehabilitation.

We added also added explanation in the background section of possible grafts to repair the ACL and the introduction to harvested tendons (Lines 70-74 in pages 3 and 4.

(9) Line 278 as well as thickness at THE mid Line

Reply: noted.

Action: Added in line 252 in page 11.

(10) 297 - why do you think the reliability is poorer in unharvested tendons? Do you think it maybe due to the reduced variability within the unharvested tendons? Please comment as this is important for understanding the reliability of relatively normal tendon scans.
Reply: Exactly. In the first version of this manuscript we mention consistency in the measurements, that’s what we meant. Variability was low for variables as thickness, tendon length, and volume.

Action: We modified the order of discussion, bringing the variables with smaller ICC together.

Further discussion was included in page 12, lines 273 to 282 In Discussion section.

(11) Line 301 –you previously note that this isn’t compulsory. How can you account for this discrepancy?

Please put a short conclusion in before your key points.

Reply: This step of drawing contours around the tendon is not the same of the skin mark by palpation that isn’t compulsory.

The skin marks are placed just to avoid wasting time with multiple scans of the same tendon. The actual final scanned image is the one to determine if that scan will be included or discarded from the analysis. The definition of an accepted scan is already included in the methods in lines 116-120.

After the scan is saved the examiner perform the analysis, which includes drawing manually the delimitation, or circumference of the patellar tendon in the transversal window of the UTC software. At this moment the examiner should draw the contours on the edge of the tendon, ensuring to be within the limits of the tendon to provide to the UTC software information of the tendon tissue exclusively. How far from the edge one examiner may decide to draw will affect the number of pixels within this area, without affecting the distribution of this pixels within the same area.

Action: Further explanation was included in lines 273-284 in page 12.

Also, in the caution section, lines 313, 314, page 14.

Conclusions chapter was included in page 12.
(12) Table and figures

You have several figures and tables. Please either place all the tables and figures at the end or place them all within the document. It is very difficult to work out which tables and figures you are referring to when they are inconsistently sequenced, and the numbering is not accurate. Further, some of the numbering does not match what you have noted in your response (e.g. Figure 1)

Reply: Noted.

We tried to keep the tables that fit into the A4 page at the right location, and the ones that fit into landscape mode at the end. Now we will upload all the tables in separate files for better visualization.

Action: Each table is saved in a different file with the corresponding name.

All the numbers have been fixed.

(13) Table 4 – the formatting has become messed up. Please correct this.

Reply: Noted. It changed into Table 5.

Action: Now table 5, has been fixed.

(14) Figures and images

Can you please provide more detailed legends to enable the reader to understand what is being represented? E.g. Figure 4 had no orientation on it (which end is the apex?) and has several lines on it that need explanation. These are provided in the text, but in isolation, are confusing.

Reply: In the methods section the areas of the patellar tendon are described in detailed. Patellar apex is (1), proximal tendon is (2), mid tendon (3), and distal tendon (4).

Action: We modified the heading of all tables to simplify them.

(15) Page numbers

When referring to changes and page numbers, please refer to the latest submission. This makes everyone’s life easier.

Reply: noted
Action: We added the numbers for pages and lines in the replies only after all the changes were performed.

REPLY TO REVIEWER 1

(1) Inclusion and exclusion criteria:

Regardless of whether your participants represented all the possible participants, you need to provide some description of what made them eligible for inclusion, and, if any didn’t participate – why was that. For example – were the 37 participants consecutive patients in your practice? Were there any patients with ACL injuries that you didn’t include (e.g. females), those that were travelling? etc…If so, how many were not included and for what reasons?

REPLY: Noted

ACTION: We modified the participants section, we have included the inclusion and exclusion criteria in page 4 lines 84-92.

(2) Difficult with understanding the number of measures done on each participant (comment 2)

The Figure 1 provided in R1 appears to be about imaging, not participant participation. It appears that Figure 2 is the one about tendons that have been assessed. Please correct this. Further, this figure does not provide any information about how many participants were scanned twice. This is important because there is an increased chance of more similarities when you are assessing the same person more than once, and, the right and left legs of one person are more similar than the right and left legs of different people.

REPLY: It is important to highlight that no comparisons between right and left leg were done.

Always a patellar tendon was compared to the same patellar tendon.

There were moments when the same tendon was scanned twice and analyzed twice, by the same examiner and by different examiners.

As when a single examiner acquired information of the same tendon twice with one day interval to perform the test-retest reliability to calculate the minimal detectable change. The goal was to
observe the normal fluctuation of the patellar tendon (intra-rater reliability of harvested and unharvested tendons).

In other moments, a tendon was acquired once but analyzed twice by different examiners.

**ACTION:** We included a table to discriminate in which analysis each participant took part, which graft was used in the ACLR, and which time of the rehabilitation they were at, when their patellar tendon was scanned. Table 1

Numbering of Figures and Tables were fixed.

(3) Can you please provide a figure that describes how many participants were used more than once, and in what way? Alex Scott suggested a Venn diagram or a table. I agree with him – I think a table with the participants numbered and which tests were done on each of them, by which assessor, and how many times, what surgery they had etc would make this information much clearer.

**REPLY:** Noted

**ACTION:** Table 1 describes which participant was included in which analysis, graft type, if harvested or unharvested patellar tendon, which phase of their rehabilitation the participant was at when the tendon was scanned.

(4) Duplicated analysis

You state that this is described on page 8. I am unable to locate this information. Maybe it was inadvertently deleted?

**REPLY:** “In case of selecting a single sample from a patellar tendon that has had multiple acquisitions and analysis, the data from the first acquisition of the intra-rater reliability study done by the most experienced examiner was selected for further analysis.”

However, we think that this sentence was confusing, so we have removed it. We believe that the new Table 1 and the more detailed description of the methods may address this point.
ACTION: We removed the sentence that was confusing.

(5) Explanation of UTC mapping to histopathology Please provide citations to support this assertion.

REPLY: Noted.


REPLY TO REVIEWER 2

(1) What was the method of placing the skin markers, palpation or us? Explain If the investigator didn’t use palpation, how did they identify the underlying anatomy? In other words, how do you know that the investigators were looking at the same thing and from the same angle?

REPLY: The skin markers were done by palpation.

The underlying anatomy was visualized in the transverse screen of the UTC. The examiner can see the US image while placing the tracker on top of the patellar tendon. The examiner will press start to start swiping the tendon only when the image of the tendon is centralized in the transverse window of the acquisition software.

Besides, the skin markers are placed just to avoid wasting time with multiple scans of the same tendon. The 3D final scanned image is the one to determine if that scan will or will not be included for analysis. The definition of an accepted scan is already included in the methods in lines 118-122.
In case the tracker was not placed satisfactory, the 3D image will not show the requirements described in the methodology. Examiner then perform another scanning of the tendon.

**ACTION:** Further explanation and another 2 figures were added in the methods section. Page 5, lines 102-106. Fig 1 and Fig 2.

(2) 600 sequential images would give a range length of 12cm, so I would guess only approximately 300 sequential images was used by the PT. Both reviewers have commented on this. Please provide some explanation within the manuscript as to how many images were used, or how this was calculated.

**REPLY:** Yes, approximately.

Number of images is related to the length of the tendon.

**ACTION:** We added 2 new figures, 1 and 2 to clarify the length of the patellar tendon and number of transverse images included in the analysis. Lines 131-150 in page 6 and 7 include full explanation of the length of interest. In lines 149-150 explain the images that are included in the patellar tendon analysis.

(3) Participants and subjects

The reviewer is pointing out that you are not being consistent with your use of terms. Please use participants throughout.

**REPLY:** There are two circumstances where the word subject is used instead of participant (Key points – Findings, line 293 and 295, page 13). In these occasions in our opinion the word participant is not suitable.

**ACTION:** We replaced subject for patients.