Author's response to reviews

Title: Impact of leg lengthening on viscoelastic properties of the deep fascia

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Author's response to reviews: see over
Dear Editor,

Thank you very much for your suggestions in our manuscript entitled “Impact of leg lengthening on viscoelastic properties of the deep fascia” (MS 1677819625335357). We are also grateful to the reviewers for their important and salient comments. Having read them carefully, we made every effort to improve the manuscript. Accordingly, the manuscript has been changed substantially. The corrections and amendments correspond to your suggestions and comments as shown in the attached pages. We also highlight the changes of our manuscript by using yellow colored text. Hope these will make it more acceptable for publication.

Kindest regards.

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Encl.
Corrections and changes according to the suggestions and comments by the editor and reviewers

We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional copyediting service. Examples are those provided by the Manuscript Presentation Service (www.biomedes.co.uk), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/).

We have asked a native English speaking friend to help us copyedit the paper.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We ensure that our revised manuscript conforms to the journal style.

Reviewer: Raffaele Da Caro

I have read this paper with interest. The authors are well known for their field of research about fascia. The paper is well structured and covers an interesting area.

Thank you.

I would classify this article as important in its field, but before publication, it needs some modifications:

1. in the “Background”, the Authors write that the deep fascia is continuous with the superficial fascia, but there aren’t Literature supporting this idea. Besides, if it is so, how they sampled the deep fascia for the experimental test, without taking the superficial fascia and without damage the deep fascia?

Thank you for this point and we fully agree. The sentence has been changed to “The deep fascia is a dense connective tissue that lies beneath the superficial fascia.” Virtually, there is no apparent connections between deep fascia and superficial fascia during our experiment.

2. in the “Methods”, the Authors write that uniaxial tensile tests were performed, but they did not specify the direction of the strength. Indeed if the fascia presents anisotropic characteristics, the results could be different if the tissue is stretched along the limb axis or in the orthogonal direction. Eventually, the Authors could evaluate also the paper: Stecco et al, Surg Radiol Anat, 2009
Agree—as mentioned above, the direction of the strength has been specified as along the limb axis for uniaxial tensile tests. In addition, thank you for providing the reference. We have added “Stecco et al, Surg Radiol Anat” as a reference both in the method section as well as in the discussion section.

3. In the “Results”, the Authors have to better describe the curves of figures 4 and 5, analyzing the comparison of strain at rupture and the ultimate tension strength according to the Statistical analysis described in the Methods.

Agree—we have described the curves of figure 4 and 5 (now figure 3 and 4) in the Results according to your suggestion.

4. In the “Discussion”, the Authors affirm that the deep fascia consisted of three layers, but it is no clear what researches support this idea. Besides, if the fascia is formed by three layers, how the collagen fibres are distributed inside each layer? And how each layer relates with the adjacent ones?

Sorry for not clarifying this point clearly. In fact, our previous study supported this idea. The deep fascia consisted of three layers, i.e., two dense connective tissues outside and one loose connective tissue in the cross sections under microscopy (ref 19). The collagen fibres distribution is various according to microscopy findings.

5. In the “Discussion”, the differences among the different experimental groups have to be highlight, with particular references to the statistical findings.

Agree—we have discussed the differences among the experimental groups with particular references to the statistical findings as you suggested.

Reviewer: Thomas M Best

Minor Essential Revisions
1) The authors mention that “relatively few authors addressed chiefly deep fascia, particularly the biomechanics of deep fascia” in the background (1st paragraph, lines 10-11) without providing examples of studies that have addressed this issue. One reference to literature work would aid in understanding the scarcity of work on this.

Thank you for this point and we fully agree. We have provided references of the studies addressing this issue as you suggested.

2) In Methods, subheading on “Tests of mechanical properties of deep fascia” (Page 6, line3, Page 7, line 2), the units are missing or else does not appear correctly.
We have changed the units according to your suggestion.

3) In Methods (Page 7, Paragraph 2), what were the criteria for determining ultimate non-damaging loads? The authors mention “tests were performed on specimens of different groups of animals to determine the ultimate non-damaging load applicable on fasciae” but do not state premise for it. It is an essential biomechanical marker but should be based on literature values or previous studies.

Agree—as mentioned above, the statement has been changed as “To determine the ultimate non-damaging loads applicable on fasciae, preliminary series of load-download tests were performed on specimens of different groups of animals on the basis of previous study on analogous tissues.”

4) Details of the fatigue test or reference are needed in Methods (Page 7, Paragraph 3, Line 6).

We have added references of the fatigue test.

5) Provide details on which variables and data were evaluated using analysis of variance followed by Student’s t-test etc.

We have provided details of variables and data that were evaluated using analysis of variance.

Discretionary Revisions
1) In results, the authors can describe the Figure first and then proceed to present the results for each Figure before moving onto the next one. Currently, the description of Figure 2 and 3 are provided first followed by results of the afore-mentioned.

Thank you for your suggestion and we have made the changes.

2) Error bars should be shown in the Figures 4 and 5.

We have added error bars in the figures.

Reviewer: Robert Schleip

I have read this paper with interest. The paper is well structured, it investigates an important area, and the applied investigation methods are appropriate for this context and questions. I would classify this article as important in its field.

Thank you.
Before publication it nevertheless needs few modifications:

Major

1. A brief explanatory sentence is needed why the “load for pre-conditioning for the hysteretic tests was 200g for control fasciae; whereas the load was 400g for the experimental groups.” Why did you choose to apply different loads for these?

Thank you for this point. Given the ultimate non-damaging loads obtained from the preliminary series for control and experimental groups were different, we chose to apply different loads for these so as to explicitly show the biomechanics of fasciae.

2. The 4 experimental groups (A,B,C,D) to which the result sections refers should be briefly described in the method section. It is too cumbersome for readers having to look these up in previously published papers in order to understand e.g. the meaning of Figure 3. A least a very brief description is needed, what differentiates these 4 groups.

Sorry for the inconvenience we brought. We have added a brief description of the 4 experimental groups in the method section as follows. In brief, four leg lengthening schemes, i.e., 1 mm/d with 10% and 20% increment in the tibia length, 2 mm/d with 10% and 20% increment in the tibia length, correspond to group A, B, C and D, respectively.

3. In the result section or discussion: indicate whether the majority of tissue ruptures occurred near the contact clamps (which could indicate a slight weakness of this contact clamping method) or also within other locations within the tissue.

Thank you for this important point. We have added such statement in the discussion section as follows. “However, the majority of tissue ruptures occurred near the middle-third part within the tissue. The phenomenon verifies the contact clamping method as a reliable alternative to study the biomechanics of soft tissue as fasciae. This might be due partly to the relatively low tensile rate we utilized”.

4. If correctly understood, the load-displacement behaviour at 20% tibia distraction was more close to that of normal fascia than at 10% distraction. If true, this counter intuitive (and interesting!) result deserves being more clearly described in the result section as well as being addressed in the discussion section.

Yes, the load-displacement behaviour at 20% tibia distraction was more close to that of normal fascia than at 10% distraction. As you suggested, the result has been clearly described in the result and discussion sections.

5. Figures 4 and 5: please include an indication for the variance of the data in each column (e.g. standard deviation, or similar).
We have added standard deviation bar for each column in the figures according to your suggestion.

**Minor**

6. Please include a description from which exact fascia layer and location the tissue samples were excerpted. Also how the long and short axis of the tissue samples were oriented in relation to the animal’s anatomy (probably with the long axis parallel to the limb axis).

Thank you for this point and we have added the description as follows. “The deep fasciae of different groups were excised from the gastrocnemius fasciae of legs of animals at the different time points.” “The direction of the strength was along the long axis of specimens, which was parallel to the limb axis.”

7. Figure 1: the photograph of the Instron machine is not ideal, as the reader needs to either guess or have familiarity with these devices in order to understand how the tissues were positioned in relation to it. I suggest substituting it with a technical drawing or photo that includes the tissue positioning.

Alternatively the paper would even be improved (in its professional appearance and reader-friendliness) if the authors decided to leave that figure 1 out all together.

We decided to leave the figure 1 out all together as you suggested.

8. Figure 3: please indicate which curve represents which experimental group?

We have indicated each curve (A, B, C, D) in the figure.

**Discretionary Revision**

9. As a recommendation only: if possible, describe not only the absolute force values in the result section (e.g. 2.69 mN), but also relate them as per square mm cross sectional area (e.g. 0.35 mN/mm²). This way future usefulness of your data will be improved, as your force values can more easily compared with other measurements.

Thank you for your important suggestion. We have added the relative value in the result section. Hope the future usefulness of our data will be improved as you mentioned.

**Quality of written English: Needs language correction by a native speaker before being published.**

We have asked a native English speaking friend to help us copyedit the paper.