Author's response to reviews

Title: Effect of training and sudden detraining on the patellar tendon and its enthesis in rats

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Author's response to reviews: see over
Specific Comments:

Background

I understand the reason for the measuring bone volume, when I read the response letter to the reviewer’s comment. In the revised manuscript, however, the authors stated the reason in the last sentence in Introduction (Page 4, line 10-11). The readers cannot understand the author’s intension.

We could not add the reason of the bone volume measurement in the Abstract because of editorial requirements on the number of words. We better explained the reason of bone volume measurement at the end of the Introduction paragraph (page 4 lines 85-88)

Methods

Page 6, Histology and Histomorphometry

As mentioned previously, these analyses (evaluated subjectively) were considerably vague. For example, sore of stained (Page 6, line 12-14; slightly, partially,,,), fiber bundle evaluation (Page 6, line 17-21; partial, slight,,,), I considered that these analyses were not objectively (“objectivity” is important for the science).

We agree with the referee on the importance of objectivity in research. Following the reviewer comments we improved the analysis of the PT metachromasia scoring as now described in the manuscript in the Methods section (page 6, lines 137-145). We used the hexadecimal RGB (Red, Green, Blue) colorimetric method as described by other authors (Fermin CD, Degraw S: Colour thresholding in video imaging. J Anat. 1995, 186:469-81. Fassina L, Visai L, Benazzo F, et al: Effects of electromagnetic stimulation on calcified matrix production by SAOS-2 cells over a polyurethane porous scaffold. Tissue Eng. 2006, 12(7):1985-99.).

The metachromatic dye (Toluidine Blue) binds to the ECM proteoglycans (PGs) coloring the PT matrix blue [Fermin CD, Degraw S: Colour thresholding in video imaging. J Anat. 1995, 186:469-81]; “blue” was defined, on the red-green-blue (RGB) scale [Fassina L, Visai L, Benazzo F, et al: Effects of electromagnetic stimulation on calcified matrix production by SAOS-2 cells over a polyurethane porous scaffold. Tissue Eng. 2006, 12(7):1985-99.], as (R=24; G=15; B=70; hexadecimal RGB value=180F469). The analyses were carried out at 10x magnification. The method provides discrete numbers which we assign a score: 0 for unstained tendons (70±10% of pixels having hexadecimal RGB value=DAC3B1: R=218; G=195; B=177), 1 for slightly stained tendons (70±10% of pixels having hexadecimal RGB value =8E5671: R=142; G=86; B=113), 2 for partially and unhomogeneously stained tendons (70±10% of pixels having hexadecimal RGB value=5C426F: R=92; G=66; B=111) and 3 for tendons with total and homogeneous staining (70±10% of pixels having hexadecimal RGB value=180F46: R=24; G=15; B=70). (paragraph “Methods” histology and histomorphometry, page 6, lines 137-145)

Scores obtained by the RGB method were similar to those previously obtained by the microscope evaluation carried out by two experienced investigators and reported in previous Table 2.
Indeed, I cannot understand the presented results by the authors, when I looked the presented figures carefully.

Following the comment of the reviewer in order to better explain our results, the Result section was improved by changing the order of histological data presentation (page 9, from line 200 to line 215) and consequently Figure citation order and Figure legend (pag. 17, lines 399-419).

Furthermore, the comments to avoid biases (Page 7, line 6-8) were very vague, and cannot be solved by the above mentioned problems (as mentioned previously).

See answer to the comment on Histology Histomorphometry.

Results (Figures)
In the pictures, the numbers of figure (Figure 1-9) were mentioned.

A total of 9 figures are inserted in the text as in the legend (Figures: 1a, 1b, 2a, 2b, 2c, 3, 4a, 4b, 4c). As previously explained, to better clarify the presented results and figures, the legend, the figure number and order in the text were modified (pag. 17, lines 399-419).

Following the previous and the present comments, the manuscript was revised and the revised parts are underlined.