Author's response to reviews

Title: Validation of quantitative magnetic resonance imaging-based apparent bone volume fraction in peri-articular tibial bone of cadaveric knees

Authors:

Jeffrey B Driban (jdriban@tuftsmedicalcenter.org)
Mary F Barbe (mbarbe@temple.edu)
Mamta Amin (mamta@temple.edu)
Neil S Kalariya (tuc58124@temple.edu)
Ming Zhang (mzhang@tuftsmedicalcenter.org)
Grace H Lo (ghlo@bcm.edu)
Anna M Tassinari (aniat@bu.edu)
Daniel Harper (dharper3@tuftsmedicalcenter.org)
Lori L Price (lprice1@tuftsmedicalcenter.org)
Charles B Eaton (charles_eaton@mhri.org)
Erika Schneider (schneie1@ccf.org)
Timothy E McAlindon (tmcalindon@tuftsmedicalcenter.org)

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Author's response to reviews: see over
We would like to thank the reviewers for offering their time and feedback on the manuscript. We have modified the manuscript based on the reviewers’ comments and offered a response below in bold.

Reviewer: Ko Chiba

Major Compulsory Revisions

Methods

1) "10% formalin fixation": Does this affect the microstructure of the trabecular bones?
We apologize for the confusion. We updated our abstract (methods) to indicate that we used a 4% paraformaldehyde in phosphate buffer, not a 10% formalin fixation. The 4% paraformaldehyde in phosphate buffer has minimal effect on trabecular bone structure.

2) "MRI signal intensity": Does the temperature of the specimen influence signal intensity? If so, how was it corrected?
Yes, storing bone at 4°C for 24 hours or more can alter the signal intensity of bone marrow (Bolan G 2010, reference 26).
We noted in the discussion (3rd paragraph) that “To minimize post-mortem changes in bone marrow MR-signal characteristics and signal-to-noise after freezing and thawing [26], we did not freeze the cadaver and evaluated the intact knees as quickly as possible (within 36 hrs of death). We also minimized the influence of tissue temperature changes by initiating the MR imaging within 15 minutes of removing a knee from its shipping containers.”

3) "spatial resolution": "0.23 mm x 0.23 mm in-plane spatial resolution" (Methods), "spatial resolution (1000µm x 230µm x 230µm) of MR" (Discussion), "that of microCT (9µm x 9µm x 9µm)" These are not spatial resolution but voxel size.
We have clarified in the discussion (3rd paragraph) that these dimensions describe voxel size: “…the lower spatial resolution (voxel size: 1000µm x 230µm x 230µm) of MR acquisition compared to that of microCT (voxel size: 9µm x 9µm x 9µm)…”

4) "ROIs" Do the ROIs in the MRI and micro-CT images correspond accurately? Were there any anatomical landmarks?
We clarified the anatomical landmarks and the steps we took to ensure the MRI and microCT ROIs corresponded (Micro-Computer Tomography Analyses section): “The osteochondral specimens were extracted by identifying the center weight-bearing zone of each medial plateau. We then measured 10 mm into the anterior and posterior planes, each, from this center point – this strategy replicated the 20 consecutive central MR images. Next, we measured 15 mm medial and lateral from this center point. In addition, several study team members (JBD, TEM, GHL, and MFB) reviewed the final microCT volumes to ensure that they corresponded to the MR ROI.”

5) "Threshold: An upper threshold of 255 and a lower threshold of 80 were used." How did the authors determine these threshold values?
We have clarified how we determined the threshold in the Methods, Micro-Computed Tomography Analyses: “Thresholding or “segmentation” was performed using simple global methods. The binary grayscale range of the Skyscan instrument is from 0 (air, black) to 255 (most solid structure, white), and is indicative of the resorptive properties of the structure scanned, in this case bone. Thus, we used an upper threshold of 255, which captures the densest bone. We also chose a lower threshold of 80 using the grayscale histogram feature of the software, which showed a clear dip in detection of
bone versus non-bone structures. We have also used this lower threshold in a number of other publications examining bone structures [24]. The density range of the system is regularly calibrated against "phantoms" of known bone mineral density content and thus Hounsfield units, in which the lower grayscale density of air (0) is equal to -1000 HU, and the highest density of 255 is equal to 9200 Hounsfield units. We computed BV/TV using a marching-cubes algorithm.

**Results**

6) "Table 1" MRI based BV/TV was too low. Was the threshold value of the MR image determined properly?

We clarified in the Methods, Magnetic Resonance Analyses section that “We used a standardized cortical bone signal intensity as a conservative threshold. We hypothesized that this conservative threshold would cause MR-based apparent BV/TV to systematically underestimate microCT-based BV/TV but also minimize the influence of abnormal bone marrow signal on MR imaging.”

Could you show a binalized image of it?

We created a supplementary file to show examples of how we placed the regions of interest for determining our threshold. This file also includes a binary image after we applied the threshold. We opted to include these in a supplementary file so that they can be viewed full size, which will help readers see some of the smaller details.

7) "Figure 2-B": The reviewer wonders why Figure 2-B is not mentioned in the results and methods section.

We have referenced figure 2B, which helps demonstrate that “…MR-based apparent BV/TV was systematically lower than BV/TV values from microCT images (Figure 2A and 2B).”

**Table & Figure**

8) "Figure 1": In Figure 1, the MR image seems to have dense trabecular bone rather than the micro-CT, even though Table 1 shows that the MR images had more bones. How do the authors explain this?

The conservative threshold, based on cortical bone signal intensity, creates a conservative estimate of BV/TV that is systematically lower than micro-CT BV/TV but well correlated. While the MR image seems to have dense trabecular bone only a few voxels are below our conservative threshold. The binary image and clarification to item 7 above help clarify this explanation.

Which cases (cadavers No. 1-5, right or left) do these images show?

*Figure 1 is the right knee of the 4th cadaver. We have noted this in the figure legends.*

9) "Figure 2-B": The reviewer cannot understand what Figure 2-B means.

*Figure 2B helps demonstrate that “…MR-based apparent BV/TV was systematically lower than BV/TV values from microCT images (Figure 2A and 2B).”* Adding the reference to this figure in the results will help the reader understand the key take home message from this figure.

**Reviewer: Alberto Tagliafico**

**Minor Essential Revisions:**
In the Methods, MRI sequences have to be described in more details. 
**We have expanded the details of our MRI sequences in the Methods (Magnetic Resonance Imaging section).**

The Authors should add some more comments in the Discussion about clinical usefulness of the results. 
**We have noted in the discussion (first paragraph) that these results validate MR-based apparent BV/TV, which “may be advantageous for large studies in which the participants are already undergoing MR imaging. Future studies using MR-based apparent BV/TV may help us improve our understanding of the role of subchondral bone changes in osteoarthritis and how bone changes in response to different interventions or exposures.”**

I have no more comments. The paper is interesting and well done.

**Editorial Requirement:**

**Could you please clarify and include in the text that NDRI, who you acquired the specimens from, requires that participating hospitals get informed consent for the bodies to be used for research purposes from the patient or family.** 
**At the end of the Methods, Cadaveric Specimen section we noted that “The National Disease Research Interchange requires that participating hospitals obtain informed consent for the bodies to be used for research purposes from the patient or family.”**