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# In-vivo micro-ct radiation does not affect bone structure in rats

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## Introduction

Recently developed animal in vivo high-resolution micro-CT scanners offer the possibility to monitor longitudinal changes in bone micro-structure of small rodents. They do, however, impose a relatively high ionizing radiation dose, which may lead to tissue damage [1].

Our goal was to determine whether an intensive 8 weeks in-vivo scanning regime of the rat proximal tibia would lead to bone damage.

## Methods

Nine female, 30 weeks-old Wistar rats were kept under normal conditions (approved by Animals Ethics Committee). Eight weekly in-vivo CT scans of the right proximal tibia were made using a Scanco vivaCT 40 scanner. During scanning the animals were anesthetized using isoflurane. The total scan length was 6 mm. A voxel resolution of 15 microns was used, resulting in a local CT dose index (CTDI) of 0.49 Gy (according to the manufacturer). Two weeks after the last weekly scan a final scan was made.



Figure 1 Rat in CT-scanner, leg is fixated

The left tibia was only scanned on the first and last time point. Image processing included filtering and segmentation. From the metaphyseal bone the structural parameters were determined. The right/left ratio for all parameters was determined for the first and the last scan. It was assumed that the effects of the radiation dose on the structural parameters would be negligible if the right/left ratio of a parameter after 9 weeks would not differ significantly from that at baseline. The animals were sacrificed after the last scan. The bone marrow was flushed out of the scanned part of both tibias and was tested for cell viability using trypan blue staining.

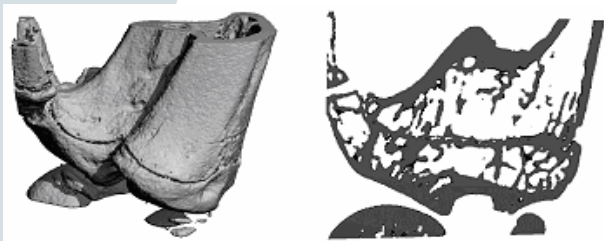


Figure 2 Left: a typical 3-D reconstruction of a tibia and fibula in-vivo obtained from the CT-scanner. Right: A section through the CT-scan showing the individual trabeculae and remains of the growth plate.

## Results

Figure 2 shows a typical 3-D scan and segmented slice, obtained for the tibia in-vivo. Figure 3 shows the bone volume fraction for all rats longitudinally, missing line segments indicate excluded measurements due to movement artefacts.

For the structural parameters, no significant difference was seen between the ratio right/left of the first and the last measurement ( $p > 0.05$ ), except for the connectivity density. No significant difference was seen between the percentage and the absolute number of living cells of the left and right tibia ( $p > 0.39$ , based on paired student t-test).

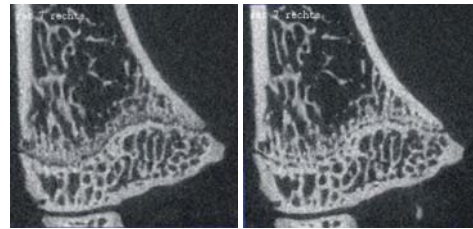


Figure 3 Scan from a rat at week 0 and 9. No differences can be seen indicating correct image registration and no radiation damage

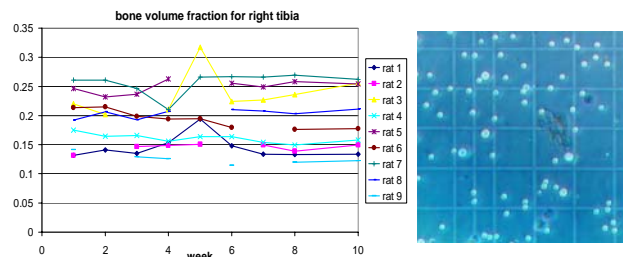


Figure 4 Left: Bone volume fraction for all rats during 9 weeks. Right: Cell trypan blue staining shows mostly living (white) cells

## Discussion

Eight weekly scans resulted in no significant radiation bone damage with the exception of the connectivity density. This parameter, however, had shown to have a low reproducibility in a previous study.

No significant difference was found between the percentage and number of viable cells of the right and left tibia, indicating neither long nor short term effects on cell viability, which is not unlikely considering the CTDI [2]. In conclusion: the results suggest that the scanning frequency used here could be applied when performing a long-term experiment. However, careful consideration should be made when determining the number of scans and the CTDI.

## References:

- [1] Waarsing 2003 Bone 34: 163-169
- [2] Dare 1997 J. Dent. Res. 1997: 658-664