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Effect of micro-computed tomography voxel size and segmentation method on trabecular bone microstructure measures in mice

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ABSTRACT

Micro-computed tomography (μCT) is currently the gold standard for determining trabecular bone microstructure in small animal models. Numerous parameters associated with scanning and evaluation of μCT scans can strongly affect morphologic results obtained from bone samples. However, the effect of these parameters on specific trabecular bone outcomes is not well understood. This study investigated the effect of μCT scanning with nominal voxel sizes between 6–30 μm on trabecular bone outcomes quantified in mouse vertebral body trabecular bone. Additionally, two methods for determining a global segmentation threshold were compared: based on qualitative assessment of 2D images, or based on quantitative assessment of image histograms. It was found that nominal voxel size had a strong effect on several commonly reported trabecular bone parameters, in particular connectivity density, trabecular thickness, and bone tissue mineral density. Additionally, the two segmentation methods provided similar trabecular bone outcomes for scans with small nominal voxel sizes, but considerably different outcomes for scans with larger voxel sizes. The Qualitatively Selected segmentation method more consistently estimated trabecular bone volume fraction (BV/TV) and trabecular thickness across different voxel sizes, but the Histogram segmentation method more consistently estimated trabecular number, trabecular separation, and structure model index. Altogether, these results suggest that high-resolution scans be used whenever possible to provide the most accurate estimation of trabecular bone microstructure, and that the limitations of accurately determining trabecular bone outcomes should be considered when selecting scan parameters and making conclusions about inter-group variance or between-group differences in studies of trabecular bone microstructure in small animals.

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1. Introduction

Micro-computed tomography (μCT) is the gold standard for quantifying trabecular and cortical bone microarchitecture in small animal models (Bouxsein et al., 2010). MicroCT is able to directly measure trabecular bone architecture without having to rely on stereological models that were previously utilized for histological assessment of bone structure (Hildebrand et al., 1999; Weibel, 1980). However, there are numerous variables associated with the data acquisition, processing, and evaluation of μCT scans that can affect morphologic results obtained from bone samples. Bouxsein et al. published guidelines for μCT studies in small animal models (Bouxsein et al., 2010), which has helped to standardize the reporting of study parameters and results, however the effects of various scan parameters on the morphologic results obtained are not fully known.

The voxel size for a μCT scan can strongly affect trabecular or cortical bone results if the voxel size is not appropriately small compared to the dimensions of the structure being measured (Kim et al., 2004). Voxel size has a negligible effect for analysis of structures with relatively high thickness relative to the nominal voxel size (<10:1). However, when analyzing small structures such as mouse trabeculae (20–70 μm), which have dimensions on the same order as the smallest voxel size of most commercially available μCT systems (1–10 μm), voxel size can have significant effects on the results (Muller et al., 1996). Ideally, the smallest voxel size (highest scan resolution) available would be used for all μCT scans. However, high-resolution scans are not always desirable since they require longer acquisition times and generate large data sets. Additionally, if μCT scans are performed on live animals *in vivo*, long scan times and higher radiation dose become important concerns.

Segmentation, the process of binarizing images to "bone" and "non-bone" is also an important process in μCT analysis that can strongly affect trabecular bone morphology results. Most studies of small animal trabecular bone utilize a "global threshold" which is applied to all samples in a study. However, the methods for selecting this threshold are not consistent between research groups, and are not always clearly communicated. Some studies utilize quantitative threshold selection, for example using the midpoint of the "bone" and "non-bone" peaks of the histogram of the local voxels of a sample (Dufresne, 1998).

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Regardless of the segmentation used, it is recommended to visually compare segmented and grayscale images to confirm that the segmentation is representative of the "physiologic" structure of the trabecular bone (Bouxsein et al., 2010).

This study investigated the effect of μCT voxel size on trabecular bone morphology indices quantified in mouse vertebral body trabecular bone. Additionally, two methods for determining segmentation threshold based on either qualitative assessment of 2D images, or quantitative assessment of image histograms were compared. Results from this study will help guide future studies of small animal trabecular bone using μCT, and will help researchers compare results from studies that used different voxel sizes.

2. Methods

L5 vertebrae from six adult (12 week-old) male C57BL/6N mice (Harlan Sprague Dawley, Indianapolis, IN) were scanned using a commercially available micro-computed tomography system (SCANCO μCT 35, Brüttisellen, Switzerland) according to the guidelines for μCT analysis of rodent bone structure (Bouxsein et al., 2010): X-ray tube potential = 55 kVp, current = 114 μA, integration time = 900 ms, number of projections = 1000/180°. Serial scans were performed on the same bone samples with isotropic nominal voxel sizes of 6, 10, 15, 20, and 30 μm (Fig. 1). The trabecular region of the vertebral body (excluding posterior elements) was designated using manually drawn contours inside the cortical shell on two-dimensional transverse slices by a single experienced operator, encompassing the entire vertebral body enclosed by the growth plates.

Segmentation threshold for image analysis was determined for scans of each voxel size using two methods. First, threshold was selected qualitatively by an experienced operator by comparing segmented trabecular bone to original grayscale images, with the goal of obtaining a physiologically accurate representation. Second, segmentation threshold was determined quantitatively from the histogram of the trabecular compartment as previously described (Dufresne, 1998). For this method the threshold was set at the midpoint between the "bone" and "non-bone" peaks of the histogram (Fig. 2).

Trabecular bone volume fraction (BV/TV), connectivity density (Conn.Dens), structure model index (SMI), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), bone tissue mineral density (Tissue BMD; mg HA/cm^3 BV), and apparent mineral density (Apparent BMD; mg HA/cm^3 TV) were directly measured using the manufacturer's 3-D analysis tools. All outcomes were compared using ANOVA to determine differences from "true" values (defined as values obtained for the 6 μm voxel size).

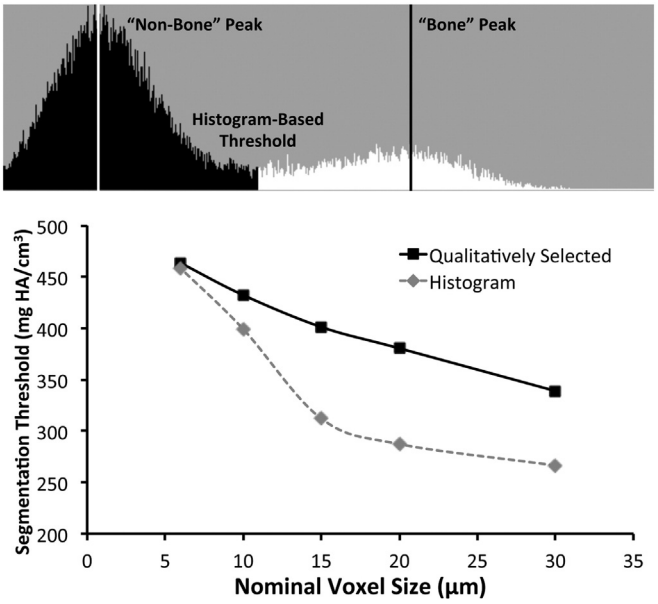

Fig. 2. (Top) For the histogram-based segmentation method, the "bone" and "non-bone" histogram peaks were identified, and the midpoint between these peaks was selected as the global segmentation threshold (histogram from a 6 μm voxel size scan). (Bottom) For smaller voxel sizes (6–10 μm), the two segmentation methods selected similar thresholds (1–8% difference), while for larger voxel sizes (15–30 μm) the two methods selected considerably different thresholds (21–25% difference).

3. Results

"Qualitatively Selected threshold" based on subjective selection by an experience operator for physiologic representation, and "Histogram threshold" based on the voxel brightness histogram were both strongly dependent on scan voxel size (Fig. 2). For larger voxel sizes (15–30 μm) the two methods selected considerably different segmentation thresholds (21–25% difference), while for smaller voxel sizes (6–10 μm) the

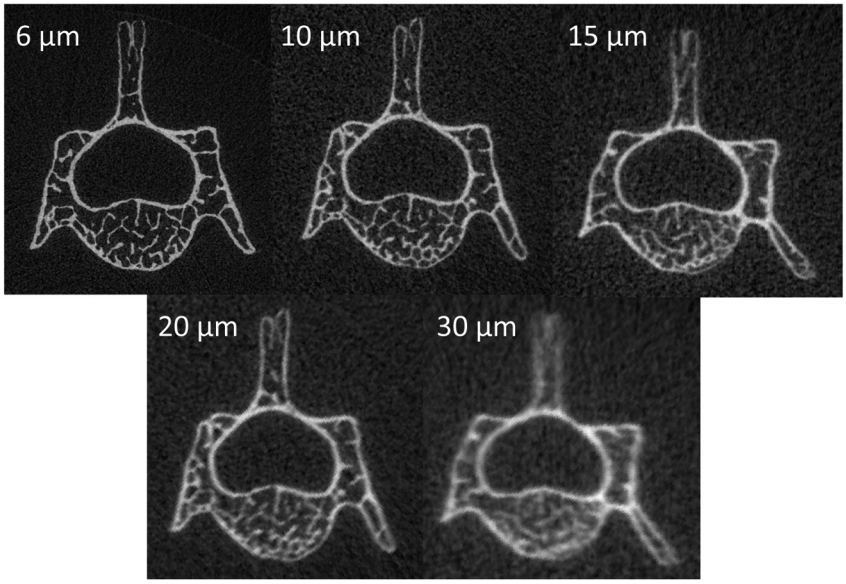

Fig. 1. Raw (unsegmented) micro-computed tomography (μCT) images of the same mouse lumber vertebra scanned with nominal voxel sizes from 6–30 μm.

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