

Technical Note

Accuracy of cancellous bone volume fraction measured
by micro-CT scanning

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Abstract

Volume fraction, the single most important parameter in describing trabecular microstructure, can easily be calculated from three-dimensional reconstructions of micro-CT images. This study sought to quantify the accuracy of this measurement. One hundred and sixty human cancellous bone specimens which covered a large range of volume fraction (9.8–39.8%) were produced. The specimens were micro-CT scanned, and the volume fraction based on Archimedes' principle was determined as a reference. After scanning, all micro-CT data were segmented using individual thresholds determined by the scanner supplied algorithm (method I). A significant deviation of volume fraction from method I was found; both the y -intercept and the slope of the regression line were significantly different from those of the Archimedes-based volume fraction ($p < 0.001$). New individual thresholds were determined based on a calibration of volume fraction to the Archimedes-based volume fractions (method II). The mean thresholds of the two methods were applied to segment 20 randomly selected specimens. The results showed that volume fraction using the mean threshold of method I was underestimated by 4% ($p = 0.001$), whereas the mean threshold of method II yielded accurate values. The precision of the measurement was excellent. Our data show that care must be taken when applying thresholds in generating 3-D data, and that a fixed threshold may be used to obtain reliable volume fraction data. This fixed threshold may be determined from the Archimedes-based volume fraction of a subgroup of specimens. The threshold may vary between different materials, and so it should be determined whenever a study series is performed. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Measurement of volume fraction is of primary importance for the evaluation of trabecular microstructure. Traditionally, volume fraction measurements have been based on Archimedes' principle (Sharp et al., 1990) or on using stereological techniques on histological sections (Kelvin, 1969). As a non-invasive method, microtomography for 3-D reconstruction of cancellous bone has attracted much interest. The bone volume fraction can easily be calculated from voxel based 3-D reconstructions of micro-CT images (Kuhn et al., 1990; Rügsegger et al., 1996; Odgaard, 1997).

Kuhn et al. (1990) reported that micro-CT (Feldkamp et al., 1989) measured volume fractions on a small sample

of 14 specimens was 1.35% different from those measured from histologic sections. Although the difference was not statistically significant, the slope of the linear regression line was significantly different from unity, and the y -intercept was significantly different from zero. More recently, a different high-resolution microtomographic system has been developed by Rügsegger et al. (1996). A strong resolution dependency of structural parameters calculated from micro-CT images was found (Müller et al., 1996), where volume fraction remained constant between the resolutions of 14 and 100 μm . The accuracy of volume fraction measurement has, however, not been quantified for this system by comparison to externally determined volume fraction. Moreover, the segmentation algorithms for both systems are based on imaging information alone, without external calibration to an Archimedes-based volume fraction which does not involve arbitrary threshold.

Our aims were therefore: (1) to assess the accuracy of volume fraction measurements from micro-CT, and to

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compare two different approaches/algorithms: segmentation based on imaging information alone with segmentation based on an external measurement; and (2) to provide guidelines for segmentation of micro-CT data.

2. Material and methods

Forty human autopsy proximal tibiae, one from each individual, without macroscopical pathological changes or history of musculoskeletal diseases, were removed and stored at -20°C . All donors, 10 females and 30 males, aged 16–85 years, were Caucasian, and each had died suddenly either due to trauma or acute disease.

Two randomly localized cylindrical specimens were removed from both medial and lateral condyles of proximal tibiae, yielding a total of 160 specimens (Ding et al., 1997). All specimens were drilled out of frozen bone using a trephine with an inner diameter of 7.5 mm. They were cut 1 mm below the subchondral bone plate and at the distal end to achieve specimens with a diameter = length = 7.5 mm.

The specimens were scanned with a high-resolution microtomographic system ($\mu\text{-CT 20}$, Scanco Medical AG., Zürich, Switzerland) in multislice mode. Each 3-D image data set consisted of approximately 350 micro-CT slide images (512×512 pixels) with 16-bit-gray levels. A detailed description of this system can be found elsewhere (Rüeggsegger et al., 1996). Our specimens were scanned in standard resolution mode, with a x -, y - and z -resolution around $40\text{ }\mu\text{m}$. The voxel size was $22 \times 22 \times 22\text{ }\mu\text{m}^3$. Scanning time for each specimen was approximately 3 h.

After scanning, the 3-D data sets were segmented by using individual global thresholds, above which all pixels are considered bone, and below which all pixels are considered non-bone. The threshold of each data set was determined using an adaptive method, where the gray-level data set is segmented at different levels, e.g. 50 different thresholds. The threshold, where the volume fraction changes the least, i.e. the steepest gradient of gray levels, is chosen as the threshold for the data set (Scanco Medical AG, 1997). This segmentation method will in the following be called *method I*.

A second segmentation was performed using individual thresholds forcing BV/TV (bone voxels per total specimen voxels) to the Archimedes-based (see description below) volume fractions (method II). After segmentation, the volume fraction measurements were done in a cylindrical volume-of-interest such that the entire cylindrical specimen was analyzed, excepting a 0.1 mm exterior border (Fig. 1).

The mean thresholds from methods I and II were applied to segment 20 randomly selected micro-CT image data (10 from low density, volume fraction 9.8–20%; and 10 from high density, volume fraction 30–39.8%),

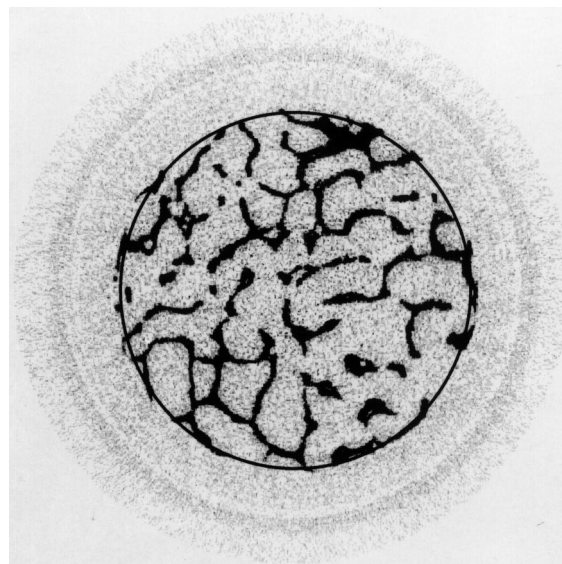


Fig. 1. Typical micro-CT images of cylindrical cancellous bone specimens. After threshold procedure, from the 3-D data sets, the volume fractions were determined in a cylindrical volume-of-interest, which was only slightly smaller than the specimen.

and volume fractions were calculated from the segmented 3-D data. Furthermore, the connectivity densities of these 20 specimens were determined (Odgaard and Gundersen, 1993).

To test the precision of volume fraction measurement, 10 specimens with volume fraction 10.7–39.9% were scanned twice, each scanning on a different day.

After scanning, an accurate protocol was conducted for measuring Archimedes-based volume fraction as suggested by Sharp et al. (1990), with a slight modification. Sharp et al. used toxic trichlorethylene to defat specimens, we used a much less toxic alcohol and acetone 1 : 1 mixture. Volume fraction and tissue density were calculated (Archimedes' principle) as described (Ding et al., 1997).

For statistic analyses, linear regression analysis was used to assess the different methods and paired t -tests were used to compare differences between groups. A p -value < 0.05 was considered significant.

3. Results

The histologic analysis of a pilot study of additional 10 specimens proved that all soft tissues had been completely removed from inter-trabecular spaces by the defatting procedure (Ding et al., 1997). The tissue density of all 160 specimens was constant (1.96 ± 0.08 , mean $\pm$ SD).

A small, yet significant, underestimation of volume fraction was found by using method I (2%, $p = 0.03$), using the Archimedes-based volume fraction as reference.

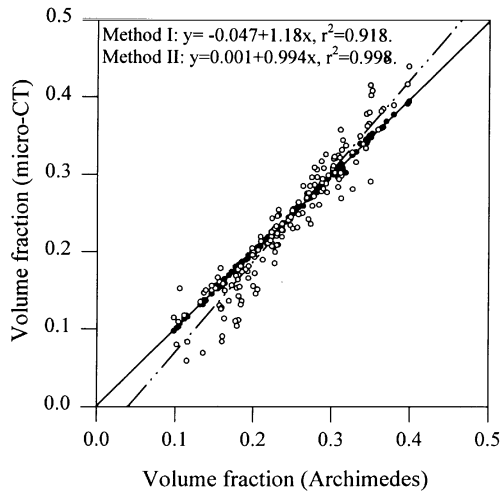
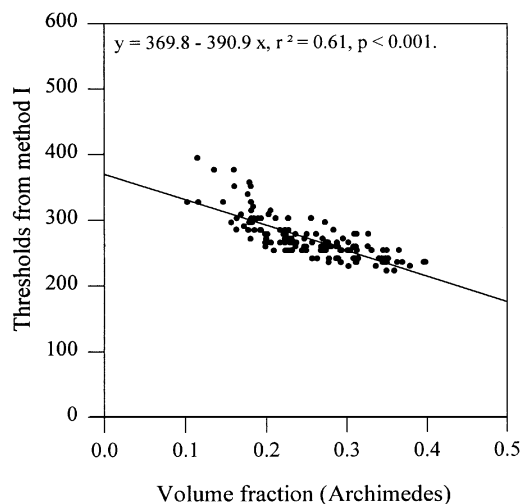


Fig. 2. Correlations of volume fractions (micro-CT vs. Archimedes) of all specimens obtained by applying individual thresholds from method I (dash line and open circles) and method II (solid line and filled circles).

Linear regression on the correlations of volume fractions obtained by applying individual thresholds from method I and method II are shown in Fig. 2. The slopes of the two regression lines were significantly different ($p < 0.001$). For method I, the y -intercept (constant coefficient) of the linear model was significantly different from zero ($p < 0.001$), and the slope was significantly different from unity ($p < 0.001$). Due to its definition, method II resulted in a y -intercept equal to 0 ($p = 0.37$), and slope equal to 1 ($p = 0.75$).

A significant dependence on volume fraction was found for the thresholds determined from method I ($r^2 = 0.62$, $p < 0.001$), while the thresholds determined from method II did not depend on volume fraction ($r^2 = 0.002$, $p = 0.053$) (Fig. 3).



The mean volume fraction of the 20 specimens segmented by using the mean threshold from method I (272) showed a 4% underestimation ($p = 0.001$), whereas the volume fractions by using the mean threshold from method II (262) was not different from Archimedes-based measures ($p = 0.80$). The y -intercept of the linear model of method I was not significantly different from zero ($p = 0.85$), and the slope was not significantly different from unity ($p = 0.09$). The y -intercept of the linear model of method II was not significantly different from zero ($p = 0.73$), and the slope was not significantly different from unity ($p = 0.63$) (Fig. 4). The mean connectivity densities of the 20 specimens using thresholds 272 and 262 were $7.25 \pm 2.29 \text{ mm}^{-3}$ and $7.02 \pm 2.17 \text{ mm}^{-3}$, respectively ($p < 0.002$).

The reproducibility of the volume fraction measurement was excellent. Statistic analysis on the spread of the measurement showed that there was not substantial difference between two measurements (99% confidence interval: 0.2265 ± 0.0057 , variation coefficient: 1.389%). The small standard error (0.0036), and the slope (1.03) of linear regression analysis ($r^2 = 0.999$) also reflected the precision of the measurement.

4. Discussion

An accurate protocol was conducted for the measurement of cancellous bone volume fractions based on Archimedes' principle (Sharp et al., 1990). Our data showed that the tissue density of these specimens was very constant. These results together with histomorphometry suggest that volume fraction measured based on Archimedes' principle is very reliable.

Our data showed that segmentation using method I underestimated volume fraction for low density

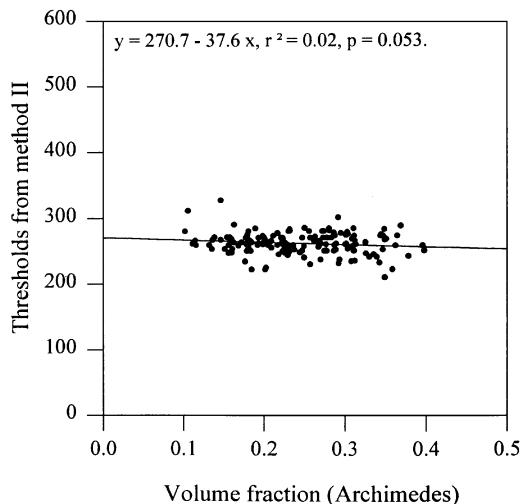


Fig. 3. Associations between volume fraction (Archimedes) and the thresholds from method I (mean = 272, left) and method II (mean = 262, right).

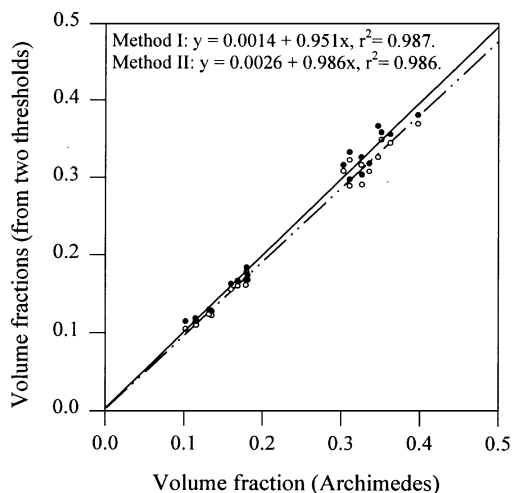


Fig. 4. Correlations between volume fractions: Volume fraction from Archimedes vs. volume fraction from method I (dash line and open circles), and vs. volume fraction from method II (solid line and filled circles).

specimens, but overestimated volume fraction for high density specimens. Subgroup data showed that the underestimation for low-density specimens (volume fraction 9.8–20%) was 12.3%, $p < 0.001$. The overestimation for high-density specimens (volume fraction 30–39.8%) was 7.2%, $p < 0.001$. Therefore, it is necessary to correct these values back to true values obtained from the technique based on Archimedes' principle. No difference was found between middle density specimens (volume fraction 20–30%), $p = 0.45$ (Fig. 2).

The results of all specimens showed a significant dependence of method I threshold on volume fraction from Archimedes, whereas no relationship was found between method II threshold and volume fraction from Archimedes (Fig. 3). The results of 20 specimens suggest that by applying the mean threshold from method I will cause a systematic underestimation. Because its regression line always lies below the diagonal line, a slope significantly different from unity might probably be achieved by increasing specimens in analysis (Fig. 4).

Our data demonstrate that care must be taken when applying thresholds in generating 3-D data sets, if one is interested in an accurate investigation of trabecular bone microstructure. Minor differences in thresholds will result in significant connectivity differences. Our results suggest that an optimal threshold may be determined from a small amount of specimens only.

Consequently, we recommend the following algorithm for calibrating the segmentation of 3-D micro-CT data.

First, a small amount of specimens (e.g. 20 specimens) should be micro-CT scanned. Second, volume fractions of these specimens should be obtained based on an accurate external method. Third, an optimal threshold for each specimen corresponding to the externally determined volume fraction should be calculated. From the mean value, a fixed optimal threshold should be applied to the segmentation of micro-CT data. The threshold will probably depend on the material studied, and a threshold determined from, say, canine cancellous bone, may not be applicable to human cancellous bone. Different anatomical locations may also require different thresholds, but these aspects are as yet unknown.

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