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Technical Note

Specimen size and porosity can introduce error into μCT-based tissue mineral density measurements

Roberto J. Fajardo [a,*], Esther Cory [a], Nipun D. Patel [a], Ara Nazarian [a], Andres Laib [b], Rajaram K. Manoharan [a,c], James E. Schmitz [a,c], Jeremy M. DeSilva [d], Laura M. MacLatchy [d], Brian D. Snyder [a,e,f], Mary L. Bouxsein [a]

[a] Orthopedic Biomechanics Laboratory, Beth Israel Deaconess Medical Center and Harvard Medical School, N 115 Boston, MA, USA
[b] Scanco Medical AG, Bruettisellen, Switzerland
[c] Department of Biomedical Engineering, Boston University, Boston, MA, USA
[d] Department of Anthropology, University of Michigan, Ann Arbor, MI, USA
[e] Children's Hospital, Boston, MA, USA
[f] Harvard Medical School, Boston, MA, USA

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ABSTRACT

The accurate measurement of tissue mineral density, ρ_m, in specimens of unequal size or quantities of bone mineral using polychromatic μCT systems is important, since studies often compare samples with a range of sizes and bone densities. We assessed the influence of object size on μCT measurements of ρ_m using (1) hydroxyapatite rods (HA), (2) precision-manufactured aluminum foams (AL) simulating trabecular bone structure, and (3) bovine cortical bone cubes (BCt). Two beam-hardening correction (BHC) algorithms, determined using a 200 and 1200 mg/cm^3 HA wedge phantom, were used to calculate ρ_m of the HA and BCt. The 200 mg/cm^3 and an aluminum BHC algorithm were used to calculate the linear attenuation coefficients of the AL foams. Equivalent ρ_m measurements of 500, 1000, and 1500 mg HA/cm^3 rods decreased ($r^2>0.96$, $p<0.05$ for all) as HA rod diameter increased in the 200 mg/cm^3 BHC data. Errors averaged 8.2% across these samples and reached as high as 29.5%. Regression analyses suggested no size effects in the 1200 mg/cm^3 BHC data but differences between successive sizes still reached as high as 13%. The linear attenuation coefficients of the AL foams increased up to approximately 6% with increasing volume fractions ($r^2>0.81$, $p<0.05$ for all) but the strength of the size-related error was also BHC dependent. Equivalent ρ_m values were inversely correlated with BCt cube size ($r^2>0.92$, $p<0.05$). Use of the 1200 mg/cm^3 BHC ameliorated the size-related artifact compared to the 200 mg/cm^3 BHC but errors with this BHC were still significant and ranged between 5% and 12%. These results demonstrate that object size, structure, and BHC algorithm can influence μCT measurements of ρ_m. Measurements of ρ_m of specimens of unequal size or quantities of bone mineral must be interpreted with caution unless appropriate steps are taken to minimize these potential artifacts.

Introduction

Measurement of equivalent bone tissue mineral density (ρ_m) using polychromatic planar radiography and computed tomography are established techniques whose precision, accuracy, and potential sources of error have been well studied [e.g., 1,2–6]. Polychromatic micro-computed (μCT) tomographic analyses of bone were originally focused only on structural assessments of trabecular and cortical bone tissue [7,8] and not measurements of ρ_m. Recently, methods for the measurement of ρ_m have been developed for polychromatic μCT and several studies have incorporated these techniques [9–22]. However, only a few published studies of the precision and accuracy of μCT-based equivalent ρ_m have been performed to date [23–26], in contrast to the numerous studies completed for clinical CT [1–6,27–38].

The accuracy and precision of μCT-based measurements of ρ_m can be affected by factors related to the scan settings, tissue samples, and scan artifacts. Recent studies have examined the influence of factors such as the X-ray tube voltage, current intensity, and sample dimensions [24,25,39,40]. Beam-hardening related artifacts such as streaking [41], dark banding [low attenuation spots between two higher density objects; [42–44]], cupping [26,45], as well as ring artifacts [41,46] can introduce errors in measured attenuation values. One topic of specific interest is the effect of sample dimensions or bone mass differences on the accurate measurement of ρ_m since (1) object thickness (size) is known to impact linear X-ray attenuation independent of ρ_m [1,42,44] and (2) a wide range of orthopedic and bone biology studies incorporate specimens of varying size or quantities of bone including animal models of osteoporosis (disease), bone biomechanics, bone tissue engineering, aging, and interspecies studies of bone structure and function [21,22,47–57]. For polychromatic computed tomography X-ray systems, the measured

* Corresponding author. Fax +1 617 667 7175.
E-mail address: rfajardo@bidmc.harvard.edu (R.J. Fajardo).

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attenuation coefficient value of rod-like objects made of hydroxyapatite, for example, will be lower in larger rods compared to smaller ones. This results from the cupping beam-hardening artifact [42] wherein the lower energy photons of a polychromatic source are absorbed more than higher energy photons as they pass through the center of the object (full thickness). This alters the energy spectrum of the X-ray beam, causing an increase in its mean energy. A polynomial correction determined using a calibration wedge phantom of known density and increasing thicknesses can be used to mitigate or correct size-related artifact in measurements of ρ_m. It should be noted that when a polynomial beam-hardening correction is chosen and applied to a scan, an assumption about the material density of the scanned region is made (e.g., the average mineral density of the specimen is assumed to be similar to the density of the specific phantom used, for example 200 mg HA/cm^3 or 1200 mg HA/cm^3).

Size-dependency of μCT-based measurements of ρ_m has previously been reported [24]. Mulder et al. used standards of dipotassium phosphate solution (K_2HPO_4) varying in concentration (up to 800 mg/cm^3) and diameter (12 mm, 20 mm, and 36 mm) to assess the accuracy of ρ_m measurements. The authors noted that μCT-determined ρ_m of similar phantom concentrations varied as the phantom size increased; accuracy errors averaged over all results (all different scan settings) reached up to 10%. Further questions and concerns remain regarding the size-related artifact in equivalent ρ_m measurements. First, while Mulder et al. [24] first reported an average of 10% error or less, some of their results showed errors as high as 15% and a later report indicated that errors as high as 25% were possible [58]. If size-dependent error in ρ_m is that high, it is important to further document this error because biologically- and mechanically-relevant differences in ρ_m are reported to be on the order of 5% or less [53,59–61]. A situation where size-related error is this large might lead to results driven by bone mass differences between groups being misinterpreted as biologically significant. In addition, expansion on the work of Mulder et al. [24,58] is necessary because they used K_2HPO_4 phantoms that ranged greatly in absolute dimensions (12 to 36 mm) and which were scanned at different voxel dimension settings. Size-dependency of μCT measurements of ρ_m was not assessed within a single scan/voxel setting and across smaller absolute dimensions, which better approximate the dimensions of small animal bones commonly assessed in μCT studies. Furthermore, it is important to document the size effect with real bone samples and models structurally similar to trabecular bone to understand how the interaction of size and structure may affect ρ_m measurements.

To address these limitations and extend earlier work assessing the size-dependency of μCT ρ_m measurements, we conducted a study to address two research questions. First, will specimen dimensions or mass differences introduce error into μCT-measured ρ_m when scan settings are held constant, and if so, are results dependent on the structure of the specimens? Second, will the choice of beam-hardening correction algorithms affect the results in a size-dependency study? Systematic analysis of μCT-based measurements of ρ_m in three different bone or bone-like models and using three different density-targeted beam-hardening corrections, including a 200 and 1200 mg HA/cm^3 correction and an aluminum correction as well, were used address these research questions.

Materials and methods

Three different models were used to assess the effect of specimen size on μCT-based ρ_m measurements: (1) hydroxyapatite rods, (2) aluminum porous foams, and (3) bovine cortical bone cubes.

Hydroxyapatite rods

Solid hydroxyapatite (HA) rods (CIRS, Virginia, USA) of increasing diameter were initially used to assess the effect of size on bone tissue density measurements. Rod diameters included 3, 7, 12, and 14 mm (Fig. 1a). The effect of size was assessed at six different densities: 0, 50, 150, 500, 1000, and 1500 mg HA/cm^3. These rods contained HA crystals embedded in resin (Fig. 1a). Rods were immersed in saline and scanned using a high-resolution desktop μCT system (μCT-40, Scanco Medical AG, Switzerland) equipped with an aluminum filter 0.5 mm thick. Scan settings included 70 kV, 114 mA, 30 mm field of view, 1024×1024 pixel matrix, and an isotropic voxel size of 0.030 mm. Rods were scanned individually and placed in the center of the field of view. Fifty slices were acquired beginning 3 mm below the top of all rods. Previous precision assessments indicated that coefficients of variation of repeat scans and intra-phantom variability were lower than 6%. Data were filtered through two second-order polynomial beam-hardening corrections (BHC) provided by the manufacturer (Scanco Medical AG), determined using a 200 and 1200 mg HA/cm^3 wedge phantom, prior to the measurement of ρ_m. Briefly, the BHCs were created by scanning sections of increasing thickness in step-wedges comprised of HA in resin at a density of 200 mg/cm^3 or 1200 mg/cm^3. A polynomial algorithm was then derived to correct for the non-linearity in the plot of linear attenuation and wedge thickness that is due to beam-hardening. The lower density target was originally designed for analyses including cancellous bone and the higher density BHC for analyses of teeth and other dense objects.

Circular regions of interest (ROI) were created on each of the 50 acquired image slices. ROI were placed centrally within the boundary of the rod, resulting in a ROI diameter smaller than that of the rod.

Aluminum precision-made porous foams

Nine precision-manufactured aluminum (AL) foams 6101-T6 (ERG Materials and Aerospace Corp. California, USA) were scanned immersed in saline. Three different volume fractions of aluminum (AV/TV) were included (n=3/group): 4–6%, 7–8% and 10–12%. The outer dimensions of all specimens were 15.01 mm×15.01 mm×20.95 mm (Fig. 1b). The density provided by the manufacturer for the AL foams was 2700 mg AL/cm^3. Scan settings were 70 kV, 114 μA, 300 ms integration time, 30 mm field of view, and a 2048×2048 pixel matrix, resulting in approximately 0.015 mm isotropic voxels. Fifty-three slices were acquired one millimeter below the superior edge of the aluminum foam. Image data were processed with a 200 mg HA/cm^3 BHC and a BHC determined using an aluminum wedge phantom (AL-BHC).

Square ROI 890×890 pixels (13.35×13.35 mm) were positioned within the boundaries of the aluminum foam across all slices. After thresholding, the average linear attenuation coefficient of the solid phase was measured in the VOI after peeling away two pixels off every surface of the aluminum to avoid the influence of partial volume averaging at the boundary of the material. Since the range of average column thickness in the aluminum lattice was 0.157–0.391 mm, after the two pixel surface peel, at least 0.097 mm (6 pixels or 61% of the object at that site) remained of the object to measure linear attenuation coefficient of the solid phase. We recorded the linear attenuation coefficient instead of a ρ_m value since it was nonsensical to report the density of aluminum in milligrams of hydroxyapatite as would be the case with the 200 mg HA/cm^3 BHC. Since this is a porous solid, the measurement of the linear attenuation coefficient required the implementation of a threshold to segment the aluminum foam from the background. The application of a threshold presents a different set of problems and assumptions that can impact measurements of structure and/or density. Two different threshold protocols were implemented on the same image data to assess the effect of object size/mass on μCT-based measurements of density. First, an adaptive, iterative algorithm (AIT) that selects a threshold based on the image grayscale histogram was used to determine the threshold for each specimen [48,51,54,62–67]. Next, a single, fixed grayscale