INTRODUCTION: Micro-computed tomography (µCT) has been used extensively to generate high-resolution images of cancellous and cortical bone morphology from normal and pathologic specimens excised from human and animal bones. Most studies have used µCT to quantify the bone microstructural architecture, however recently manufacturers of µCT system have developed solid hydroxyapatite phantoms to be used with their systems, so that µCT can be used to measure the bone tissue mineral density non-invasively. While the use of these phantoms has become commonplace, the technical and physical factors that influence the analytic relationship for converting X-ray attenuation coefficients (µ) to equivalent bone mineral density (ρEQUIV) have not been specified. Algorithms that correct for polychromatic beam hardening have removed an important technical obstacle affecting bone tissue density measured by µCT, yet the effect of varying the energy (keV), current (µA), integration time (ms), pixel resolution (µm) and fluid media/tissue surrounding the bone specimen on the analytic relationship for converting µ to equivalent bone mineral density have received little attention. Solid hydroxyapatite phantoms can have a heterogeneous distribution of resin and mineral crystal that contributes to the variation in the µ at the microscopic level. HKPO4 liquid phantoms are similar in atomic weight and X-ray attenuation to hydroxyapatite but are more homogeneous at the microscopic level. Therefore, our objectives were to investigate the effects of physical and technical constraints on the conversion relationship between X-ray attenuation coefficient and bone mineral tissue density; to establish a technique to measure the equivalent bone mineral density using liquid phantoms of hydrogen disodium phosphate (HKPO4) and to validate the calculated bone tissue density measured non-invasively using µCT with HKPO4 phantoms to the bone mineral ash density measured directly.

METHODS: HKPO4 phantoms of 50, 150, 500 and 1000 mg/cm3 equivalent mineral densities were prepared in sterile glassware using 18.2 MΩ double distilled water. Eppendorf vials were filled and capped while submerged in HKPO4 to avoid the introduction of air. A 1.8 mm trans-axial slice was imaged through each phantom using a Scanco 40 (Scanco Medical AG, Bassersdorf, Switzerland) µCT. Imaging through the top, middle and bottom of the phantom tested the homogeneity and spatial variation of the X-ray attenuation coefficient throughout the phantom. Serial µCT scans obtained weekly over three months tested the conversion relationship for the HKPO4 phantom over time. Using the phantoms as a surrogate for bone, parametric analyses were performed to evaluate the effect of varying: the scanning energy (45, 55 and 70 keV), resolution (18 and 36 µm) and surrounding media (air, saline and ethyl alcohol) on the relationship between the linear X-ray attenuation coefficient and the equivalent mineral density for the phantoms. Twenty-three identical cubes of bovine cortical bone (average bone mass 0.467 g ± 0.016) were cut, weighed and randomly assigned to one of 7 groups of 3 cubes each that were then decalcified in EDTA for 0, 2, 4, 6, 8, 10 or 12 days. After imaging each cube using µCT, the bone mineral density was derived using the HKPO4 phantom relationship to convert the X-ray attenuation coefficient to an equivalent mineral density. The specimens were then dried in air, weighed and ashed (Furnace 48000, Thermolyne, Dubuque, Iowa) to determine the true mineral ash density (mASH/V) and ash content (mASH/mDRY). The µCT derived bone mineral density measured non-invasively was compared to the true mineral ash density for each specimen. A one-way analysis of covariance (ANCOVA) was performed to test the effect of spatial variation and time on the slope of the fit relationship between the X-ray attenuation coefficient and equivalent bone density. Two-way analysis of variance (ANOVA) was used to study the effects of surrounding media, energy, current, integration time, and resolution for all equivalent densities and variables, with equivalent density and specific parameter variation (e.g. 45, 55 and 70 keV E variation) as fixed factors and ρ as dependent variable. Bonferroni post-hoc was applied for multiple comparisons.

RESULTS: The HKPO4 phantom equivalent densities were homogeneous throughout the vials (p = 1.00, F = 0.000). The relationship between the HKPO4 X-ray attenuation coefficient and ρEQUIV were unaffected by time over three months of observation [p = 1.00, F = 0.002 w/ p = 1.00 for all post-hoc cases], Variations in current [p = 0.58, F = 0.703] and integration time [p = 0.85, F = 0.506] also had no effect on the conversion relationship between ρ and ρEQUIV for the phantoms. However, as expected, variations in scanning energy [p<0.01, F = 21.97] resolution [p < 0.01, F = 8.005] and the surrounding media [p = 0.05, F = 2.91] where the effects of ETOH and saline on the attenuation coefficient were similar to one another (p = 0.580) but different from air (p < 0.01 for saline and p=0.04 for ETOH), significantly affected the conversion relationship between the X-ray attenuation coefficients and equivalent mineral density for the phantoms (Figure 1). Multivariate linear regression approach was used to estimate ρEQUIV based on attenuation, energy, resolution and surrounding media. This model explaining over 90% of the variation in ρEQUIV (R²=0.91, p<0.001) was of the form:

\[
\rho_{EQUIV}=\frac{14.09(E)+3.32(M)+217.23(\rho)-90.87}{\rho_{ETOH}+\rho_{SALINE}}
\]

where E represents energy in keV, RES represents resolution in µm, M represents media (1=air, 2=saline, 3=ETOH) and µ represents attenuation coefficient in cm⁻¹. The ρEQUIV of the bovine cortical bone samples decalcified over time were highly correlated (R²=0.92) with the with the ash densities of the progressively decalcified samples in this study.

DISCUSSION: These findings indicate that the relationship used to convert the linear X-ray attenuation coefficient measured by µCT to an equivalent bone mineral density for the phantom is significantly influenced by the energy level and pixel resolution that the µCT was obtained and is also affected by the media surrounding the phantom (which should be equivalent to that surrounding the bone). Therefore any time one of these scanning parameters is changed, the phantom must be re-scanned and a new conversion relationship generated to properly derive the bone mineral tissue density from the X-ray attenuation coefficient. Failure to do this will result in erroneous µCT based bone density measurements. Further, HKPO4 liquid phantoms provide a stable and convenient means to perform quantitative µCT analysis using any µCT system. If the proper conversion relationship is generated using these phantoms, the bone mineral tissue density can be accurately specified.

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