

## ACCURACY OF BONE DENSITY AND AREA MEASUREMENTS FROM PQCT

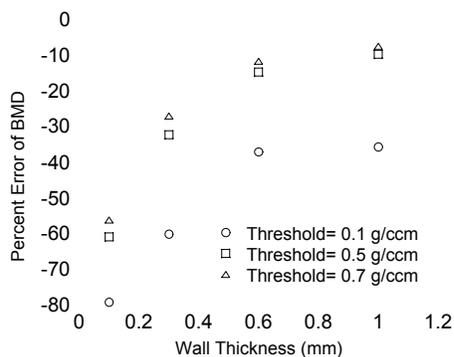
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**Introduction-** Peripheral quantitative computed tomography (pQCT) is increasingly used in research studies to measure densitometric and geometric properties of rat and mouse long bones [1,2]. While excellent precision and accuracy have been demonstrated for standard specimens the size of rat bones [3], the accuracy of pQCT has not been demonstrated for specimens the size of mouse bones. Due to the small cortical thickness of mouse bones (0.10-0.50 mm) [2,4] and the relatively large voxel size available with commercial scanners (0.07 mm or larger), volume averaging may be an important confounding factor for accurate measurement of bone density and area. Consequently, differences between study groups in cortical thickness due to differences in animal age or strain may lead to artifactual differences in density and area values. Software thresholding may be used to compensate for those differences, but may also introduce errors.

**Objectives-** 1) Determine the accuracy of pQCT density measurements made on standard aluminum phantoms of varying thickness. 2) Determine the effect of software threshold on the accuracy of cross-sectional area measurements of mouse femora from pQCT relative to histomorphometric measurements.

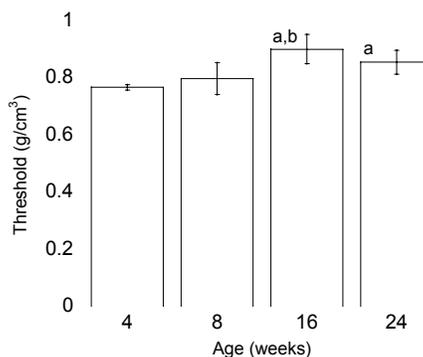
**Methods- Densitometric Measurements:** Four tubes (outer diameter: 0.9, 1.6, 3.5, 3.4, wall thickness: 0.1, 0.3, 0.6, 1.0 mm) and one solid cylinder (3.2 mm diameter) were machined out of 6061 aluminum for use as standard phantoms. Dimensions were chosen to represent femora of animals ranging from 4-week old mice to mature rats. Aluminum was chosen because it has a similar x-ray attenuation as bone [5]. In contrast to bone, aluminum also has a homogeneous composition and can be machined to precise dimensions. The phantoms were scanned at 5 locations using pQCT (XCT Research M, Norland Stratec) with a voxel size of 0.07 mm. Images were then analyzed using the manufacturer's software to determine average density (BMD) at three different thresholds (0.1, 0.5, 0.7 g/cm<sup>3</sup>). The solid cylinder BMD was 2.25 g/cm<sup>3</sup> and was independent of threshold. Percent error relative to the solid phantom density was determined as a function of wall thickness and threshold value for the remaining phantoms. **Bone Area Measurements:** Femurs from female C57BL/6 mice at four ages (4, 8, 16, 24 weeks; n= 5 per group) were embedded in acrylic and scanned at 50% of total bone length (voxel size 0.07 mm). Bones were embedded to allow for precise alignment during scanning and subsequent sectioning for histomorphometric analysis. The bones were then sectioned at 50% of total bone length and area was determined using a standard protocol [4]. pQCT images were analyzed by adjusting the threshold value until the area of the image matched the area measured by histomorphometry. The precision of area measurements was less than 1% for both pQCT and histomorphometric techniques. ANOVA was used to determine the effect of age on threshold value.



**Figure 1-** Percent error in density of aluminum tubes relative to the solid phantom. Error decreases with increasing threshold value and wall thickness.

**Results-** Despite being made from the same material, the measured density of the aluminum tubes varied greatly with both wall thickness and threshold value (Fig. 1). Although increasing the threshold decreased the error in

density, at a threshold of 0.70 g/cm<sup>3</sup> the density of the thickest tube was still significantly different from the solid phantom (p< 0.001). A threshold of 1.9 g/cm<sup>3</sup> used on the thickest tube did result in zero error, however using threshold values higher than 0.70 g/cm<sup>3</sup> on the thinnest walled tube caused significant erosion of the wall leaving an open cross-section. Average threshold values used to match pQCT bone area with histomorphometric bone area changed significantly with age (p< 0.005, Fig. 2). The threshold values for the 16 and 24-week old mice were significantly different from the 4-week old values. Based on these findings the optimal 4-week old threshold value (0.770 g/cm<sup>3</sup>) was used to recalculate area for the other three age groups (Table 1). Paired t-testing indicated that the pQCT area was not statistically different from histomorphometric area at 4-8 weeks, but suggested differences at 16-24 weeks (p< 0.10).



**Figure 2-** Average threshold used to match pQCT bone area with histomorphometric area changed significantly with age (p< 0.005). a= different from 4 weeks, b= different from 8 weeks.

**Table 1-** Mean geometric data for the female C57BL/6 femurs. pQCT area determined at 0.770 g/cm<sup>3</sup>. \* p< 0.1 compared to histomorphometric area.

Age (weeks)	4	8	16	24
Histomorphometric	0.156	0.170	0.183	0.190
Cortical Thickness (mm)	(0.016)	(0.022)	(0.017)	(0.010)
Histomorphometric Area (mm <sup>2</sup> )	0.578	0.664	0.730	0.783
	(0.034)	(0.043)	(0.039)	(0.031)
pQCT Area (mm <sup>2</sup> )	0.555	0.668	0.818*	0.842*
	(0.005)	(0.053)	(0.042)	(0.043)

**Discussion-** pQCT has become widely used to measure density and area in mouse long bones because it has excellent precision and can be used nondestructively both ex-vivo and in-vivo. However, the results of our accuracy study suggest that researchers interpret pQCT results with some caution. We conclude that: 1) Wall thickness significantly affects BMD measurements. While increasing the threshold can decrease the error in accuracy it may produce the undesirable effect of an open cross-section. To minimize errors in BMD, we advocate using the highest threshold possible that does not produce an open section. 2) Slight differences in cortical thickness of the bones measured, and/or age of the animals studied, can introduce errors in BMD and area if a single threshold is used across study groups. This suggests that both absolute and relative differences between groups may be compromised. 3) While pQCT can be used to determine accurate values of area, validation of an optimal threshold must be done by comparison to histomorphometric measurements and be specific to the size of bones being studied.

**References-** 1. Ferretti, Bone 17:353S, 1995. 2. Beamer, Bone 18:397, 1996. 3. Gilsanz, JBMR 11:1518, 1996. 4. Brodt, JBMR 14:2159, 1999. 5. Martin, CTI 47:82, 1990.