

The X-ray attenuation characteristics and density of human calcaneal marrow do not change significantly during adulthood

C.M. Les^{a,*}, R.T. Whalen^a, G.S. Beaupré^c, C.H. Yan^{b,1}, T.M. Cleek^a, J.S. Wills^{a,2}

^a Musculoskeletal Biomechanics Laboratory, NASA Ames Research Center, Moffett Field, CA, USA

^b Lucas MRS Imaging Center, Stanford University, Stanford, CA, USA

^c Rehabilitation Research and Development Center, VA Palo Alto Health Care System, Palo Alto, CA, USA

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Abstract

Changes in the material characteristics of bone marrow with aging can be a significant source of error in measurements of bone density when using X-ray and ultrasound imaging modalities. In the context of computed tomography, dual-energy computed techniques have been used to correct for changes in marrow composition. However, dual-energy quantitative computed tomography (DE-QCT) protocols, while increasing the accuracy of the measurement, reduce the precision and increase the radiation dose to the patient in comparison to single-energy quantitative computed tomography (SE-QCT) protocols. If the attenuation properties of the marrow for a particular bone can be shown to be relatively constant with age, it should be possible to use single-energy techniques without experiencing errors caused by unknown marrow composition.

Marrow was extracted by centrifugation from 10 mm thick frontal sections of 34 adult cadaver calcanei (28 males, 6 females, ages 17–65 years). The density and energy-dependent linear X-ray attenuation coefficient of each marrow sample were determined. For purposes of comparing our results, we then computed an effective CT number at two GE CT/i scan voltages (80 and 120 kVp) for each specimen. The coefficients of variation for the density, CT number at 80 kVp and CT number at 120 kVp were each less than 1%, and the parameters did not change significantly with age ($p > 0.2$, $r^2 < 0.02$, power > 0.8 where the minimum acceptable $r^2 = 0.216$). We could demonstrate no significant gender-associated differences in these relationships. These data suggest that calcaneal bone marrow X-ray attenuation properties and marrow density are essentially constant from the third through sixth decades of life. © 2002 Orthopaedic Research Society. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Bone marrow; Quantitative computed tomography; Cancellous bone; Aging; Calcaneus; Bone density

Introduction

The measurements of X-ray attenuation and ultrasound propagation are currently the two most widely used techniques in bone densitometry studies [6,29,30]. However, accurate interpretation of these measurements in cancellous bone requires a thorough characterization of the bone marrow at the particular anatomic site of interest. For instance, the accuracy and long-term precision of single-energy quantitative computed tomogra-

phy (SE-QCT) in measuring bone density are dependent in part upon uncertainties in the linear X-ray attenuation characteristics of the marrow [18,20,22–24,26,35, 41,61]. If the density and X-ray attenuation characteristics of bone marrow at a particular site were known to change predictably with age, or better yet, to be constant with age in the adult, then precise long-term bone densitometry studies would be improved by the knowledge that changes in X-ray attenuation or ultrasound propagation could be reasonably ascribed solely to material or structural changes in the mineral component.

The change in marrow composition with age from hematopoietic to adipose tissue can result in an underestimation in cancellous bone mineral density of up to 15% [20,41]. Dual-energy quantitative computed tomography (DE-QCT) can correct for unknown relative contributions of yellow and red marrow when measuring bone density in normal and osteoporotic subjects

* Corresponding author. Present address: Bone and Joint Center, 2015 ERB, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA. Tel.: +1-313-916-3166; fax: +1-313-916-8064.

E-mail address: les@bjc.hfh.edu (C.M. Les).

¹ Present address: DSO National Laboratories, 20 Science Park Drive, Singapore 118230, Singapore.

² Present address: Accenture, Charlotte, NC 28269, USA.

[1,2,9,19,23,28,34,46,55,57,58], and can also be used to evaluate changes in marrow fat content with disease [40,48,49]. Most of these techniques are based upon post-processing calibration procedures; an alternative approach suggested by Alvarez and Macovski [2] is based instead on a characterization of the physics of the X-ray beam itself.

However, DE-QCT achieves this improved accuracy at the cost of reduced precision and at a higher radiation dose to the patient or subject [9]. In addition, most clinical CT scanners are not equipped for simultaneous DE-QCT.

It is known that the constituents of marrow change dramatically between birth and old age, as a function of the change from hematopoietic to adipose marrow [12,14,16,20,31,43,47,50,73]. The sequence, pattern, and timing of this change is characteristic for the specific bone. If the X-ray attenuation of marrow changes significantly and systematically with age, as is the case in the vertebrae, then accurate and precise long-term evaluation of bone density requires that the change be thoroughly characterized so that adequate correction may be made in reconstruction algorithms. If, on the other hand, the X-ray attenuation characteristics of marrow for select bones are known and do not change within a given age range, then bone density can be measured at these sites with SE-QCT within this age range without experiencing errors caused by changes in marrow composition.

The calcaneus (os calcis) has several features that make it a useful site for bone densitometry. Calcaneal architecture and local mechanical conditions have been well-characterized in humans and other animals [27,32,33,37,51–53,71], and changes in bone properties at this site have been associated with material, architectural, or mechanical changes at other, more clinically-important but difficult-to-assess sites such as the spine or hip [3,6,13,38,44,54,59,66]. The calcaneus is a peripheral bone surrounded by a thin layer of soft tissue. The radiation dose involved with multiple, serial examinations would not expose vital internal organs or areas of high hematopoietic activity, and the signal-to-noise ratio would be high.

In addition to this use as a sentinel bone for changes at other sites, the calcaneus has immense potential in and of itself as a site for evaluating the effects of changes in mechanical forces on bone tissue, architecture, and structure. For several years, we have been developing a model of skeletal adaptation using QCT of the calcaneus, mathematical modeling of adaptation [4,5,10,11,36,56,62], and estimates of daily skeletal (specifically, calcaneal) loading obtained from a custom-designed ground reaction force monitoring system [7,8,63]. Clearly, an improvement in accuracy and precision in non-invasively evaluating bone density in vivo at this site would dramatically improve our ability to test these

theories, and to discern small changes to the structure in response to changes in loading conditions, nutritional alterations, or pharmaceutical interventions.

In a study based largely on data from other primary sources, Christy [12] showed that there was essentially no hematopoietic marrow in the ankle and foot bones after age 10. No specific mention was made of the calcaneus, and the study did not address changes in attenuation properties or physical density in adulthood.

The objective of this study was to determine the physical density and energy-dependent linear X-ray attenuation coefficients of marrow extracted from adult calcanei, and to evaluate the relationship between these parameters and the age of the donor. Our hypothesis was that there would be no significant change in these parameters with age in adults.

Methods

After obtaining institutional review board approval, 34 pairs of human calcanei were acquired from a local tissue bank. Only the left calcanei were used in this study. Gender and age data were recorded (Table 1; 28 males, 6 females, mean age 44.2 years, SD 12.2 years, range 17–65 years) and the calcanei were stored at -80°C .

Marrow extraction

Calcanei were removed from the -80°C freezer and positioned in a custom holding fixture. A 10 mm thick frontal-plane section was cut on a low-speed saw under ice water irrigation (Isomet Plus, Buehler, Lake Bluff, IL, USA), centered at a point 15 mm posterior to the talar articulation. Cancellous bone and marrow was removed from the section with a rongeur, warmed to 60°C in a water bath, and centrifuged at 1835g for 10 min through a 300 μm stainless-steel mesh (Spectra/Mesh 145 821, Spectrum, Laguna Hills, CA, USA; Fig. 1) into a 20 cc syringe, separating marrow from bone. Extracted marrow volumes ranged from approximately 1 to 6 cc. The syringe containing the marrow was uncapped, air was expelled, and the cap was replaced before storage at -80°C .

Density

A 0.5 cc aliquot of each sample was measured at 37°C and weighed to calculate marrow density (ρ).

CT imaging

At the time of imaging, the syringe was warmed to 37°C , gently agitated in a vortex mixer, and scanned (CT/i, General Electric, Milwaukee, WI, USA) with the syringe inserted into the bore of a 9.21 cm diameter cylindrical acrylic holder. For each sample, 15 images were obtained at 80 kVp, 190 mA, 1 s, with a display field of view of 15 cm, and a slice thickness of 3 mm. The voxel size was approximately $0.30 \times 0.30 \times 3.00$ mm. These images were averaged to reduce quantum noise. The procedure was then repeated at 120 kVp, 190 mA, 1 s.

Attenuation coefficients

The mean energy-dependent linear attenuation coefficient, $\mu_{\text{marrow}}(E)$, was determined for each marrow specimen using a new dual-energy CT technique [70]. Using the GE CT/i X-ray beam spectra ($S(E)$) for 80 kVp and 120 kVp, determined experimentally [69], we computed two constants 'a' and 'b' at each voxel that determine the X-ray attenuation characteristics of the material within the voxel. Mean values, \bar{a} and \bar{b} , were computed from the voxels contained within the syringe. With this approach, the mean linear attenuation coefficient of the marrow sample is expressed as a linear combination of known

Table 1
Donor summary

Age (years)	Gender	Height (cm)	Mass (kg)	Cause of death
19	Female	173	52	Trauma, motor vehicle accident
23	Female	170	91	Trauma, motor vehicle accident
35	Female	157	52	Cerebrovascular accident
38	Female	152	52	Trauma, motor vehicle accident
49	Female	160	80	Myocardial infarction
52	Female	152	50	Cerebrovascular accident
17	Male	178	68	Trauma, motor vehicle accident
30	Male	183	86	Trauma, motor vehicle accident
30	Male	170	91	Trauma, gunshot wound, head
30	Male	178	80	Aortic valve disorder
32	Male	183	108	Trauma, asphyxiation
33	Male	178	68	Asphyxiation
38	Male	165	102	Trauma, gunshot wound, head
38	Male	180	70	Myocardial infarction
40	Male	180	93	Myocardial infarction
42	Male	178	80	Trauma, head
44	Male	170	68	Cerebrovascular accident
45	Male	183	98	Trauma, gunshot wound, head
45	Male	183	82	Myocardial infarction
46	Male	168	85	Trauma, motor vehicle accident
47	Male	191	80	Myocardial infarction
51	Male	180	86	Intracranial hemorrhage
52	Male	180	77	Apparent myocardial infarction
54	Male	175	86	Myocardial infarction
54	Male	185	84	Myocardial infarction
54	Male	178	85	Apparent myocardial infarction
55	Male	178	73	Myocardial infarction
55	Male	180	68	Trauma, motor vehicle accident
56	Male	178	86	Apparent myocardial infarction
58	Male	180	77	Apparent myocardial infarction
58	Male	183	77	Subdural hematoma
59	Male	170	68	Atherosclerotic heart disease
60	Male	178	73	Apparent myocardial infarction
65	Male	173	86	Cerebrovascular accident

water and aluminum attenuation functions (<http://physics.nist.gov>)

$$\mu_{\text{marrow}}(E) = \bar{a}\mu_{\text{water}}(E) + \bar{b}\mu_{\text{al}}(E).$$

For the purpose of comparing our results, we calculated an effective linear attenuation coefficient and an effective CT number [42], designated CT(80) and CT(120), for each specimen at each scan energy:

$$\text{CT\#} = 1000 \frac{\mu_{\text{marrow, effective}} - \mu_{\text{water, effective}}}{\mu_{\text{water, effective}}},$$

where

$$\mu_{\text{marrow, effective}} = \frac{\int^E \mu_{\text{marrow}}(E)S(E) dE}{\int^E S(E) dE}$$

and

$$\mu_{\text{water, effective}} = \frac{\int^E \mu_{\text{water}}(E)S(E) dE}{\int^E S(E) dE}.$$

Validation procedures

We evaluated the accuracy of our analytical technique by scanning a syringe filled with distilled water at 37 °C inserted into the acrylic holder. The water scan was processed along with the marrow samples. The linear attenuation coefficient functions for the water and acrylic holder were determined following the procedures described previously [70] and compared to known X-ray attenuation values for water and acrylic obtained from the National Institute of Standards and Technology (<http://physics.nist.gov/lab.html>). For each beam spec-

trum, effective linear attenuation coefficients were computed for the measured and known functions ($\mu(E)$) of each material and compared. Finally, to ensure CT scan conditions did not change throughout the scan session, we compared the effective linear attenuation coefficients (at 80 and 120 kVp) of acrylic from the first and last marrow scans.

Statistics

Gender-associated differences in age, height, weight, body mass index ($\text{BMI} = \text{mass}/\text{height}^2$), ρ , CT(80), or CT(120) were evaluated using *t*-tests. Linear regression analyses were performed using ρ and age as the independent variables, and ρ (where appropriate), CT(80), and CT(120) as dependent variables. Stepwise multiple linear regressions were performed using ρ , CT(80), and CT(120) as dependent variables, and allowing ρ (where appropriate), age, height, weight, and BMI as the independent variables. Analyses were performed for all subjects, and then were divided by gender.

Wherever $p > 0.05$, the power and minimum sample size required to achieve a power of 0.8 were calculated. For linear regressions, the minimum acceptable r^2 values required to achieve a power of 0.8 were also calculated (SigmaStat 2.0 for Windows, SPSS Science, Chicago, IL, USA).

Results

The measured and known energy-dependent linear attenuation coefficient functions for water and acrylic

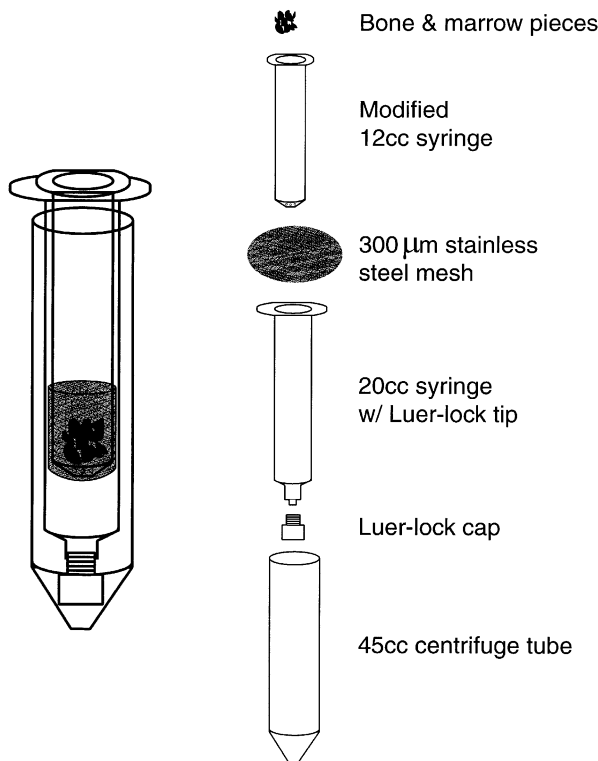


Fig. 1. Marrow extraction apparatus, schematic.

are shown in Fig. 2. The measured effective attenuation coefficients of water and acrylic for the 80 kVp beam spectrum were within 3.8% of the value computed from NIST data, and within 0.8% for the 120 kVp beam spectrum. Effective attenuation coefficients for the 80 and 120 kVp spectra were within 1.0% between the first and last marrow scan.

For the population of marrow samples, the coefficient of variation (CV) was ($< 1\%$) for ρ , CT(80), CT(120)

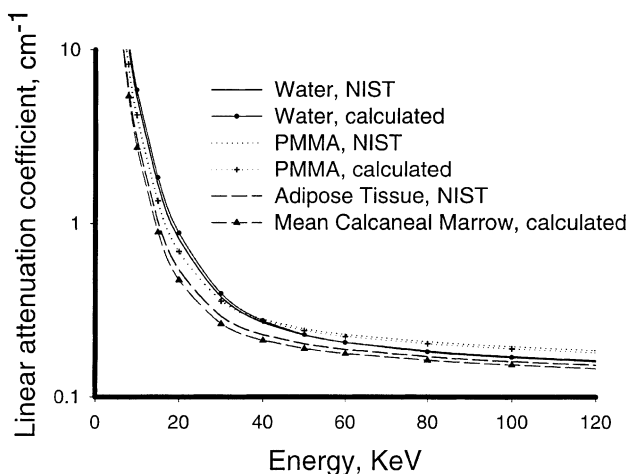


Fig. 2. Energy-dependent linear attenuation coefficients for water, polymethylmethacrylate, and adipose tissue from the NIST standards (lines), and as determined experimentally (circles and crosses) for water, polymethylmethacrylate, and bone marrow.

and parameter 'a', and less than 3% for parameter 'b' (Table 2). The regression slope for ρ , CT(80), and CT(120) as a function of age was not significantly different from zero (Table 3, Figs. 3 and 4). There was no demonstrable correlation between ρ and CT(80), or between ρ and CT(120) (Table 3, Fig. 5). Regressions were not significantly improved through the use of stepwise multiple linear analyses. The variation in energy-dependent linear attenuation coefficients was remarkably small for these specimens (Figs. 5 and 6).

Statistical power was less than 0.5 for all linear regression analyses (Table 3). For $n = 34$ and $\alpha = 0.05$, a minimum r^2 value of 0.216 is necessary to achieve a power of 0.8 [72]. For these data, $p \geq 0.24$, and $r^2 \leq 0.01$ (Table 3, Figs. 3,4,6). The minimum sample size for the power to exceed 0.8 at the calculated r^2 values ranged from 178 to 1647 (Table 3).

There were significant differences between genders in height ($p < 0.001$, mean female height = 1.61 m, mean male height = 1.78 m), and weight ($p = 0.001$, mean female weight = 63 kg, mean male weight = 82 kg), but not necessarily for age, BMI, ρ , CT(80), or CT(120) ($p > 0.15$, power < 0.32). There were no significant correlations between ρ and CT(80) or CT(120), or between age and ρ , CT(80), or CT(120) when the data were separated by gender ($p > 0.12$, power < 0.32).

Discussion

The calcaneus has become an increasingly important bone site for monitoring skeletal changes with age and activity level. Changes in calcaneal bone mineral content have been used as sentinel values, as statistical surrogates, for use in following the process of bone loss at other, more clinically relevant sites such as the hip or spine [3,6,13,38,44,54,59,66]. As an anatomical site, the calcaneus has the advantage of being peripheral, and of being surrounded by a smaller and more consistent amount of soft tissue than these other sites. Our results suggest another advantage of this site, that of a constancy of marrow constituents not seen at the spine or hip. Such a feature would improve remarkably the long-term precision of such screening protocols.

The results of this study have important implications beyond computed tomography as well. Clearly, the assumption that calcaneal marrow density may be considered as constant may also be used in studies that utilize X-ray absorptiometry or ultrasound propagation as a means of examining bone mineral density. Any measured changes in X-ray attenuation or ultrasound propagation at this site with time could be assumed to be a result of changes in bone density or structure, but not to changes in marrow.

While a number of factors contribute to the accuracy and precision of repeated measurements, our objective

Table 2
Summary data

Parameter	Mean	Standard deviation	Coefficient of variation	Range
ρ (gm/cc)	0.922	0.007	0.007	0.911 to 0.940
CT(80)	782.5	5.6	0.007	766.7 to 795.6
CT(120)	852.3	4.3	0.005	836.7 to 859.7
Parameter 'a'	1.002	0.004	0.004	0.986 to 1.009
Parameter 'b'	-0.036	0.001	0.029	-0.038 to -0.033

Table 3
Linear regression data

Variables		Slope (standard error)	r^2	p	Power	Minimum sample size
Independent	Dependent					
Age	ρ	-9.8×10^{-5} (9.9×10^{-5})	< 0.01	0.32	0.16	261
	CT(80 kV)	-0.094 (0.078)	0.01	0.24	0.43	178
	CT(120 kV)	-0.058 (0.061)	< 0.01	0.35	0.15	287
ρ	CT(80 kV)	-67.6 (140.2)	< 0.01	0.63	0.07	1647
	CT(120 kV)	-72.1 (108.4)	< 0.01	0.51	0.10	572

Minimum sample size is that required at the calculated r^2 to achieve a power of 0.8. Alternatively, with $n = 34$, $\alpha = 0.05$, an r^2 of at least 0.216 is required to achieve a power of 0.8. See Figs. 3–5.

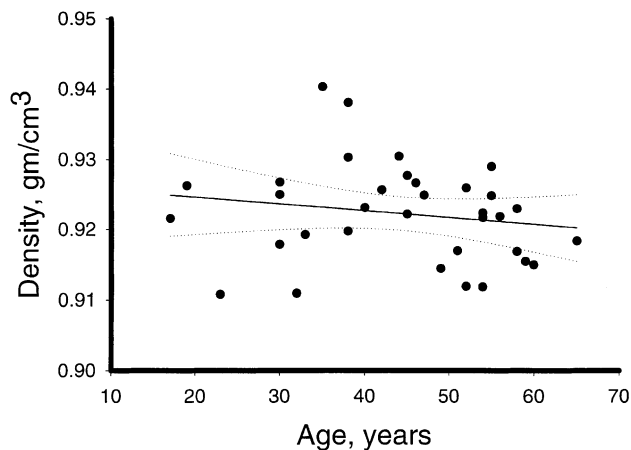


Fig. 3. Calcaneal marrow density, as a function of donor age. Line indicates linear regression and 95% confidence interval. No significant change in marrow density with age could be demonstrated with these samples.

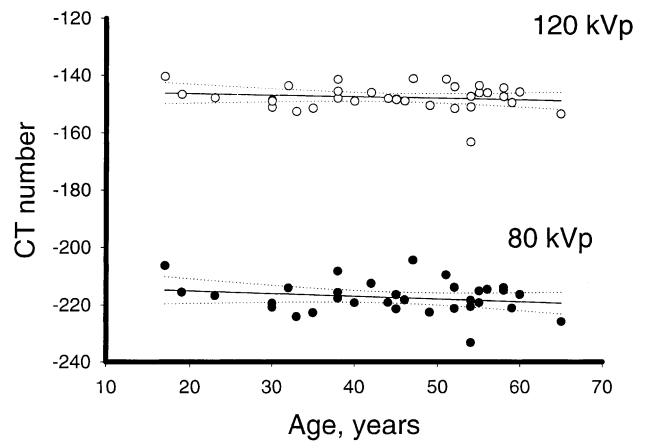


Fig. 4. Calcaneal marrow attenuation, Hounsfield units, at 80 and 120 kVp, as a function of donor age. Lines indicate linear regressions and 95% confidence intervals. No significant change in attenuation with age could be demonstrated with these samples.

with this study was to determine those material properties of bone marrow, the dominant non-bone constituent comprising the calcaneus, that significantly impact interpretation of results obtained from different imaging modalities. If marrow properties are constant, or if changes are systematic with age, then a potential source of error affecting long-term measurement precision has been eliminated. Another objective was to obtain accurate estimates of material properties that could then be used to further enhance the accuracy and precision of bone density and ultrasound measurements.

Our concern over the possibility of age-related changes in composition of adult calcaneal marrow, with

concomitant changes in material properties, arose from trends that have been documented in the marrow of vertebral bodies, where the conversion of marrow from hematopoietic to adipose tissue can continue throughout adult life [18,21,60,61]. In general, the conversion of marrow from hematopoietic to adipose occurs first in the periphery of the appendicular skeleton, moves towards the axial skeleton, and is essentially complete in these peripheral sites by the end of the second decade [12,14,31,43,47,50,73]. A second concern was whether the additional vascular network and blood volume within the calcaneus would significantly affect the variability in measured properties among specimens.

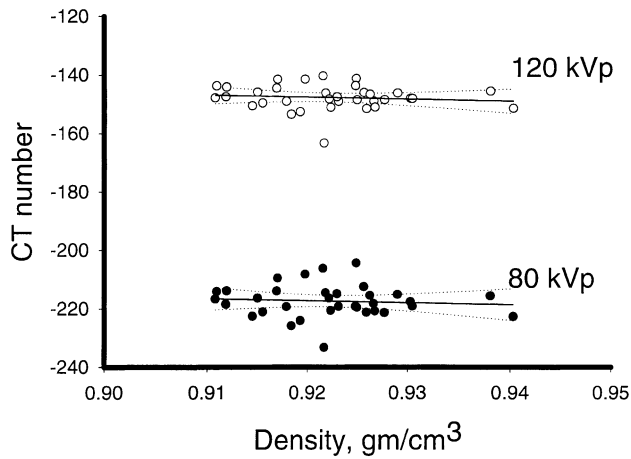


Fig. 5. Calcaneal marrow attenuation, Hounsfield units, at 80 and 120 kVp, as a function of density. Lines indicate linear regressions and 95% confidence intervals. No significant correlation was demonstrated between density and attenuation with these specimens.

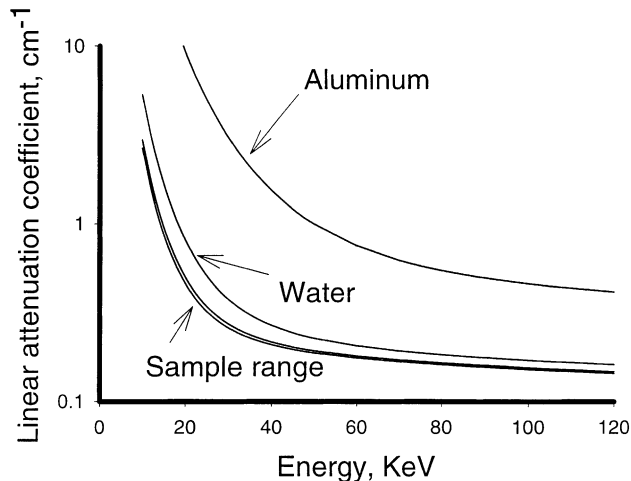


Fig. 6. Energy-dependent linear attenuation coefficients for aluminum, water, and the marrow samples. The variation in attenuation coefficient at a given energy level for the 34 specimens was quite small.

It appears from our results that neither was a source of significant variation in the data, at least not in the 10 mm mid-frontal slab from which we extracted marrow. Because the marrow extraction process homogenized our marrow sample, there may be regional intra-sample variations that we could not detect. From a practical standpoint, however, it is clear that calcaneal marrow density and calcaneal marrow X-ray attenuation characteristics may be considered as a constant throughout adulthood. The low power and prohibitively high minimum sample sizes of the statistical analyses preclude us from stating definitively at the observed r^2 value that there is *no* relationship between age and marrow density, or between age and X-ray attenuation characteristics. If, however, we are willing to accept 0.216 as a minimum acceptable r^2 value in regression analyses, then our

sample size is indeed sufficient to establish an hypothesis of no correlation. Moreover, the low coefficients of variation, high p values, low r^2 values, and low slopes strongly suggest that there is no significant, systematic change in these parameters with age in adults.

We also found a lack of a demonstrable correlation between marrow sample mass density and CT attenuation. Theoretically, we should have been able to demonstrate a positive correlation between these parameters [42]. Given the very short range of the independent variable in our sample (mass density 0.91–0.94 gm/cc), it should not be entirely surprising that we were not able to establish a positive correlation, and indeed, the 95% confidence interval for the calculated slopes (Fig. 5, Table 3) did include positive as well as negative slopes. It is possible that the accumulation of experimental errors in the density and X-ray attenuation measurements, combined with this very small range of values, may have obscured the expected significant correlation. For instance, any air introduced during the pre-scanning vortexing of the sample could have contributed to error in the CT attenuation measurement; if such an error was systematically skewed towards either high- or low-density marrow, this might explain the lack of a significant correlation between density and attenuation (Fig. 5). Since air was expelled manually from the syringe before vortexing, such error was probably minimal. Vacuuming the sample before scanning might have also helped to remove any residual gas, but would have allowed enough time between mixing and scanning so that the sample might not have been entirely homogeneous. Errors in the mass density measurement may also have contributed to this lack of significant correlation between density and attenuation. Alternative means of measuring tissue density include pycnometry [15,17] and the use of a density gradient [39]. The former technique works best in situations in which the sample is either a solid or has a density greater than that of the reference fluid, and in which the sample is not miscible with the reference fluid. None of these criteria were met with our specimens. The accuracy and precision of the latter technique depends strongly on details of gradient preparation and maintenance, as well as on considerations given to the protein and lipid constituents of the specimen (which were not determined in our specimens). With proper equipment and training, both of these alternative techniques can give a precision that is an order of magnitude greater than that of the technique that we used, with smaller samples. Our technique was essentially that of the pycnometer, in which the reference fluid was air. The technique was tested with distilled water and found to be accurate to within 0.006 gm/cc.

Our CT validation procedures provide us with a measure of the accuracy and reliability of our data. The technique of Yan et al. [70] computes the energy-

dependent linear attenuation coefficient function of the material contained within each voxel in the scan field of view. Accuracy is difficult to assess without a comparison to an appropriate “gold standard”. We used the computed linear attenuation coefficients of the known materials, water and acrylic, in the scan image to obtain an estimate of the accuracy of the linear attenuation function of the marrow sample. We also used the computed effective attenuation coefficient of the acrylic in the holder as a measure of the stability of the beam spectra.

We have plotted the marrow mean linear X-ray attenuation coefficient function in Fig. 2 along with water and acrylic. Adipose tissue (dotted line, <http://physics.nist.gov/lab.html>), also plotted, is slightly offset from and higher than our experimentally-derived value. While there may be differences in composition between our marrow and adipose tissue, some of the difference in attenuation can be attributed to a difference in marrow density. We measured a mean density of 0.92 gm/cc, whereas IRCU reports a density of 0.95 gm/cc for adipose tissue [25] (<http://physics.nist.gov/PhysRefData/XrayMassCoef/tab2.html>). Interestingly, NIST also reports an adipose tissue density of 0.92 gm/cc (<http://physics.nist.gov/cgi-bin/Star/compos.pl?refer=ap&matno=103>). Woodard and White [65] report a mean density of 0.950 ± 0.020 (SD) gm/cc for adipose tissue, 1.030 gm/cc for red marrow, and 0.980 gm/cc for yellow marrow, all of which are higher than our measured values. Fat, the major constituent of adipose tissue, was measured at 0.92 gm/cc by White [64], which suggests that our specimens may consist largely of that material. Obviously, these differences could be the result of alternative measuring techniques, or of real variation in material properties between tissues at different anatomic sites. They do, however, emphasize the importance of measuring critical properties directly.

Of course, in order to fully take advantage of these findings in long-term, longitudinal studies using single-energy CT, other issues must be addressed. The problem of beam hardening can be important when measuring bone density [19,42]. In addition, great care must be taken during longitudinal densitometry studies to achieve repeatable positioning between CT sessions, in order to be sure of comparing the same anatomic site from study to study. The development of image registration techniques to address this issue is an area of active research [45,67,68].

In conclusion, our data suggest that the physical density and X-ray attenuation properties of bone marrow in the adult calcaneus do not change significantly between the third and sixth decades in life. In this system, therefore, we propose that single rather than DE-QCT can be accurately used for quantitative determination of bone density, thereby increasing precision and decreasing subject radiation exposure.

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