Estimating the density of femoral head trabecular bone from hip fracture patients using computed tomography scan data

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Abstract
The purpose of this study was to compare computed tomography density ($\rho_{CT}$) obtained using typical clinical computed tomography scan parameters to ash density ($\rho_{ash}$), for the prediction of densities of femoral head trabecular bone from hip fracture patients. An experimental study was conducted to investigate the relationships between $\rho_{ash}$ and $\rho_{CT}$ and between each of these densities and $\rho_{bulk}$ and $\rho_{dry}$. Seven human femoral heads from hip fracture patients were computed tomography–scanned ex vivo, and 76 cylindrical trabecular bone specimens were collected. Computed tomography density was computed from computed tomography images by using a calibration Hounsfield units–based equation, whereas $\rho_{bulk}$, $\rho_{dry}$ and $\rho_{ash}$ were determined experimentally. A large variation was found in the mean Hounsfield units of the bone cores (HUcore) with a constant bias from $\rho_{CT}$ to $\rho_{ash}$ of 42.5 mg/cm³. Computed tomography and ash densities were linearly correlated ($R^2 = 0.55, p < 0.001$). It was demonstrated that $\rho_{ash}$ provided a good estimate of $\rho_{bulk}$ ($R^2 = 0.78, p < 0.001$) and is a strong predictor of $\rho_{dry}$ ($R^2 = 0.99, p < 0.001$). In addition, the $\rho_{CT}$ was linearly related to $\rho_{bulk}$ ($R^2 = 0.43, p < 0.001$) and $\rho_{dry}$ ($R^2 = 0.56, p < 0.001$). In conclusion, mineral density was an appropriate predictor of $\rho_{bulk}$ and $\rho_{dry}$, and $\rho_{CT}$ was not a surrogate for $\rho_{ash}$. There were linear relationships between $\rho_{CT}$ and physical densities; however, following the experimental protocols of this study to determine $\rho_{CT}$, considerable scatter was present in the $\rho_{CT}$ relationships.

Keywords
Computed tomography, femoral head, trabecular bone, bone density, X-ray attenuation

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Introduction
The success of surgical interventions in orthopaedics depends heavily on the quality of the bone in the region of interest.1–5 Bone quality, which refers to the physical and mechanical properties of the bone, has been estimated clinically through densitometry measurements using dual-energy X-ray absorptiometry (DXA).6 DXA measures bone mineral content (expressed in grams) and areal bone mineral density (expressed in grams per square centimetre) and is widely used clinically to diagnose osteoporosis in the hip and spine.7 Despite its widespread clinical use, and the low radiation exposure for patients, the main limitation of DXA is that it is a projected two-dimensional measurement of X-ray attenuation.
Computed tomography (CT) has emerged as a clinical and research tool to evaluate bone quality and as a basis for finite element (FE) analysis of bone. It provides three-dimensional (3D) distributions of X-ray attenuation (Hounsfield units, HUs) allowing not only the analysis of a 3D geometry but also the measurement of volumetric mineral content;8–12 and thus, the bone mineral density can be estimated using solid calibrated standards of known density. Previous studies have shown that in addition to mineral density, bone quality is affected by the spatial distribution of mineral.12,13 These characteristics have motivated studies aimed at relating mineral density derived from CT data ($\rho_{CT}$) to other physical and mechanical properties.11,14–17

Ultimately, the goal is to assess the mechanical properties for fracture risk evaluation,13,18–21 surgical planning22 or subject-specific FE modelling.19–21,23–28 While patient-specific FE models have been used in pre-surgical planning20 and to assess a clinical condition,21 patient-specific models are not yet reliable enough to consistently use clinically because of their limited accuracy in modelling the non-homogeneous, anisotropic mechanical properties of bone.23 These FE models incorporate two independent relationships, one from HU to density and a second from density to modulus of elasticity, to define material properties from CT scans. These two relationships are independent and can each act as a source of error in the FE model.23

Several researchers have related HU or $\rho_{CT}$ directly to elastic modulus or strength,2,15,16,29–32 while others have analysed the relationship between $\rho_{CT}$ and bone density for subsequent estimation of mechanical properties.17,26,33 Lotz et al.31 determined a linear relationship between $\rho_{CT}$ and rehydrated apparent density ($\rho_{app}$) of trabecular bone from the human proximal femur ($R^2 = 0.73$), as have other groups for human trabecular bone ($R^2 = 0.60–0.89$).2,15 In addition, mineral ash density ($\rho_{ash}$)33,34 and dry apparent density ($\rho_{dry}$)33,35 have been considered as effective predictors of bone strength and stiffness and linearly related to $\rho_{CT}$.17,26,36 For example, Schileo et al.26 found a strong relationship ($R^2 = 0.937$) between mineral density and $\rho_{CT}$ for trabecular bone. However, many of the reported relationships for predicting density from image data have a wide variation in their coefficients, particularly the predicted $\rho_{app}$ at 0 CT density (13.2–170 mg/cm$^3$), as well as the statistical strength of the relationships ($R^2 = 0.60–0.99$) (Table 1). This variation in the mathematical relationships limits the use of medical image data as a predictor for density and mechanical properties to investigate bone quality. Additionally, most previous studies have been performed on cadaveric bones with no radiographic evidence of bone disease. Therefore, the purpose of this study was to quantify the ability of CT density obtained using typical clinical CT scan parameters to predict the density of femoral head trabecular bone from hip fracture patients.

In this study, four types of densities, including bulk, dry apparent, ash and CT densities, were measured and their empirical relationships were critically examined. The purpose of this study was to compare CT and ash densities for the prediction of dry and bulk densities of bone cores from the human femoral head, explicitly: (1) the relationships between $\rho_{ash}$ and both $\rho_{bulk}$ and $\rho_{dry}$ and (2) the clinically relevant relationship between $\rho_{CT}$ and both $\rho_{bulk}$ and $\rho_{dry}$. In addition, this study assessed the coefficient of variation (CV) of the mean HUs of the bone cores (HU$_{core}$) as a statistical parameter to represent texture and heterogeneity. CT scan data were assessed in empirical relationships to predict physical properties of human trabecular bone (Table 3 in Appendix 2).

Methods

Scan calibration

In a previous study, we validated a method for determining a repeatable scanner-specific density calibration to determine the relationship between HUs and density. Details for this calibration procedure can be found elsewhere.37 In brief, four hydroxyapatite mineral content standards (phantoms) were imaged in air using a helical multi-slice CT scanner (LightSpeed Plus; General Electric Medical Systems, Milwaukee, WI, USA) with the following parameters: bone reconstruction algorithm, 120 kVp, 250 mA s, 2.5 mm slice thickness, 1.25 mm spacing and 200 mm field of view resulting in a pixel size of 0.422 $\times$ 0.422 mm$^2$. This is the standard clinical CT scan protocol used for hip fractures at the collaborating hospital, Kingston General Hospital, Kingston, ON, Canada. The mineral contents of the standards were 100, 400, 1000 and 1750 mg/cm$^3$ (CIRS Inc., Norfolk, VA, USA), encompassing the density range of human long bone.38,39 The two standards with the lowest mineral content were custom-made (32.4 mm base diameter, 1.5% taper and 80 mm long) and the remaining two were manufactured as plugs (10 mm diameter and 80 mm long) within a water-equivalent material (1000 mg/cm$^3$, part 06217; 1750 mg/cm$^3$, part 06221).

To measure the mean HU of the standards, the CT data from the four standards were segmented from the images using Mimics (version 11.00; Materialise, Ann Arbor, MI, USA). The segmented regions were edited to close any open internal holes and then the surface voxels were removed from the mask. These edits ensured that the mean HU was measured without any partial volume effects.37 A repeatability study determined no significant difference in the mean HU when scanned seven times over 19 weeks (inter-class correlation coefficient, ICC(2,1) = 0.9998, 95% lower limit = 0.9993). A linear regression was used to determine the
relationship between mean HU and mineral content for each standard.

Patient recruitment and specimen retrieval

This study was approved by the Queen’s University Research Ethics Board. Over the course of 5 months, patients who suffered a low energy subcapital or transcervical femur fracture were scheduled for hip arthroplasty. Seven patients (five females; age range 66–87 years) provided written informed consent to participate in this study. During surgery, the exposed femoral head was notched on the superior surface, excised from the acetabulum using an extraction screw and wrapped in saline-saturated gauze for storage at −40 °C.

Specimen preparation, scanning and registration

Each femoral head was thawed for 24 h and CT-scanned ex vivo in air along with the standards, using the same clinical scan parameters as were used for the scan calibration. Although it is known that soft tissue surrounding bone affects X-ray attenuation, the femoral heads were scanned in air. To obtain a reasonable HU to CT density calibration, the standards were also scanned in air. The calibration equation from HU to CT density is specific to the parameters of this study including the scanner, scan parameters and the environment in which the objects were scanned. The effects of scanning media were investigated in a previous study, which provided experimental support for the

<table>
<thead>
<tr>
<th>Study</th>
<th>Bone</th>
<th>Equation</th>
<th>$R^2$</th>
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<tbody>
<tr>
<td>Hannson (1986)</td>
<td>3 subjects, 78 ± 6.5 yo, 12 vertebrae, $n = 231$, 10 $\times$ 10 mm, 1000 mm$^2$</td>
<td>$\rho_{\text{dry}} = 1.614\rho_{\text{ash}} + 2$</td>
<td>0.988</td>
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<tr>
<td>Mosekilde (1989)</td>
<td>17 m, 13f, 43–95 yo, 60 vertebrae, $n = 30$, whole vertebral body, 140 kV, 60 mA, 3 s, 2 mm$^2$</td>
<td>$\rho_{\text{ash}} = 0.4\text{HU} + 63 \text{(L3)}$</td>
<td>0.66</td>
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<td>Schileo (2008)</td>
<td>5 m, 5 f, 60–83 yo, 10 p. tibiae, 7.5 dia $\times$ 7.5 mm, 331 mm$^3$, 120 kVp, 50 mA</td>
<td>$\rho_{\text{ash}} = 0.688\text{HU} + 61.3$ ($n = 215$)</td>
<td>0.91</td>
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<td>Rho (1995)</td>
<td>7 m, 1 f, 45–68 yo, 8 p. femurs, $n = 128$, 10 $\times$ 10 mm, 1000 mm$^3$, 120 kVp, 150 mA s, 10 mm thickness</td>
<td>$\rho_{\text{dry}} = 1.20\text{HU} + 101$ ($n = 215$)</td>
<td>0.88</td>
</tr>
<tr>
<td>Carelli (1991)</td>
<td>4 m, 4 f, 62–92 yo, 8 p. femurs, $n = 49$, 9.5 dia $\times$ 5 mm, 354 mm$^3$, 120 kVp, 5 mm thickness</td>
<td>$\rho_{\text{dry}} = 1.81\rho_{\text{ash}} - 17.0$ ($n = 255$)</td>
<td>0.97</td>
</tr>
<tr>
<td>Mosekilde (1989)</td>
<td>16 17 m, 13 f, 43–95 yo, 60 vertebrae, $n = 30$, whole vertebral body, 140 kV, 60 mA, 3 s, 2 mm$^2$</td>
<td>$\rho_{\text{ash}} = 0.4\text{HU} + 88 \text{(L2)}$</td>
<td>0.58</td>
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<td>Keyak (1994)</td>
<td>1 m, 1 f, 40–45 yo, 4 p. tibiae, $n = 36$, 15 $\times$ 15 mm, 3375 mm$^3$, 140 kVp, 70 mA, 3 s, 1.5 mm thickness, 1.08 mm pixels</td>
<td>$\rho_{\text{dry}} = 0.953\rho_{\text{CT}} + 45.7$</td>
<td>0.986</td>
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<td>Lotz (1990)</td>
<td>2 m, 2 f, 25–81 yo, 4 p. femurs, $n = 49$, 9.5 dia $\times$ 5 mm, 318 mm$^3$, 120 kVp, 240 mA s, 0.7 mm pixel</td>
<td>$\rho_{\text{dry}} = 1.2\rho_{\text{CT}} + 170$</td>
<td>0.73</td>
</tr>
<tr>
<td>Schileo (2008)</td>
<td>3 subjects, 3 femurs, 4% formalin large: $n = 15$, 10 dia $\times$ 19 mm, 1490 mm$^3$ small: $n = 5$, 10 dia $\times$ 5 mm, 393 mm$^3$, 120 kVp, 160 mA, 0.625 mm spacing and thickness, 0.3125 mm pixels</td>
<td>$\rho_{\text{ash}} = 1.02\rho_{\text{CT}} + 51.0$</td>
<td>0.937</td>
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<tr>
<td>This study</td>
<td>2 m, 5 f, 66–87 yo, 7 p. femurs, $n = 76$, 10 dia $\times$ 5 mm cores, 393 mm$^3$, 120 kVp, 250 mA s, 2.5 mm thickness, 1.25 mm spacing, 0.422 mm pixels</td>
<td>$\rho_{\text{dry}} = 1.07\rho_{\text{CT}} + 147$</td>
<td>0.562</td>
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$m$: male; $f$: female; yo: years old; p.: proximal; d.: distal.

All density values are in milligram per cubic centimetre.
 practice of imaging the calibration standards in the same environment as the target bone.37

After the initial ex vivo CT scan, the femoral heads were sectioned perpendicular to the femoral neck under slow, continuous water irrigation using a diamond-coated band saw (EXAKT 311; Norderstedt, Germany). This orientation was identified using the fovea, the angle of the femoral neck fragment and the superior surgical notch. One to three 7-mm thick slices were obtained from each head. Each slice was submerged in cool water and held using a custom-made jig. A diamond-tipped coring bit was used to remove 10-mm-diameter cylindrical cores from each slice. The position of each core within the slice was mapped with respect to the anterior and superior surfaces of the femoral head. The cores were then placed in a custom-made stainless steel clamp and milled (BF400; Präzi Inc., Plymouth, MA, USA) under cool water to a height of 5 mm (1 mm removed from each end) to ensure parallel planes. A total of 86 cores were machined; 10 were excluded due to damage during machining, resulting in 76 cores. From each femur head, 5–19 cylindrical cores were extracted and subsequently tested (Table 2).

To find the X-ray attenuation of cores as located within the femoral head, the CT data from the initial ex vivo scan were used to determine the mean of the voxel HUs within a bone core (HUcore) for each core. After the cores were removed, the slices of the femoral head were re-assembled, and the remaining bone was CT-scanned (LightSpeed 16; General Electric Medical Systems) using the same imaging parameters as the pre-machined, ex vivo scan. Image segmentation was performed using HU-based thresholding, and 3D geometries were generated from the pre- and post-machined scans with the cores removed (Mimics version 11.00; Materialise). A combination of in-house, pair-point and surface matching algorithms was used to align the pre- and post-machined geometries.40 The latter was registered to the pre-machined scan using point registration and subtracted from the pre-machined, ex vivo model. The 3D solid of the post-machined head without the cores was subtracted from the 3D solid of the pre-machined head. This resulted in 3D solids of only the cores and the machining remains (Figure 1), thus enabling identification of each core. The number of voxels, HUcore mean and standard deviation for each core were recorded, excluding boundary voxels to avoid edge artefacts.26

To determine the average magnitude of alignment errors between the two CT datasets, pre- and post-machined, the root mean square error between 160 evenly distributed points on the surfaces (pre- and post-
machined) of each femoral head was calculated. A sensitivity analysis was performed on the HUcore of three cores from different femoral head specimens to determine the effect of altering the position of the core by three increments of 1 mm (in each slice) and three increments of 1.25 mm between slices.

Physical density measurements

To determine the wet bulk volume and bulk densities, the bulk dimensions and wet weight of the cores were measured using vernier calipers (resolution 0.01 mm; Canadian Tire Corporation, Ltd, Toronto, ON, Canada) and an analytical scale (resolution 0.1 mg; Mettler-Toledo, Inc., Columbus, OH, USA). The diameter, height, and weight of each core were determined from the mean of three measurements. Hence, bulk density (ρbulk) was calculated by dividing wet mass by bulk volume. Envelopes of folded filter paper were placed in a muffle oven (Blue M Electric, Watertown, WI, USA) at 65 °C for 1 h to dry any excess moisture and then placed in a desiccator (Corning Incorporated, Lowell, MA, USA; desiccant: anhydrous calcium sulphate; W.A. Hammond Drierite Company, Xenia, OH, USA) for 12 min, after which each envelope was weighed. The cores were then secured inside the corresponding envelopes and dried in the oven at 70 °C for 24 h. Enveloped cores were placed in the desiccator for 12 min, immediately after which each core and envelope were weighed. The dried cores were defatted by wrapping the enveloped cores in cheese cloth and placing them in a modified Soxhlet extractor (Corning Incorporated) with ethyl ether for 24 h. After 24 h, the cores were removed from the Soxhlet and left in a fume hood for a further 24 h. The cores were left in the oven at 70 °C for another 24-h period and then placed in the desiccator, as described above, prior to weighing. The dry, defatted bulk dimensions and mass were measured and used to determine the dry apparent density (ρdwy) of each core.

To determine the mineral density (ρash), the ash weight of the cores was determined. Each core was contained in a pre-weighed crucible and placed in the oven at 100 °C oven for 24 h. Following this initial drying, crucibles with cores were placed in the desiccator for 25 min and then removed and weighed immediately. This step was then repeated for another 24-h period at 100 °C, followed by desiccation and weighing. The crucibles were left in the 700 °C oven for 24 h and then left to cool for 5 h, following which they were placed in a 100 °C oven for 1 h. Once removed from the oven, the crucibles were placed in the desiccator for a period of 25 min and then weighed immediately. This mass, corrected for the mass of the crucible, was divided by dry, defatted bulk volume to calculate ρash.

Data analysis

All results were expressed as mean ± standard deviation and analyses were conducted using the statistics package Minitab 14 (Minitab Inc., State College, PA, USA). Two methods for obtaining mineral density, namely, ρash and ρCT, were compared by using the Bland–Altman (BA) analysis with ρash as the reference measurement. The difference between the two measurements was plotted against the mean of the two measurements. The difference between the means was calculated to represent the bias, and 95% limits of agreement were determined. The Shapiro–Wilk normality test was used to test the assumption of normality of the difference between means.

The empirical relationships between ρash and both ρbulk and ρdwy were analysed with a linear regression model. For each regression, the regression coefficients (slope and intercept) were estimated by the least-squares fit method. Analysis of variance (ANOVA) was performed to test the significance of the valid regression models, whereas the strength of each model was analysed by their coefficient of determination (R²). In all cases, significance level was set at p = 0.05. For each regression, the standardized residuals were verified as independent normally distributed random variables with 0 mean and constant variance. The significance of slope coefficients with their respective confidence intervals was also reported.

The CV of the HUcore was calculated from the standard deviation over the mean of the voxel HUs within each core. The HUs of each voxel from ex vivo, registered scans were converted to ρCT using equation (1). A negative ρCT was considered not representative of bone tissue and was therefore set to 0.43,44 The mean ρCT was found for each of the cores and plotted with respect to ρbulk and ρdwy. Linear regressions were performed to determine the predictability of ρbulk and ρdwy from ρCT using the same method described above. As a measure of texture and heterogeneity, the HUcore CV was included in a multiple regression model in conjunction with ρCT to determine predictors of ρdwy and ρash.

Results

The linear regression between HU and mineral content from the standards was found (equation (1)) (R² = 0.99, p < 0.05)

\[ ρ_{CT} = 0.751 \, \text{HU} - 19.3 \]

where ρCT has units of milligram per cubic centimetre.

The root mean square error between points on the surfaces of the registered CT datasets for all the femoral heads ranged from 0.589 to 0.993 mm. The sensitivity analysis performed on the core positioning yielded a change in the HUcore by 0.095%–19.4%, for changes in the position of up to 3.75 mm.

Although the mean HUcore and CT density for the female cores were significantly lower (25%) than the male cores (p = 0.002), there were no statistically significant differences between sexes for the bulk (p = 0.474), dry (p = 0.648) and ash (p = 0.693) densities.
A pairwise comparison found that all mean densities of the two male hips were significantly different ($p = 0.000–0.012$) from each other, and the mean densities of the bone cores from the 86-year-old female’s hip were significantly lower ($p < 0.00001$) than the other hips.

Two of the samples had drastically different HU$_{\text{core}}$ CVs than the other samples (CV of $3.41$ and $212.4$ versus a mean CV of $0.486$). These cores also had more than 10% of their voxels with HU less than that of fat (approximately $-80$) and therefore were considered as unrepresentative of bone tissue and excluded. Consequently, for the CT density evaluation, only 74 cores were considered. The overall HU$_{\text{core}}$ of 74 cores was $260$ ($\pm 115$) with a range of $45–564$ (Table 2).

The comparison of $\rho_{\text{ash}}$ and $\rho_{\text{CT}}$ methods to determine mineral density is shown in the BA plot (Figure 2). The difference between methods was constant and consistent as the average $\rho_{\text{ash}}$ increased with a bias of $42.5$ mg/cm$^3$ across the mean values. This comparison shows that the $\rho_{\text{CT}}$ value falls between $74.9$ and $160$ mg/cm$^3$ for a given $\rho_{\text{ash}}$ (95% confidence interval).

A strong, linear relationship was observed between $\rho_{\text{ash}}$ and $\rho_{\text{bulk}}$ ($R^2 = 0.78$, $p < 0.05$; Figure 3)

$$\rho_{\text{bulk}} = 1.19 \rho_{\text{ash}} + 769$$

Similarly, $\rho_{\text{ash}}$ was strongly related to $\rho_{\text{dry}}$ ($R^2 = 0.99$, $p < 0.05$; Figure 3)

$$\rho_{\text{dry}} = 1.52 \rho_{\text{ash}} - 19.2$$

In contrast, the relationships were moderate between $\rho_{\text{CT}}$ and $\rho_{\text{bulk}}$ ($R^2 = 0.43$, $p < 0.05$; Figure 4), $\rho_{\text{dry}}$ ($R^2 = 0.56$, $p < 0.05$; Figure 4) and $\rho_{\text{ash}}$ ($R^2 = 0.54$, $p < 0.05$; Figure 5)

$$\rho_{\text{bulk}} = 0.799 \rho_{\text{CT}} + 909$$

$$\rho_{\text{dry}} = 1.07 \rho_{\text{CT}} + 147$$

$$\rho_{\text{CT}} = 1.52 \rho_{\text{ash}} - 19.6$$

$\beta$ and $\rho_{\text{CT}}$ measurements plotted with respect to the CT density ($\rho_{\text{CT}}$) calculated from the mean HU$_{\text{core}}$ of each core and fitted with a linear regression. CT: computed tomography.
As expected, the relationship between $\rho_{\text{ash}}$ and $\rho_{\text{dry}}$ ($R^2 = 0.99$) was stronger than the relationship between $\rho_{\text{ash}}$ and $\rho_{\text{bulk}}$ ($R^2 = 0.78$). The $\rho_{\text{bulk}}$ measurements have increased variability due to inclusion of collagen, fat and water in the cores compared to $\rho_{\text{ash}}$ measurements of mineral content only. Similarly, high variability in the data was observed in the relationships between $\rho_{\text{CT}}$ and $\rho_{\text{bulk}}$ and $\rho_{\text{dry}}$. Again, this was expected due to the trabecular bone’s heterogeneity. Moreover, the CT scan resolution was able to capture some of this variation within each core. Correlation equations between CT data and physical density vary greatly among research studies reported in the literature. Table 1 presents several relationships determined between $\rho_{\text{bulk}}, \rho_{\text{app}}, \rho_{\text{dry}}$ or $\rho_{\text{ash}}$ of human trabecular bone samples and HU of $\rho_{\text{CT}}$; the corresponding coefficients of determination; the sites of the bone samples; the sizes of the material test specimens and the CT scan parameters. In most studies, voxel HUs were converted into mineral density $\rho_{\text{CT}}$ by a respective calibration curve. This table illustrates the variation in the reported relationships, the slopes ($m$) and particularly the $y$-intercepts ($b$), as well as the variation in the correlations ($R^2$) of the relationships. For example, Mosekilde et al. \(^{16}\) found a good correlation between $\rho_{\text{ash}}$ and the HU$_{\text{core}}$ of cores of trabecular bone from vertebral bodies, concluding that CT data yielded valid predictions of the vertebral trabecular bone mass, as have others for trabecular bone ($R^2 = 0.58–0.91$, $m = 0.4–0.7$, $b = 61–88$ mg/cm$^3$). \(^{17,26,33}\) Strong correlations between $\rho_{\text{ash}}$ and $\rho_{\text{CT}}$ for human trabecular bone have been found by several research groups ($R^2 = 0.94–0.99$, $m = 0.79–1.02$, $b = 46–188$). \(^{17,26,36}\) Strong correlations between $\rho_{\text{app}}$ and $\rho_{\text{CT}}$ for trabecular bone have also been reported ($R^2 = 0.60–0.89$, $m = 0.98–3.7$, $b = 13–188$). \(^{2,13,26,31,36}\) In comparison, the results of this study found similar regression coefficients, but lower correlations for predicting density from $\rho_{\text{CT}}$. The two unique features of this study that could contribute to the lower $R^2$ values are as follows: (1) the bone came from hip fracture patients and (2) the CT scan protocol was adopted from the standard protocol used in the clinic. Previous studies have chosen bone and CT scan protocols with the aim of reducing variation; in contrast, this study used bone and scan parameters from the clinic. Several limitations are acknowledged in this study. The trabecular specimens in this study were retrieved from proximal femurs of elderly, hip fracture patients; therefore, results and conclusions are to be applied to this particular population. It is possible, due to the specific patient group, that the marrow space had a higher fat content, which may have decreased the HU$_{\text{core}}$ and increased its variation. The machining, drying and ashing protocols may have caused errors in the measurements of volumes and weights; however, the high correlations found between the bulk, dry apparent and ash densities (equations (2) and (3)) indicate that the errors in these densities, especially $\rho_{\text{ash}}$ and $\rho_{\text{dry}}$, were small. The machining of the bone specimens resulted in

$$\rho_{\text{ash}} = 0.694 \rho_{\text{CT}} + 111$$

All density values in equations (2)–(6) are in milligram per cubic centimetre.

**Discussion**

This study evaluated the ability of CT data to predict the experimentally measured density of human trabecular bone, focusing on the relationships of $\rho_{\text{CT}}$ versus $\rho_{\text{ash}}$ and $\rho_{\text{dry}}$. A bias was found between the mineral density measurement methods. Strong relationships were found between $\rho_{\text{ash}}$ and both $\rho_{\text{bulk}}$ and $\rho_{\text{dry}}$. On the other hand, correlations of CT density with respect to experimentally measured densities, although statistically significant, only explained between 40% and 60% of the variance. The strongest relationship was found for predicting $\rho_{\text{dry}}$ from $\rho_{\text{CT}}$.

The BA analysis is a statistical method to present and compare data collected by two different methods\(^{42}\) to evaluate whether the methods are interchangeable. In this study, $\rho_{\text{ash}}$ was assumed as the reference measurement, and a constant bias of 42.5 mg/cm$^3$ was found between $\rho_{\text{ash}}$ and $\rho_{\text{CT}}$ such that $\rho_{\text{ash}}$ measures of mineral density exceed $\rho_{\text{CT}}$ measures of mineral density. This result was expected since voxels in the CT data included non-bone material, such as fat. Due to averaging effects, HUs of such voxels were lower and yielded a lower $\rho_{\text{CT}}$. Limits of agreement show the certainty at the 95% level that $\rho_{\text{CT}}$ values are within 75 mg/cm$^3$ below and 160 mg/cm$^3$ above $\rho_{\text{ash}}$. An underestimation of $\rho_{\text{CT}}$ by 42.5 mg/cm$^3$ would cause a decrease of 14% in the estimate of $\rho_{\text{dry}}$, based on equation (5). Using similar linear relationships from previous studies,\(^2,31\) and for example, by Rho et al.\(^{32}\) for elastic modulus versus apparent density in the proximal femur (mediolaterally) $E = 0.01 \rho_{\text{app}}^{1.80}$, the estimate of the elastic modulus would have a decrease by 24% with a decrease in $\rho_{\text{CT}}$ of 42.5 mg/cm$^3$. Based on this analysis, it was concluded that the two methods for mineral density measurement, $\rho_{\text{ash}}$ and $\rho_{\text{CT}}$, were not interchangeable. Schileo et al.\(^{26}\) drew similar conclusions from regression analyses of their data.

The results from this study illustrated that $\rho_{\text{ash}}$ was an excellent predictor of $\rho_{\text{dry}}$, as has been found in several previous studies.\(^{33,35,36}\) Several researchers have shown a relationship between $\rho_{\text{app}}$ and elastic modulus,\(^{32,39,45,46}\) therefore, the prediction of $\rho_{\text{app}}$ from $\rho_{\text{ash}}$ allows for estimation of mechanical properties of bone, useful, for example, in the definition of mechanical properties in bone FE models. However, it has been reported that $\rho_{\text{dry}}$ yields better correlation to mineral density, as specimens for $\rho_{\text{app}}$ are prone to error due to specimen size.\(^{26}\) Also, Keyak et al.\(^{36}\) found that there was no significant difference between the use of $\rho_{\text{dry}}$ and $\rho_{\text{app}}$, with the latter introducing an additional level of experimental complexity and source of error. Taken together, these findings support the measurement of mineral density to estimate $\rho_{\text{dry}}$.\(^{42}\)
loss of bone due to the slicing, coring and milling procedures. The post-machined, re-assembled femoral heads would therefore have lost height (the dimension perpendicular to the plane of the slices) in comparison to their pre-machined geometry. This change in geometry is reflected in the root mean square error (0.589–0.993 mm) between the registration of the pre- and post-machined scans. The effect of the registration error on determining a core’s $H_{Ucore}$ was determined (0.095%–19.4%) assuming a worst-case positioning error of 3.75 mm. Each core’s $H_{Ucore}$ was determined from the mean $H_U$ of all voxels completely within a core (885–5141 voxels per core); however, due to the bone’s heterogeneity, the bone cores had large variances associated with their mean $H_{Ucore}$. The mean of all the cores CVs was 62%. The main sources of error in the $H_U$s of the voxels were the scan parameters, slice spacing and thickness. These parameters were chosen to mimic clinical scan parameters, not to minimize voxel size partial volume effects. The combination of the inherent variance in the material and sources of error in determining $H_{Ucore}$ would have directly affected $p_{CT}$ (the dependent variable in Figures 4 and 5) and contributed to the low coefficients of determination in equations (4)–(6).

Another potential weakness of $p_{CT}$ is the limitations in defining property–density relationships from in vitro rather than in vivo bones.20 Due to these limitations in defining physical and mechanical properties from $H_U$, an alternate material mapping strategy has been proposed by Helgason et al.24 They found decreased error in surface strain predictions when mechanical properties including elastic modulus are distributed within the elements as compared to the conventional method of constant mechanical properties per element.

This study assessed the CV in $H_{Ucore}$ as a statistical parameter to represent texture and heterogeneity of the trabecular cores. This parameter was included in a multiple regression model in conjunction with $p_{CT}$ to determine predictors of $p_{dry}$ and $p_{ash}$. In both cases, $H_{Ucore}$ CV obtained from CT data was not a significant independent predictor of density. Thus, it was concluded that the CT data from the trabecular cores of this study did not convey important structural information for density estimation.

**Conclusion**

In conclusion, this study determined a strong linear relationship between the mineral density and bulk and dry apparent predictor of both. Through a BA analysis, it was concluded that $p_{CT}$ and $p_{ash}$ were not adequate surrogates of each other. The relationships between CT density and physical densities were linear; however, due to the limitations of this study, considerable scatter was present. Following the experimental protocol of this study, it was concluded that CT density could not be implemented to accurately predict bulk or dry densities. Therefore, the limitations of this study, in implementing CT data as a means of estimating bone physical properties, lie in the relationship between the CT data and the mineral density estimate and not in the use of the mineral density to estimate the material properties of trabecular bone.

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**Declaration of conflicting interests**

The authors declare that there is no conflict of interest.

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**References**


Appendix 1

**Notation**

- $b$: $y$-intercept of regression equation
- $\text{HU}_{\text{core}}$: mean of the voxel Hounsfeld units within a bone core
- $m$: slope of regression equation
- $R^2$: coefficient of determination
- $\rho_{\text{app}}$: wet apparent density considered mass of intact core excluding fat, over bulk volume
- $\rho_{\text{CT}}$: computer tomography density derived from Hounsfield units by means of a calibration equation from mineral standards (equation (1))
- $\rho_{\text{ash}}$: ash density considered mineral mass over bulk volume
- $\rho_{\text{dry}}$: dry apparent density considered mass of the intact core excluding fat and water, over specimen bulk volume
- $\rho_{\text{bulk}}$: bulk density defined as the mass of the intact core, including fat and water, over specimen bulk volume

### Table 3. CT scan data, number of voxels, mean, standard deviation and coefficient of variation in HUs and different densities expressed in milligram per cubic centimetre of human trabecular bone specimens.

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