Computed tomography topographic mapping of subchondral density (CT-TOMASD) in osteoarthritic and normal knees: methodological development and preliminary findings

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Summary

Objectives: To develop a precise imaging tool which measures three-dimensional (3D) subchondral bone mineral density (BMD), and investigate its ability to distinguish subchondral bone properties in osteoarthritic and normal cadaveric tibiae.

Methods: We developed a novel imaging tool [Computed tomography topographic mapping of subchondral density (CT-TOMASD)], which employs a surface projection image processing technique to map 3D subchondral BMD measured in relation to depth from the joint surface. Sixteen intact cadaver knees from 10 donors (8 M:2F; age: 77.8 ± 7.4) were scanned using quantitative computed tomography (QCT). Projections of average BMD to normalized depths of 2.5 mm and 5.0 mm were acquired, with regional analyses including: (1) medial and lateral BMD, (2) anterior/central/posterior compartmental BMD, (3) max BMD contained within a 10 mm diameter ‘core’, and (4) medial/lateral BMD ratio. Precision was assessed using coefficients of variation (CV%). Osteoarthritis (OA) severity was assessed by examination of computed tomography (CT) and fluoroscopic radiographic images, and categorized using modified Kellgren–Lawrence (mKL) scoring.

Results: Precision errors for CT-TOMASD BMD measures were focused around 1.5%, reaching a maximum CV% of 3.5%. OA was identified in eight compartments of six knees. Substantial qualitative and quantitative differences were observed between the OA and normal knees, with the medial/lateral BMD ratio and peak core regional analyses demonstrating differences greater than 4.7 standard deviations (SDs) when compared with normals. Preliminary results revealed effect sizes ranging from 1.6 to 4.3 between OA and normal knees.

Conclusions: CT-TOMASD offers precise 3D measures of subchondral BMD. Preliminary results demonstrate large qualitative and quantitative differences and large effect sizes between OA and normal knees. This method has the potential to identify and quantify changes in subchondral BMD associated with OA disease progression.

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Key words: Subchondral bone, Bone mineral density, Computed tomography, Proximal tibia, Osteoarthritis.

Introduction

Subchondral bone changes have become a controversial and growing area of focus in osteoarthritis (OA) research. Various imaging studies have shown direct relationships between apparent bone mineral density (BMD), a measure of bone mass per unit volume (or area), and the presence or severity of knee OA. These studies have generally assessed BMD at sites remote from the affected joint, though evidence from both human and animal studies suggests that subchondral bone located nearest to the overlying articular cartilage may play a significant role in the initiation and/or progression of OA. Research examining direct associations between proximal tibial BMD and knee OA offers conflicting results. These conflicting results may be due to the imaging tools used to assess BMD combined with selected analysis regions containing cortical and/or trabecular bone which may both be affected differently by OA.

The subchondral bony region is made up of three principal regions: (1) subchondral cortical endplate; (2) subchondral trabeculae which are attached to, and support, the overlying subchondral cortical endplate; and (3) epiphyseal trabeculae within the proximal epiphysis. Cortical and trabecular bone located less than 5 mm away from the articular cartilage/subchondral bone interface (subchondral surface) offer the largest resistance to loading and are capable of adversely affecting the overlying cartilage. The OA subchondral endplate and subchondral trabeculae demonstrate increased thickness and volume, and resulting in increased apparent density. Conversely, OA epiphyseal trabeculae distal to the subchondral surface demonstrate reductions in thickness and density to the point of being labeled osteoporotic. Imaging studies of osteoarthritic proximal tibial subchondral bone have typically included epiphyseal trabeculae with subchondral cortical and trabecular bone in the image analyses, or have ignored the critically important cortical endplate and trabecular bone located nearest to the subchondral surface.
Dual-energy X-ray absorptiometry (DXA), the most commonly used technique for assessing associations between BMD and OA, is poorly suited for analyzing the subchondral region because its two-dimensional (2D) projection nature cannot characterize a complex three-dimensional (3D) region, results are sensitive to patient positioning, and DXA is limited to imaging in the coronal and sagittal planes. Quantitative computed tomography (QCT) is an imaging method capable of assessing 3D BMD, with the specific advantage of differentiating between cortical and trabecular bone. Few studies have however utilized QCT to assess subchondral bone density, solely within the osteoarthritic human proximal tibia. Computed tomography osteoabsorptiometry (CT-OAM) uses QCT imaging methods and maximum intensity projection image processing to map 3D subchondral density in relation to depth (measured relative to the subchondral surface) directly at the joint surface. Our research questions were:

1. Can CT-TOMASD provide precise measures of subchondral bone density within human cadaveric specimens?
2. Using preliminary cadaveric results, does CT-TOMASD demonstrate potential to discriminate differences in subchondral bone density between normal and osteoarthritic populations?

**Methodology**

**SPECIMENS**

Sixteen intact fresh human cadaver knee specimens, including tissues 25 cm proximal and distal to the tibio-femoral joint line (~50 cm length), were obtained from the UBC Anatomy Department and an anatomical tissue bank (LifeLegacy, St. Paul, MN, USA). Specimens came from 10 donors (eight males and two females; ages ranging from 67 to 88 years; mean ± standard deviation (SD): 77.8 ± 7.4), including six left:right pairs, two right and two left knees. The specimens were wrapped in saline soaked towels, hermetically sealed and stored at -20 °C prior to imaging. Sealed specimens were thawed in air (18 h at 20 °C) prior to all preparation or scanning. Study approval was provided by the UBC Clinical Research Ethics Board.

**QCT IMAGE ACQUISITION**

Knee specimens were imaged via single-energy QCT using a clinical computed tomography (CT) scanner (multi-slice helical Aquilion 64, Toshiba Medical Systems, Tokyo, Japan). The QCT image volume included the patella, distal femur and proximal tibia, though only QCT images of the proximal tibia were used in this analysis. CT scanning parameters included: bone standard reconstruction algorithm, axial scanning plane, 120 kVp tube voltage, 300 mA tube current, ~3.7 second scan time, ~300 slices, 0.5 mm isotropic voxel resolution (0.5 mm slice thickness, 0.5 × 0.5 mm in-plane pixel resolution). The primary advantage of isotropic voxels is the ability to perform reliable data reformatations in any plane of choice – a process unsuitable for voxels with dissimilar slice thicknesses and in-plane pixel resolutions. To simulate a physiologic setting, left and right knees were orientated in a supine position and imaged simultaneously with the knee of interest located centrally within the CT gantry. A solid diotassium phosphate (K2HPO4) phantom, used to convert grayscale CT Hounsfield units (HU) to an equivalent volumetric BMD (mg/cm² K2HPO4), was included in each image (Model 3T, Mindways Software Inc, Austin, TX, USA) [Fig. 1(A)]. Half of the knees (n = 8) were repositioned in a different supine orientation and rescanned for a total of three scans.

**CT-TOMASD IMAGE ANALYSIS**

**BMD conversion**

CT HU values were converted to BMD using a custom-written algorithm (Matlab 2007a; MathWorks, Natick, MA, USA). Circular regions of interest (ROI) were overlaid within each of the reference phantom cylinders, and mean HU values were recorded for all pertinent images [Fig. 1(A)]. A linear regression equation (r² = 0.96), derived from the mean HU values and known reference cylinder densities, was used to convert HU to equivalent volumetric BMD for each individual axial image.

**Segmentation**

The proximal tibia was segmented in the sagittal plane with a combination of manual and semi-automated segmentation techniques using commercial segmentation software (Analyze6.0; Mayo Foundation, Rochester, MN, USA) [Fig. 1(B)]. A region growing segmentation technique was initially employed using a subject-specific threshold value defined by the Half-Maximum Height (HMH) method. Correct definition of the subchondral surface is difficult due to limited CT slice thickness capabilities (0.5 mm) and subsequent partial volume effects at the subchondral bone/cartilage interface. The HMH method defines an optimum threshold value for characterizing cortical periosteal surfaces using histogram line profiles of imaged density to define the 50% density midpoint between low density soft tissue (predominantly articular cartilage) and high density bone. The HMH value was used both as an input for the region growing technique and as an input for assisting with manual correction of the segmentations using a stylus and interactive touch-screen tablet (Cintiq 21UX, Adobe, Krefeld, Germany). Manual correction was included to ensure that segmentations omitted osteophytes and represented the cartilage/bone interface as a smooth, consistent surface.

**Surface projection**

Landmark boundary points outlining the outer periphery (n = 5) of the medial and lateral plateaus [Fig. 1(C)] were manually selected from sagittal, coronal, and axial CT images using commercial software (Analyze6.0). Prominent anterior and posterior points were also selected for each of the medial and lateral plateaus, with anterior points defining the medial—lateral axis. Best-fit planes were matched to the boundary points for each plateau using singular value decomposition (Matlab 2007a) [Fig. 1(D)]. Segmented proximal tibias were realigned and reconstructed (cubic interpolation) relative to the respective medial and lateral plateaus, with the medial—lateral axis serving as the x-axis (Matlab 2007a). Beginning at the superior segmented joint surface, proximal tibia BMD was measured in increments of 0.5 mm to a depth of 6.5 mm beyond the defined segmented subchondral surface, resulting in a series of 2D surface projection images [Fig. 1(E)]. The 2D projection images of the medial and lateral plateaus were then segmented using natural cubic splines fit to the previously selected boundary points (Matlab 2007a) while permitting manual adjustment of knot points [Fig. 1(E)]. Care was taken to ensure that the segmented 2D regions did not overlay high density cortical edges of the proximal tibia, tibial spine, or osteophyte locations.

**Normalization**

For subject-to-subject comparisons the depth to which BMD was assessed was controlled based upon a user-defined normalized depth (e.g., 2.5 mm) and relative tibial volumes and areas. Proximal tibial volume superior to the lateral inferior ridge of the proximal tubular head (repeatability landmark) was calculated after aligning the tibia in a neutral physiologic orientation (tibial plateau angled posteriorly 10°) in sagittal plane; tibial long axis vertically oriented in coronal plane). Specific depth measures were determined using the following equation:

\[
d = \frac{d_{\text{area}} \cdot \text{area}_{\text{subject}} \cdot \text{volume}_{\text{subject}}}{\text{volume}}
\]

where \(\text{volume}_{\text{subject}} / \text{volume}\) is a relative volume ratio defined by dividing each subject-specific specimen proximal tibial volume by the average proximal tibia volume of all specimens, \(\text{area}_{\text{subject}}\) is the subject-specific (segmented) area of the medial and lateral plateaus, \(\text{area}\) is the mean area of all subjects, \(d\) is the desired user-defined normalized depth, and \(d_{\text{area}}\) is the actual subject-specific depth corresponding to the user-defined normalized depth. Average subchondral densities from the subchondral surface to normalized depths of 2.5 and 5.0 mm were selected for analysis. Average density values were computed as the average of a series of 0.5 mm incremental 2D surface projection images beginning at the superior surface and ending at the specific normalized depth. Fractional depths (e.g., 4.7 mm) were computed by incorporating the percentage of a specific distal surface projection (i.e., for above case, 0.2 multiplied by the surface projection corresponding to 4.5–5.0 mm depth).
Compared with linear normalization measures (medial:lateral and anterior:posterior dimensions), the volume measure offered greater repeatability (0.5% vs 1.4% for linear measures), smaller differences between left:right paired knees (1.9% vs 2.5% for linear measures) and was less influenced by landmark uncertainties due to osteophyte presence. The area measure offered similarly small repeatability errors (2.2%). As such, the combination of tibial volume and area served as a conservative normalization measure with nominal uncertainties due to OA disease severity.

Regional analyses

Regional analyses were performed for the normalized 2.5 and 5.0 mm depths, including: (1) total average BMD of both the medial and the lateral plateaus combined; (2) average BMD of each plateau; (3) anterior/central/posterior compartment BMD, assessed by dividing the anterior/posterior dimension of each plateau into three equally spaced subregions; (4) medial:lateral (M:L) BMD ratio; and (5) average BMD of a 10 mm diameter ‘core’ which searched each plateau for a maximum value [Fig. 1(F)].

OA ASSESSMENT

Each knee was assessed for OA by the participating surgeon (BAM) using CT and fluoroscopic radiographic evidence of osteophytes and sclerosis with a modified Kellgren–Lawrence (mKL) scoring scale (modified due to non-weight bearing status of cadaver specimens):

- 0 = normal, no osteophytes;
- 1 = possible osteophytic lipping;
- 2 = definite osteophytes, possible joint space narrowing;
- 3 = moderate or multiple osteophytes, definite joint space narrowing, some sclerosis and possible bony attrition;
- 4 = large osteophytes, marked joint space narrowing, severe sclerosis and definite bony attrition.

The subjects were then subdivided into three OA categories: normal (mKL = 0); early OA (mKL = 1.2); and late OA (mKL = 3.4). An estimation of varus/neutral/valgus knee alignment from CT images was provided by the participating surgeon based upon knee orientation, presence of joint space narrowing, near bone-on-bone contact, and ligament soft tissue laxity.

RESULTS

PART I: METHODOLOGICAL PRECISION

CT-TOMASD precision was gauged using three separate assessments for eight specimens by root-mean-square coefficients of variation (CV%) according to procedures outlined by Gluer et al. Precision was assessed for measures of HMH values, proximal tibia volume, subchondral surface area, and average BMD values. Due to the methodological and descriptive nature of this study, combined with a limited sample size, statistical analyses gauging the validity of observed differences between OA and normal knees were largely avoided. Basic descriptive statistics were provided for large sample sizes (≥10), with data expressed as mean ± SD. Isolated differences between OA and normal knees were assessed using standard z-scoring, which presents differences as the number of SD above or below normal mean values. Effect and sample size calculations were performed (α = 0.05, power = 0.90) using Cohen’s d52 to determine the sample sizes necessary for a full-scale study to detect significant differences in subchondral BMD between OA and normal tibiae. A Cohen’s d above 0.8 was considered a large effect size53 with statistical/clinical significance53.

PART II: OA VS NORMAL SUBCHONDRAL BONE DENSITY

OA was identified in eight compartments of six knees using CT, including two cases of late OA (OA1 and OA2) and...
four cases of early OA (OA3–OA6). CT images demonstrated that knee OA1 (mKL = 3) was in valgus alignment and showed osteophytes and sclerosis in the lateral compartment and osteophytes in the medial compartment. Knee OA2 (mKL = 3) was in varus alignment and showed medial sclerosis and osteophyte presence. Knee OA3 (mKL = 1–2) was in neutral alignment and showed osteophytic lipping in the anterior lateral compartment and possible lipping in the anterior medial compartment. Knee OA4 (mKL = 1) was in neutral alignment and demonstrated medial lipping. Knee OA5 (mKL = 1) was in neutral alignment and showed lipping in the medial and lateral compartments. Knee OA6 (mKL = 1) was in neutral alignment and demonstrated lipping in the medial posterior compartment. Only one case of late OA (mKL = 3) was identified using fluoroscopic imaging (Knee OA1). Nine cases of possible early OA (mKL = 1) were identified using fluoroscopy with three closely matching CT-based mKL characterizations (Knees OA3, OA4 and OA6). The remaining 10 knees were categorized as normal (mKL = 0).

Substantial qualitative differences were noted between the osteoarthritic and normal knees at both normalized 2.5 and 5.0 mm depths (Figs. 2 and 3). In general, the bone in normal knees was most dense anteriorly/centrally in the medial compartment and posteriorly/centrally in the lateral compartment. These patterns were not observed in the early- and late-OA knees, which tended to have more high dense bone than normal knees (Figs. 2 and 3).

Regional analyses demonstrated large quantitative differences between normal and OA knees, particularly for the

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<th>Table I</th>
<th>Precision results for the CT-TOMASD analysis expressed as a percentage coefficient of variation (CV%)</th>
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<td>Depth (mm)</td>
<td>M&amp;L Medial plateau</td>
</tr>
<tr>
<td>Total</td>
<td>Anterior</td>
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<td>2.5</td>
<td>1.1%</td>
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<td>5.0</td>
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Fig. 2. CT-TOMASD topographic density maps of proximal tibial subchondral bone using the surface projection image processing technique, assessed across a depth of 2.5 mm from the subchondral surface. Results are displayed for two cases of late OA (valgus-aligned lateral OA1, varus-aligned medial OA2), one case of early OA (neutral-aligned lateral OA3), and five asymptomatic subjects displaying no radiographic evidence of OA. All knees are displayed as left knees for comparative purposes.
5.0 mm depth with the late-OA knees (Table II). The M:L BMD ratio— a measure related to OA disease severity and varus/valgus alignment— was approximately 1.2 in normal knees while OA1 demonstrated an M:L BMD ratio of 0.77, which was 4.9 SD lower than normals. The peak core in knee OA1 was 4.7 SD higher than normals.

At the 5 mm depth the varus-aligned knee OA2 demonstrated an M:L BMD ratio 3.1 SD higher than normals. The peak core at this depth was 3.3 SD higher than normals. Similar differences were noted at the 2.5 mm depth between knees OA1 and OA2 with the normal knees, but the differences were not as pronounced.

Early-OA knee OA3, which was in neutral alignment, demonstrated a similar M:L BMD ratio to normal knees at both the 2.5 and 5.0 mm depths, as well as a similar peak core density at the 5.0 mm depth. The peak core analysis at the 2.5 mm depth, however, revealed a BMD 3.5 SD higher than normals and higher than both late-OA knees. Knees OA4 through OA6 demonstrated moderately higher M:L BMD ratios and peak density cores when compared with normals, though less pronounced than other OA knees.

Statistical power analyses (\(\alpha = 0.05\), power = 0.90) using peak core densities estimated that six subjects, per group, are required to differentiate between OA (pooled early and late OA) and normal tibial subchondral bone density. Eight subjects, per group, are however required to differentiate between early-OA and normal knees. Cohen’s \(d\) effect sizes ranged from 1.6 (early OA and normals) to 4.3 (late OA and normals).

**Discussion**

CT-TOMASD measures 3D BMD to specified depths from the subchondral surface, with repeatability errors many times smaller than the variance in the normal population sample. The high precision is due to the 3D isotropic nature of the method, and the ensuing ability to reconstruct the imaged tibia dataset in various orientations. Using small isotropic voxel sizes, combined with digitized points defining the periphery and inner regions of the medial and lateral plateaus, the effects of patient positioning are minimized.

A key advantage of CT-TOMASD is its ability to image thin layers near the joint surface. This is important because there may be a variable density transition zone located somewhere between the higher density subchondral bone and lower density trabecular bone found with OA subjects. While it is accepted that both the subchondral endplate and the nearby trabeculae increase in thickness and density with OA, trabeculae located more distal to the surface decrease in density—possibly due to structural stress shielding by high density bone near the subchondral surface. Fractal signature...
analysis studies have shown that horizontal plates and vertical rods of trabecular bone in regions periarticular to OA affected regions are thinner (i.e., bone is less dense) than in knees without OA. Similarly, animal studies simulating secondary OA have shown decreases in periarticular trabecular bone density which precede thickening of the subchondral plate. Based on our results and previous findings we estimate this transition zone to be between 2.5 and 5.0 mm from the subchondral surface.

CT-TOMASD addresses some of the limitations of the DXA, CT-OAM, and QCT methods that have been used to study BMD near the joint. A key limitation of DXA is that it represents any 3D bony structure as a 2D projection image; making it prone to errors due to patient positioning and physical size. DXA studies of BMD at the knee have used a range of different sized ROIs at various locations, typically positioned away from the subchondral surface (> 10 mm) or too large to obtain useful spatial information. An ROI containing both the subchondral endplate and the distal trabeculae likely contains bone experiencing both increased and decreased density; possibly canceling one another and imaged BMD increases (or decreases) with OA are subsequently missed. The maximum intensity projection algorithm used by CT-OAM primarily focuses on peak densities contained with the subchondral endplate; thus, nearby trabecular changes are likely ignored. Past CT-OAM and QCT subchondral bone studies have predominantly used a 1–4 mm slice thickness, possibly leading to significant partial volume effects at the subchondral bone/cartilage interface and within the thin subchondral endplate. Usage of a large slice thickness also limits accurate data reformating due to the large voxel size, which may lead to repeatability errors due to patient positioning. These limitations are largely omitted with our imaging technique due to the usage of a small 0.5 mm isotropic voxel size.

Study parameters were chosen to minimize radiation dosage while permitting usage of a small isotropic voxel size and QCT reference phantom. Voxel size is dependent both upon CT slice thickness capabilities and the physical dimensions of the QCT reference phantom, which must be contained within each knee image. We chose the smallest available isotropic voxel based upon slice thickness capabilities of the CT machine. Single-energy QCT was utilized instead of dual-energy QCT due to the lower radiation dosage and lower marrow fat variability in older OA populations. Due to the low presence of radiosensitive tissues at the knee joint the effective dosage using the CT-TOMASD analysis is about 0.15 mSv, estimated from scan parameters (17 mGy CTDIvol dosage, 15 cm length, 255 mGy cm dose length product) using shareware software (CT-DOSE; National Board of Health, Herley, Denmark). CT-TOMASD dosages are comparable with Henckel et al., who calculated an effective dosage of 0.12 mSv at the knee joint using similar CT scanning parameters. This compares with an exposure of 0.05 mSv during a transatlantic flight from Europe to North America and 0.7 mSv for an anterior–posterior pelvic radiograph or long-leg standing radiograph.

The CT-TOMASD imaging method has possible sources of error. First, partial volume effects are high both at the subchondral surface — due to the high density gradient between articular cartilage and subchondral bone — and through the thin subchondral endplate. We minimized these effects by using the smallest clinically available slice thickness (0.5 mm) and characterized the subchondral surface using patient-specific HMH threshold values combined with manual segmentation correction for each individual sagittal CT image. Second, the reference phantom used in this study was not designed for subchondral or cortical density measurements, and density values higher than those in the reference phantom were linearly extrapolated using regression equations. Phantoms containing higher density reference materials increase imaged sensitivity to X-ray scatter and beam hardening artifacts; therefore, phantoms similar to ours have been widely used for the evaluation of subchondral bone.

Third, beam hardening is an inherent limitation of CT imaging. However, expression of subchondral BMD in relation to densities present within a reference phantom minimized beam hardening effects since the reference phantom was subjected to the same beam hardening and scanning conditions as the proximal tibia.
Lastly, the accuracy of CT-TOMASD measures of BMD requires consideration. CT-TOMASD measures are obtained using QCT, a validated technique used to assess trabecular, cortical26,37, and subchondral BMD.40 Since a QCT reference phantom of known bone mineral densities is contained within each image, direct conversions from image intensity to physical density can be made. Human and animal studies have verified that QCT density measures are accurate representations of true BMD.44,62–67. The BMD accuracy of an individual voxel, or a thin structure less than 2 mm thick, is however questionable61,68,69. Importantly, analyses which of an individual voxel, or a thin structure less than 2 mm thick, were associated with OA disease progression.

Conclusions
We have developed a precise 3D imaging technique which measures subchondral BMD in relation to depth from the subchondral surface, projecting results to a 2D topographical density map directly at the joint surface. Our preliminary results demonstrate quantitative and qualitative differences and large effect sizes between OA and normal knees, though larger studies of OA populations in vivo are required to investigate these differences further. This method has the potential to identify and quantify changes in subchondral BMD that may be associated with OA disease progression.

Conflict of interest
The authors have no conflict of interest.

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