Introduction

- Adults with type 2 diabetes have a greater risk of hip fracture\(^1\) compared to non-diabetic adults, despite higher areal bone mineral density (aBMD).
- Areal BMD can be confounded by bone size\(^2\), degenerative changes in the spine\(^3\), and overlying fat\(^4\), therefore using aBMD as a measure of bone health in adults with type 2 diabetes may not be predictive of fracture in this group.
- Tissue mineral density (TMD) reflects the mineralization of bone only; unlike aBMD and volumetric BMD (vBMD) measurements, which are acquired by quantifying X-ray attenuation by both bone and non-bone soft tissue.
- Adults with type 2 diabetes have greater vBMD and lower bone volume fraction (BV/TV)\(^5\), however TMD of human samples has not been investigated using μCT.

Study Objectives:
1) determine whether there are differences in TMD and BV/TV in excised samples of bone from adults with and without type 2 diabetes;
2) determine correlates of TMD using bone mineralization density distribution (BMDD) measurements and type 2 diabetes-related variables, chosen a priori.

Methods

Study Design:
- Cross-sectional, ex vivo study using proximal femur specimens obtained from elective total hip arthroplasty patients (HHS Orthopedic Program, Jarvisville Hospital).

Inclusion/Exclusion Criteria:
- Men, women ≥65 yr, undergoing total hip arthroplasty due to end-stage osteoarthritis.
- Exclusion criteria: currently taking/known osteoporosis-related medication in past 24 months; metastatic cancer in past 10 years; taking systemic glucocorticoids for 3 months ≥7.5mg/day; renal disease (Ccr< 30ml/min); hyper/hypophosphataemia, Paget’s disease, Cushings Syndrome, osteogenesis imperfecta.

Sample Preparation:
- A 5mm thick sagittal section of the femoral neck was cut at the most distal end of the proximal femur and the section was further divided along anterior/posterior axis (Figure 1).
- Both sections were fixed in sodium cacodylate buffer, degreased in methanol/chloroform, dehydrated in graded alcohol (70-100%) and dried at 60°C for 4h.

Microcomputed Tomography:
- A 5mm thick section was taken from the middle of each posterior sample using diamond blade saw.
- Dried samples were placed in a tray and imaged using a μCT system (General Electric [GE] explore Locus 120, GE Medical Systems, London, Canada).
- Imaging parameters: applied electrical potential = 80 kVp, tube current = 450 μA, integration time per projection = 2000 ms, 720 views, image nominal isotropic voxel size = 21μm\(^3\). Calibration: scanning a solid-state phantom with simulated air, water and HA.
- Reconstructed images uploaded to MicroView: ABA v 2.1.2 (GE Medical Systems).
- Standard threshold value used for segmenting bone from non-bone for all samples, and a slice-wise comparison between the gray scale and segmented images.
- TMD (mg of HA/cm\(^3\)) was calculated as the average attenuation value of bone tissue, and bone volume fraction (BV/TV, %) was calculated as the ratio between segmented bone volume to the total volume of the region of interest, which have been previously validated by others.

Bone Mineralization Density Distribution (BMDD)
- Quantitative backscattered electron imaging and image analysis completed with scanning electron microscope (Vega II LSU, Tescan USA Inc. Cranberry Township, USA) and ImageJ v 1.44o (NIH, Bethesda, USA) to yield BMDD measurements: \(Ca_{\text{MEAN}}\), \(Ca_{\text{PEAK}}\), \(Ca_{\text{WIDTH}}\)

Statistical Analysis:
Between-group differences in TMD and BV/TV determined with independent Student’s t-test. Pearson correlation coefficients were calculated for the relationships between TMD and both BMDD measures and other correlates (a priori).

Table 1. Descriptive characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>n=20</td>
<td>n=14</td>
</tr>
<tr>
<td>Male</td>
<td>13 (65)</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>Female</td>
<td>7 (35)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>BMI</td>
<td>28.8 (5.7)</td>
<td>30.7 (7.4)</td>
</tr>
<tr>
<td>Calcium intake, mg/d(^*)</td>
<td>368 (479)</td>
<td>463 (560)</td>
</tr>
<tr>
<td>Vitamin D intake, IU/d(^*)</td>
<td>470 (511)</td>
<td>785 (1242)</td>
</tr>
<tr>
<td>Taking insulin, n (%)</td>
<td>-1</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Taking biguanide, n (%)</td>
<td>-7</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Values are mean (SD), unless indicated. \(p\)-value < 0.05 significant

Conclusions

Summary of findings:
- No difference in TMD or BV/TV between groups, but greater variation in values in type 2 diabetes women.
- In all adults, TMD is associated with less mineralization heterogeneity (\(Ca_{\text{WIDTH}}\)).
- suppressed bone turnover in adults with type 2 diabetes\(^5\) may explain relationship between TMD and number of years with diabetes diagnosis and \(Ca_{\text{WIDTH}}\).
- Association between TMD and biguanide use may be explained by in vitro research demonstrating osteogenic action of metformin on osteoblasts\(^6\).

Limited to:
- No bone turnover markers measured, μCT and BMDD measurements made on different sections of bone, all patients had OA therefore not generalizable to all.
- Due to small sample size, did not adjust for covariates in correlation analyses and did not perform regression analyses.

Study provides insight into potential mechanisms of higher bone mineralization in patients with type 2 diabetes, which may be detrimental to bone strength.

References:
1. Veerling P. Osteoporos Int. 2007;18:427
8. The authors have no conflicts of interest to declare

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This study was approved by McMaster University HIREB.