ECTS PP459

Correlates of tissue mineral density (TMD) of bone samples from total hip arthroplasty patients with type 2 diabetes: an *ex vivo* study

Pritchard JM[1], Papaioannou A[1], Hurtig M[2], Giangregorio LM[3], Atkinson SA[4], Beattie KA[1], Adachi JD[1], DeBeer J[5], Winemaker M[5], Avram V[5], Schwarcz HP[6]

McMaster University, Dept. of Medicine[1], Dept. of Pediatrics[4], Dept. of Surgery[5], Dept. of Geography and Earth Sciences[6]
[2] University of Guelph, Dept. of Clinical Studies [3] University of Waterloo, Dept. of Kinesiology

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)[5,6], 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, Juravinski Hospital).

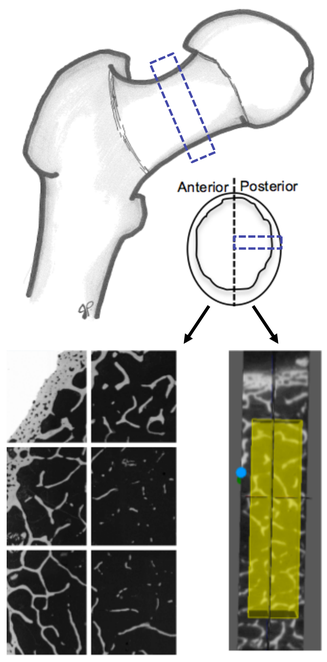
Inclusion/Exclusion Criteria:

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

Sample Preparation:

- A 5 mm 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.

Figure 1. Bone specimen and sectioning



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_{MEAN}, Ca_{PEAK}, Ca_{WIDTH} [7].

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).* *P*-value of 0.05 was significant.

This study was approved by McMaster University HIREB.

Results

Table 1. Descriptive characteristics of study participants.

	control n=20	type 2 diabetes n=14	*p-value*
Age, years	76.1 (6.6)	73.9 (6.0)	0.306
Female, n(%)	13 (65.0)	6 (42.9)	0.201
BMI, kg/m^2	28.8 (5.7)	30.7 (7.4)	0.426
Age-adjusted Charlson Index	2.2 (2.4)	4.4 (0.8)	**0.003**
Calcium intake, mg/d*	368 (479)	463 (560)	0.601
Vitamin D intake, IU/d*	470 (511)	785 (1224)	0.313
# years since diabetes diagnosis, y	-	13.5 (7.4)	-
Taking insulin, n (%)	-	4 (28.6)	-
Taking biguanide, n (%)	-	7 (50.0)	-

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

Figure 2. Comparison of BV/TV and TMD between groups.

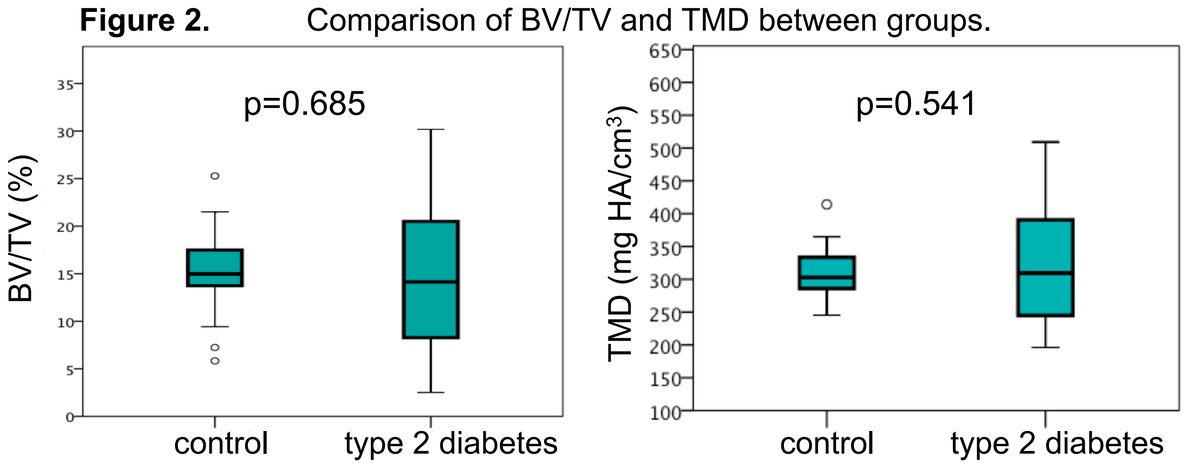


Table 2. Correlates of TMD in participants with and without type 2 diabetes.

	Pearson r	*p-value*
Ca_{MEAN}	0.234	0.306
Ca_{WIDTH}	-0.617	**0.003**
Ca_{PEAK}	0.228	0.320
Age	-0.250	0.153
BMI	-0.042	0.815
Number years since diabetes since diagnosis*	0.410	**0.020**
Taking insulin (no=0, yes=1)*	-0.161	0.363
Taking biguanide (no=0, yes=1)*	0.463	**0.006**

* correlation coefficients calculated for participants with type 2 diabetes only

Conclusions

Summary of findings:

- No difference in TMD or BV/TV between groups, but greater variation in values in type 2 diabetes group.
- In all adults, TMD is associated with less mineralization heterogeneity (Ca_{WIDTH}).
- In adults with type 2 diabetes, TMD is associated with greater number of years with type 2 diabetes and biguanide use.

- Suppressed bone turnover in adults with type 2 diabetes[8] may explain relationship between TMD and number of years with diabetes diagnosis and Ca_{WIDTH}[7].
- Association between TMD and biguanide use may be explained by *in vitro* research demonstrating osteogenic action of metformin on osteoblasts[9]
- *Limitations:*
  - 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.

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The authors have no conflicts of interest to declare

Funding sources: Osteoporosis Canada Tim Murray Award, Ontario Graduate Scholarship Program, Amgen Canada, Natural Sciences and Engineering Research Council of Canada