

## In Search of Bone Quality Deficits in Type 2 Diabetes

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**INTRODUCTION:** Bone fractures are a widespread and costly problem [1]. Clinical meta-analyses conclude that people with type 2 diabetes (T2D) are at a higher risk of a hip fracture than those who do not have the disease [2]. Current clinical tools, such as T-scores from dual-energy X-ray absorptiometry (DXA) and the online FRAX calculator, underestimate fracture risk for individuals with diabetes suggesting disease-related changes to trabecular bone microarchitecture and matrix quality. We hypothesize that T2D for at least 10 years has detrimental effects on the material properties of the trabecular bone, altering the matrix bound water. While matrix bound water has previously been shown to be a predictor of fracture resistance in cortical bone [3], its role in trabecular bone has yet to be determined.

**METHODS:** To test this hypothesis, we acquired and analyzed fresh-frozen cadaveric femurs from 40 donors [T2D: 6 males (70.7±12.6 yrs, T2D duration: 20.4±9.9 yrs) and 14 females (70.3±13.7 yrs, T2D duration: 22.5±9.7 yrs) and non-T2D: 7 males (76.9±9.4 yrs) and 13 females (69.7±13.5 yrs)]. The T2D donors were screened for relevant comorbidities, such as stage 3 or higher chronic kidney disease (CKD) [1 male, 90 yrs; 4 females, 76-85 yrs]. The intact femurs were scanned on a clinical DXA machine (Hologic Horizon series W) to obtain the femoral neck (FN) and hip T-scores. Next, two trabecular bone cores (24.0 mm length x 10.1 mm diameter) were extracted from each of the proximal femurs using a drill press and diamond-embedded trephine under hydration (Fig.1B). The coring axis was aligned with the primary direction of the trabeculae. The excised cores were flushed with distilled water to remove the marrow and subsequently machined using a low-speed, water irrigated circular saw. The core from the posterior quadrant of the proximal femur was cut at either ends to normalize the length (20.8±0.53 mm) before imaging with micro-computed tomography (µCT50, Scanco Medical AG). The mid-region of the posterior core (12.07 mm in length) was scanned at an isotropic voxel-size of 14.8 µm to characterize the trabecular bone microarchitecture (Fig.1C): bone volume fraction (BV/TV), trabecular number, thickness, and separation (Tb.N, Tb.Th, Tb.Sp, respectively), and tissue mineral density (Tb.TMD). Using Scanco's linear-elastic, finite element analysis software (µFEA), the reconstructed 3D scans were converted to finite element models (direct voxel-to-hexahedral element) and prescribed boundary conditions that simulated uniaxial, high-friction, compression testing (Fig.1D). Based on linear elastic failure criteria (2% of BV exceeding 0.007 equivalent strain), the µFEA predicted compressive failure force in addition to the apparent modulus. The neighboring core (anterior) was cut at the distal end to obtain an approximately 5 mm x 5 mm x 5 mm sample for matrix-bound water measurements using <sup>1</sup>H-nuclear magnetic resonance relaxometry (NMR, Varian 4.7T) [4] (Fig.1E). The NMR sample was also scanned with µCT50 at an isotropic voxel size of 34.4 µm to obtain bone volume and normalize the NMR signal. The data were checked for normal distribution using Shapiro-Wilk test, and statistical significance (p<0.05) in the difference between groups was determined using two-tailed, unpaired t-test or Mann-Whitney test.

**RESULTS:** The T-scores, both hip and femoral neck (FN), were not significantly different between T2D and control donors (Table 1). For the trabecular cores, there were also no differences in trabecular bone volume fraction, Tb.TMD, or microarchitecture between groups (Table 1). Consistent with the µCT data, the predicted compressive failure force, as determined by µFEA, showed no difference between T2D and control (Table 1). With respect to matrix-bound water of trabecular bone, we found a significant difference in the bound water concentration (C<sub>bw</sub>) between control and T2D donors when excluding one outlier (Fig.2A) and a trend toward C<sub>bw</sub> being lower when including all donors (Table 1). C<sub>bw</sub> decreased with age in only the Control group (Fig. 2B).

**DISCUSSION:** Both matrix-bound water [5] and strength [6] of cortical bone decrease as donor age increases. Whether matrix-bound water in trabecular bone decreases with age and T2D is unknown. The present study suggests that T2D and age affect C<sub>bw</sub> but that the linear relationship differs between T2D and control. A limitation of the study is the insufficient number of donors to determine if CKD is associated with low bound water and BV/TV. The lack of difference in trabecular architecture between the groups in the present is similar to previous studies comparing trabecular bone from discarded proximal femurs in total hip arthroplasty between T2D and control patients [7,8].

**SIGNIFICANCE:** Matrix-bound water of trabecular bone is a potential indicator of the elevated fracture risk in type 2 diabetes.

**REFERENCES:** [1] Lewiecki EM. *Clin Mol Allergy*. 2004, [2] Janghorbani M. *Am J Epidemiol*, 2007, [3] Manhard MK et al., *Bone*, 2016, [4] Horch RA et al., *Magn Reson Med*, 2010, [5] Wang X et al., *JMBR PLUS*, 2018 [6] Horch RA et al., *PLoS One*, 2011, [7] Nilsson AG et al., *J. Bone Miner. Res.* 2017 [8] Karim L et al. *Bone*, 2018.

**ACKNOWLEDGEMENTS:** This study was supported by AR063157 from NIH NIAMS and BX004297 from VA BLR&D.

Fig.1: Schematic outlining the experimental methods.

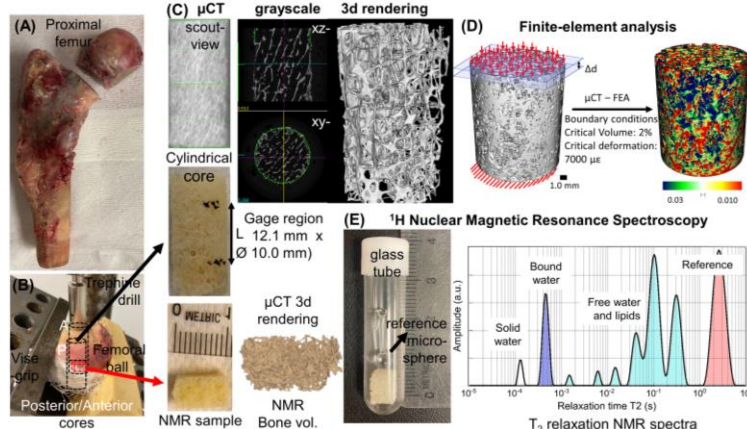


Table 1: Mean ± SD of selected outcomes per group

Characteristic	Unit	Control (n=20)	T2D (n=20)	p-value
Age	years	72.2 ± 12.5	70.4 ± 13.1	0.6584
BMI	kg/m <sup>2</sup>	26.6 ± 8.0	32.5 ± 11.2	0.1674
C <sub>bw</sub>	mol <sup>1</sup> /H/L	25.8 ± 2.0	24.7 ± 2.0	0.0529
FN T-score	-	-1.5 ± 1.0	-1.1 ± 1.2	0.3572
Hip T-score	-	-1.2 ± 1.2	-0.6 ± 1.2	0.1148
BV/TV	-	0.267 ± 0.078	0.291 ± 0.066	0.2995
Tb.N	1/mm	1.467 ± 0.193	1.560 ± 0.145	0.0915
Tb.Th	mm	0.188 ± 0.030	0.195 ± 0.032	0.4628
Tb.Sp	mm	0.627 ± 0.097	0.588 ± 0.071	0.1600
Tb.Conn.D	1/mm <sup>3</sup>	6.954 ± 2.235	7.969 ± 1.831	0.1244
Tb.TMD	mg HA/cm <sup>3</sup>	881 ± 27	877 ± 21	0.6103
Failure load est.	N	546.5 ± 274.1	587.0 ± 241.6	0.6228

Figure 2 consists of two panels. Panel (A) is a box plot showing the bound water concentration (mol <sup>1</sup>H Bone) for Control and T2D groups. The Control group has a median bound water concentration of approximately 26.5 mol <sup>1</sup>H Bone, while the T2D group has a median of approximately 24.5 mol <sup>1</sup>H Bone. The p-value for the difference between groups is 0.0194. Panel (B) is a scatter plot showing the bound water concentration vs. Age (years) for CKD donors. The plot shows a negative correlation between age and bound water concentration. The p-value for the correlation is 0.0340 for T2D donors and 0.4787 for Control donors. The plot also indicates that outliers were excluded.

Fig.2:(left) Bound water concentration (right) correlation with age.