Femoral Neck Bone Tissue Composition is Altered in Cortical but not Trabecular Bone in Postmenopausal Women with Long-Duration Type 1 Diabetes

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Disclosures: SRE (N), SG (N), EP (N), NH (N), IW (N), SVTJ (N), MGY (N), HSS (N), GLK (8: Sunstar Foundation, Thomas Beatson, Jr. Foundation), FJ (N), EWY (4: Opko; 5: Amgen; 8: JBMR, Bone; 9: ASBMR), MLB (3B: Keros Therapeutics, Beryl Therapeutics; 4: Keros Therapeutics; 9: ASBMR)

INTRODUCTION: Type 1 Diabetes (T1D) is associated with an increased risk of fracture compared to non-diabetics, with up to a 7-fold increased risk for hip fracture [1]. However, the factors contributing to skeletal fragility in T1D are not well understood. It is hypothesized that modifications to bone material properties induced by altered tissue composition, such as increased advanced glycation endproduct (AGE) content, could influence skeletal fragility. However, no studies have assessed bone compositional properties at the femoral neck in older adults with T1D. The aim of this study was to compare cortical and trabecular bone compositional properties via Raman spectroscopy in the excised femora of postmenopausal women with long-duration T1D and non-diabetic controls.

METHODS: Whole femora were acquired post-mortem from postmenopausal women in the Joslin Medalist Study [2,3], a unique cohort of individuals with long-duration T1D (≥ 50 years) (n = 11). Non-diabetic control femora of similar age-, race- and sex were obtained from a commercial tissue bank (n = 10). Women with end stage renal disease, lower limb amputation, or history of bone metastases were excluded. Femoral neck cross-sections were isolated from each specimen and the cortical and trabecular bone in the superior-anterior quadrant was evaluated via Raman spectroscopy (Bruker Senterra). The following parameters were calculated from the Raman spectra in each anatomical region: mineral/matrix ratio, mineral crystallinity, relative lipid content, glycosaminoglycan content (GAG), pyridinioline (enzymatic collagen crosslink), three AGEs – CML (ε-N-Carboxymethyl-L-lysine), pentosidine, and glucosepane – and glucose [4]. Non-parametric unpaired Wilcoxon rank sum tests were used to determine differences between groups (p<0.05).

RESULTS: The T1D specimens included 11 women with an average HbA1c = 8.3%, mean T1D duration = 65.2 years (range 56-76), age at onset = 12.8 years, age at death = 77.8 years (range 68-92) and BMI = 25.5 kg/m^2. The control group was comprised of 10 women; age and BMI were not significantly different between groups (p = 0.72 and 0.83, respectively). Raman analysis indicated differences in the cortical (Table 1), but not the trabecular (Table 2), compartment. Specifically, femoral neck cortical bone in T1D had lower mineral/matrix ratio (-25%, p < 0.001) and glucose content (-24%, p = 0.001) compared to non-diabetic controls. Notably, cortical bone AGE content was greater in T1D, with greater CML (+141%, p < 0.001), and glucosepane (+200%, p = 0.002) versus control. In the trabecular bone, Raman indices were not different between T1D and non-diabetic controls.

DISCUSSION: The results of this study suggest that long-duration T1D in postmenopausal women leads to altered bone tissue composition, as measured by Raman spectroscopy of femoral neck bone specimens. Consistent with previous work [4], our study shows lower glucose content in postmenopausal women with T1D, thought to be due to reduced blood flow due to microvascular complications. Furthermore, differential Raman indices between cortical and trabecular bone likely stem from distinct bone turnover rates in each tissue. Importantly, we previously demonstrated that long-duration T1D compromised the post-yield energy absorbing capacity (i.e., toughness), but not elastic behavior, of cortical bone in the femoral mid-diaphysis [5]. In bone, reduced toughness and post-yield behavior are attributed to altered matrix collagen properties such as increased AGE accumulation [6-8]. Greater cortical AGE content in the femoral cortical bone may help to explain the reduced post-yield energy absorbing capacity in T1D. Together, these data suggest a significant deterioration of bone material properties and compositional properties in long-duration T1D compared to non-diabetic controls.

SIGNIFICANCE/CLINICAL RELEVANCE: Although the growing issue of skeletal fragility in patients with T1D is widely recognized, there is a lack of information regarding how T1D increases fracture risk. The current observations indicate that altered femoral neck cortical bone composition may contribute to the increased fracture risk in patients with T1D.

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ACKNOWLEDGEMENTS: This work was supported by NIH awards DK122593, DK124710, AG023480, DK036836, EY026080, HL161864, the Mary K. Iacocca Fellowship, the Beatson Foundation, the Dianne Nunnally Hoppes Fund, AUVA and OEGK.

Table 1: Raman indicies in the femoral neck cortex of postmenopausal women with long-duration type 1 diabetes (T1D) and non-diabetic controls.

	Control N = 10[1]	T1D N = 11[1]	p-value[2]
Mineral Crystallinity	0.0445 (0.0439, 0.0451)	0.0444 (0.0434, 0.0451)	0.605
Mineral / Matrix	1.03 (0.97, 1.09)	0.78 (0.67, 0.84)	**<0.001**
Pyridinoline	0.0177 (0.0171, 0.0182)	0.0182 (0.0175, 0.0191)	0.314
Lipids	0.070 (0.065, 0.074)	0.066 (0.060, 0.076)	0.654
GAGs	0.044 (0.042, 0.050)	0.047 (0.041, 0.064)	0.557
CML	0.017 (0.014, 0.020)	0.041 (0.031, 0.050)	**<0.001**
Pentosidine	0.023 (0.018, 0.025)	0.026 (0.022, 0.042)	0.197
Glucosepane	0.012 (0.008, 0.017)	0.036 (0.027, 0.047)	**0.002**
Glucose	0.038 (0.037, 0.042)	0.029 (0.020, 0.033)	**0.001**

[1] Median (IQR); n (%)
[2] Wilcoxon rank sum test; Fisher's exact test

Table 2: Raman indicies in the femoral neck trabecular bone of postmenopausal women with long-duration type 1 diabetes (T1D) and non-diabetic controls.

	Control N = 10[1]	T1D N = 11[1]	p-value[2]
Mineral Crystallinity	0.0446 (0.0443, 0.0448)	0.0447 (0.0439, 0.0450)	0.918
Mineral / Matrix	0.76 (0.61, 0.84)	0.51 (0.43, 0.65)	0.099
Pyridinoline	0.0213 (0.0187, 0.0223)	0.0222 (0.0206, 0.0247)	0.173
Lipids	0.108 (0.089, 0.131)	0.110 (0.094, 0.129)	0.705
GAGs	0.034 (0.026, 0.038)	0.040 (0.038, 0.045)	0.061
CML	0.021 (0.011, 0.027)	0.023 (0.016, 0.025)	0.705
Pentosidine	0.017 (0.015, 0.025)	0.019 (0.017, 0.024)	0.426
Glucosepane	0.019 (0.014, 0.020)	0.018 (0.013, 0.036)	0.863
Glucose	0.031 (0.024, 0.037)	0.032 (0.029, 0.034)	0.654

[1] Median (IQR); n (%)
[2] Wilcoxon rank sum test; Fisher's exact test