

Differential Effects of Type 2 Diabetes and RAGE Signaling in Male and Female Femoral Mechanics

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INTRODUCTION: Type 2 diabetes (T2D) is a widespread disease with numerous musculoskeletal comorbidities, including elevated bone fracture risk. Patients with T2D often present with increased bone mineral density yet experience a much higher incidence of fracture¹. Therefore, the mechanisms behind increased fracture risk in T2D patients is an area of active research. The receptor for advanced glycation endproducts (RAGE) is a member of the immunoglobulin superfamily and can bind multiple ligands, including advanced glycation endproducts (AGEs) and high mobility group box 1 protein (HMGB1), both of which are overexpressed in T2D^{2,3}. RAGE is upstream of the NF- κ B inflammatory pathway and has been shown to dysregulate bone cell function, which could lead to detrimental effects on bone mechanics⁴. In this study, we investigate the effects of RAGE ablation on the mechanical and structural properties of diabetic femurs in male and female mice.

METHODS: All procedures were performed in accordance with Washington University SOM IACUC. The genetic model included female and male wild type mice (wt), RAGE knockout mice (RAGE^{-/-}), leptin receptor-deficient mice (db/db), and double mutants (RAGE^{-/-};db/db). All mice were euthanized at one of three time points: 3-4 months (Early), 6-8 months (Middle), and 10-14 months (Late). Following sacrifice, right femurs were dissected and stored at -20C. First, the femurs were scanned via microCT analysis using Scanco vivaCT40 (8um voxel size, 70keV, 170uA, and 300ms integration time). The first 100 slices distal to the regression of the third trochanter were isolated and used to measure the morphometric properties and tissue mineral density (TMD) of the mid-diaphysis using a custom Matlab code. Next, the femurs were subjected to three-point bending using an Instron ElectroPuls E1000 (preload of -0.3N, loading rate of -0.1mm/s). A span length of 7.5mm was used, with the anterior surface of the femur facing down. Separate 3-way ANOVAs were ran for each sex in Graphpad Prism to determine the effects of age, diabetes, and RAGE on femur mechanical, material, and morphological properties.

RESULTS: Experimental procedures and data analysis were performed on n=5-10 mice per group. Whole-bone mechanics: Age was a significant factor in maximum load (female: p<0.0001, male: p=0.0037) and stiffness (female: p<0.0001, male: p<0.0001), with the middle and late groups displaying increased results compared to the early group. In female mice, diabetes was associated with decreased maximum load (p=0.0311), and stiffness was trending lower (p=0.0535). In male mice, diabetes was associated with reduced stiffness (p=0.0087). RAGE ablation protected stiffness (p=0.0030) and maximum load (p=0.0006) from deterioration due to diabetes in female animals. Tissue-level mechanics: Age group was not a significant factor in ultimate stress or Young's modulus in either female or male mice. In female mice only, diabetes was associated with decreased ultimate stress (p=0.0262) and Young's modulus (p=0.0259). RAGE ablation did not improve tissue-level mechanical properties in diabetic female or male mice. Material properties: Age group was a significant factor in TMD (p<0.0001) in both female and male mice, with the middle and late groups displaying increased results compared to the early group. In female mice only, diabetes was associated with significantly decreased TMD (p<0.0001). RAGE ablation protected TMD from deterioration due to diabetes (p=0.0004) in female animals. Morphological properties: Age group was a significant factor in cortical thickness (p=0.0005) in female mice, with the early and late groups displaying decreased results compared to the middle group. In female mice only, diabetes was associated with decreased cortical thickness (p=0.0057). RAGE ablation was not significantly associated with cortical thickness in female or male mice.

DISCUSSION: In both female and male mouse femurs, diabetes was associated with deteriorations in whole-bone mechanical properties, notably maximum load and stiffness. Female mice also exhibited impairments at the tissue level in ultimate stress, Young's modulus, and TMD. RAGE ablation protected against the declining maximum load and stiffness only in female T2D mice. This suggests that there may be sex-based differences in how RAGE ablation and/or T2D affects diabetic bone that warrants further investigation. These data suggest that RAGE ablation appears to improve bone quality through its effects on bone TMD. The beneficial effects of RAGE ablation on bone mechanics and microstructure in female db/db mice was not observed in male mice or in mice without T2D. We hypothesize that the diminished level of RAGE signaling (due to reduced binding of its ligands) in wt mice and potentially male db/db mice does not negatively affect bone cells and thus does not significantly decrease bone mechanical properties. Future work will help further elucidate the relationship between RAGE signaling and T2D bone mechanics.

SIGNIFICANCE/CLINICAL RELEVANCE: RAGE may be a promising therapeutic target for alleviating the mechanical deficits of bone in individuals with chronic type 2 diabetes.

REFERENCES: [1] Liu et al, BMC Endocrine Disorders, 2023. [2] Meerwaldt et al, Diabetologia, 2004. [3] Wang et al, PLOS One, 2006. [4] Zhou et al, Journal of Experimental Medicine, 2006.

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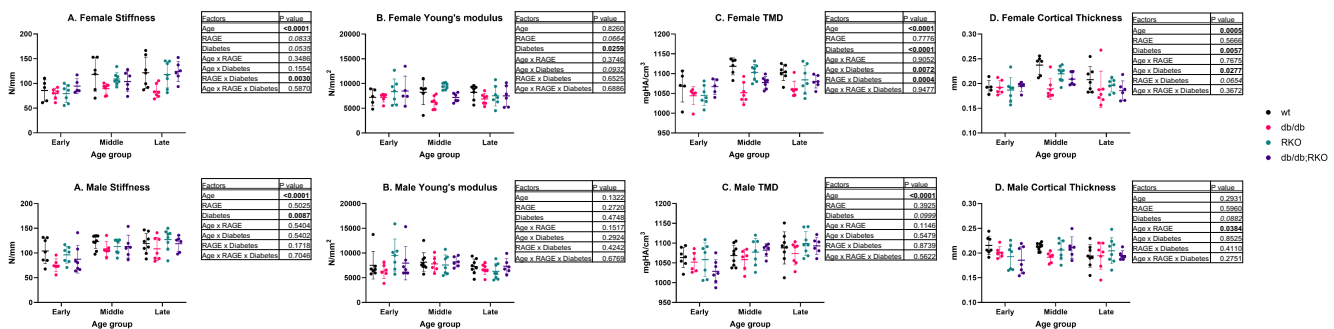


Figure 1. (A) RAGE ablation rescues loss of stiffness due to diabetes in female mice. (B) RAGE ablation does not protect against reduction in Young's modulus due to diabetes. (C) RAGE ablation protects against decrease in TMD due to diabetes in female mice. (D) RAGE ablation does not protect against loss of cortical thickness due to diabetes.