Short-term inhibition of RAGE protects against osteoclast impairment in a mouse model of type 2 diabetes

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INTRODUCTION: Patients with type 2 diabetes experience more fragility fractures in spite of increased bone mineral density.[1,2] One likely contributor is the accumulation of advanced glycation endproducts (AGEs) in the bone of T2D patients which have been shown to impair bone mechanics.[3] AGEs have also been shown to be detrimental to bone cell function both *in vitro*[4] and *in vivo*[5,6] via activation of the receptor for AGEs (RAGE). Previous work in our lab has shown improved osteocyte density and trending improvements in osteoclast and osteoblast activity, along with reduced AGE accumulation in a RAGE knockout mouse model with type 2 diabetes (db/db) in the vertebrae.[7] We hypothesize that the pharmacological inhibition of RAGE will improve bone cell function and in turn the bone matrix in a mouse model of T2D. This study is testing the effects of 60 days of RAGE inhibitor treatment (FPS-ZM1) on bone cell activity, bone AGEs, and mechanical properties in a high AGE environment.

METHODS: Female leptin receptor deficient mice and littermates at 5 months of age were injected daily with 1mg/kg/ml of FPS-ZM1 in 0.05% DMSO or with vehicle (n=4-5/group) for 60 days. Body weight was monitored biweekly. L4 vertebra were scanned by microCT (Scanco vivaCT 40, 10um, 70 keV, 170uA, and 300ms integration time) before treatment and then again immediately prior to sacrifice. This data was visualized in 3D using time-lapse microCT. Representative initial and final microCT scans were uploaded to Dragonfly ORS for image registration and quantification of formation to resorption ratio to measure bone homeostasis. L3 vertebra were stained with TRAP against H&E to measure osteoclast activity (osteoclast surface per bone surface, Oc.S/BS) and osteocyte density. Mice were injected with calcine 2 days prior to sacrifice to measure osteoblast activity (mineralizing surface per bone surface, MS/BS). L5 vertebra were scanned by microCT and tested in compression and an AGE fluorometric assay was performed.

RESULTS: Body weight for all groups remained consistent throughout the treatment period. BV/TV stayed consistent throughout treatment in both wt groups but increased in the vehicle db/db group. These changes in BV/TV are characterized by an increase in Oc.S/BS in the db/db treatment group compared to the vehicle db/db group, with a trending effect of treatment on MS/BS. Diabetes showed an effect on osteocyte density (reduced), vertebral AGEs (increased), and mechanical properties (decreased) with no effect of treatment.

DISCUSSION: The results show that inhibition of RAGE for 60 days in db/db mice increases osteoclast activity and prevents the non homeostatic bone accumulation observed in non-treated db/db animals. No significant effects of treatment were noted on bone matrix AGEs or osteocyte lacunae density, though osteoblast function trended towards increased mineral apposition. Our previous study revealed that systemic RAGE ablation in db/db mice rescued osteocyte lacunar density, enhanced bone mineralizing surface and osteoclast function. Taken together, these data indicate that long term RAGE inhibition could help restore bone cell function and in turn bone matrix quality.

SIGNIFICANCE/CLINICAL RELEVANCE: This study investigates the efficacy of RAGE inhibition in treating bone fragility in type 2 diabetes.

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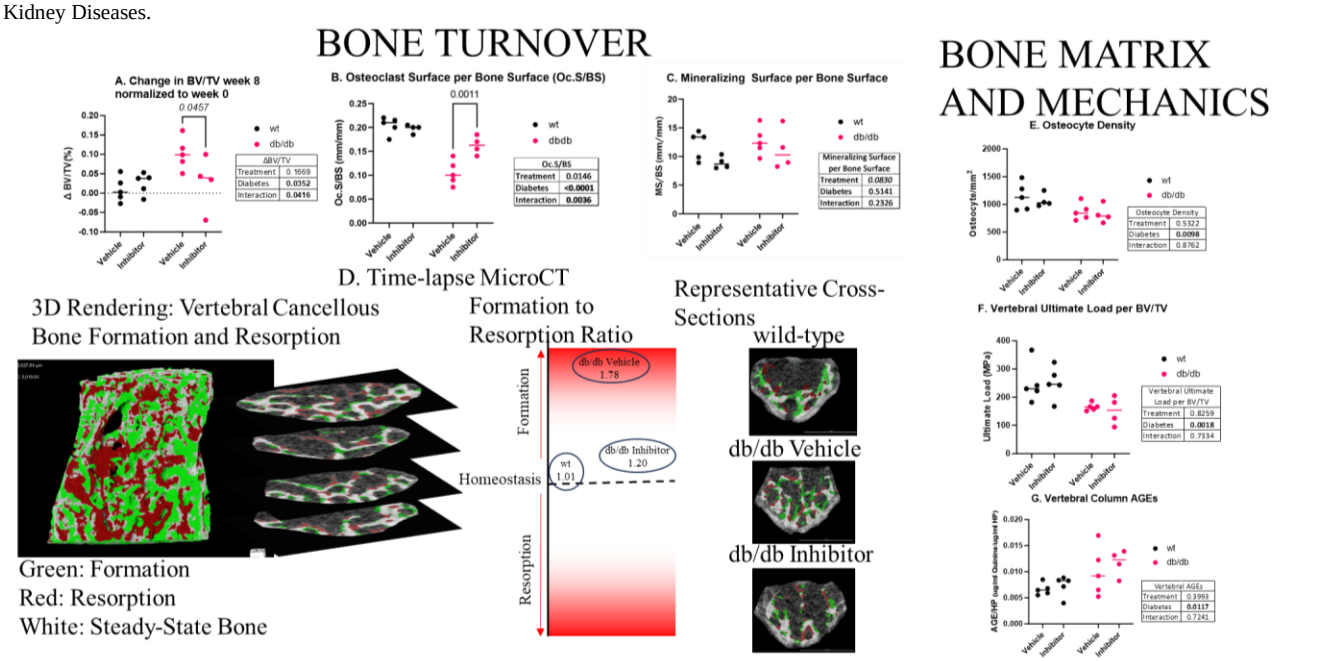

Figure 1. Bone Turnover: [A] Change in BV/TV normalized to week 0, increased BV/TV noted in db/db vehicle treated mice which is trending towards baseline in the db/db inhibitor group, db/db animals overall showed an increase in Δ BV/TV with a trending on significant interaction term. These changes can further be characterized by bone cell activity; [B] osteoclast surface per bone surface was significantly reduced in the db/db animals with a significant increase in osteoclast activity with treatment. Furthermore, [C] osteoblast activity appears to not be altered by diabetes or treatment. [D]Visualization of bone changes can also be done in 3-D using time-lapse microCT. Areas highlighted in red represent areas of bone resorption, areas in green show bone formation, and white is steady-state bone. The formation to resorption ratio in these samples confirms what was seen in the longitudinal microCT data (A). Bone matrix and mechanics: [E] Osteocyte density is reduced in db/db animals with no effect of treatment, [F] vertebral ultimate load per BV/TV is reduced due to diabetes with no effect of treatment, and [G] AGE density is increased with diabetes with no effect of treatment.