Romosozumab partially rescues impaired bone mass and strength in a murine model of diabetic kidney disease

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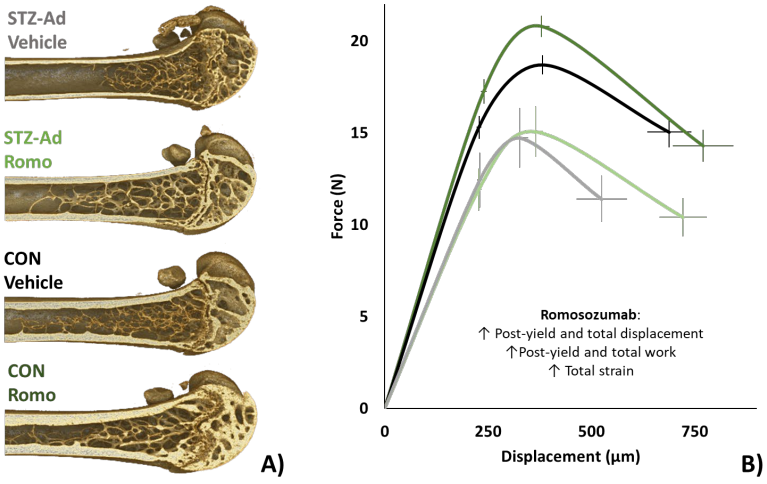
INTRODUCTION: As the incidence of diabetes and diabetes-driven comorbidities such as chronic kidney disease (CKD) continue to climb, interventions are needed that address the high-risk skeletal fragility of what is a complex disease state. Diabetes alone increases fracture risk via mechanisms that are still poorly understood but appear to be related to bone quality, with accumulation of advanced glycation end-products commonly believed to increase tissue stiffness and fragility. Meanwhile, late-stage CKD can induce a huge loss of bone mass, as accumulated serum phosphorus leads to elevated parathyroid hormone which causes rapid bone resorption, drastically reducing bone integrity. Despite the increasing prevalence of combined diabetes and CKD (DKD), little is known about how best to decrease fracture risk in this population due to regular exclusion of these patients from clinical trials. Current best practice is to refer patients to osteoporotic therapies on a case-by-case basis, however, these interventions may not address the adverse impact of diabetic hyperglycemia on bone quality. Romosozumab (Romo) is an FDA-approved sclerostin inhibitor that has been shown to increase bone mineral density and decrease fracture rates in osteoporotic patients with mild to severe CKD, but its effect on diabetes-weakened bone is unknown. We hypothesized that Romo would still be able to improve bone mass and strength in DKD, and aimed to test its performance in a recently developed murine model.

METHODS: 6-week old female C57BL/6 mice were randomly divided into DKD (STZ-Ad) and control groups (CON). STZ-Ad mice were given 5 daily intraperitoneal injections of streptozotocin (90 mg/kg) to impair beta cell function and mimic the insulin deficiency of type 1 diabetes. Blood glucose and body weight measures were taken weekly to assess development of hyperglycemia, and all mice that did not become hyperglycemic within 2 weeks were given additional STZ booster shots (90 mg/kg). Approximately 2/3 of animals developed hyperglycemia. Starting at 11 weeks, mice were then fed a purified casein diet with altered mineral ratio (0.9% phosphorous, 0.6% calcium) which, for the first 6 weeks, also contained 0.2% adenine to induce kidney damage and development of mineral bone disorder. CON mice were maintained on standard chow during the entirety of the study. After 6 weeks, STZ-Ad mice were switched to casein diet without adenine, and both CON and STZ-Ad groups were subdivided into two treatment groups and given weekly subcutaneous injections of 100 µL vehicle (phosphorus buffered saline, PBS) or 10 mg/kg Romo. Mice were euthanized after 4 weeks of treatment via cardiac exsanguination and cervical dislocation. Hindlimb bones were cleaned of soft tissue, wrapped in PBS-soaked gauze and stored at -20 °C, then later thawed and scanned via microcomputed tomography (µCT) in groups of 4. Scans were performed using an isotropic voxel size of 8 µm (Skyscan 1272, Bruker), scanned through a 0.5 mm Al filter (V = 60kV, I = 167µA) with a 0.7-degree angle increment and two frames averaged. Images were reconstructed (nRecon) and rotated (Data Viewer) before calibrating to hydroxyapatite-mimicking phantoms (0.3 and 1.25 g/cm^3 CaHA). A 1 mm trabecular region of interest (ROI) was selected starting from the growth plate of each bone, extending distally in tibiae (T) and proximally in femora (F). Cancellous architecture in each ROI was quantified using CT Analyzer (CTAn). A 0.1 mm cortical ROI was selected at central midshaft of each bone (50% of bone length). Cortical ROIS were analyzed with a custom MATLAB (MathWorks, Inc. Natick, MA) program. Tibiae were then tested to failure in four-point bending (lower span at 9mm; upper span at 3mm), with the medial surface in tension. Bones were loaded at a displacement control rate of 0.025 mm/s while the sample was kept hydrated with PBS. After each test, fracture location was measured with calipers and used to select cortical ROIs of the fracture site from µCT scans, which were analyzed using the methods described above. Fracture-site geometry was used to map load-displacement data into stress-strain data using standard engineering equations to estimate tissue level properties. Two-way ANOVA tests were performed to assess the contributions of disease and Romo treatment to bone morphology and mechanics at a significance level of $\alpha = 0.05$, with Tukey post-hoc tests used for any significant interactions.

RESULTS: Romo treatment significantly increased cortical and trabecular bone mass in both STZ-Ad and CON animals. Cortical bone had significantly reduced marrow area (T: p = 0.0001, F: p = 0.0005) with increased cortical thickness (T: p = 0.0008, F: p = 0.008) and bone area ratio (T: p = 0.0005, F: p = 0.0036) while trabecular bone had substantial increases in bone volume fraction (T: p = <0.0001, F: p = <0.0001), with more (T: p = 0.0002, F: p = <0.0001) and thicker (T: p = <0.0001, F: p = <0.0001) trabeculae spaced closer together (T: p = 0.0025, F: p = 0.0309). There were significant interaction effects for bone volume percent (p = 0.04554) and trabecular number (p = 0.0267) in femora, and post-hoc interactions showed no statistical difference between Romo-treated STZ-Ad and CON mice. Mechanical testing showed higher energy absorption prior to fracture, with displacement (postyield: p = 0.0366, total: p = 0.0335), work (postyield: p = 0.0372, total: p = 0.0392), and total strain (p = 0.0283), all increased in Romo-treated specimens.

DISCUSSION: Romo produced similar relative improvements to bone mass and strength in STZ-Ad mice and CON mice, suggesting that Romo's efficacy is not impaired by this disease state. While cortical reinforcements led to increased bone strength, Romo-driven improvements were especially pronounced in trabecular bone. In general, Romo helped STZ-Ad mice recover cortical bone measures to match those of untreated CON mice, while all trabecular measures in Romo-treated STZ-Ad mice exceeded those of untreated CON mice. While mechanical properties did not improve to the same extent, Romo-treatment did allow bone to recover some fracture resistance, as seen by increased displacement, work and strain to fracture.

SIGNIFICANCE/CLINICAL RELEVANCE: Romosozumab could be used to decrease fracture risk for patients with DKD, especially by reinforcing trabecular bone in high-risk sites such as lower vertebrae and the femoral head and neck.



A. Representative distal femoral cross-sections for different treatment groups in female cohort showing how reduced trabecular and cortical bone mass in disease group is partially rescued by Romo treatment. **B.** Mean force and displacement values from 4-pt tests of female bones, plotted at yield, ultimate, and failure points for each group, with error bars showing standard error (SEM). Text insert summarizes main effects from two-way ANOVA test.
For contrast: ASBMR Abstract (approved by Amgen)