

Six months of canagliflozin treatment improves bone morphology in young and adult C57Bl/6J mice

Carolyn Chlebek¹, Casey McAndrews², Samantha Costa¹, Erin M. MacFarlane², Shoshana Yakar³, Clifford J. Rosen¹

¹MaineHealth Institute for Research, Scarborough, ME, ²University of New England College of Osteopathic Medicine, Biddeford, ME, ³New York University College of Dentistry, New York, NY
Carolyn.Chlebek@mainehealth.org

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INTRODUCTION: Canagliflozin lowers serum glucose by inhibiting sodium glucose transporter 2 (SGLT2), which is primarily expressed in the kidney. SGLT2 inhibitors are used to treat Type II Diabetes and cardiovascular disease, despite increased risk of fracture¹. In preclinical models, short-term treatment with canagliflozin negatively affected trabecular bone² whereas long-term treatment reduced cortical bone mineralization in male but not female mice³. We aimed to determine the effect of an intermediate period of canagliflozin treatment on bone quality in nondiabetic mice.

METHODS: Under IACUC approval, male and female C57Bl/6J mice aged 3- or 6-months received either control or canagliflozin-containing diet (180 ppm) for six months (n = 8-10/group). Body mass, composition, and bone mineral density (BMD) were assessed monthly using dual energy x-ray absorptiometry (Faxitron UltraFocus-DXA). Changes in body mass, composition, and BMD were calculated as the difference between final and baseline scans. Prior to euthanasia, mice received calcein injections (-10 days, -3 days). Animals were housed in metabolic cages one week before euthanasia. Mice were euthanized following six months of treatment, at 9 months or 12 months of age. At euthanasia, fasting blood glucose was recorded and right femurs were collected for micro-computed tomography (μCT). Following μCT, femurs from young animals were embedded in plastic, sectioned, and imaged for dynamic histomorphometry. Cortical bone was sectioned in the transverse plane and trabecular bone was sectioned in the coronal plane. Mineral apposition rate and bone formation rate were calculated in Osteomeasure. All young animals were evaluated for differences in cortical bone formation (n=8-10/group) whereas only a subset was completed to evaluate trabecular bone formation indices (n = 5/group). To assess differences in the bone morphology and changes in body mass, composition, and bone mineral density, we used a three-way ANOVA with factors of drug, sex, and age. For data collected from metabolic cages, treatment differences using age- and sex-matched controls were determined with t-tests. Dynamic histomorphometry differences were determined with a two-way ANOVA with factors of drug and sex.

RESULTS: Canagliflozin did not alter body weight, but significantly lowered blood glucose levels (drug, p < 0.0001). The change in areal bone mineral density over six months of treatment was greater in canagliflozin-treated females compared with controls (drug*sex interaction, p = 0.0055). Young males treated with canagliflozin also had a greater increase in areal bone mineral density compared with age-matched controls (drug*age interaction, p = 0.0044). Trabecular bone volume fraction and number were increased with canagliflozin treatment in males, but not females (Fig. 1, trabecular number: drug*sex interaction, p = 0.0097). Trabecular thickness and BMD increased with canagliflozin treatment in all groups (trabecular thickness: drug, p = 0.0058; trabecular BMD: drug, p = 0.0052). Canagliflozin reduced trabecular separation (drug, p = 0.0305). In a subset of young mice, canagliflozin did not change trabecular mineral apposition rate or bone formation rate. All canagliflozin-treated animals had increased femoral cortical thickness compared with their age- and sex-matched controls (Fig. 2). In females, canagliflozin increased cortical bone area (drug*sex interaction, p = 0.0348). SGLT2 inhibition reduced marrow area in adults but not young animals (drug*age interaction, p = 0.0408). Moment of inertia was not different with treatment. In young animals, canagliflozin treatment reduced endocortical mineral apposition rate (drug, p = 0.0235) and bone formation rate (Fig. 3). Periosteal bone mineral apposition rate and bone formation rate were not different with canagliflozin. Six months of canagliflozin treatment had limited effects on systemic metabolic activity and body composition. Compared with sex- and age-matched controls, canagliflozin-treated animals had greater water and food intake. Females starting treatment at 3-months-of-age had greater nighttime energy expenditure compared with controls (p = 0.001). Males starting treatment at 6-months-of-age had greater daytime energy expenditure compared with controls (p = 0.0028). Young animals had greater increases in lean mass compared with adults. In adult mice, canagliflozin treatment increased fat mass and percent fat over six months of treatment.

DISCUSSION: Despite the lack of SGLT2 expression in bone, in this study both cortical and trabecular bone morphology were improved with 6 months of canagliflozin treatment. More benefits to cortical bone were measured in females whereas males had more trabecular improvements. The increased risk of bone fracture observed in patients receiving canagliflozin treatment is primarily driven by data collected in the CANVAS clinical trial¹; non-CANVAS studies did not record increased fractures with canagliflozin treatment⁴. Variability in canagliflozin-associated fracture risk may be due to variation within patient populations or differences in fracture risk at the time of treatment initiation. CANVAS patients had Type II Diabetes, likely increasing their baseline fracture risk. Use of canagliflozin has expanded to non-diabetic populations to treat cardiovascular disease. This study demonstrated that in nondiabetic C57Bl/6J mice, reduced serum glucose via canagliflozin treatment may improve bone morphology.

SIGNIFICANCE/CLINICAL RELEVANCE: The increased use of canagliflozin in nondiabetic patients warrants further investigation into the effects of this treatment on bone morphology.

REFERENCES: ¹Zhou+ 2019 ²Thraikill+ 2016 ³Yildirim+ 2023 ⁴Watts+ 2016

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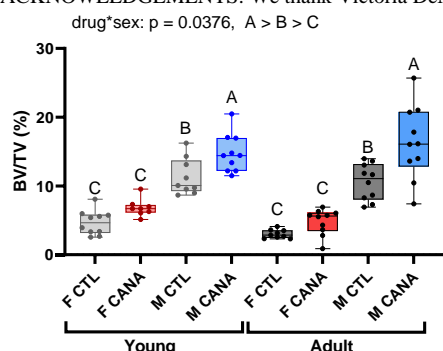


Figure 1. Canagliflozin increased bone volume fraction in male but not female mice.

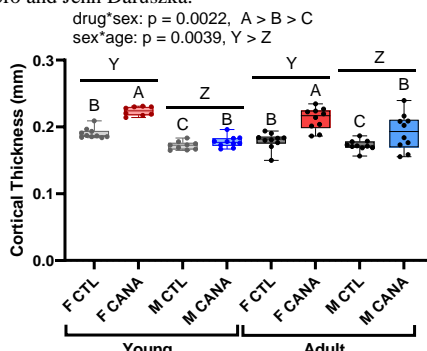


Figure 2. Canagliflozin increased cortical thickness in both males and females. Females had greater cortical thickness compared to age-matched males.

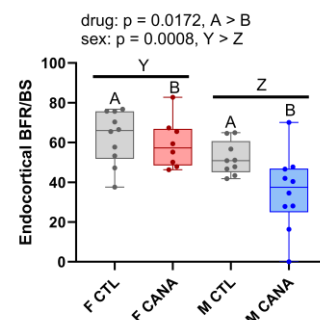


Figure 3. In young mice, canagliflozin reduced endocortical bone formation rate. Males had reduced endocortical bone formation rate compared with females.