



Supplementary Figure 1: The UM-HET3 mouse model. The UM-HET3 mice are generated via a 4-way cross of BALBc/J, C57BL6/J, C3H/HeJ, and DBA/2J strains. The specific crossing strategy yields an offspring in which each mouse is genetically unique but shares 50% identity with any other mouse in the offspring. Importantly, this “controlled” genetic heterogeneity enhances the relevance of the findings to human populations, in which genetic diversity is a significant factor, improves the generalizability of the results, and makes the UM-HET3 mice particularly valuable for studying complex traits (such as bone morphology and BMD). This model provides a rigorous, reproducible, alternative breeding structure.

The UM-HET3 mice were selected by the **National Aging Institute (NIA)**-sponsored **Intervention Testing Program (ITP)** to test therapeutic interventions. CANA treatment of the UM-HET 3 mice extended lifespan in males (PMID: 32990681). We have previously reported that long term CANA treatment (of 17 months) compromised bone morphology and integrity in male mice. In the current study we aimed at understanding the short and long-term effects of CANA treatment on bone metabolism.

CANA dose selection was based on previous studies examining the glucose-lowering effects of CANA and toxicological calibrations performed by multiple groups, including the ITP pharmacology core (PMID: 26970780, 30886225, 38801647, 38753230, 39004654). Concentrations of SGLT2 inhibitors ranging from 0.1 to 1,000 mg/kg/day have been used in mice and rats, with several studies using a daily gavage. In studies with longer treatment durations CANA was incorporated into diets at a fixed percentage (PMID: 24944269, 25344694, 23751087, 25000147). Several studies have evaluated multiple concentrations simultaneously, demonstrating a clear dose-response effect. Based on the dose-response data and the estimated glucose excretion target per day, as well as the goal of reducing postprandial glucose excursions and minimizing body fat and weight gain, the ITP program selected a body-weight-adjusted dose to achieve a target of 30 mg/kg/day in feed. We adopted this ITP study design.