Mineralocorticoid receptor inhibition mildly blunts age-dependent decline in vertebral bone in female C57BL/6 mice

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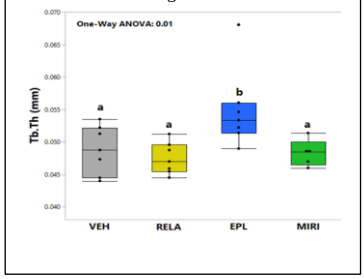
INTRODUCTION: Aging is typically characterized by the deterioration of the structural and functional characteristics of an organism over time. Osteoporosis is an age-associated metabolic bone disease affecting both men and women, although it is more prevalent in women due to hormonal changes that occur during menopause. Abnormal bone turnover occurs in the pathophysiology of osteoporosis, where the balance between bone formation and resorption is disrupted. Glucocorticoids (GCs) are positioned to play a critical role in the development of osteoporosis by influencing the behavior of many cells within the skeletal niche, including the propensity of GC to cause bone marrow mesenchymal stem cells (BMSCs) to differentiate into the adipogenic lineage at the expense of osteogenic differentiation. Although great attention has been paid to the negative skeletal effects of exogenous GC in causing glucocorticoid-induced osteoporosis, the effects of endogenous, physiological GC have been less extensively studied. GCs can exert downstream effects by activating the glucocorticoid receptor (GR), a transcription factor encoded by the NR3C1 gene. Interestingly, GCs can also bind with higher affinity to the mineralocorticoid receptor (MR; gene name NR3C2). We recently reported that mRNA expression of the MR was significantly and substantially increased in the bone of aged mice, with a ~70-fold increase in 23- as compared to 12-month-old animals [1]. The purpose of the current study was to explore the contribution of endogenous GC signaling mediated by either the GR or MR on the skeleton in aged female mice through the use of receptor-specific pharmacological inhibitors *in vivo*.

METHODS: Forty 18-month-old C57BL/6 female mice were obtained from the NIA rodent colony (NIA, Bethesda, MD, USA); two mice died prior to the onset of studies due to unknown causes. All mice had access to food and water *ad libitum* and were maintained on a 12/12-hour light-dark cycle in a standard temperature and humidity environment. Dual x-ray absorptiometry (DXA; Kubtec Digimus) analysis of bone mass and body composition, glucose tolerance tests, hang time tests of muscle endurance and muscle grip tests of muscle strength were collected at baseline prior to the onset of treatment. Mice were randomly assigned to receive chow containing the selective MR antagonist Eplerenone (EPL; 200 mg/kg/day), the selective GR antagonist Relacorilant (RELA; 60mg/kg/day) [2], the dual GR/MR antagonist Miricorilant (MIRI; 60mg/kg/day) [3], or a control base diet (VEH; Teklad 2018) for eight weeks. Body mass and food consumption rate were monitored throughout the treatment. DXA, hang time, and muscle grip tests were repeated during the 4th and 8th week of treatment and glucose tolerance tests were repeated during the 8th week of treatment. During the 7th week of treatment, mice were given access to running wheels (Columbus Instruments) to quantify voluntary physical activity. Calcein was injected on days 7 and 1 prior to sacrifice to label mineralizing bone surfaces for dynamic histomorphometry. Mice were sacrificed at the conclusion of the 8th week of treatment. Serum, hindlimb bones, lumbar vertebrae, skeletal muscles, interscapular brown adipose tissue, gonadal white adipose tissue, livers, and hearts were collected at sacrifice. Femurs and L5 vertebrae were analyzed by microCT (Skyscan 1272, 9-micron resolution) to measure cortical and trabecular bone architecture. Variables of interest were compared between groups via one-way ANOVA followed by Student's t-test post-hoc pairwise comparisons.

RESULTS: After sacrifice, eight mice were excluded from further study upon the discovery of non-study-related aging pathologies at necropsy (e.g., tumors; VEH: 2, RELA: 2, MIRI: 3, EPL: 1). An additional four mice were removed from further study for having lost over 14% body mass during the study (VEH: 0, RELA: 1, MIRI: 1, EPL: 2); final sample sizes were VEH: 7, RELA: 7, MIRI: 5, EPL: 7. The drug-treated mice tended to lose weight over the course of the study, showing trends for more negative changes in body mass as compared to VEH-fed mice in terms of percent changes in body mass at 4- and 8-wks of study against baseline (p_{ANOVA} = 0.056 at 4 wks, 0.046 at 8 wks). DXA scans revealed that body composition was altered at 4 wks of treatment, showing reduced %lean and increased %fat in the RELA- and MIRI-treated mice as compared to the EPL and VEH-treated animals (p_{ANOVA}=0.012), but these differences resolved by 8 wks of treatment. There were no effects of treatment on muscle grip strength, muscle endurance, or voluntary physical activity at either 4- or 8 wks of treatment (p_{ANOVA} >0.195). Lumbar vertebrae (L4-6) BMD measured by DXA demonstrated a mild beneficial effect from EPL treatment, showing a +4.6% increase of L4-6 BMD between 0 and 4 wks of treatment as compared to the other groups that had either a net loss or minimal net gain (VEH: -4.8%, RELA: -10.4% and MIRI: +0.1%, p_{ANOVA}=0.012), although this difference was no longer statistically significant by 8 wks of treatment (EPL: +0.3%, VEH: -9.4%, RELA: -5.0%, MIRI: -2.0%; p_{ANOVA}=0.181). However, higher resolution microCT analysis of L5 trabecular bone revealed that mice treated with EPL presented with a significant increase in Tb.Th as compared to other treatment groups (p_{ANOVA}= 0.010; post hoc testing of EPL vs. other groups: p<0.011) **(Figure 1).** No differences were observed between groups in terms of mid-femoral BMD (p_{ANOVA} >0.162), cortical bone architecture by microCT (p_{ANOVA} >0.642), or trabecular bone in the distal femoral metaphysis (p_{ANOVA} >0.142), except for a mild trend for elevated trabecular bone volume fraction in RELA-treated mice (p_{ANOVA} =0.096) without significant changes in Tb.Th, Tb.Sp, or Tb.N. Similarly, no differences were observed between group in terms of organ weights (p_{ANOVA} >0.162), except for an increase in BAT mass normalized to body mass in MIRI-treated mice (p_{ANOVA} =0.007; post-hoc testing of MIRI vs. other groups: p<0.008).

Figure 1



DISCUSSION: We recently reported that mRNA expression of MR is upregulated in murine bone with age [1], suggesting that MR may be available to mediate effects of endogenous GC in the aged skeleton [7]. Results presented here partially support this hypothesis, as mice treated with the MR antagonist EPL demonstrated a mild but statistically significant benefit of treatment in lumbar vertebral trabecular bone. EPL is an FDA-approved MR antagonist used in management of hypertension [4], and both GR and MR are expressed in the human skeleton [2,3]. Interestingly, MR inhibitors have been reported to reduce fracture risk in hypertensive patients [5], although it is unclear whether this benefit is from a direct effect on bone or secondary to improving cardiac function. RELA and MIRI are investigational drugs currently in clinical trials, where RELA is being tested for efficacy in treating the effects of GC excess in Cushing syndrome and adrenal adenomas (e.g., NCT03604198, NCG04308590) and MIRI is being tested for efficacy in blunting weight gain from antipsychotic medications [3], and in patients with nonalcoholic steatohepatitis (e.g., NCT05117489, NCT05553470).Neither RELA nor MIRI showed a robust beneficial effect on bone, and both RELA and MIRI altered body composition, decreasing %lean and increasing %fat mass at 4 wks of treatment, consistent with reported effects of deleting GR in the skeleton with Osx1-Cre [7], supporting the importance of proper GC-GR signaling for musculoskeletal health. It is important to note that, unlike genetic models, the pharmacological inhibitors employed in the current study are broadly acting, making it more difficult to determine their specific mechanisms of action in discrete tissues; confounding effects of weight loss may have also affected the measured properties. Site-specific histological analyses and *in vitro* studies with isolated cell types are ongoing to refine direct mechanisms of action for these drugs in bone as well as bone-initiated crosstalk with other body systems.

SIGNIFICANCE/CLINICAL RELEVANCE: GC-MR signaling may represent a novel therapeutic target for improving musculoskeletal health during aging.

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REFERENCES: [1] Bensreti et al., ORS Annual Meeting 2022; Oral Presentation # 1812. [2] Viho et al., J Endocrinol 2022; 256(2): e220263. [3] Hunt et al., J Clin Psychopharmacol 2021; 41(6): 632. [2] Beavan et al., JBMR 2001; 16(8): 1496. [3]. Fumoto et al, BBRC 2014; 447(3): 407 [4] Pitt et al., Circulation (108):2003. [5] Carbone et al., JACC 52(2): 2008. [6] Bensreti et al., COR (21):2023 [7] Pierce et al., JBMR (37) 2022. IMAGES: Figure 1