Bone loss after acute sex-hormone removal via gonadectomy prior to skeletal maturity most striking in male but not female animals

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Abstract

Menopause, an age-related loss of sex hormone production in women, is one of the most common causes of osteoporosis. Previous work has established that this loss of sex hormones, in particular estrogen, causes dramatic loss of bone volume and strength. Similarly, removal of sex steroids results in acute loss of bone mass in adult animals. Mouse models of sex steroid-deficiency include surgery removing the sex organs (orchidectomy, ORX, for males; ovariectomy, OVX, for females) are commonly used to understand the role of sex steroids in bone, but are typically preformed at animal maturity (16 weeks of age) and are analyzed six weeks post-operation. This study aimed to determine whether acute removal of the male or female sex hormones prior to maturity would impact the cortical and trabecular bone volume. Gonadectomy or sham operations were performed on mice at 11 weeks of age, and femurs were then harvested either 2 weeks (13 weeks of age) or 4 weeks post-surgery (15 weeks of age). Analysis of the cortical and cancellous bone volume of the femur were assessed by microCT. In cancellous bone, male animals two and four weeks ORX demonstrated decreases in the following parameters compared to sham operated, age-matched controls (2 week; 4 week): bone volume (BV/TV, -70.9%; -86.6%), tissue mineral density (V-TMD, -8.69%; -17.9%), trabecular thickness (TbTh, -31.9%; -27.8%), and trabecular number (TbN, -57.5%; -81.4%). In cancellous bone, female animals two and four weeks OVX demonstrated decreases in the following parameters compared to sham operated, age-matched controls: BV/TV (-61.2%; -41.0%), V-TMD (-30.7%; -15.6%), and TbN (-64.0%; -42.4%). In cortical bone, male animals four weeks ORX demonstrated decreases in the following parameters compared to sham operated, age-matched controls: cortical area (-13.8%), Endocortical bone surface (-7.10%), and TMD (-10.2%). In cortical bone, female animals four weeks OVX demonstrated decreases in only TMD (-7.50%) compared to sham operated, age-matched controls. In summary, the acute removal of sex hormones has a larger impact on cancellous bone in both males and females, with male animals showing increasing bone loss as time progressed. Further studies are needed to understand the underlying mechanisms behind the progressive bone loss seen in males after sex hormone depletion.

Background

Females undergo a rapid depletion of their estrogen levels once they enter menopause. It is understood that this decline in estrogen levels leads to increased activity of osteoclasts, more bone resorption, and ultimately a higher likelihood of developing osteoporosis. Males do not undergo a similar rapid loss of testosterone. This difference in depletion of sex hormones explains why women age 50 and older have a higher lifetime risk of fracture than men age 50 and older (1). However, it is common for males to lose testosterone levels as they age, and this loss of testosterone impacts bone mineral density. A previous study found that older men with higher testosterone levels better maintained their bone mineral density and had lower fracture risks (2). Other studies have postulated that testosterone stimulates osteoblasts to produce trabecular bone and aids osteocytes in preventing trabecular bone loss (3). While this provides insight into how chronic loss of testosterone impacts bone health, this study was aimed at discovering how acute removal of sex hormones impacted male and female mice.

Materials & Methods

Images of the femurs were obtained using a SkyScan 1176 micoCT. The microCT images of the individual femurs were then reconstructed using Nrecon and DataViewer software. To analyze the cortical and trabecular bone separately, region of interests (ROIs) were drawn around the trabecular and cortical areas of each femur. Analysis programs were then executed to report the data of the cortical and trabecular bone volume. Tissue mineral density (TMD) was normalized to a phantom using houndsafield units.

Figure 1 – Trabecular bone loss in both male and female animals after both 2 and 4 weeks post gonadectomy, but only males have decreased volumetric tissue mineral density

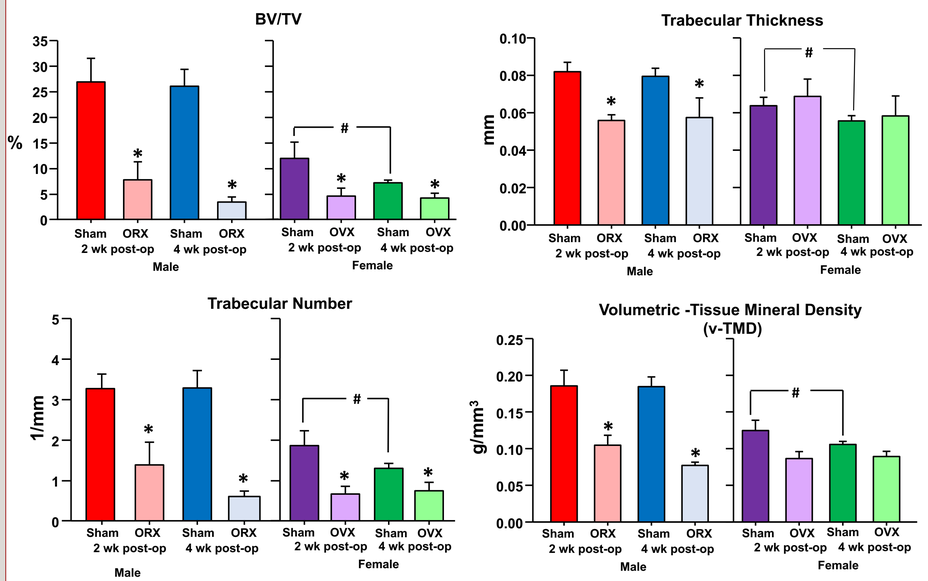
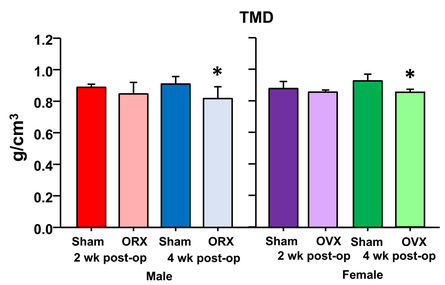


Figure 1 – Bone Volume/ Tissue Volume, Trabecular Thickness, Trabecular Number, and Volumetric Tissue Mineral Density (v-TMD) from male and female sham and operated animals 2 and 4 weeks post gonadectomy. N = 4-5/group.

*** = p <0.05 via Two-way ANOVA vs. Sham , #= p <0.05 via Two-Way ANOVA.**

Figure 2 – 4 weeks post-gonadectomy, males lose cortical bone volume, but both males and females lose TMD



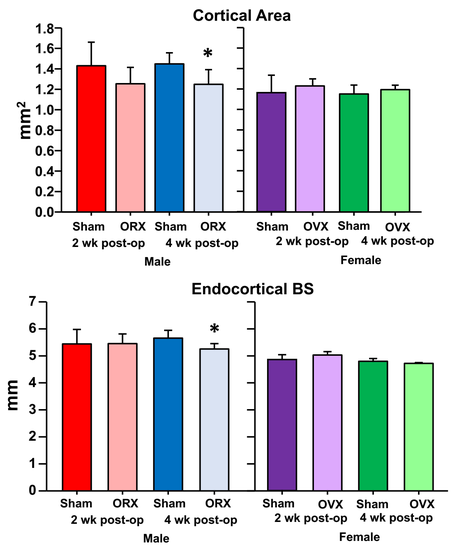


Figure 2 –Tissue Mineral Density (TMD), Cortical Area, and Endocortical Bone Surface from male and female sham and operated animals 2 and 4 weeks post gonadectomy. N = 5/group.

*** = p <0.05 via Two-way ANOVA vs. Sham , #= p <0.05 via Two-Way ANOVA.**

Summary

- **The acute removal of sex hormones prior to skeletal maturity negatively impacts the tissue mineral density of bone in both male and female mice.**
- **Cancellous bone is more sensitive than cortical bone to the decline in circulating sex hormones.**
- **Male mice demonstrated a larger decline in both cancellous and cortical bone after removal of sex hormones.**

Conclusions

Sex hormones, particularly androgens in males, are crucial for proper development of the skeletal system. Testosterone seems to play a role in maintaining cancellous bone in males. The large decreases in bone volume and tissue mineral density suggest without proper exposure to sex hormone levels, bone achieves a lower peak bone mineral density.

Future studies will be needed to assess the cellular mechanisms responsible for this sex-dependent bone volume and mineral density loss with acute sex hormone removal.

References

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