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Bone Strength and Distal Femur Trabecular Thickness in Sleep-Deprived Ovariectomized Rats Treated with Zoledronate

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Introduction

Approximately 200 million people worldwide suffer from osteoporosis, a disease characterized by the degradation of bone leading to decreased bone strength and bone mineral density (BMD) and increased risk of fracture^[1]. Data suggested that 25% of Americans reported insufficient sleep (>6 hr over more than 15 days/month),^[2] with individuals who logged less than 6 hours of sleep per night suffering a significantly lower BMD than those who reported 6-8 hours of sleep per night^[3]. Heightened adverse physiological effects, including lowered BMD, resulting from sleep deprivation were identified in menopausal females^[4]. With nearly a 2-fold increase in the number of females who have filled executive level business positions, the physiological response to the heightened levels of stress may leave females subject to greater bone health concerns.^[5] Previously, our lab examined the BMD of sleep deprived animals, but this work focused upon trabecular thickness as an additional tool in the evaluation of osteoporosis. BMD can be evaluated via trabecular thickness. To minimize the bone degradation occurring in sleep-deprived individuals, bisphosphonate-based medications like Zoledronate are often prescribed. Zoledronate works to inhibit osteoclastic activity and function by blocking a portion of the mevalonate pathway through the inhibition of farnesyl pyrophosphate synthase which restricts the prenylation of certain GTPases^[6]. These GTPases alter the signaling for osteoclastic function, thus reduce the bone degrading effects of osteoporosis^[6]. The purpose of this study was to evaluate the consequences of sleep deprivation on bone metabolism with and without the protective effects of Zoledronate in sleep-deprived, estrogen-deficient rats.

Methodology

Wistar female rats (mean weight of 341g) were ovariectomized to induce estrogen deficiency. These rats were randomly separated into four groups. The control (C, n=6) group was injected with 0.9% saline before the study and allowed 12 hours light and 12 hours dark. The Zoledronate (Z, n=9) group received an intraperitoneal injection (50 µg/kg) of Zoledronate at the same time. One sleep-deprived group (SD, n=9) was housed in the MMPM tank (Figure 1) that prevented sleep for 18 hours, then moved to a sleep chamber for 6 hours. A second sleep-deprived group (SDZ, n=9) was treated as the SD group but with the addition of 50 µg/kg Zoledronate before the study. Tibiae and femora were collected, wrapped in saline-soaked gauze and stored at -80 F. Subsequently, a DEXA scan, and a 3-point bending test were performed.

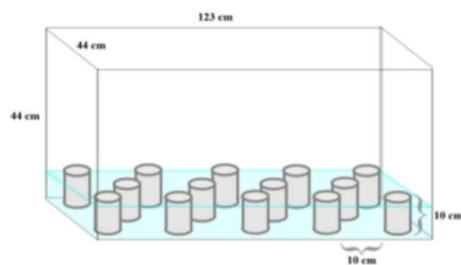
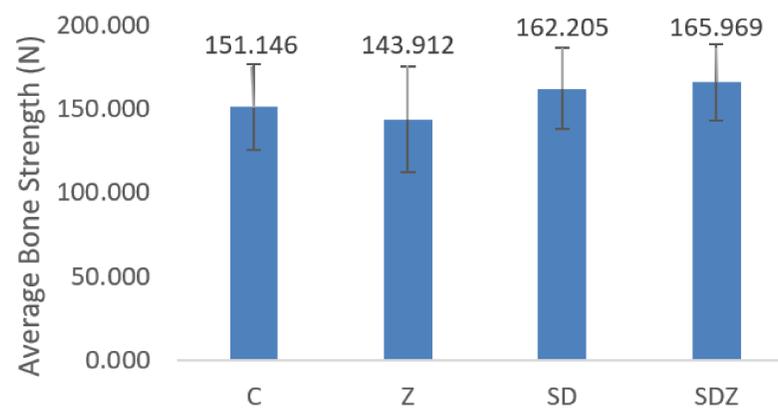


Figure 1: Modified Multiple Platform Method (MPPM)

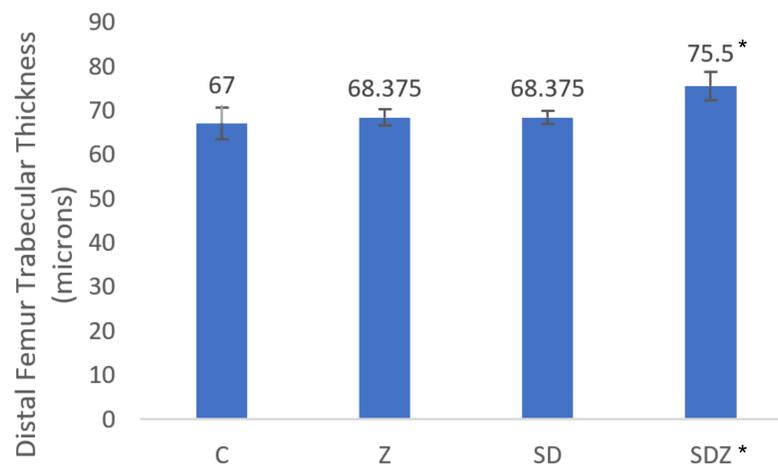
Results

Average Bone Strength of OVX Rats



Graph 1. Bone strength was assessed in control (C) group, animals receiving Zoledronate (Z), those animals sleep deprived (SD), and sleep deprived animals receiving Zoledronate (SDZ). There were no significant differences between the groups ($P>0.05$).

Distal Femur Trabecular Thickness of OVX Rats



Graph 2. Distal Femur Trabecular Thickness was assessed in control (C) group, animals receiving Zoledronate (Z), animals who were sleep deprived (SD), and sleep-deprived animals who received Zoledronate (SDZ). The SDZ group showed significance ($P=0.05$) compared to the SD group.

Discussion

The purpose of this study was to determine the effects of sleep deprivation on bone metabolism with and without Zoledronate treatments. Zoledronate was shown to increase both strength ($P>0.05$) and distal femur trabecular thickness ($P<0.05$). The study was conducted with a total of 29 animals which made the statistical analysis sensitive to variance (Graphs 1 and 2). Our results may have been more illuminating had we used a greater number of animals. Explanations for the increased bone strength and distal femur trabecular thickness of subjects in the sleep-deprived groups were derived based on Wolff's Law stating that mechanical load from the environment can induce bone remodeling. It was believed that the sleep-deprived rats were subjected to training effects from extended periods of standing on the narrow pillars (Figure 1) which would strengthen the trabeculae and cortical bone in response to the additional load. Great lengths were taken to construct a trial which equated the relative lifespan of rats to humans as determined by Sengupta where the relative age for humans during a postmenopausal period over 1 year was equal to 11.8 days in rats^[7]. It was determined based on the relative proportions that a 5-week trial would simulate the common 3-year design of many bisphosphonate clinical trials. It should be noted that even though the relative age between humans and rats was considered, rats may not be the preferred model for human physiology. Future studies may be fruitful if a different animal model is used. There is much work remaining that examines the consequences of sleep deprivation in postmenopausal women.

Citations

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