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The Effects of Zoledronate and Sleep Deprivation on the Distal Femur Trabecular Thickness of Ovariectomized Rats: Application of Different Statistical Methods

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The Effects of Zoledronate and Sleep Deprivation on the Distal Femur Trabecular Thickness of Ovariectomized Rats: Application of Different Statistical Methods

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Introduction

Osteoporosis is a disease of the skeletal system in which bone is compromised leading to increased risk of fracture [1]. One in three women will experience a fracture due to osteoporosis in their lifetime, with postmenopausal women being the most susceptible [1]. One of the main intrinsic factors that increase the risk of osteoporotic fracture in postmenopausal women is discontinuities in the microarchitecture of bone [1]. Since trabecular bone is 3-4 times more metabolically active than cortical bone [2], distal femur trabecular thickness was used as the parameter for bone quality in this study. Data suggested that 25% of Americans reported insufficient sleep (>6 hr over more than 15 days/month) [3]. Heightened adverse physiological effects, including lowered BMD and decreased bone quality, resulting from sleep deprivation were identified in menopausal females [4], therefore, this study aimed to incorporate sleep-deprivation as a variable in bone health. Bisphosphonates, such as Zoledronate, are a common treatment for osteoporosis. Zoledronate attempts to inhibit osteoclastic activity by blocking a portion of the mevalonate pathway through the inhibition of farnesyl pyrophosphate synthase which restricts the prenylation of certain GTPases [5]. 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.

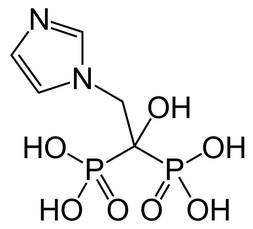

Figure 1: Zoledronate

Methodology

Ovariectomized Wistar female rats (n=31) were received from the Wistar Institute and given a 1-week adjustment period in standard conditions. Standard conditions included a 12-hour light/dark cycle and food and water provided ad libitum. The rats were randomly assigned into 4 groups including a Control (C, n=4), Zoledronate (Z, n=9), Sleep-deprived (SD, n=9), and Sleep-deprived + Zoledronate (SDZ, n=9). The C and SD groups were given a one-time intravenous injection of 0.45mL of 0.9% saline. The Z and SDZ group received a one-time intravenous injection of 50ug/kg body weight of 10% Zoledronate. After the injection, the 5-week protocol was started. The C and Z groups were kept in standard housing. However, the SD and SDZ groups were housed in MMP tanks *(Figure 2)* which prevented sleep for 18 hours, then moved to the sleep chambers for 6 hours. After 5 weeks, the rats were exsanguinated and tibiae and femora were collected, wrapped in saline-soaked gauze, stored at -80°C, and shipped to Novartis Institute for Biomedical Research where high-resolution micro-computed tomography scans were performed *(Figure 2)*. Statistical analysis included ANOVA, Tukey, Bonferroni, and Kruskal Wallis tests as well as the creation of an interaction graph using ANCOVA in R Studio.

Figure 2: Modified Multiple Platform Tank

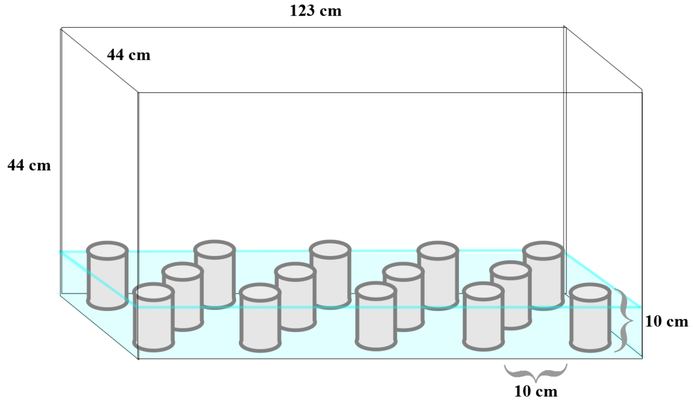

Figure 3: Ex vivo location of µCT

Results

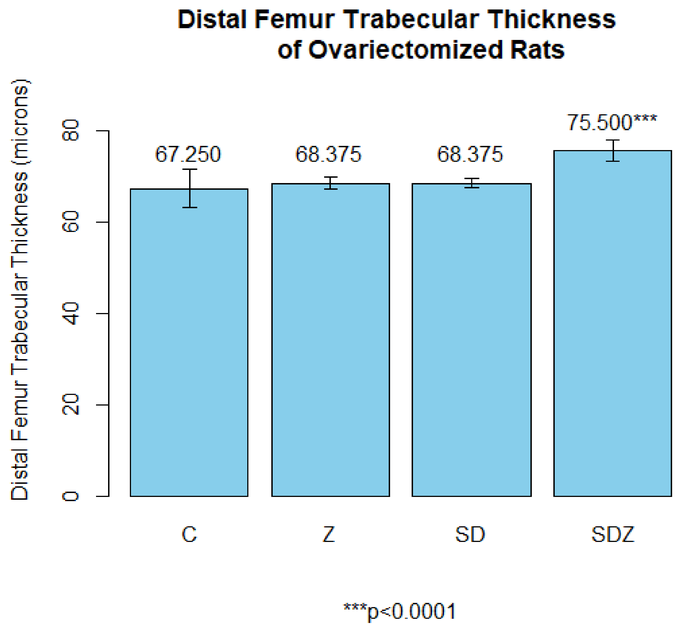

Figure 4: The average distal femur trabecular thickness and SE of the C, Z, SD, and SDZ groups. The SDZ group had a significant difference in distal femur trabecular thickness compared to the Control group (75.5 and 67.25 microns, respectively; p<0.0001).

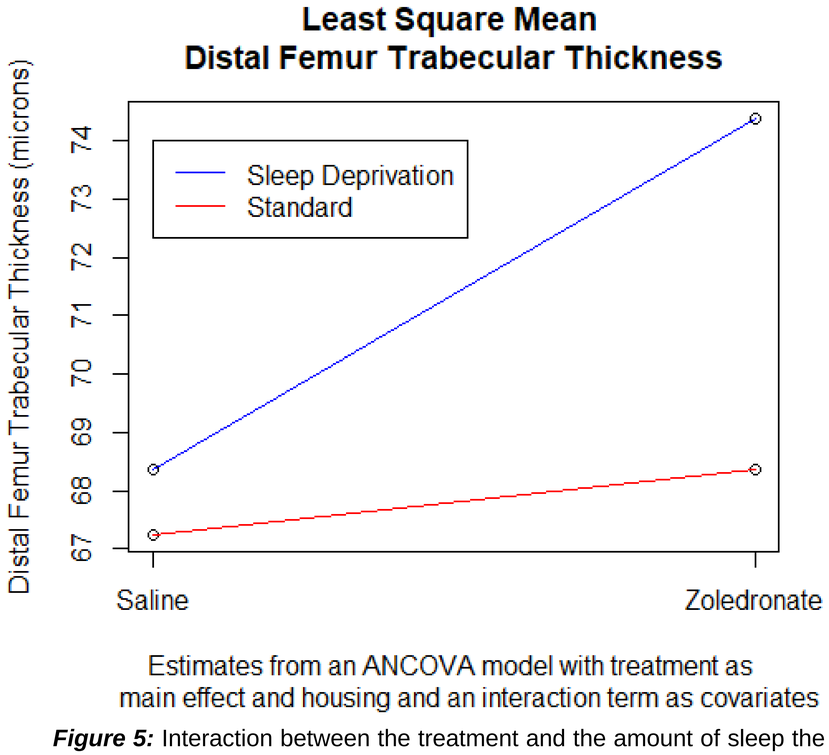

Figure 5: Interaction between the treatment and the amount of sleep the rats received (p=0.0078; R^2=0.612)

The purpose of this study was to evaluate the trabecular thickness using a commonly prescribed bisphosphonate in attempts to explore potential advantages in sleep-deprived, ovariectomized rats. An analysis of covariance (ANCOVA) model was used to predict trabecular thickness with treatment group as a main factor and housing as a covariate, including a factor for the interaction of treatment and housing. There was a significant treatment and housing effect on trabecular thickness (each p<0.0001), with the interaction also being a significant (p=0.0078), suggesting treatment affected mean trabecular thickness differently by which housing rats were in. Overall the model achieved an R^2 of 0.612. Assumptions of normality of the residuals were not investigated further, but a non-parametric Kruskal Wallis test was run to verify the pattern of the ANCOVA results (p<0.001). Further investigation using Tukey (confirmed with Bonferroni) multiple comparisons tests revealed there were significant differences between SDZ and C (p<0.0001), SD and SDZ (p<0.0001), and the Z and SDZ groups (p<0.0001).

Discussion

Using more robust statistical analyses on previously gathered data has revealed a possible physiological interaction between the amount of sleep and the use of Zoledronate. Further studies are needed to determine the mechanism behind the physiological interaction, but results could have a profound impact on the use of Zoledronate in modern society where many people are getting less sleep because of work demands.

Citations

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