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The Effects of Chronic Sleep Deprivation on Tumor Necrosis Factor Alpha and Bone Strength in Menopausal Rats

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INTRODUCTION

Sleep deprivation has become increasingly prevalent among industrialized nations, and the average sleep duration for people in the United States of America has decreased from 9 hours per night in 1910 to 7 hours per night in 2010. Sleep deprivation has been linked to osteoporosis, a chronic metabolic skeletal disease characterized by low bone mass and microarchitectural degradation of bone tissue as an imbalance of osteoblasts and osteoclasts. Sleep deprivation also disrupts the Circadian rhythm of humans, which not only challenges the immune system, but compounds the deleterious effects of post-menopausal estrogen deficiency. Immunologic cytokines, such as tumor necrosis factor alpha-type (TNFα) are elevated in periods of stress associated with sleep deprivation. TNFα is involved in systemic inflammation, fever, cell apoptosis, and it has been shown to directly and indirectly increase osteoclastic activity. Bisphosphonates, like Zoledronate, are the most commonly-prescribed treatment for osteoporosis because they decrease osteoclastic activity. Bisphosphonates are a class of bone-seeking agents that are selectively internalized by osteoclasts and inhibit bone resorption through accumulation of toxic metabolites within osteoclasts, leading to apoptosis. This study evaluates the effects of chronic sleep deprivation and Zoledronate on the concentration of tumor necrosis factor alpha (TNFα), and the relative bone strength of female, ovariectomized (OVX) Wistar rats (simulating menopause) subjected to a five-week sleep deprivation protocol.

METHODS

Thirty-two female, ovariectomized (OVX) Wistar rats ($\bar{x}$ mass = 386± 6.74 g) were randomly placed in control (C), Zoledronate (Z), sleep deprived (SD), sleep deprived with Zoledronate (SDZ) groups. SD and SDZ groups placed in MMPM tank (figure below). C and Z groups placed in control tank. Groups were administered injections of either 0.9% saline solution or Zoledronate (50 µg/ml). Rats were given a one week adaptation period prior to the start of the sleep deprivation protocol. Sleep deprivation groups were randomized into 3 different sleep deprivation protocols. Following experimental protocol, rats were humanely sacrificed and blood serum was collected and was added to TNFα ELISA Assay. Rat femurs were harvested, cleaned with a saline (0.9%NaCl) and dried, then placed on apparatus to perform 3-Point Bending Test. Force displacement data was collected.

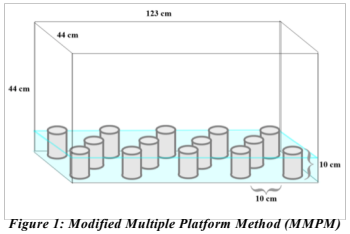

Figure 1: Modified Multiple Platform Method (MMPM) utilized for rats in sleep deprivation group.

RESULTS

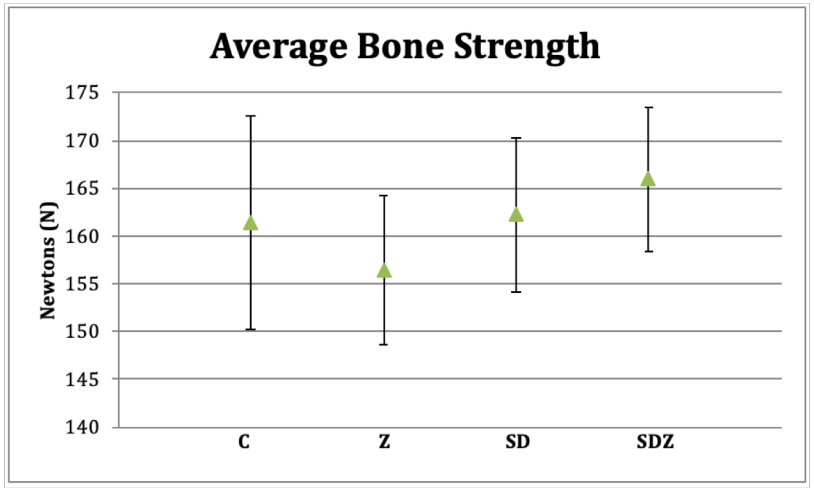

Figure 2: Average bone strength (N) determined by 3 point bending test for the 4 different groups.

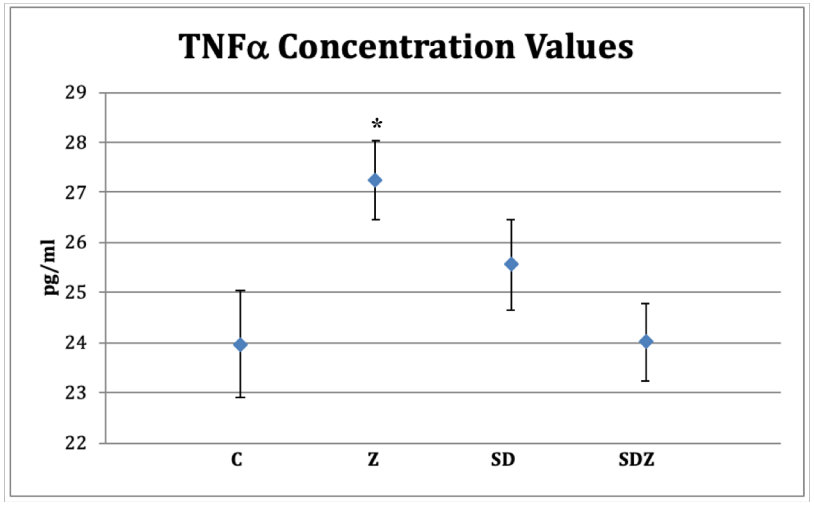

Figure 3: TNFα concentration values across the 4 different groups

DISCUSSION

Significant changes in overall bone strength were not found as a result of sleep deprivation or Zoledronate injection over the course of this five-week study. The lack of significant changes may be attributed to the short time frame of the study and was not long enough to detect significant microarchitectural differences in the bone. However, TNFα levels in the blood were significantly higher in the Z group compared to the other groups; this may be attributable to Zoledronate's propensity to induce transient fever for twenty-one days after injection. SD and SDZ groups were comparable with C group, which may may be attributable to a challenge of the animals' immune systems. Further research measuring the differences in average bone strength for a longer experimental period will determine if Zoledronate and sleep deprivation play a significant role in the degradation of bone in rats. As sleep deprivation and a post-menopausal status is common in women with career and family, research involving sleep-deprived, ovariectomized rats may lay the foundation for a better understanding of the factors affecting bone health among aging executive women.

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