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The Effects of Sleep Deprivation on TNF- Alpha in Menopause-Induced Rats

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Abstract
Post-menopausal osteoporosis as a consequence of estrogen depletion is a growing concern for women in the United States. As more women take on executive positions and experience sleep deprivation, there is the potential for up regulation of pro-inflammatory cytokines, such as Tumor Necrosis Factor-Alpha (TNF-α). It follows that the homeostatic imbalance of osteoclastic and osteoblastic activity leads to a greater risk of disease. Bisphosphonates generally, and Zoledronate specifically, works by attempting to correct the imbalance of osteoblasts and osteoclasts decreasing the number of osteoclasts. This current study investigated the impact of Zoledronate on the concentrations of TNF-α in 32 ovariectomized Wistar rats. Throughout a five-week period of sleep deprivation cycles, the concentrations of TNF-α were collected and examined. It was originally hypothesized that the sleep-deprived group of rats would have the highest concentration of TNF-α due to the biological stress associated with insomnia. However, TNF-α levels were significantly higher in the Zoledronate group than both the control and sleep-deprived groups, as well as the sleep-deprived with Zoledronate groups ($p<0.01$). We ascribe this to bisphosphonate induced transient fever seen in Zoledronate usage in previous studies (Dicuonzo, 2003). It is also suspected that the low concentrations of TNF-α in the sleep-deprived groups are seen due to the short time frame of this experiment along with a challenged immune system in the animals. Future studies should address this issue by involving a longer cycle of sleep deprivation along with a greater sample size.

Keywords: concentrations, deprivation, osteoporosis, necrosis

Introduction
Osteoporosis is a metabolic bone disorder characterized by a loss of bone mass and a deterioration of the tissue that can ultimately lead to compromised bone strength and an increased risk of fractures. The disease can affect a wide range of individuals of both sexes but is most prevalent among older women, particularly Caucasians (Cosman et al., 2014). Post-menopausal women are considerably more susceptible to the disease due to their significant decreased levels of estrogen. Estrogen deficiency directly causes a notable decrease in bone due to the role estrogen plays in osteoclast apoptosis. Without the ability to regulate osteoclast apoptosis, bone resorption goes unmediated and results in a decrease in bone strength (Kameda et al., 1997).

Chronic sleep deprivation is a growing concern in recent years with most individuals reporting less than 6.5 hours of sleep per night. Studies have confirmed that a deficiency in sleep has adverse effects of metabolic and endocrine function (Specker et al., 2007). Approximately 67% of adults over the age of 65 have at least one sleep-related complaint (Foley et al., 2003). Although the mechanism is not yet well understood, studies are supporting a link between sleep duration and bone metabolism (Fu et al., 2011). As more women, who are at a higher risk for bone degradation, are taking on executive professions that increase levels of stress and decrease the amount of sleep per night, there could be a great impact on bone health.

TNF-α is a pro-inflammatory cytokine that is directly involved in bone turnover by inhibiting mature osteoblasts (Kotrych., 2016). Cytokines are proteins that are able to interact and carry out integral functions between cells when released. Cytokines can have autocrine functions,

when the action is performed on the cell that released it, they have paracrine actions, when they have a specific function with a neighboring cell, and they can also have endocrine actions when the action is on distant cells in the body (Zhang et al., 2009). When the body is deficient in estrogen, it will produce rapid resorption of bone caused by TNF-α and will result in lower bone density and strength. TNF-α inhibits bone-forming cells, osteoblasts, from maturing and consequently no more bone will form (Azuma et al., 2000). This cytokine decreases bone matrix production and mineralization. TNF-α has also been seen in recent findings to promote the formation of bone breaking cells through osteoclastogenesis (Osta, Benedetti, & Miosecc, 2014). When the body is in a state of inflammation and TNF-α is being secreted in high amounts, the effects on the bone can be detrimental due to the upregulation of the osteoclasts to tear down the bone and the inhibition of the osteoblasts to rebuild causing an imbalance.

Methods

As cited in earlier studies (Nolte, Frisch, & Lopez, 2020), thirty-two female, ovariectomized Wistar (321 ± 28 g) rats were randomly placed in Control (C), Zoledronate (Z), Sleep-Deprived (SD), and Sleep-Deprived with Zoledronate (SDZ) groups. The SD and SDZ groups underwent a five-week sleep deprivation protocol using a MMPM tank while the C and Z groups were placed in control tanks. The C group was given an injection of 0.9% saline solution and the Z and SDZ groups were administered a dose of 10% mL/g Zoledronate. The experimental group rats were randomly placed into one of three possible sleep schedules following a one-week adaptation period. After the five-week sleep deprivation protocol, the rats were injected with ketamine and humanely sacrificed. Blood was collected from each animal for use in the TNF-α ELISA Assay.

Experimental and Control Groups

Animals were randomly placed in Control (C), Zoledronate (Z), Sleep-Deprived (SD), and Sleep-Deprived with Zoledronate (SDZ) groups. The control group and Zoledronate groups were in the control tank for an average of eighteen hours a day. (Table 1) In this tank they were able to comfortably sleep, exercise, and socialize. The SD and SDZ groups were each placed in their own experimental tank following the Modified Multiple Platform Method. (Figure 1) Each group was either injected with Zoledronate or .9% saline with a dose of 10% ml/g. The rats were then given a one-week adaption period. Saline was administered to the Control group and Sleep-Deprived groups to control for the stress of the injection. Both the saline and the Zoledronate were injected into the tail vein using a standard insulin needle. All animals were weighed once a week.

Table 1: Table of time allowed for sleep chosen by number generator

Day	Time Awake (hr)	Time Asleep (hr)
1	17	7
2	18	6
3	19	5
4	17	7
5	18	6
6	19	5
7	18	6
8	17	7
9	19	5
10	17	7
11	17	7
12	17	7
13	19	5
14	17	7
15	17	7
16	17	7
17	17	7
18	19	5
19	19	5
20	19	5
21	18	6
22	19	5
23	18	6
24	19	5
25	18	6
26	19	5
27	19	5
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30	18	6
31	18	6
32	18	6
33	18	6
34	17	7
35	17	7

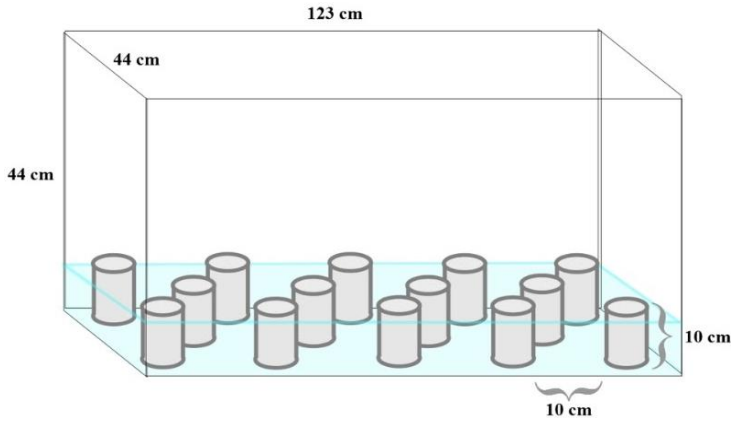

Fig 1: Diagram of Modified Multiple Platform Method experimental tank

Sleep Deprivation Protocol

Thirty-two female ovariectomized Wistar rats were obtained from Charles Rivers Laboratories International, Inc.. Upon reception, the rats were allowed an adaptation period of one week. During this week, the animals were kept on a twelve-hour light/dark cycle (lights on at 7:00am). Food and water were available to the rats, ad libitum.

After the completion of the one-week adaptation period the rats were subjected to CPSD. To simulate irregular sleep cycles experienced by women in executive positions, the rats were placed into one of three variable sleep cycles each night for five weeks. The values for these sleep cycles were chosen in order to resemble the sleep deprivation protocol that can be found in various sleep studies. The entire sleep schedule for the five-week study was generated by the use of a random number generator. The rats were woken at 14:00 each day and placed in their respective tanks. After undergoing the designated amount of sleep deprivation, the rats were placed back into their individual cages and allowed to sleep for the allotted amount of time. Food and water were available ad libitum for both of these periods.

Serum Collection and Analysis

Following the conclusion of the Sleep Deprivation protocol, animals were injected with ketamine and humanely sacrificed via puncturing their heart. Blood was collected from each animal and centrifuged at 14,000 RPM for 5 minutes. Following centrifugations, supernatant (plasma) was decanted and flash frozen using liquid Nitrogen. Serum samples were stored at -80 C° for approximately two months before utilization in a TNF-α ELISA assay.

The left and right femurs were obtained from each animal and cleaned. Femurs were wrapped in saline-soaked gauze and frozen at -80 C°.

TNF-α ELISA Assay

A Rat TNF-α ELISA MAX™ Deluxe kit was obtained from Biolegend, Inc. (San Diego, Ca, USA). PBS and Tween-20 (Wash Buffer) were provided by the University of California Irvine Institute of Immunology. Stop Solution ($2NH_2SO_4$) was created using reagents provided from Chapman University. Serum samples stored at -80 C were thawed, and diluted (1:8) prior to use. Serum samples were diluted prior to use in the assay (1:8). The absorbance for the ELISA samples was measured at 450 nm.

Results

The TNF-α serum values were obtained and measured in pg/mL. The control group's average value for TNF-α concentration was 23.97 with a standard deviation of 1.87. The Zoledronate treated group's average value for TNF-α concentration was 27.26 with a standard deviation of 2.22. The sleep-deprived group's average value for TNF-α concentration was 25.56 with a standard deviation of 2.56. The sleep-deprived Zoledronate treated group's average value for TNF-α concentration was 24.02 with a standard deviation of 2.17.

Discussion

It was expected that the concentration of TNF-α would be increased in the rats that experienced sleep deprivation and lower in those that were sleep-deprived but given the Zoledronate treatment. This was the outcome seen as the sleep deprivation group had a mean of 25.56 pg/mL with a standard deviation of 2.56 and the sleep deprivation group treated with Zoledronate had a mean of 24.02 pg/mL with a standard deviation of 2.17. Although the difference was not great, we believe that sleep deprivation protocol may have been too brief to elicit significant change. With a longer time frame, based on previous research, sleep-deprived groups would have a significantly larger concentration of the TNF-α.

The Zoledronate group's results were contrary to what was expected. The rats treated with bisphosphonates were seen to have the greatest concentration of TNF-α although it was expected to have the lowest concentrations. As seen in Figure 2, TNF-α levels were significantly higher in the Zoledronate group than both the control and SD, and SDZ groups ($p<0.01$). In humans, Zoledronate has been shown to produce symptoms of transient fever for twenty-one days after injection of the drug. It is plausible that the TNF-α levels could be elevated due to the stress induced by transient fever. There was a modest depression of the Zoledronate Sleep-Deprived group compared to the Sleep-Deprived group who did not receive Zoledronate, but did not reach a critical value of significance. It is possible that while the values between these two groups were not significantly different, further study is warranted to examine the effects of a longer exposure to sleep deprivation or perhaps variances in bisphosphonate dosage.

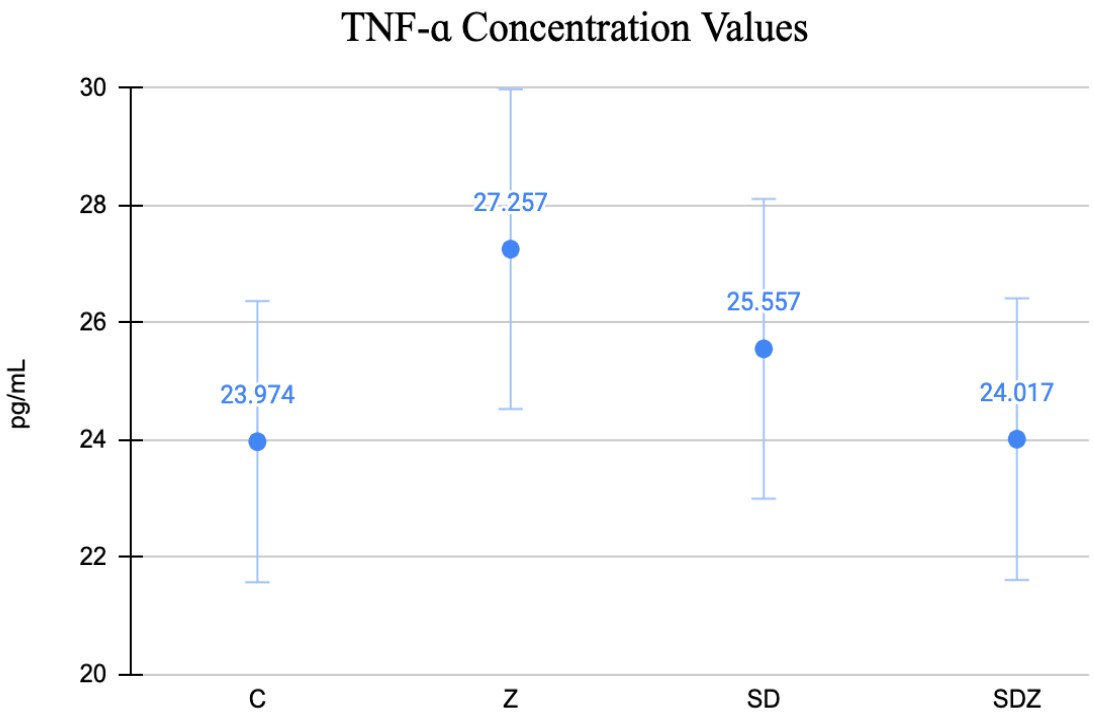

Fig 2: Graph of average TNF-α concentration values (± pg/mL)

Table 2: Average TNF-α Concentration Values for Control, Zoledronate, Sleep-Deprived, and Sleep-Deprived Zoledronate Treated (± pg/mL)

Group	Mean (pg/mL)	Standard Deviation (pg/mL)
Control (C)	23.97	1.87
Zoledronate (Z)	27.26	2.22
Sleep Deprived (SD)	25.56	2.56
Sleep Deprived Zoledronate Treated (SDZ)	24.02	2.17

Conclusion

Significant changes in TNF-α concentrations were not seen as a result of the sleep deprivation or Zoledronate injection. One limitation of this study was the short time frame of the sleep deprivation protocol and small sample size. Future studies regarding sleep deprivation should address this issue and allow for a longer time frame along with a larger sample size in order to explore the relationship further between sleep deprivation and loss of estrogen. There is a growing population of women who hold executive positions, balance family and work and do so at the expense of sleep. This group deserves more intense scrutiny into the ways bone health may be sustained.

References

1. Akira, Shizuo et. al (1990). Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF-α). Institute for Molecular and Cellular Biology, Osaka University, 1-3.
2. Azuma, Y., Kaji, K., Katogi, R., Takeshita, S., & Kudo, A. (2000). Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. *The Journal of Biological Chemistry*, *275*(7), 4858–4864.
3. Cosman, F., de Beur, S. J., LeBoff, M. S., Lewiecki, E. M., Tanner, B., Randall, S., & Lindsay, R. (2014). Clinician's Guide to Prevention and Treatment of Osteoporosis. *Osteoporosis International: With Other Metabolic Bone Diseases*, *25*(10), 2359.
4. Dicuonzo, G., Vincenzi, B., Santini, D., Avvisati, G., Rocci, L., Battistoni, F., Gavasci, M., Borzomati, D., Coppola, R., & Tonini, G. (2003). Fever after zoledronic acid administration is due to increase in TNF-alpha and IL-6. *Journal of Interferon & Cytokine Research: The Official Journal of the International Society for Interferon and Cytokine Research*, *23*(11), 649–654.
5. Du, D., Zhou, Z., Zhu, L., Hu, X., Lu, X., Shi, C., Chen, F., and Chen, A. (2018) TNF-α-suppresses osteogenic differentiation of MSCs by accelerating P2Y2 receptor in estrogen-deficiency induced osteoporosis. *Bone.* 117(2018), 161-170.
6. Everson, C. A., Folley, A. E., & Toth, J. M. (2012). Chronically inadequate sleep results in abnormal bone formation and abnormal bone marrow in rats. *EXPERIMENTAL BIOLOGY AND MEDICINE -MAYWOOD NJ-*, (9), 1101.
7. Fu, X., Zhao, X., Han, L., Jiang, F., Ma, X., & Zhu, S. (2011). Association between sleep duration and bone mineral density in chinese women. *Bone*, 2011(49), 1062-1066.
8. Hu, J., Chen, Z., Gorczynski, C. P., Gorczynski, L. Y., Kai, Y., Lee, L., Manuel, J., & Gorczynski, R. M. (2003). Sleep-deprived mice show altered cytokine production manifest by perturbations in serum il-1ra, TNF-α, and il-6 levels. *Brain, Behavior, and Immunity*, 2003(17), 498-504.

9. Hughes, FJ., Howells, GL. (1993). Interleukin-6 inhibits bone formation in vitro. Bone and Mineral, (1):21-8.
10. Kameda, T., Mano, H., Yuasa, T., Mori, Y., Miyazawa, K., Shiokawa, M., … Kameda, A. (1997). Estrogen Inhibits Bone Resorption by Directly Inducing Apoptosis of the Bone-resorbing Osteoclasts. *JOURNAL OF EXPERIMENTAL MEDICINE*, (4), 489.
11. Kotrych, D., Dziedziejko, V., Safranow, K., Sroczynski, T., Staniszewska, M., Juzyszyn, Z., & Pawlik, A. (n.d.). TNF-alpha and IL10 gene polymorphisms in women with postmenopausal osteoporosis. *EUROPEAN JOURNAL OF OBSTETRICS & GYNECOLOGY AND REPRODUCTIVE BIOLOGY*, *199*, 92–95.
12. Li, X., Zhou, Z. Y., Zhang, Y. Y., & Yang, H. L. (2016). IL-6 Contributes to the Defective Osteogenesis of Bone Marrow Stromal Cells from the Vertebral Body of the Glucocorticoid-Induced Osteoporotic Mouse. PloS one, 11(4), e0154677.
13. Nolte, E., Frisch, F., Lopez, O. (2020) The Physiological Interaction of Sleep Deprivation and Zoledronate on Distal Femur Trabecular Thickness of Ovariectomized Rats. *Arch Epidemol* 4: 145.
14. Osta, B., Benedetti, G., & Miossec, P. (n.d.). Classical and paradoxical effects of TNF-alpha on bone homeostasis. *FRONTIERS IN IMMUNOLOGY*, *5*.
15. Romano et. al (1997). Role of IL-6 and Its Soluble Receptor in Induction of Chemokines and Leukocyte Recruitment. Immunity, Vol. 6, 315-325.
16. Sozen, T., Ozisik, L., & Basaran, N. C. (2017). An overview and management of osteoporosis. *European Journal of Rheumatology*, (1), 46.
17. Specker, B. L., Binkley, T., Vukovich, M., & Beare, T. (2007). Volumetric bone mineral density and bone size in sleep-deprived individuals. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *18*(1), 93–99.
18. Tanaka et. al (2014). IL-6 in Inflammation, Immunity, and Disease. Coldspring Hard Perspec Biol, 6(10): a016295.
19. Zhang, J. and An, Jianziong. (2009) Cytokines, Inflammation and Pain. *International anesthesiology clinics*, *45*(2), 27-37.