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Combination of Volitional Exercise and Bisphosphonate Treatment is Able to Restore Bone Loss Induced by Hindlimb Suspension

Timothy L. Norman
Cedarville University, tnorman@cedarville.edu

N. Mukherjee

C. Cutlip

O. Wirth

N. Clovis

COMBINATION OF VOLITIONAL EXERCISE AND BISPHOSPHONATE TREATMENT IS ABLE TO RESTORE BONE LOSS INDUCED BY HINDLIMB SUSPENSION

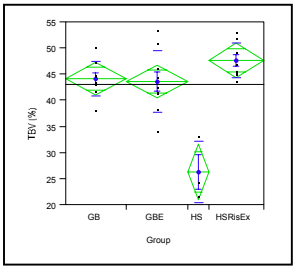
+*Norman, TL; *Mukherjee, N; **Cutlip, RG; **Wirth, O; *Clovis, NB
+West Virginia University, Morgantown, West Virginia; **National Institute for Occupational Safety and Health, Morgantown, West Virignia

Introduction: It has been previously established in numerous radiodensitometric and biochemical studies of human bone and animal models that paralysis, immobilization and weightlessness result in loss of bone mass or disuse osteoporosis. Loss of bone due to microgravity has raised considerable concern about the increased fracture risk of astronauts, especially post-menopausal females.[1] Despite the serious implications of bone loss and fracture fragility of the human skeleton, no countermeasure has been clearly established that would maintain bone mass during periods of weightlessness. Data from many laboratories suggest that exercise[2,3,4] and pharmaceuticals (both anti-resorptive and anabolic agents)[5,6] are capable of restoring bone mass/quality lost due to a variety of conditions like aging, menopause/ovariectomy and skeletal unloading/disuse. The objective of this research was to determine the efficacy of combined interventions of volitional resistance (weight lifting) exercise and anti-resorptive pharmacological therapy in reversing bone loss in a rat hindlimb suspension model.

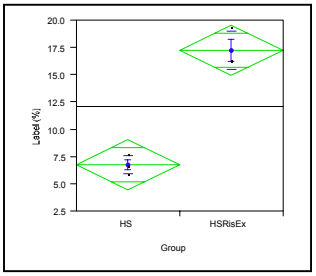
Methods: Male Sprague Dawley rats were obtained from Hilltop (Scottdale, PA) at 22 weeks of age. During a training and acclimation period of four weeks, the rats were reduced to their proper weight (80% of *ad libitum* feeding weight) and trained to exercise on a previously validated volitional resistance-training model.[7] At approximately 26 weeks of age, the rats were assigned randomly to test groups of hindlimb suspended (HS) and hindlimb suspended with exercise and Risedronate (HSRisEx). After rats were trained, they were hindlimb suspended[8,9,10] using a harness made of a polyester mesh fabric (Harvard Biosciences). The exercised rats were released from the suspension system and the lift response exercise began and lasted daily for 60 minutes or 80 full lifts, whichever came first. The exercise protocol was maintained 5 days per week. The hindlimb suspension protocol was continued for 21 days. Twice per week, Residronate was injected subcutaneously at a dosage of 5 mg/kg. Six days prior to sacrifice, Calcein (15 mg/kg) and thirteen days prior to sacrifice Democlocycline (15 mg/kg) was administered to label bones. The animals were sacrificed by AMVA approved methods. The femurs and tibias were excised. The right femur was wrapped in saline dampened gauze and frozen for mechanical testing to measure breaking load of the femoral neck.[11] The left tibia was cut at the mid-section and both portions placed in formalin for histology. The proximal third of the tibias was fixed in 70% ethanol and then embedded undecalcified in methyl methacrylate. Frontal sections of the tibia were cut to approximately 5 microns using a microtome (RM 2155, Leica, Bannockburn, IL). For histomorphometry, a digital image was obtained using an Olympus BH-2 microscope and image analysis software (Optimas, Edmonds, WA) of each tibial section. Trabecular bone volume (TBV) was measured in the primary and secondary spongiosa. These results were compared to ground-bearing (GB) and ground-bearing rats exercised on a treadmill (GBE) groups that were done as part of a different study.[11] A one-way analysis of variance was used to statistically compare groups.

Essential Results: Mechanical testing for strength of the femoral neck revealed significant differences between groups. The ground bearing (GB) and ground bearing exercise (GBE) control group had ultimate load of 125 N (SD±21.26 N) and 114.55 N (SD±24.28 N), respectively. The hindlimb suspended (HS) group had an ultimate load of 82.76 N and the combined treatment of exercise and bisphosphonates (HSRisEx) was able to bring the ultimate load up to 148.89 N (SD±4.26 N). Histomorphometric analysis indicated that TBV was significantly less ($p<0.05$) in HS than in all other groups, which was restored by a combination treatment of Exercise and Risedronate (HSRisEx). Mean TBV of HSRisEx was slightly greater than in GB and GBE groups but the difference was not significant (Figure 1a). An analysis of the fluoroscopic images (Figures 1b,c,d) indicated significantly ($p<0.0008$) greater new bone formation in the HSRisEx group than the HS group.

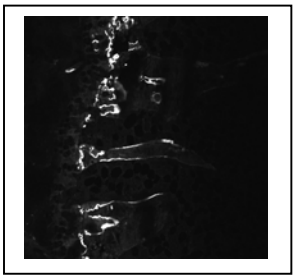
Discussion: Few studies have looked at the effects of combining different therapies. As far as we are aware, only one such study exists for rats where exercise and bisphosphonates are combined. Tamaki et al.[12] examined the effects of treadmill training (TR) following treatment with the disphosphonate etidronate (E) in rats with and without ovariectomy (ovx). Results indicated that the bone mineral density of the femur and the trabecular bone area of the tibia was significantly greater in E and/or Tr compared to ovariectomized groups. They concluded that the etidronate treatment for two weeks beforehand influenced the effects of subsequent exercise training on maintaining BMD in the proximal femur and the trabecular bone area of the femur. In another study investigating the combined effect of exercise and bisphosphonates in humans, Grigoriev et al.[13] tested nine bedrest subjects using the exercise protocol used by cosmonauts with four of the subjects receiving the bisphosphonate ethane-1-hydroxy-1-disphosphonate. The bisphosphonates combined with exercise reduced negative calcium balance over the entire 360-day test period. Exercise alone had no effect until the second 120 days. Our results seem to support these studies that show that bisphosphonates can be effectively used together with exercise to reduce calcium loss. This pilot study indicated that combination of therapies appears to be feasible to reverse bone loss due to disuse/microgravity.



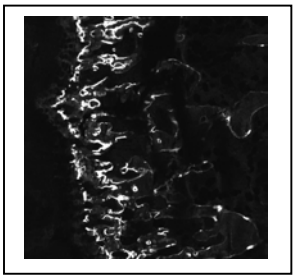
a)



b)



c) HS



d) HSRisEx

Figure 1. Exercise combined with Risedronate treatment was able to rescue trabecular bone volume loss due to hindlimb suspension (a). There was also a significant increase in the amount of fluorescent label incorporation, indicating enhanced mineral deposition and bone formation in the HSRisEx group (b). Fluorescent images of hindlimb suspended (c) and HSRisEx (d) bones are shown to emphasize increased bone formation.

References:

1. Dempster and Lindsay, *Lancet* 341:797-801, 1993
2. Shackelford et al., *Proc 1st Biennial Space Biomed Invest Workshop*, p. 235, 1999a
3. Shackelford et al., *NASA Life Sci Prog Tasks & Biblio for FY 1999 Details*, 1999b
4. Lanyon and Skerry, *J Bone Miner Res* 16:1937-1947, 2001
5. Fleisch, *Ann Med* 29:55-62, 1997
6. Russell and Rogers, *Bone* 25:97-106, 1999
7. Wirth and Cutlip, Advances in Occupational Ergonomics and Safety, IOS, 2001
8. Schulthesis et al., *45th Annual Meeting ORS*, p. 784, 1999a
9. Schulthesis et al., *45th Annual Meeting ORS*, p. 569, 1999b
10. Schulthesis et al., *46th Annual ORS*, p. 724, 2000
11. Norman et al., *Aviation, Space and Environ Med* 71:593-598, 2000
12. Tamaki et al., *Bone* 23:147-153, 1998
13. Grigoriev et al., *J Bone Miner Res* 7:S449-S455, 1992

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