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Equal BMD After Daily or Triweekly Exercise in Growing Rats

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Abstract
The purpose of this study was to examine the efficacy of continuous resistance training (3 days/week) compared to interrupted resistance training where 20-24% separated an exercise bout (i.e. 6 days/week) for enhancing bone mineral density (BMD) in growing male rats. The total volume of work performed per week between the two resistance training programs was equivalent by design. Young male rats were randomly divided into Control (Con, n=9), 3 days/week resistance trained group (RT3, n=9), and 6 days/week resistance trained group (RT6, n=9). The RT3 and RT6 groups were condition to climb a vertical ladder with weights appended to their tail for a total of 6 weeks. All 6 weeks, RT6 (assessed via DXA) from the left tibia was significantly greater for RT3 ([0.24±0.004] and RT6 [0.24±0.004]cm²) compared to GC, 0.226±0.003 cm². Further, serum osteocalcin (OC, in ng/ml) was significantly greater for RT3 (75.8±6.4) and RT6 (73.5±3.8) compared to Con (53.4±2.9). There was no significant differences BMD or serum OC between RT3 and RT6 group. The results indicate that both resistance training programs were equally effective in elevating bone mineral density in young, growing rats.

Introduction
The amount of peak bone mass accrued during childhood and adolescence, as well as the amount of bone mass lost during senescence, are important factors in assessing the potential development of osteoporosis. The number of studies that have focused upon the impact of exercise in the elderly, to further our knowledge of attenuating bone loss; has outweighed the number of reports examining the impact of exercise during the growth period, to encourage understanding of elevating peak bone mass. The hormonal milieu associated with the maturation process elicits a positive impact upon bone metabolism and function. Incorporating exercise to stimulate an osteogenic response during the growth period would be beneficial in elevating peak bone mass. Thus, determining the optimal exercise regimen for the maximal stimulation of bone accrual during the growth period could minimize the deleterious effects of osteoporosis later in life. Using an anesthetized rat model, Robling et al. [18, 19] introduced a protocol that had the potential to maximize the osteogenic effects from training. They reported that partitioning burst loading bouts into multiple sessions (separated by rest periods) during a training day was more effective in elevating bone formation rates compared to a single loading session on a given day (i.e. 6 days/week). Since ladder climbing has previously been observed to elicit an osteogenic response.

Materials and Methods
Animals
The experimental protocol for this study was pre-approved by the Chapman University Institutional Review Board and in accord with the Public Health Service policy on the use of animals for research. Thirty-six male Sprague Dawley rats (initially weighing 17.3±1.4 g), 28 days old, were acclimated to their living conditions for one week. During the acclimation period, the animals were returned to the breeding colonies. Thirty-six animals were acclimated to their living conditions for one week. During the acclimation period, the animals were returned to the breeding colonies.

Experimental protocol
Animals were sacrificed 48 h after their final training session to minimize any residual effect of the last training bout. The flexor hallicus longus (FHL, [15]), an intramuscular muscle, was isolated and weighed, and immediately frozen in liquid nitrogen for the subsequent determination of protein content. We chose the FHL since ladder climbing has previously been observed to elicit an osteogenic response. The muscle was removed from the right hindlimb, weighed, and immediately frozen in liquid nitrogen for the subsequent determination of protein content. We chose the FHL since ladder climbing has previously been observed to elicit an osteogenic response in the FHL. The RT6 group performed 6 consecutive ladder climbs on a given training day. The 6 ladder climbs constituted the maximum amount of consecutive repetitions that the RT6 animals could achieve during the exercise session. The maximal amount of ladder climbs was based upon the animals’ refusal to climb despite motivation attempts. For the RT6 group (i.e. 6 days/week), the animals performed 6 ladder climbs on a given training day.

Results
The results indicate that both resistance training programs were equally effective in elevating bone mineral density and bone strength compared to controls. The total volume of work performed per week as the triweekly exercised group, but performed half the work on a given day, separated by 20-24% of recovery before completing the other half of work on the next day. For the daily exercised group, the significant recovery period between exercise bouts (i.e. 20-24%) is not as effective as continuous, uninterrupted resistance training. Thus, if the mechanosensors were able to rest then, according to the hypothesis proposed by Turner and Robling [18, 19], we would anticipate that the daily exercised group would demonstrate more bone formation culminating in greater BMD compared to the triweekly group. To further assess the impact of any resistance training-induced alterations to the bone, we also performed three-point bending tests to measure bone mechanical properties (i.e. bone bending strength). Based upon our prior reports [6, 7] and in contrast to the hypothesis proposed by Turner and Robling [18, 19, 22-24], we postulated that during the growth period both resistance training protocols (i.e. daily and triweekly) would be equally effective in stimulating an elevation in bone mineral density and bone strength compared to controls.

Discussion
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the distance covered. The total training volume (i.e. work) per week for the RT3 and RT6 group was expressed in Joules. For comparison of weekly total training volume, a Student’s t-test was used to determine statistical significance. Total protein in the FHL was calculated as the product of protein concentration and muscle mass. Deoxypyridinoline (DPD) was corrected for urine concentration (or dilution) by dividing by the creatinine content, which was measured using the standardized enzymatic method. 

**Results**

The initial body mass was not significantly different between groups (Table 2). After the 6-week resistance training program, the final body mass was similarly not significantly different between groups (Table 2). The total training volume (work) per week for the RT3 and RT6 group was 133.7 ± 8.2 × 10^6 Joules and 113.3 ± 13.1 × 10^6 Joules, respectively. The maximum force to failure, energy to failure, and bone stiffness were significantly greater for all groups compared to Baseline (Table 2). The bone mineral density from the left tibia was significantly elevated for Con (i.e. 14.1% increase), RT3 (i.e. 22.2% increase), and RT6 (i.e. 44.5% increase) compared to Baseline (Fig. 2). Further, the BMD from the RT3 and RT6 groups was significantly greater (i.e. 7.5% increase) than the Con group (Fig. 2). However, the BMD was not significantly different between RT3 and RT6 groups. Serum osteocalcin was not significantly different between Con compared to Basal, but was significantly greater for Con compared to Baseline, supporting animal growth over the 7-week period. Incorporating resistance training during this growth period provided an additional osteogenic stimulus culminating in greater elevations in bone mineral density at maturity to young adulthood alone. The osteogenic response RT3 and RT6 may be attributable to an elevation in osteoblast activity, as indicated by an increase in serum OC compared to controls, and not a decrease in serum OC activity as seen in equivalent levels of dietary uric acid among groups. Further, both RT3 and RT6 groups demonstrated augmented bone strength when compared to Baseline (Table 3).

**Discussion**

The Con, RT3, and RT6 groups demonstrated elevations in body mass, PHL, mass, PHL, protein, BMD, and bone strength compared to the baseline group, supporting animal growth for the 7-week period. Incorporating resistance training during this growth period provided an additional osteogenic stimulus culminating in greater elevations in bone mineral density at maturity to young adulthood alone. The osteogenic response RT3 and RT6 may be attributable to an elevation in osteoblast activity, as indicated by an increase in serum OC compared to controls, and not a decrease in serum OC activity as seen in equivalent levels of dietary uric acid among groups. Further, both RT3 and RT6 groups demonstrated augmented bone strength when compared to Baseline (Table 3). In addition, resistance training resulted in significant increases in bone stiffness for RT3 (i.e. 24.4% increase) and RT6 (i.e. 28.0% increase) compared to controls. However, the mass.

**Chemical analyses**

Protein concentration in the FHL was assessed [13] as an indirect indicator of bone turnover. We measured the protein concentration in the FHL as the average of three scans and the coefficient of variation (CV) between scans was less than 0.99. Finally, a Dual Energy X-ray Absorptiometer (DEXA, GE Lunar Prodigy, Madison, WI) employed the small animal software module (version 6.81) to assess the BMD of the whole left tibia. Briefly, the left hindlimb was thawed, positioned, and the entire tibia was scanned. Conﬁdence and malleolus markers of the tibia were used as anatomical markers and the tibia was positioned to prevent twisting so that the tibia was not exaggerated or obliterated. Scans were allowed to run to completion between each scan. The reported BMD was the average of three scans and the coefficient of variation for repeated scans (mean ± standard error) that included all hindlimbs was 0.85 ± 0.07.

**Biomechanical three-point bending tests**

The mechanical properties of bone were measured at room temperature using a three-point bending rig placed onto the stage of a texture analyzer instrument (TA-XT2, Texture Technologies, Ramona, CA). Prior to testing, the right tibia was rinsed in saline and then dried in saline for 24 h at room temperature. The instrument was calibrated using a standard weight and then the tibia was potted and secured to the rig. The span of the two support points was 15.7 mm for the baseline group (to account for the smaller tibial length due to the age of the animals) when the span of the two support points was 18.0 mm for the remaining groups who were now 7 weeks older. The deformation rate was set at 0.9 mm/dsec for all groups. A medial to lateral force was applied to the tibia at the load of the peak, and the deformation rate was measured using Texture Expert v. 1.22, Stable Micro Systems Ltd., Surrey, England, UK.

**Calculations and statistics**

Work (i.e. training volume) was calculated as the product of the total weight lifted by the animal (body mass plus the amount of weight appended to the tail), the acceleration due to gravity, and the amount of work performed for each training week by the animals. Total work was kept constant between RT3 vs. RT6 groups. The osteogenic response RT3 and RT6 may be equally effective in stimulating an increase in tibial BMD and bone strength in young, growing male rats.

**Acknowledgements**

The development of osteoporosis is a low peak bone mineral density at skeletal maturity [11,27]. Elevating peak bone mass during childhood and adolescence has been advocated as a method to attenuate the risk of osteoporosis.
during senescence [1]. In like manner, resistance training has traditionally been promoted as a method to stimulate an osteo-

dence in bone mineral density [2, 3]. Since the maturation period already provides a hormonal milieu conducive to skeletal growth, this would be an opportune time to maximize bone mass and bone strength through adequate exercise load.