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Resistance Training and Bone Mineral Density During Growth

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Resistance Training and Bone Mineral Density during Growth


Abstract

This study examined the efficacy of two different resistance training programs in enhancing bone modeling and bone mineral density (BMD) in maturing rats. One exercise mode involved lifting a lighter weight with more repetitions (LI), while the other regimen involved lifting a heavier weight with fewer repetitions (HI) where the total volume of work between exercise programs was equivalent by design. Twenty-three male rats were randomly divided into control (Con, n = 8), LI (n = 7), and HI (n = 8) groups. The LI and HI groups were conditioned to climb a vertical ladder with weights appended to their tails 4 days/week for 6 weeks. After training, serum osteocalcin (OC) was significantly lower in both LI (45.2 ± 1.7 ng/ml) and HI (39.3 ± 2.7 ng/ml) when compared to Con (29.9 ± 0.9 ng/ml). Left tibial BMD was significantly lower in the LI group (0.80 ± 0.04 g/cm²) compared to both HI (0.213 ± 0.003 g/cm²) and Con (0.208 ± 0.005 g/cm²) with no significant difference between LI and Con. The results indicate that both HI and LI are effective in elevating serum OC, implicating an osteogenic response; however only HI resulted in a significant elevation in BMD.

Introduction

Exercise is commonly accepted as an important factor for bone accrual during growth and attenuating bone loss during senescence. Of the two primary modes of exercise, resistance training has been recognized to be more effective in eliciting an osteogenic response when compared to endurance training [9]. Given the recognized benefits of resistance training programs have reported in children where growth is relatively rapid, matching the growth velocity between LI and HI groups throughout the 6-week training period. It should be noted that one of the animals in the LI group refused to climb the ladder at the beginning of the second week. This animal was eliminated from the LI group which accounts for the decrease in sample size for LI (n = 7).

Materials and Methods

Animals

The experimental protocol for this study was approved by the Chapman University Institutional Review Board and in accord with the Public Health Service policy on the use of animals for research. Twenty-four male Wistar rats (initially ~225 grams, 8 weeks old) obtained from Charles River Laboratories (Wilmington, MA, USA) were housed individually and maintained in a 12/12-hour light/dark cycle. The animals were acclimated to their living conditions for 1 week with food and water ad libitum. Then they were randomly assigned to either the control group (Con, n = 8), a resistance trained group where the animals lifted a low amount of weight with high repetitions (LI, n = 8), or another resistance trained group where the animals lifted a high amount of weight with fewer repetitions (HI, n = 8).

Resistance training

The strength training regimen consisted of a vertical ladder climbing task in which weights were appended to the rat’s tail. For each training session there were 30% of body weight appended to the tail, and each week the resistance was elevated by 30% BW until they were carrying 150% of their body weight by the beginning of week 5, where they similarly maintained this resistance until the end of week 6. The number of ladder climbs (i.e., total repetitions) for the LI group was twofold higher than the HI group. The resistance (3% body weight appended to their tails plus their body weight) and the number of repetitions served to equate the total volume of work between LI and HI groups throughout the 6-week training period. It should be noted that one of the animals in the LI group refused to climb the ladder at the beginning of the second week. This animal was eliminated from the LI group which accounts for the decrease in sample size for LI (n = 7).

Experimental protocol

To minimize any residual effects of the last bout of exercise, animals were sacrificed 48 hours after the last training session. To help substantiate a resistance training effect, and consistent with Hornberger and Farrar [8], the Flexor Hallucis Longus (FHL) was rapidly dissected from the right hindlimb, weighed, and immediately frozen in liquid nitrogen for the subsequent determination of protein content. The left hindlimb was rapidly amputated, positioned, and frozen in liquid nitrogen for the assessment of bone mineral density of the tibia. Finally, blood samples were collected, placed on ice, allowed to clot, and the sera was frozen for the subsequent measurement of osteocalcin and pyridinoline cross-links. All tissue and serum samples were kept at -80°C until its analyses.

Fig. 1. The ladder climbing apparatus. A rat is shown climbing the 1-meter, 90° incline ladder with weights appended to the tail.

References

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Hornberger and Farrar [8] later verified the efficacy of a similar model, i.e., vertical ladder climbing task, for producing muscle hypertrophy that was confirmed in the Flexor Hallucis Longus. In a preliminary study we also employed a modified version of this vertical ladder climbing task and found it to be an effective resistance training stimulus for rats [9].

Key words

○ tibia
○ femur
○ pyridinoline
○ BMD

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Bibliography

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Fig. 1. The ladder climbing apparatus. A rat is shown climbing the 1-meter, 90° incline ladder with weights appended to the tail.
Chemical analyses
A protein assay [11] was used to determine the protein concentration in the FHL, as an indirect indicator of training (i.e., muscle hypertrophy). A sandwich ELISA (rat osteocalcin kit from Bio-Medical Technologies, Inc., Stoughton, MA, USA) was employed to determine serum osteocalcin levels (an indicator of osteoblast activity). The inter-assay variation was <4% and the intra-assay variation was <7%. Serum pyridinoline cross-links (an indirect indicator of osteoclast activity) were measured using a competitive enzyme immunoassay (PVD EIA kit from Quidel Corp., San Diego, CA, USA). The intra-assay variation was <6% and the inter-assay variation was <8%. A microplate reader (MaxLine, Molecular Devices Corp., Sunnyvale, CA, USA) was used with the absorbance set at 450 nm for the ELISA or 405 nm for the EIA. Finally, a Dual Energy X-ray Absorptiometer (DXA - GE Lunar Prodigy, Milwaukee, WI, USA) employing the small animal software module (version 6.81) was used to assess the BMD of the left tibia. The frozen left hindlimb was positioned in the machine and was scanned. Three consecutive measurements were performed with reporting between each scan. The average was used as the BMD and the coefficient of variation for repeated scans was <1.4% for each group.

Calculations and statistics
Total protein in the FHL was calculated as the product of protein concentration and muscle mass. Work (i.e., training volume) was determined as the product of the weight lifted by the animal (body weight plus the amount of weight appended to the FHL) by the number of repetitions (8). Where BW = body weight and FHL = FHL weight, the BMD and the coefficient of variation for repeated scans was 90.86±2.1%. The current study demonstrated that a high-intensity resistance training program effectively induced both hypertrophy in the FHL and a concomitant osteogenic response in maturing rats. While the site of bone mass could be attributable to differences in the amount of bone worked, the current study demonstrated that a high-intensity resistance training program significantly increased BMD in the midfemur and proximal tibia compared to sedentary controls [14]. Given the success of a climbing platform in animals to mimic resistance exercise, we modified the training program of Hornberger and Farrar [8] and climbed the ladder. In this study, we observed a significant elevation in serum osteocalcin (an indicator of bone turnover) in the LI group. Conclusively, we found that the BMD of the left tibia from the HI group was significantly greater than from the LI group (Fig. 4). In contrast, the BMD from LI was not significantly different from Con (p = 0.32).

Discussion
The current study demonstrated that a high-intensity resistance training regimen effectively induced both hypertrophy in the FHL and an increase in BMD. However, previous studies have demonstrated that an increase in BMD is elicited by our high-intensity resistance training program. Since the site of bone deposition is specific to the mechanical loads placed upon it [21], we chose to examine the tibial BMD in accord with the location of the FHL. Using a bone biochemical marker of an osteogenic response, human studies have demonstrated that high-intensity resistance training led to an increase in BMD. However, the serum osteocalcin levels were also augmented in the LI group, there was no significant enhancement of total protein in the HI or HILBMD. As such, our results suggest that muscle hypertrophy may not be required for elevations in bone formation such as osteocalcin. In support of our results, adaptations (e.g., improved synchronization of motor units) could also elicit strength gains in the absence of muscle hypertrophy [23] and still provide the requisite stimulus for bone modeling. Thus, while the increase in FHL muscle mass and concomitant increases in serum osteocalcin and tibial BMD observed from only the HI group, suggests that 6 weeks were appropriate, we recognize that more time (i.e., more than 6 weeks) of training might be required to eventually observe the increase in BMD. As such, our results suggest that muscle hypertrophy may not be required for elevations in bone formation such as osteocalcin.

Although our result demonstrating an increase in BMD was consistent with prior reports, the mechanism for bone deposition appears to be capricious. Ven et al. [23] examined the impact of
A 6-week exercise (treadmill) program and determined that the training-induced bone modeling in their maturating female rats was the result of a decrease in bone resorption. In contrast, we failed to observe a decline in osteoclast activity as indicated by serum pyridinoline cross-links. In fact, our results suggest that the increase in BMD in male rats is attributable to an elevation in osteoblast activity as indicated by the elevation in serum osteocalcin. As such, the significant increase in BMD for the HI group appears to be the result of more bone deposition rather than a decline in bone resorption. The discrepancy suggests either sex differences in the bone modeling response to exercise, the type of exercise employed (i.e., treadmill vs. ladder climbing), or the potential for oscillatory effects between bone resorption and bone deposition. Despite the mechanistic discrepancies between animal studies, our results support the few prospective studies in prepubertal boys [3] and premenarchal girls [12] that high-intensity, strength training is an effective means for eliciting a bone modeling response. Further, given the equivalent training duration, training frequency, and volume of work between HI and LI, we conclude that training intensity is an essential factor for this type of exercise contributing to the elevation in BMD in our maturating animals. The significant difference in carrying weight between HI (i.e., 150% of BW) and LI groups (i.e., 25% of BW) support greater loads upon the skeleton for the HI animals. Whether this will similarly apply to maturating humans remains to be determined and should be examined with caution. However, it offers a potential insight into the type of training program that would optimize bone accrual during growth.

Finally, we recognize several limitations of our study. First, while numerous reports have employed the DXA in animals [2, 7, 9, 13, 14], there are limitations associated with using a DXA for the determination of BMD. Specifically, bone mineral density, as determined by the DXA is expressed as an area (i.e., g/cm²) and may not account for changes in bone size where increases in height could contribute to elevations in BMD [4]. While we recognize the limitations associated with the DXA, we also note that it was sensitive enough to detect the alterations in BMD despite differences in body weight between groups. In support, the HI group had the lowest body weight, ostensibly implicating a lower bone size, yet also had the greatest BMD. Further confirmation of an osteogenic response in the HI group was the serum biochemical markers of bone formation, which were elevated in the HI group as compared to controls. Whether this will similarly apply to maturating humans remains to be determined and should be examined with caution. However, it offers a potential insight into the type of training program that would optimize bone accrual during growth.

In summary, using animals and a mode of exercise that mimics human progressive resistance training where the volume of work was equivalent between HI and LI groups, we provide evidence that high-intensity resistance training elicits the more effective stimulus to elicit an osteogenic response in maturating rats. This is supported by concomitant elevations in FHL protein, serum osteocalcin levels, and tibial BMD. We note that the time point we used within our 6-week time frame, given the significant elevation in serum osteocalcin from LI as well as the trend toward an augmentation in FHL protein, we cannot rule out the possibility that more time (i.e., >6 weeks) might be required to finally observe the increase in skeletal muscle hypertrophy and BMD as a result of the LI resistance training program.

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