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# Interrupted Vs. Uninterrupted Training on BMD During Growth



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- osteocalcin
- deoxypyridinoline
- 3-pt bending test

## Abstract

This study compared a resistance training program where the exercise was uninterrupted (UT, i.e., continuous repetitions) against a resistance training program where the exercise was interrupted (IT, i.e., 3 exercise sessions during a training day) for enhancing bone modeling and bone mineral density (BMD) in maturating animals. The total volume of work performed between the two resistance training programs was equivalent by design. 24 young male rats were randomly divided into Control (Con, n = 8), UT (n = 8) and IT (n = 8) resistance trained groups. The UT and IT groups were conditioned to climb a vertical ladder with weights appended to their tail 3 days/wk for 6 wks. After the 6-wk program, serum osteocalcin was not significantly different between groups, whereas the adjusted urinary deoxypyridinoline (DPD) was significantly lower for both UT (81.03 ± 5.53) and IT (88.30 ± 7.29) compared to Con (128.13 ± 9.99). Tibial BMD (assessed via DXA) was significantly greater for UT (0.222 ± 0.005 g/cm$^2$) and IT (0.219 ± 0.003 g/cm$^2$) when compared to Con (0.205 ± 0.004 g/cm$^2$). There was no significant difference in DPD or BMD between UT and IT groups. The results indicate that both interrupted and continuous, uninterrupted resistance training programs were equally effective in stimulating bone modeling.

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## Introduction

Bone cells can respond to mechanical stress, especially dynamic physical exercise. The external forces imposed upon the bone via exercise needs to be of a sufficient magnitude to create a fluid flow within the lacunar-canalicular network to stimulate bone formation [3]. In this regard, resistance training (e.g., strength exercise) or high-impact activities (e.g., jumping) have been recognized to be more effective in stimulating bone formation when compared to endurance training [9,26]. In support, numerous human [1,4,16,19, 22,27] and animal studies [7,9,13 – 15,20,28] have demonstrated the effectiveness of resistance training or high-impact exercise in stimulating an osteogenic response and elevating bone mineral density. However, only a few studies have sought to determine the most effective training program to elicit elevations in bone accrual during growth. Prior reports in prepubertal boys [2] and premenarcheal girls [12] following various exercise programs demonstrated increases in bone mineral accrual compared with sedentary children. However, cross-sectional comparisons in humans are subject to various confounding variables such as: genetics, dietary intake, and activity levels (to name a few). Additional challenges when studying children include matching the growth velocity between the exercise and control groups [2]. In this regard, the use of maturating animals can minimize many of these confounding variables, but identifying a mode of exercise to mimic resistance training had previously been a significant obstacle.

In a prior study in maturating rats, we employed a vertical ladder climbing task with weight appended to an animal's tail and reported an elevation in bone mineral density [20]. In this study, we observed that lifting a heavy weight with fewer repetitions was more efficacious for increasing tibial bone mineral density compared to lifting a lighter weight with more repetitions, despite an equivalent amount of work performed by each exercised group [20]. While our prior animal study [20] provided evidence regarding the effectiveness of high-intensity resistance training in stimulating the osteogenic response, this type of training may not be suitable during the formative years, especially toward the end of a given set of repetitions when fatigue might be a contributing factor for elevating the risk of injury.

In elaborate studies by Robling et al. [17,18], they characterized a potential exercise protocol that could minimize the risk of injury and optimize a bone formation response. Specifically, they reported in anesthetized animals that loading the bone at discrete intervals during a day (separated by 3 hours) was more effective in eliciting an osteogenic response than a single loading session per training day [17,18]. Turner and Robling [25] have interpreted these findings to suggest that bone cells exhibit desensitization to a prolonged mechanical loading bout. As such, the bone cells become saturated and will not provide an additional response despite the continuous loading stimuli. From these experiments, Turner [23] noted two important factors pertaining to bone mechanosensitivity; the mechanical loading sessions do not have to be long, and incorporating recovery periods can restore the mechanosensitivity. While these studies [17,18,24, 25] are extremely promising, they were performed on animals under ether-induced anesthesia. In this regard, Stanek et al. [21] previously reported alterations in hemodynamics and blood flow distribution using ether as an anesthetic agent that could inadvertently influence experimental observations. Currently, the potential benefit of using interrupted bouts of exercise in conscious animals during maturation remains to be elucidated. To date, we are aware of only the study by Umemura et al. [26] who specifically investigated the potential for using intervals between exercise bouts in conscious maturating female rats to augment the osteogenic response. They failed to observe any difference in bone mass from rats exposed to two bouts of exercise within a training day compared to a single bout of exercise in a training day, where the total number of repetitions was equivalent between the training programs [26]. Umemura et al. [26] employed a jumping protocol, where the animals were initially motivated with use of electric shock. However, there could be independent effects of electric shock upon the bone that can similarly influence experimental observations.

The purpose of the current study was to determine if a resistance training program, where sessions were separated into discrete bouts during an exercise training day, was more effective than a continuous bout of resistance training, on markers of bone modeling and bone mineral density. Specifically, one resistance trained group performed continuous repetitions of work on a training day (abbreviated for this paper as Uninterrupted) while the other resistance trained group performed the same amount of work, but on a given training day the work was separated into 3 discrete bouts with 4 – 5 hours of recovery between exercise sessions (abbreviated for this paper as Interrupted). Three discrete bouts of exercise within a training day were chosen in an attempt to maximize the potential stimulation for bone formation while approximating the protocol used by Robling et al. [17, 18]. We employed a vertical ladder climbing task which has been shown to mimic resistance training [8]. In addition, we used a 6-week exercise protocol previously observed to elicit an osteogenic response [20]. All animals were conscious and no electric shock was used to motivate the animals to climb. Thus, we sought to examine the effectiveness of using interrupted resistance training exercise rather than interrupted high-impact exercise (i.e., jumping), as previously reported by Umemura et al. [26]. To further relate any resistance training-induced alterations in bone mineral density to bone strength, we also performed three-point bending tests to measure bone mechanical properties. We hypothesized that interrupted resistance training would induce a greater increase in bone mineral density than uninterrupted resistance training in maturating male rats, culminating in added mechanical bone strength.

## Materials and Methods

### Animals

The experimental protocol for this study was preapproved 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 Sprague Dawley rats (initially ~ 225 grams, ~ 8 weeks old) obtained from Charles River Laboratories (Wilmington, MA, USA) were housed individually and maintained on a reverse 12/12 hour light/dark cycle. The animals were acclimated to their living conditions for 1 week with food and water provided *ad libitum*. Then they were randomly assigned to either a Control group (n = 8), a resistance trained group where the animals performed continuous uninterrupted repetitions on a given training day (Uninterrupted, n = 8), or another resistance trained group where the animals performed repetitions that were interrupted at 3 separate times during a training day (Interrupted, n = 8). The group size of 8 was determined in a scientific manner (Stat View software, SAS Institute Inc., Cary, NC, USA) based upon prior experience and using potential means and standard deviations from previous reports.

### Resistance training

The strength training regimen has previously been described [20]. Briefly, the animals engaged in a vertical ladder-climbing task in which weights were appended to the rat's tail. One repetition along the 1 meter length of the ladder required 26 total lifts by the animal (or 13 lifts per limb). The resistance trained animals were operantly conditioned to climb the ladder in order to avoid a vat of water beneath them. The exercised animals trained 3 days/week for a total of 6 weeks. The control animals were handled on the same days as the trained groups in order to minimize any stress attributable to handling. All animals were weighed at the beginning of the week to monitor weight gains and, for the resistance trained animals, to determine the amount of weight to append to their tails for the remainder of the week. The resistance trained animals started with 30% of their body mass appended to their tail, and each week the resistance was elevated by 30% of their body mass until they were carrying 150% of their body mass by the beginning of week 5, where they maintained this resistance until the end of week 6. For the Uninterrupted group, the animals performed 6 consecutive ladder climbs on a given training day. The 6 ladder climbs constituted the maximum amount of consecutive repetitions the Uninterrupted animals could achieve. For the Interrupted group, the animals performed 2 ladder climbs 3 times during a training day where 4 – 5 hours separated a bout of exercise. Thus, the total number of ladder climbs (i.e., total repetitions) in a given day was equivalent between the Uninterrupted and Interrupted groups. The resistance (% body mass appended to their tail plus their body mass), the distance covered, and the total number of repetitions served to equate the total volume of work between Uninterrupted and Interrupted groups throughout the 6-week training period.

**Table 1** Body mass and flexor hallucis longus protein content

| Group | Initial BM (g) | Final BM (g) | FHL Mass (g) | FHL Protein (mg/muscle) | FHL Protein (mg/muscle/100 g BM) |
|---|---|---|---|---|---|
| Con | 277.1 ± 4.5 | 403.1 ± 14.1 | 0.230 ± 0.009 | 46.98 ± 1.16 | 11.71 ± 0.34 |
| UT | 284.1 ± 2.5 | 392.5 ± 4.6 (p = 0.612) | 0.249 ± 0.006 (p = 0.151) | 52.70 ± 2.10 (p = 0.084) | 13.41 ± 0.45* (p = 0.041) |
| IT | 282.5 ± 5.0 | 378.2 ± 20.3 (p = 0.239) | 0.245 ± 0.011 (p = 0.261) | 51.54 ± 3.02 (p = 0.162) | 13.69 ± 0.58* (p = 0.025) |

Con = control group (n = 8), UT = uninterrupted resistance trained group (n = 8), and IT = interrupted resistance trained group (n = 8). BM = body mass (in grams) and FHL = flexor hallucis longus (in grams). p values are indicated for UT and IT vs. Con. * Significant difference vs. Con

### Experimental protocol

To minimize any residual effects of the last bout of exercise, animals were sacrificed 72 hours after the last training session. The flexor hallucis longus 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 and frozen in liquid nitrogen for the assessment of bone mineral density of the tibia and bone mechanical properties. Blood samples were collected, allowed to clot, centrifuged, and the serum was frozen for the subsequent measurement of serum osteocalcin. Finally, a syringe was used to extract urine directly from the bladder and immediately frozen for the subsequent measurement of deoxypyridinoline and creatinine. All tissue, serum, and urine samples were kept at – 80 °C until its analyses.

### Chemical analyses

Protein concentration in the flexor hallucis longus was assessed [10] as an indirect indicator of training (i.e., muscle hypertrophy). A sandwich enzyme-linked immunosorbent assay (Biomedical Technologies, Inc., Stoughton, MA, USA) was used to determine serum osteocalcin levels (an indicator of osteoblast activity). Urinary deoxypyridinoline (an indicator of osteoclast activity) was measured using a competitive enzyme immunoassay (Quidel Corp., San Diego, CA, USA). Urinary creatinine was measured using an enzyme assay and picric acid as the color reagent (Quidel Corp). A microplate reader (MaxLine, Molecular Devices Corp., Sunnyvale, CA, USA) was used with the absorbance set at 450 nm for the enzyme-linked immunosorbent assay, 405 nm for the competitive enzyme immunoassay, or 490 nm for the microassay using picric acid. A standard curve was generated for all chemical analyses and controls were run to ensure quality. For all standard curves, the correlation coefficient was greater than 0.95. Finally, a dual energy X-ray absorptiometer (GE Lunar Prodigy, Chicago, IL, USA) employing the small animal software module (version 6.81) was used to assess the bone mineral density of the left tibia. The left hindlimb was thawed, positioned, and the tibia was scanned. Three consecutive measurements were performed with the hindlimb repositioned between each scan. The reported bone mineral density was the average of three scans and the coefficient of variation for repeated scans was < 1.0% for each group.

### Biomechanical three-point bending tests

The mechanical properties of bone were measured using a three-point bending rig placed onto the stage of a texture analyzer instrument (TA-XT2, Texture Technologies, Ramona, CA, USA). Following the dual energy X-ray scans, hindlimbs were thawed and all soft tissues were removed from the left tibia. The bone was submerged in saline for 20 hours prior to testing at room temperature. Prior to testing, the instrument was calibrated using a standard weight. Then the tibia was patted dry and secured to the rig. The span of the two support points was 18 mm and the deformation rate was 0.9 mm/sec. A medial to lateral force was applied to the midshaft of the bone. The maximal load to failure (units = N) and energy to failure (determined from the area under the load-deformation curve to the fracture point, units = N × mm) was determined using Texture Expert (v. 1.22, Stable Micro Systems Ltd., Surrey, England, UK).

### Calculations and statistics

Work (i.e., training volume) was determined as the product of the total weight lifted by the animal (body weight plus the amount of weight appended to the tail), the acceleration due to gravity, and the distance covered. The total training volume (i.e., work) for Uninterrupted and Interrupted groups was expressed in Joules. For total training volume, a Student's *t*-test was used to determine statistical significance. Total protein in the flexor hallucis longus was calculated as the product of protein concentration and muscle mass. Due to the variability in the body weight from the Control and Interrupted groups, that also contributed to variances in muscle size (and hence muscle weight), we expressed the total protein in the flexor hallucis longus (mg/muscle) per 100 grams of body weight to help normalize these differences. In support, examining the flexor hallucis longus weight relative to the final body weight for each animal revealed that in resistance trained groups it was significantly greater than in controls (data not shown). Deoxypyridinoline (nmol/L) was corrected for urine concentration or dilution, by dividing by the creatinine concentration (mmol/L) and expressed as the adjusted urinary DPD. Except for the training volume, an ANOVA was employed for all comparisons, and when a significant F ratio was identified, a Fisher's PLSD post hoc test was used. The level of significance set was at $p < 0.05$ for all statistical comparisons and the results were expressed as the mean ± standard error (SE).

## Results

The initial body mass was not significantly different between groups. After the 6-week resistance training program, the final body mass was similarly not significantly different between groups (**Table 1**). The total training volume for the resistance trained animals was not significantly different between Uninterrupted vs. Interrupted groups at any time during the 6-week exercise program (**Fig. 1**). The flexor hallucis longus mass and total protein content in the flexor hallucis longus, while generally higher, was not significantly greater for either Uninterrupted or Interrupted groups when compared to controls (**Table 1**).



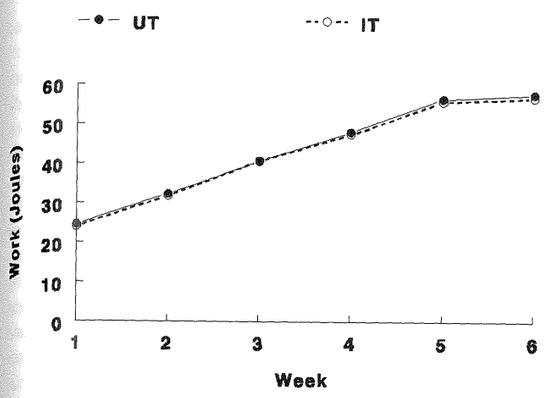


**Fig. 1** Total work (in Joule) performed for each training day during the week indicated from the Uninterrupted resistance trained group (UT, n = 8) and the Interrupted resistance trained group (IT, n = 8). No significant difference between groups.

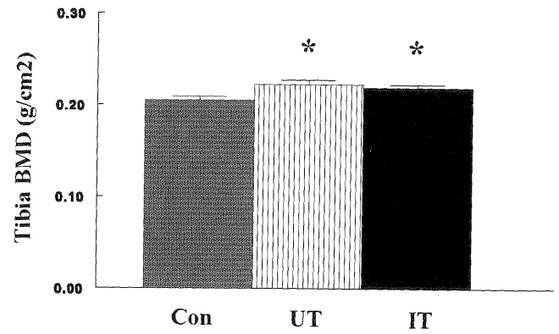


**Fig. 2** Bone mineral density (BMD) for the left tibia from Controls (Con, n = 8), Uninterrupted resistance trained group (UT, n = 8), and Interrupted resistance trained group (IT, n = 8). * Significant difference vs. Con.

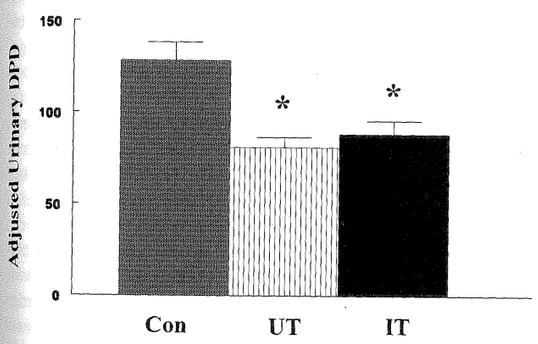


**Fig. 3** Adjusted urinary deoxypyridinoline (DPD) concentrations (see Methods) from Controls (Con, n = 8), Uninterrupted resistance trained group (UT, n = 8), and Interrupted resistance trained group (IT, n = 8). *Significant difference vs. Con.

However, when we expressed the protein content in the flexor hallucis longus per 100 grams of body mass, there were significantly higher protein concentrations from both Uninterrupted and Interrupted groups compared to the Control group (**Table 1**).

The bone mineral density in the left tibia of both the Uninterrupted ($p = 0.024$) and Interrupted ($p = 0.029$) groups was significantly greater than of the Control group (**Fig. 2**). However, the bone mineral density was not significantly different between Uninterrupted and Interrupted groups ($p = 0.930$). Serum osteocalcin did not significantly differ between the Controls (43.6 ± 1.2 ng/ml), Uninterrupted (42.6 ± 0.6 ng/ml), and Interrupted (40.4 ± 1.6 ng/ml) groups. In contrast, the adjusted urinary deoxypyridinoline was significantly lower in Uninterrupted ($p = 0.004$) and Interrupted ($p = 0.002$) groups when compared to the Control group, but did not significantly differ ($p = 0.518$) between the Uninterrupted and Interrupted groups (**Fig. 3**).

The increase in bone mineral density observed for Uninterrupted and Interrupted groups resulted in significant elevations in the mechanical properties of the left tibia compared to controls as measured from the three-point bending test. The force to failure and energy absorption prior to failure were significantly greater for Uninterrupted and Interrupted groups compared to controls (**Table 2**), but were not significantly different between Uninterrupted and Interrupted groups ($p = 0.497$ and $p = 0.436$, respectively).

**Table 2** Bone mechanical properties from 3-pt bending test

| Group | Fmax (N) | EF (N × mm) |
|---|---|---|
| Con | 75.3 ± 2.9 | 61.3 ± 3.4 |
| UT | 107.3 ± 4.1* (p = 0.002) | 116.6 ± 9.1* (p < 0.001) |
| IT | 101.1 ± 9.7* (p = 0.009) | 105.9 ± 13.2* (p = 0.003) |

Con = control group (n = 8), UT = uninterrupted resistance trained group (n = 8), and IT = interrupted resistance trained group (n = 8). Maximum load to failure = Fmax (in Newton) and energy to failure = EF (area under the load-deformation curve in Newton × millimeter). P values are indicated for UT and IT vs. Con. * Significant difference vs. Con

## Discussion

The current study demonstrated that both an uninterrupted and interrupted 6-week resistance training regimen (where the total volume of work was kept constant between Uninterrupted vs. Interrupted groups) effectively induced an increase in tibial bone mineral density in maturating male rats. After 6 weeks of training, the osteogenic response for both Uninterrupted and Interrupted groups was not attributable to an elevation in osteoblast activity, as indicated by an increase in serum osteocalcin, but a decrease in osteoclast activity, as indicated by a decline in the adjusted urinary deoxypyridinoline. The increased bone mineral density from both Uninterrupted and Interrupted groups culminated in augmented bone strength as assessed from three-point bending tests when compared to controls. While the bone mineral density and bone strength were elevated in both Uninterrupted and Interrupted groups, there was no significant difference between Uninterrupted and Interrupted groups. Thus, our results did not support our initial hypothesis

of a greater osteogenic response from Interrupted compared to Uninterrupted resistance training.

Consistent with prior studies using the ladder climbing task [8, 20], we report an elevation in skeletal muscle protein in the flexor hallucis longus to offer support of a training effect from the exercise programs. Given that bone deposition is specific to the mechanical loads placed upon it, we chose to examine the bone mineral density in the tibia in accord with the location of the flexor hallucis longus. The elevation in tibial bone mineral density in the Uninterrupted group is consistent with our prior study, where we similarly observed an increase in bone mineral density from animals carrying 150% body mass after 6 weeks of training [20]. In contrast to our hypothesis, we failed to observe more bone modeling and elevated bone mineral density when interrupting the exercise bout into three discrete sessions per training day, when compared to the continuous resistance trained group. However, our results support the observations of Umemura et al. [26] who similarly reported, in a jump exercise program, no augmented response from a 6-hour interval between two daily exercise sessions (2 × 10 jumps) compared to a continuous exercise bout (1 × 20 jumps) in maturating animals. To our knowledge, the current study constitutes only the second report to specifically examine, in growing animals, the impact upon bone mineral density when separating voluntary exercise sessions into discrete intervals rather than performing continuous repetitions in a single training bout. Umemura et al. [26] used high-impact exercise (5 days/week for 8 weeks), whereas we employed the use of resistance training (3 days/week for 6 weeks). Collectively, both of these reports in rats suggest that separating a bout of exercise into discrete sessions during a training day is equally as effective as a single bout of exercise performed on a given training day.

In contrast, Robling et al. [17,18] reported, in anesthetized animals, that a daily loading protocol of 4 bouts of 90 loading cycles separated by 3 hours produced a significantly greater osteogenic response than a single, continuous bout of 360 loading cycles. The potential mechanism(s) for the difference between our results and that of Robling et al. [17,18] is beyond the scope of this study. First, since we did not perform any strain gauging, we cannot appropriately compare the ladder climbing task we employed to the ulna loading protocol used by Robling et al. [17, 18]. Next, we note several disparities between the studies. For example, we used conscious animals and a resistance training program that incorporated bouts of exercise every other day. In contrast, Robling et al. [17,18] used anesthetized animals and a daily loading protocol. Despite the differences between our study and that of Robling et al. [17,18], we were able to achieve a ~7.5% increase in bone mineral density in the tibia after 6 weeks while Robling et al. [17,18] observed ~7% increase in bone mineral density in the ulna after 16 weeks. While an appropriate comparison between our resistance training protocol and the loading protocol implemented by Robling et al. [17,18] was not possible, we concur with the speculation submitted by Umemura et al. [26] that more time than the 4–5 hours we provided between intervals were required for the mechanosensors to recover. If the mechanosensors fail to recover, this could be the reason for the lack of difference between our exercise protocols. Last, it is conceivable that the age of the animal might be a factor for the different results. In the current study, as well as the prior report by Umemura et al. [26], maturating animals were examined, whereas Robling et al. [17,18] used adult rats. Thus, it is possible that the use of mature adult animals may yield contrasting results compared to growing animals.

Net bone deposition is dependent upon the amount of bone formation compared to bone resorption. As such, an osteogenic effect can be the result of increased bone formation compared to bone resorption, decreased bone resorption compared to bone formation, or a combination of both. Our results suggest that the training-induced increase in bone mineral density is attributable to a decline in bone resorption, whereas bone formation was maintained. Yeh et al. [29] similarly observed a training-induced elevation (via treadmill exercise) in bone modeling from maturating female rats due to a decline in bone resorption while bone formation was sustained. However, these observations are in contrast to previous studies [5,6,11,27], including our own prior report [20], where training-induced augmentations in bone mineral density were attributable to an increase in bone formation as evidenced by an elevation in serum osteocalcin. While our current results are consistent with Yeh et al. [29], we have no explanation for the contrasting observations regarding the biochemical markers. Nevertheless, we note that to minimize any residual effects of the last exercise bout we delayed the sacrifice of the animals by 72 hours. As such, it is possible that the significant postponement caused more rapid changes from exercised animals in serum osteocalcin returning it to pretraining levels, whereas the lower urinary deoxypyridinoline: (a) suggests a continuous attenuated osteoclast activity, (b) reflects a delay in glomerular filtration that would eventuate in a return to pretraining levels, (c) a longer half-life for deoxypyridinoline, or (d) represents a different point in the bone modeling cycle. As it pertains to the bone modeling cycle, a better experimental design to account for oscillatory effects would be to measure biochemical markers (i.e., osteocalcin and deoxypyridinoline) prior to and throughout the training program. While we acknowledge this potential deficiency, we note that the biochemical markers still support the elevation in bone mineral density, albeit at a single terminal time point.

We also recognize that the control animals could have initially started with less baseline tibial bone mineral density than the other groups, thereby limiting any interpretation of our data. As such, measuring the bone mineral density (via the dual energy X-ray absorptiometer) prior to and throughout the training program would similarly be advantageous. However, given that the animals were randomly separated into three groups where initial body weights were not significantly different between groups; the probability of placing only animals with less bone mineral density into the Control group was minimal. Further, only the resistance trained groups (i.e., Uninterrupted and Interrupted) demonstrated concomitant elevations in flexor hallucis longus total protein, declines in urinary deoxypyridinoline, and increases in bone strength, which add support to the effectiveness of the resistance training protocol on bone modeling.

While elevations in bone mineral density as a result of our resistance training protocol are noteworthy, the most important parameter related to the risk of fractures is the mechanical strength of the bone. The results of our three-point bending test demonstrated that relatively small elevations in bone mineral density yielded large changes in bone strength, where the maximum force to failure and the amount of energy absorbed by the bone prior to failure was increased ~38% and ~82%, respectively, in the resistance trained groups when compared to controls. These results are consistent with Robling et al. [18,25] who reported that a 5.4% increase in bone mineral density after bone loading in the rat ulna resulted in a 64% elevation in the force to failure and a 94% augmentation in the energy to failure compared to controls. Our results are also consistent with other studies in rats that examined the femur after jumping exercise [14] and tower climbing [15], confirming that relatively small increases in bone mineral density can culminate in large elevations in bone strength. Last, we note that Umemura et al. [26] similarly examined bone strength in the tibia from continuous vs. interrupted jump exercise in young rats and observed a 34% elevation in bone strength compared to controls with use of a three point bending test. Collectively, our results from uninterrupted vs. interrupted resistance training, as well as the observations by Umemura et al. [26] employing continuous vs. interrupted high-impact exercise, suggests that either exercise mode (i.e., high-impact or resistance training), performed either continuously or via interruption, are equally effective in elevating bone strength in growing rats.

Although the use of animals helps to eliminate many of the confounding variables associated with human studies, we acknowledge the limitations with use of rats. First, we recognize that the epiphyseal plates in rats do not close. As such, we chose to examine the impact of exercise specifically during the growth period in rats that could apply to maturating humans, albeit this should be done with caution. Next, there is also the prevailing concern that an observation in animals may not occur in humans. However, the findings in animals have been consistent with the observations in humans pertaining to the impact of exercise on bone. Thus, our results offer a potential insight into the type of resistance training program (i.e., continuous vs. interrupted exercise) that would optimize bone accrual during growth.

Finally, with prudence we offer a consideration based upon our observations of the exercised animals. The limiting factor in the number of repetitions performed by the Interrupted group was the amount of repetitions achieved by the Uninterrupted group. The ladder climbs were easily accomplished by the Interrupted group during each discrete exercise bout during the day, whereas the Uninterrupted group struggled during the last several ladder climbs on a given day. While the volume of work was equivalent by design, we submit that the recovery period for the Interrupted group was sufficient to easily achieve the required work for each exercise session during a training day. In this regard, to the extent that these observations can be extrapolated to an application for humans, separating a training day into discrete exercise bouts would stimulate bone accrual during growth equivalent to what is achieved via continuous exercise, while minimizing muscle fatigue and lowering the potential risk of injury.

In summary, using conscious animals and a mode of exercise that mimics resistance training, where the volume of work was equivalent between Uninterrupted and Interrupted programs, we offer evidence that both exercise regimens were equally effective in providing a stimulus to elicit an osteogenic response in maturating male animals. This is supported by a decline in adjusted urinary deoxypyridinoline levels and an increase in bone mineral density. The elevation in bone mineral density also yielded augmented bone mechanical properties assessed from three-point bending tests. We also acknowledge that while our interrupted exercise program did not optimize the osteogenic response, we cannot rule out the possibility that more recovery time between intervals was necessary to reset the mechanosensors. Therefore, more studies in conscious maturating animals employing a variety of interval durations are required to fully elucidate any maximal effectiveness of using multiple exercise sessions within a given training day.





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