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Key words
- Tibia
- DXA
- osteocalcin
- deoxypyridinoline
- 3-pt bending test

Abstract

The purpose of this study was to examine the efficacy of continuous resistance training (3 days/wk) compared to interrupted resistance training where 20–24 h separated an exercise bout (i.e. 6 days/wk) 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/wk resistance trained group (RT3, n=9), and 6 days/wk resistance trained group (RT6, n=9). The RT3 and RT6 groups were conditioned to climb a vertical ladder with weights appended to their tail for a total of 6 wks. After 6 wks, BMD (assessed via DXA) from the left tibia was significantly greater for RT3 ($0.242 \pm 0.004\,g/cm^2$) and RT6 ($0.244 \pm 0.004\,g/cm^2$) compared to Con ($0.226 \pm 0.003\,g/cm^2$). Further, serum osteocalcin (oc, in ng/ml) was significantly greater for RT3 (75.8 ± 4.4) and RT6 (73.5 ± 3.8) compared to Con (53.4 ± 2.4). There was no significant difference in BMD or serum OC between RT3 and RT6 groups. The results indicate that both resistance training programs were equally effective in elevating bone mineral density in young, growing rats.

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Introduction

The amount of peak bone mass accrued during childhood and adolescence, as well as the amount of ensuing bone loss 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 enhance our understanding of elevating peak bone mass. The hormonal milieu associated with the maturation process elicits a positive impact upon bone modeling. As such, incorporating exercise to stimulate an osteogenic response during the growth period would be beneficial in further 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 bone 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, even though the amount of force applied between loading regimens was equivalent [18, 19]. From these experiments, Turner and co-workers [22–24] suggested that bone cells become desensitized to prolonged mechanical loading, whereas recovery periods can restore the mechanosensitivity, thereby augmenting the osteogenic stimulus. While the findings of Robling and Turner [18, 19, 22–24] were promising, we previously failed to observe additional gains in bone mineral density using an interrupted resistance training protocol allowing 3–4 h of recovery between exercise bouts [7] and 10–12 h of recovery between bouts [6] in young, growing rats. In both our prior studies, after 6 weeks of training, interrupting the resistance exercise into discrete bouts (with various hours of recovery) was just as effective as continuous, uninterrupted resistance training in elevating bone mineral density in growing rats [6, 7].

Given our inability to support the innovative work of Turner and Robling [18, 19, 22–24], we previously speculated that: [1] even more time was needed between the interrupted resistance training bouts to allow the mechanosensors to recover or [2] at a certain amount of work, the mechanosensors become desensitized so that daily resistance training, where muscles perform only half the work, could similarly elicit an osteogenic response equally effective to resistance training every other day [6]. Therefore, the purpose of the current study was to determine if a daily resistance training program, where only half the work was performed on a given training day, was more effective than triweekly (i.e. 3 times per week) resistance training, where all the work was performed on a given training day, on bone mineral density (BMD) in young, growing animals. In this regard, the daily exercised group essentially executed the same amount of work per week as the triweekly exercised group, but performed half the work on a given day, separated by 20–24 h 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 h) should restore the mechanosensitivity. Thus, if the mechanosensors were able to reset then, according to the hypothesis proposed by Turner and Robling [18, 19, 22–24], 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 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 for stimulating an elevation in bone mineral density and bone strength compared to controls.

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 ~225 grams, ~8 weeks old) obtained from Charles River Laboratories (Wilmington, MA) were housed individually and maintained on a reverse l2/12 h light/dark cycle. Food and water were provided *ad libitum* throughout the experimental period. The animals were acclimated to their living conditions for one week prior to random separation into a control group (Con, n=12), a resistance trained group where the animals exercised 3 days/week (RT3, n=12), or another resistance trained group where the animals exercised daily (i.e. 6 days/week, RT6, n=12). After the random separation of animals into their respective groups and prior to any exercise training, 3 animals from each group were sacrificed to obtain baseline values (e.g. osteocalcin, deoxypyridinoline, BMD, and bone strength). The purpose of the baseline data was to ensure that we did not inadvertently place animals with more or less BMD into a specific group. Further, the baseline data allowed for an examination of the amount of bone modeling attributable to normal growth compared to any additional impact elicited by resistance training. Since there were no significant differences between 3 animals from each group, the animals in the baseline group were pooled (BL, n=9) leaving a total of nine animals in each of the three groups (i.e. Con, RT3, and RT6).

Resistance training

The strength training regimen has previously been described [21]. Briefly, the animals were required to climb a vertical ladder with weights appended to their tail. There were 26 rungs across the 1 meter ladder. The animals were positioned to ensure that they performed each sequential step, where one repetition along the ladder required 26 total lifts by the animal (or 13 lifts per limb). The resistance trained animals were operantly conditioned for one week to climb the ladder in order to avoid a vat of water beneath them. Both the RT3 and RT6 groups trained for a total of 6 weeks. The control animals were handled on the same days and times as the trained groups in order to minimize any stress attributable to handling. All animals were weighed at the beginning of each week to monitor weight gains and, for the resistance trained animals, to help determine the amount of weight to append to their tails for the remainder of the week. All resistance trained animals started with 30% body mass (BM) appended to their tail at week one. At week two they were carrying 60% BM. At week three they carried 90% BM. At week four they were carrying 120% BM and at week five they carried 135% BM. At week six, they were carrying 150% BM. For the RT3 group (i.e. 3 days/wk), the animals performed 6 consecutive ladder climbs on a given training day. The 6 ladder climbs constituted the maximum amount of consecutive repetitions that the RT3 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/wk), the animals performed 3 ladder climbs each day. As such, the RT6 group essentially executed the same number of ladder climbs as the RT3 group, but the RT6 group performed the exercise over 2 days with 20–24 h of recovery, whereas the RT3 group did all the work on a specific training day. The 20–24 h between exercise sessions for the RT6 group served to allow the mechanosensors to reset. The resistance (i.e. the weight appended to their tail plus their body mass) and the distance covered helped to equate the total volume of work performed within a given week between the RT3 and RT6 groups throughout the training period.

Experimental protocol

Animals were sacrificed 48 h after their final training session to minimize any residual effect of the last training bout. 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. We chose the FHL since ladder climbing has previously been observed to elicit hypertrophy in the FHL [6, 7, 9, 21]. All remaining soft tissues were removed from the right tibia and the bone was submerged in a scintillation vial filled with an ethanol/saline (50/50) solution, capped, and kept at room temperature. Bone strength was assessed from the right tibia within 2 weeks after dissection. The left hindlimb was rapidly amputated, positioned, and frozen in liquid nitrogen for the assessment of bone mineral density of the tibia. Blood samples were collected, allowed to clot, centrifuged, and the serum was frozen for the subsequent measurement of serum osteocalcin (OC). Finally, a syringe was used to extract urine directly from the bladder and immediately frozen for the

atinine. The FHL, left hindlimb, serum, and urine samples were kept at −80 °C until their analyses.

Chemical analyses

Protein concentration in the FHL was assessed [13] as an indirect indicator of training (i.e., muscle hypertrophy). A sandwich enzyme-linked immunosorbent assay (ELISA, Biomedical Technologies, Inc., Stoughton, MA) was used to determine serum osteocalcin levels (an indicator of osteoblast activity). The intra-assay variation was <4% and the inter-assay variation was <7%. Urinary deoxypyridinoline (an indicator of osteoclast activity) was measured using a competitive enzyme immunoassay (EIA, Quidel Corp., San Diego, CA). The intra-assay and inter-assay variation was <6%. Urinary creatinine was measured using an enzyme assay and picric acid as the color reagent (Quidel Corp., San Diego, CA). A microplate reader (MaxLine, Molecular Devices Corp., Sunnyvale, CA) was used with the absorbance set at 450 nm for the ELISA, 405 nm for the EIA, 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 (Pearson's Product for linear curves, i.e. protein and creatinine, or Coefficient of Determination for non-linear curves, i.e. OC or DPD) was greater than 0.99. Finally, a Dual Energy X-ray Absorptiometer (DXA - GE Lunar Prodigy, Chicago, IL) employing the small animal software module (version 6.81) was used to assess the BMD of the whole left tibia. Briefly, the left hindlimb was thawed, positioned, and the entire tibia was scanned. Condyle and malleolus curvatures 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 only if proper orientation was observed by the technician. Three consecutive measurements were performed with the hindlimb repositioned 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 submerged in saline for 24 h at room temperature. The instrument was calibrated using a standard weight and then the tibia was patted dry 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) whereas 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/sec for all groups. A medial to lateral force was applied to the midshaft of the bone. The maximal load to failure (Fmax, units = N), energy to failure (EF, determined from the area under the load-deformation curve to the fracture point, units = N × mm), and bone stiffness (slope of the linear portion from the load-deformation curve) were assessed 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 distance covered. The total training volume (i.e. work) per week for the RT3 and RT6 group was expressed in Joules. For the 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 (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 (see above), an ANOVA was employed and when a significant F ratio was identified, a Fisher's PLSD post hoc test was employed. 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).

Table 1 Body Mass.

Group	Initial Body Mass (grams) at 9 weeks of age	Final Body Mass (grams) at 16 weeks of age
BL	348.9 ± 4.9	not Applicable
Con	330.1 ± 7.3	466.9 ± 14.1
RT3	333.3 ± 8.3	444.3 ± 12.5
RT6	336.5 ± 8.2	445.4 ± 15.0

BL = Baseline Group (n = 9), Con = Control Group (n = 9), RT3 = 3 Days/Week Resistance Trained Group (n = 9), and RT6 = 6 Days/Week Resistance Trained Group (n = 9). No significant difference between groups for initial or final body mass

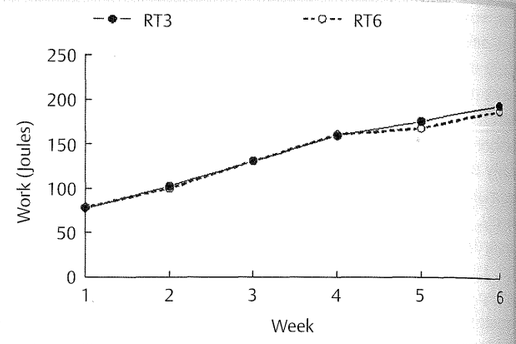

Fig. 1 Total work (in Joules) performed for each training week by the 3 days/week resistance trained group (RT3, n = 9) and the 6 days/week resistance trained group (RT6, n = 9). For each group, there were consistent body mass gains each week resulting in no significant difference between groups at any time point.

Results

The initial body mass was not significantly different between groups (◎ **Table 1**). 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 RT3 vs. RT6 for any week during the 6 week exercise program (◎ **Fig. 1**). The FHL mass and total protein content in the FHL was significantly elevated for all groups (i.e. Con, RT3, and RT6) compared to Baseline (◎ **Table 2**). In addition, the FHL mass and total protein was significantly greater for RT3 and RT6 groups when compared to the Con group (◎ **Table 2**). There was no significant difference in FHL mass or total protein in the FHL between RT3 and RT6 groups.

Table 2 Resistance Training Effect on the Flexor Hallucis Longus.

Group	FHL Mass (grams)	FHL Protein (mg protein/muscle)
BL	0.186 ± 0.006	28.62 ± 1.18
Con	0.225 ± 0.024†	35.70 ± 3.94†
RT3	0.283 ± 0.011†*	45.59 ± 1.40†*
RT6	0.290 ± 0.015†*	48.09 ± 2.84†*

BL = Baseline Group (n = 9), Con = Control Group (n = 9), RT3 = 3 Days/Week Resistance Trained Group (n = 9), and RT6 = 6 Days/Week Resistance Trained Group (n = 9). FHL = flexor hallucis longus. †Significant difference vs. BL. *Significant difference vs. Con

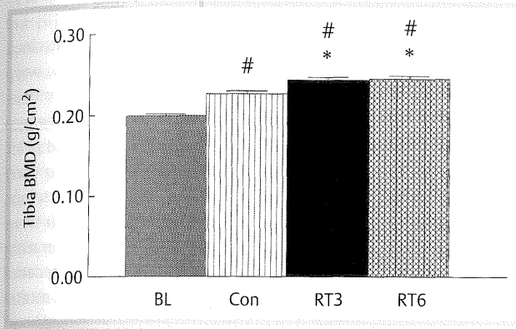

Fig. 2 Bone mineral density (BMD) for the left tibia from Baseline animals (BL, n = 9), Controls (Con, n = 9), 3 days/week resistance trained group (RT3, n = 9), and 6 days/week resistance trained group (RT6, n = 9). Significant difference vs. BL. *Significant difference vs. Con.

The bone mineral density from the whole left tibia was significantly elevated for Con (i.e. 14.1% increase), RT3 (i.e. 22.2% increase), and RT6 (i.e. 23.2% increase) compared to Baseline (◎ **Fig. 2**). Further, the BMD from the RT3 and RT6 groups were 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 BL, but was significantly greater for RT3 (i.e. 41.8% increase) and RT6 (i.e. 37.6% increase) compared to Con as well as Baseline (◎ **Fig. 3**). Serum osteocalcin concentrations were not significantly different between RT3 and RT6 groups (◎ **Fig. 3**). The adjusted urinary deoxypyridinoline did not significantly differ between the Baseline (131.9 ± 10.8), Con (108.8 ± 38.3), RT3 (102.5 ± 17.7), and RT6 (111.0 ± 30.9) groups.

The 6 week growth period resulted in significant increases in bone strength parameters. The maximum force to failure, energy to failure, and bone stiffness were significantly greater for all groups (i.e. Con, RT3, and RT6) compared to Baseline (◎ **Table 3**). Specifically, the 6 week growth period yielded a significantly greater maximum force to failure (i.e. 33% increase), energy to failure (i.e. 47% increase), and bone stiffness (i.e. 27.7% increase) from Con compared to BL groups. In addition, resistance training resulted in further increases in bone strength of the right tibia for the RT3 and RT6 compared to controls. The maximum force to failure was significantly greater for RT3 (i.e. 54% increase) and RT6 (i.e. 62% increase) compared to controls (◎ **Table 3**). Similarly, the energy to failure was significantly greater for RT3 (i.e. 56% increase) and RT6 (i.e. 68% increase) compared to controls (◎ **Table 3**). Further, resistance training resulted in significant increases in bone stiffness for RT3 (i.e. 24.4% increase) and RT6 (i.e. 28.9% increase) compared to controls. However, the maximal force, energy to failure, and bone stiffness were not significantly different between RT3 and RT6 groups (◎ **Table 3**).

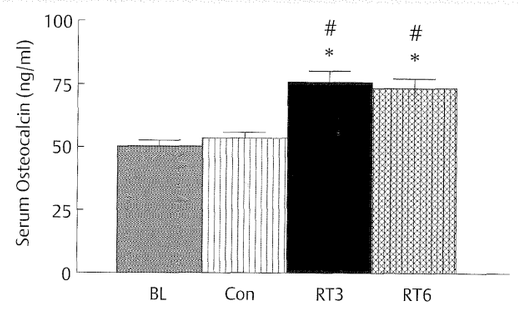

Fig. 3 Serum osteocalcin concentrations (in ng/ml) from Baseline animals (BL, n = 9), Controls (Con, n = 9), 3 days/week resistance trained animals (RT3, n = 9), and 6 days/week resistance trained animals (RT6, n = 9). Significant difference vs. BL. *Significant difference vs. Con.

Table 3 Bone Mechanical Properties from 3-Pt Bending Test.

Group	Fmax (N)	EF (N x mm)	Bone Stiffness (N/mm)
BL	65.4 ± 3.8	63.9 ± 8.2	52.7 ± 4.0
Con	87.1 ± 4.8†	93.8 ± 4.9†	67.3 ± 4.3†
RT3	133.7 ± 11.3†*	146.4 ± 10.3†*	83.7 ± 4.7†*
RT6	141.3 ± 6.9†*	157.4 ± 10.7†*	86.8 ± 5.8†*

Bone strength of the tibia from the BL = Baseline Group (n = 9), Con = Control Group (n = 9), RT3 = 3 Days/Week Resistance Trained Group (n = 9), and RT6 = 6 Days/Week Resistance Trained Group (n = 9). Fmax = Maximum load to failure (in Newtons), EF = Energy to Failure (area under the load-deformation curve in Newtons x millimeters), and Bone Stiffness (linear portion of the load-deformation curve in Newtons ÷ millimeters). †Significant difference vs. BL. *Significant difference vs. Con

Discussion

The Con, RT3, and RT6 groups demonstrated elevations in: body mass, FHL mass, FHL protein, BMD, and bone strength compared to the baseline group, 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 BMD compared to maturation to young adulthood alone. The osteogenic response for both 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 decline in osteoclast activity, as indicated by equivalent levels of adjusted urinary DPD between groups. Further, both RT3 and RT6 groups demonstrated augmented bone strength when compared to controls. While the BMD, bone strength, and serum OC were elevated in both RT3 and RT6 compared to controls, there was no significant difference between RT3 and RT6 groups. Thus, the results support our initial hypothesis where the daily and triweekly resistance training programs (where the total volume of work was kept constant between RT3 vs. RT6 groups) were equally effective in stimulating an increase in tibial BMD and bone strength in young, growing male rats.

A contributing risk factor for the development of osteoporosis is 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 osteogenic response, thereby elevating bone mineral density [3,5]. Since the maturation period already provides a hormonal milieu conducive to skeletal growth, this would be an opportune time to incorporate exercise in order to stimulate an even greater elevation in peak bone mass. In support, cross-sectional studies in prepubertal boys [2,14] and prepubertal or peripubertal girls [12,20] reported increases in bone mineral density after exercise training when compared to non-exercised counterparts. We recognize that in humans, the maturation stage and the growth rate are contributing factors to bone formation which may account for differences between groups in cross-sectional studies. Nevertheless, an examination in prepubertal monozygotic female twins [26] similarly observed increases in bone accrual in one twin after exercise training compared to the other twin who did not participate in a training program. The positive impact of exercise during growth has also been observed in mid-adolescent female tennis players who demonstrate greater bone mass in the dominant arm compared to their non-dominant arm [8]. Further, several animal studies have similarly supported additional increases in bone mass or BMD when exercise is implemented during the growth period [10,16,17,25]. In this regard, the current findings were consistent with the benefits of resistance training during the maturation period to young adulthood in rats resulting in even more bone accrual compared to growth alone. In addition, our results also confirm previous reports in humans [3,5,15] and animals [7,21,28] pertaining to the exercise-induced elevation in osteoblast activity as the likely mechanism for the elevation in BMD, supported by the significant increase in serum OC for both RT3 and RT6 groups compared to controls.

Given the beneficial effects of exercise during the growth period in further elevating bone mineral density, identifying the optimal training program for eliciting the greatest impact upon bone would be important for the prevention of osteoporosis. In this context, Turner and Robling [24] proposed a novel exercise regimen. Based upon their elaborate studies [18,19], they suggested that the osteogenic response to bone loading can be elevated with multiple sessions during a training day separated by periods of rest (i.e., at least a 3 h recovery period between loading bouts). They attributed the enhanced bone formation to the restoration of bone cell mechanosensitivity during the recovery period [18,22,24]. As such, interrupting bone loading into multiple sessions essentially provided more stimulation for bone formation compared to continuous loading [18,19,22–24]. In contrast, Umemura et al. [25] examined high impact (i.e. jumping) exercise 5 days/week for 8 weeks. They reported equivalent elevations in bone mass using a 6 h recovery between two daily exercise sessions (2×10 jumps) compared to a continuous exercise bout (1×20 jumps) in maturating female rats [25]. Further, in two prior resistance training studies where we employed the ladder climbing task as described in the current study, we also observed equal effectiveness in elevating BMD and bone strength between continuous versus interrupted resistance training after 6 weeks of exercise 3 days/week in growing male rats. In one report, we separated the exercise into 3 discrete bouts during a training day (3 bouts×2 ladder climbs, with 3–4 h of recovery between bouts) vs. continuous exercise on a training day with no interruptions (i.e. 6 consecutive ladder climbs) [7]. In another report, we replicated the resistance training protocol, but increased the amount of time between exercise sessions using 2 discrete bouts during a training day (2 bouts×3 ladder climbs, with 10–12 h of recovery between bouts) compared to continuous exercise (i.e. 6 consecutive ladder climbs) [6]. In both these prior studies, we failed to observe a greater BMD in the interrupted resistance trained group compared to the continuous exercised group. We have no explanation for the discrepant results when compared to Robling et al. [18,19], but we recognize several differences. First, Robling et al. [18,19] used anesthetized animals whereas the other studies examined conscious animals [6,7,25]. In this regard, the use of anesthetized animals could limit the physiologic response to exercise and may account for the additional length of time required before alterations in bone formation were observed by Robling et al., [18,19] i.e. loading the bone for 16 weeks, compared to the use of conscious animals where differences in bone mineral density were observed in 6–8 weeks of training [6,7,21,25]. Next, it is conceivable that the age of the animal might be a factor where growing animals were examined in the current study and prior reports [6,7,25], compared to Robling et al. [18,19] who used adult rats. Collectively, these prior reports in young, conscious animals suggested to us that (a) more time than 10–12 h were required to restore the bone mechanosensitivity in maturating rats or (b) at a certain amount of work the osteocytes became desensitized despite the additional osteogenic input via multiple exercise bouts within a training day. Given the 20–24 h we allowed in the current study for the mechanosensors to reset and our failure to observe greater elevations in BMD in the daily exercised group compared to the triweekly group, we offer an amended mechanism and propose that the hormonal milieu during the growth period in combination with the exercise reached a maximal stimulation threshold where any additional osteogenic input was ineffective, culminating in the equivalent results between resistance training protocols. This alternate hypothesis of a maximal stimulation threshold could account for the difference in results between the studies examining young, growing animals [6,7,21,25] compared to Robling et al. [18,19] in adult animals.

One of the most important factors in the prevention of fractures is bone strength. We recognize that interpretations in bone strength data represent relative rather than absolute changes given the potential differences in specimen storage, bone hydration, the temperature at which the bones are broken, etc. that can contribute to differences between studies. However, it is noteworthy that Turner and Robling [24] reported a 5.4% elevation in BMD after bone loading in the rat ulna resulting in a 64% and 94% increase in the maximal load to failure and energy to failure, respectively. In like manner, Umemura et al. [25] reported a 12% elevation in tibial bone mass culminating in a 34% increase in the maximal load to failure after jump exercise. In a prior report we reported a 7.5% increase in tibial BMD resulting in a 38 and 82% elevation in the maximal load to failure and energy to failure, respectively after ladder climbing [7]. More recently, we reported a 6.7% increase in tibial BMD culminating in a 27% and 58% elevation in the maximal load to failure and energy to failure, respectively following 6 weeks of ladder climbing [6]. In the current study, we observed a 7.5% elevation in tibial BMD resulting in a training-induced average increase of 58% and 62% in the maximal load to failure and energy to failure, respectively. In addition, we observed a training-induced elevation of ~26% in bone stiffness compared to controls. Therefore, our current results were consistent with prior animal reports demonstrating that relatively small elevations in bone mineral density can yield large increases in bone strength. Recognizing that an assessment of bone strength is not attainable in man and assuming these results can be extrapolated to humans, this would substantiate the importance of resistance exercise in the strengthening of bone and the prevention of fractures.

With caution we offer a consideration based upon our observation of the exercised animals. The number of repetitions performed by the daily exercised group was predicated upon the number of climbs performed by the triweekly exercised group. The ladder climbs were easily accomplished by the daily exercised group whereas the triweekly group struggled to complete the last 1–2 ladder climbs during a training day. Thus, rigorous exercise during the growth period may not be necessary as long as the exercise is sufficient to create a fluid flow within the lacunar-canalicular network to stimulate bone formation. However, we recognize that specific exercise intervention strategies (i.e. type and intensity) need to be further elucidated, especially in children.

Finally, we acknowledge several limitations in the interpretation of our results. First, we note that the epiphyseal plates in rats do not close where any extrapolation derived from adult rats to that of humans should be done with caution. In this regard, we chose to examine the impact of exercise during the growth period in rats that could apply to maturating humans, although this should also be done with caution. Next, while we randomly selected 3 animals from each group to represent our baseline group, we recognize that a better experimental design would be to obtain DXA measurements from all animals prior to the random separation in order to prevent inadvertent outliers within and between groups. Last, our control animals were not exposed to any activity giving rise to the dramatic differences in BMD between groups. Even in the absence of what would constitute "normal" activity for the rat we note that the control animals demonstrated appropriate elevations in body mass, BMD, and other parameters representative of growth. In addition, many other animal studies have similarly compared exercised rats to sedentary controls [10,16–19,25], which may be appropriate in the extrapolation to humans, given the current societal factors that promote a more sedentary lifestyle. Moreover, to the extent that our findings in animals can be applied to humans, our results support the importance and impact of incorporating exercise training to further augment bone mineral density during the growth period that extends into young adulthood.

In summary, using conscious animals and a mode of exercise that mimics resistance training, where the weekly volume of work was equivalent between the RT3 and RT6 groups, we provide evidence that both daily and triweekly programs were effective in elevating BMD in young male animals above the impact of growth alone. The effectiveness of the resistance training programs were further supported by elevations in: serum OC and bone mechanical properties assessed from three point bending tests. We conclude that under the conditions of the current study, allowing 20–24 h for the mechanosensors to reset (i.e. daily resistance training) failed to elicit greater elevations in BMD compared to triweekly strength training. Thus, it is possible that during the growth period there is a maximal stimulation threshold for bone mineral density. As such, we acknowledge that further investigations are warranted in growing animals to determine the amount of exercise required to reach the maximal stimulation threshold for BMD.

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