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R. A. Pierce
Chapman University

L. C. Lee
Chapman University

C. P. Ahles
Chapman University

S. M. Shdo
Chapman University

S. V. Jaque
California State University - Northridge

See next page for additional authors

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R. A. Pierce, L. C. Lee, C. P. Ahles, S. M. Shdo, S. V. Jaque, and Ken D. Sumida

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R. A. Pierce[1], L. C. Lee[1], C. P. Ahles[1], S. M. Shdo[1], S. V. Jaque[2], K. D. Sumida[1]

Affiliations

[1] Department of Biological Science, Chapman University, Orange, California, United States
[2] Department of Kinesiology, California State University, Northridge, California, United States

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

Abstract

The purpose of this study was to determine if an exercise threshold existed in stimulating an elevation in bone mineral density (BMD), via resistance training, during the growth period in male rats. 27 male rats were randomly divided into Control (Con, n=9), 3 ladder climb resistance trained group (3LC, n=9), and 6 ladder climb resistance trained group (6LC, n=9). The 3LC and 6LC groups were conditioned to climb a vertical ladder with weights appended to their tail 3 days/wk for a total of 6 wks, but the 6LC group performed significantly more work than the 3LC group. After 6 weeks, left tibial BMD (mean±SD) was significantly greater for 3LC (0.225±0.006 g/cm^2) and 6LC (0.234±0.008 g/cm^2) when compared to Con (0.202±0.013 g/cm^2). Further, bone strength (force to failure in Newtons) was significantly greater for 3LC (132.7±13.7) and 6LC (130.0±22.8) compared to Con (102.0±10.1). There was no significant difference in BMD or bone strength between 3LC and 6LC. The results indicate that both resistance training programs were equally effective in elevating BMD and bone strength in growing rats. These data suggest that during growth, there is a stimulation threshold where more work per exercise session is ineffective in promoting additional bone formation.

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Bibliography
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Correspondence
Dr. Ken D. Sumida, PhD
Department of Biological Science
Chapman University
One University Drive
92866 Orange
United States
Tel.: +1/714/997 6995
Fax: +1/714/532 6048
sumida@chapman.edu

Introduction

Incorporating exercise during childhood and adolescence has been advocated to help delay the onset or reduce the severity of osteoporosis [12,28]. Given that the hormonal milieu associated with the growth process promotes bone modeling, incorporating exercise that stimulates an osteogenic response would be beneficial in further elevations in peak bone mass. Maximizing peak bone mass during the growth period via exercise could minimize the deleterious effects of osteoporosis during senescence. However, the amount of exercise required during the growth period to maximize bone accrual remains to be elucidated.

In prior reports we have consistently demonstrated elevations in bone mineral density when growing animals engage in resistance training for 6 weeks, via ladder climbing with weights appended to their tail [6,7,13]. Further, in these previous studies, we attempted to provide an additional stimulus for bone formation by interrupting the exercise bouts as proposed by Turner and Robling [25]. In contrast to the hypothesis submitted by Turner and Robling [25], we observed equivalent training-induced elevations in bone mineral density irrespective of the resistance exercise regimen (i.e. interrupted vs. continuous bouts of exercise). This led us to speculate that a maximal threshold had been reached attributable to the hormonal milieu during the growth process in combination with resistance training. Thus, the bone had reached some type of maximal capacity where any additional osteogenic stimulation, via interrupted exercise bouts, was ineffective.

Therefore, the purpose of the current study was to determine if an exercise threshold exists where, despite additional work performed per exercise bout, no further training-induced increases in bone mineral density would be observed during the growth period in animals. Specifically, we compared 2 resistance training protocols where one exercised group performed significantly more work per training session compared to another exercised group. We also performed 3-point bending tests to measure bone mechanical properties to help relate any alterations in bone mineral density (BMD) to

bone strength. We hypothesized that during the growth period there would be a bone formation threshold where more exercise would not be effective in stimulating additional increases in BMD.

Materials and Methods

Animals

The experimental protocol for this study was pre-approved by the Chapman University Institutional Review Board and is in accord with the International Journal of Sports Medicine ethical standards on the use of animals for research [8]. 36 male Sprague Dawley rats (initially ~225 g, ~8 weeks old) obtained from Charles River Laboratories (Wilmington, MA) were housed individually and maintained on a reverse 12/12 h light/dark cycle. Food and water were provided *ad libitum* throughout the experimental period. The animals were acclimated to their living conditions for 1 week prior to random separation into a control group (Con, n = 12), a resistance trained group where the animals performed 3 ladder climbs per exercise session (3LC, n = 12), or another resistance trained group where the animals performed 6 ladder climbs per exercise session (6LC, 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 in any measured parameter between the 3 animals from each group, the animals in the baseline group were pooled (BL, n = 9) leaving a total of 9 animals in each of the 3 groups (i.e. Con, 3LC, and 6LC).

Resistance training

The strength training regimen has previously been described [6,7,13,24]. Briefly, the animals were required to climb a vertical ladder with weights appended to their tail. The animals were positioned to ensure that they performed each sequential step, where one repetition along the 1 m ladder required 26 total lifts by the animal (or 13 lifts per limb). The resistance trained animals were operantly conditioned for 1 week to climb the ladder in order to avoid a vat of water beneath them. Both the 3LC and 6LC groups trained 3 days per week for a total of 6 weeks. The vertical ladder climbing task and 6 week training period has consistently been demonstrated to be an effective stimulus for bone formation during the growth period in rats [6,7,13,24]. 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 tail for the remainder of the week. All resistance trained animals started with 30% body mass (BM) appended to their tail. Every week the carrying weight was elevated by 30% BM for the next 4 weeks (i.e. 30%, 60%, 90%, and 120%) until the beginning of week 5. At week 5 they carried 135% BM and at week 6 they were carrying 150% BM. For the 6LC group, the animals performed 6 consecutive ladder climbs on a given training day.

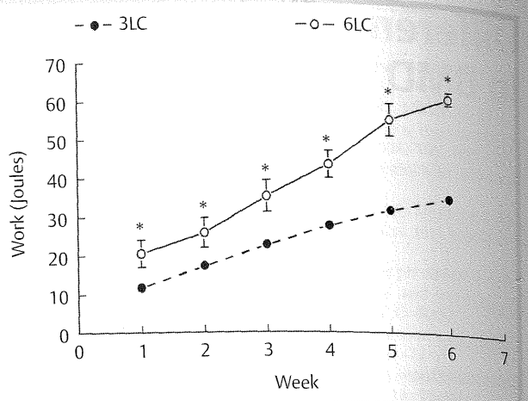

Fig. 1 Total work (in Joules) performed by the resistance trained group climbing the ladder 3 times per exercise session (3LC, n = 9) and the resistance trained group climbing the ladder 6 times per exercise session (6LC, n = 9). * Significant difference between groups.

As per our prior reports [6,7,13,24], the 6 ladder climbs constituted the maximum amount of consecutive repetitions that the 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 3LC group, the animals performed 3 ladder climbs on a given training day. As such, the attempt was to get the 6LC group to perform twice as much work as the 3LC group. However, various animals from each group did not perform all the required ladder climbs on a given training day. Further, there were body mass differences between the 3LC and 6LC groups. Thus, the 6LC group did not perform 100% more work per training bout than the 3LC group, but on any training day for a given week, the amount of work performed by the 6LC group was significantly greater than the 3LC group amounting to a nadir of 50% more work and peak of 75% more work respectively, than the 3LC group (◎ **Fig. 1**).

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 [6,7,9,13,24]. 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 1 week 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 subsequent measurement of deoxypyridinoline (DPD) and creatinine. 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 [15] 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 < 6%. 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 < 5%. 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 and 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 to ensure proper positioning of the tibia. 3 consecutive measurements were performed with the hindlimb repositioned between each scan. The reported BMD was the average of 3 scans and the coefficient of variation for repeated scans (mean ± standard error) that included all hindlimbs was 0.73 ± 0.07%.

Biomechanical 3-point bending tests

The mechanical properties of bone were measured at room temperature using a 3-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, the length was measured, 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 2 support points was 15.0 mm for the baseline group (to account for the smaller tibial length due to the age of the animals) whereas the span of the 2 support points was 18.9 mm for the remaining groups who were now 7 weeks older. The deformation rate was set at 0.9 mm/s for all groups. A medial to lateral force was applied to the midshaft of the bone. The maximal load to failure (Fmax, units = N) and energy to failure (EF, determined from the area under the load-deformation curve to the fracture point, units = N x mm) 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 exercise session for the 3LC and 6LC groups was expressed in Joules. For the comparison of 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, in nmol/L) was corrected for urine concentration (or dilution) by dividing by the creatinine concentration (in mmol/L) and expressed as the adjusted urinary DPD (no units). Except for the training volume (see above), an ANOVA was employed and when a significant F ratio was identified, a Tukey's 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 deviation.

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 not significantly different between the Con and 3LC groups, but the body mass from the 6LC group was significantly lower compared to the Con and 3LC groups (◎ **Table 1**). The total training volume for the resistance trained animals was significantly greater for the 6LC group compared to the 3LC group (◎ **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**). Since the body mass was significantly lower for the 6LC group, the total FHL protein was expressed relative to 100 g of BM. In this regard, the total FHL protein per 100 g of BM was significantly greater for the 3LC and 6LC groups when compared to the Con group (◎ **Table 2**). There was no significant difference in total FHL protein per 100 g of BM between the 3LC and 6LC groups.

The bone mineral density from the whole left tibia was significantly elevated for Con (i.e. 27.8% increase), 3LC (i.e. 42.4% increase), and 6LC (i.e. 48.1% increase) compared to the Baseline group (◎ **Fig. 2**). Further, the BMD from the 3LC and 6LC groups was significantly greater, 11.4% and 15.8% respectively, than the Con group (◎ **Fig. 2**). However, the BMD was not significantly different between 3LC and 6LC groups. Serum osteocalcin was not significantly different between Con compared to BL, but was significantly greater for 3LC and 6LC compared to Con as well as

Table 1 Body Mass.

Group	Initial Body Mass (grams) at 9 weeks of age	Final Body Mass (grams) at 16 weeks of age
BL	270.5 ± 12.3	not applicable
Con	269.2 ± 12.3	484.6 ± 52.9
3LC	268.8 ± 10.2	496.8 ± 31.9
6LC	268.5 ± 11.5	438.8 ± 23.3*

BL = Baseline Group (n = 9), Con = Control Group (n = 9), 3LC = Resistance Trained Group (n = 9) climbing the ladder 3 times per exercise session, and 6LC = Resistance Trained Group (n = 9) climbing the ladder 6 times per exercise session. * Significant difference between 6LC and all other groups

Table 2 Resistance Training Effect on the Flexor Hallucis Longus.

Group	FHL Mass (grams)	FHL Protein (mg protein/muscle)	FHL Protein/100 g (mg/100 g BM)
BL	0.182 ± 0.027	32.33 ± 5.58	10.72 ± 1.87
Con	0.249 ± 0.026†	51.52 ± 6.43†	10.70 ± 1.37
3LC	0.341 ± 0.063†*	63.19 ± 12.62†*	12.74 ± 2.61*
6LC	0.291 ± 0.031†	59.06 ± 9.53†	13.54 ± 2.69*

BL = Baseline Group (n = 9), Con = Control Group (n = 9), 3LC = Resistance Trained Group (n = 9) climbing the ladder 3 times per exercise session, and 6LC = Resistance Trained Group (n = 9) climbing the ladder 6 times per exercise session. † Significant difference vs. BL. * Significant difference vs. Con

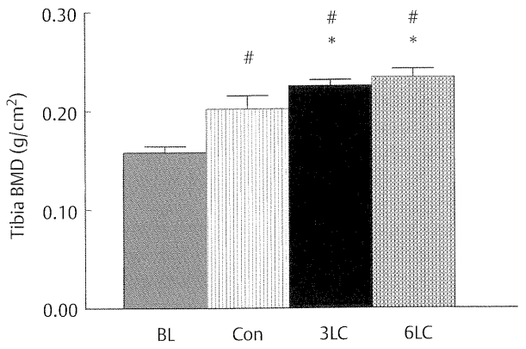

Fig. 2 Bone mineral density (BMD) for the whole left tibia from Baseline animals (BL, n = 9), Controls (Con, n = 9), the resistance trained group climbing the ladder 3 times per exercise session (3LC, n = 9), and the resistance trained group climbing the ladder 6 times per exercise session (6LC, n = 9). # Significant difference vs. BL. * Significant difference vs. Con.

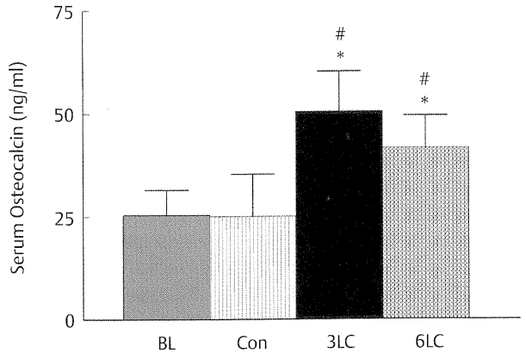

Fig. 3 Serum osteocalcin (OC) concentrations from Baseline animals (BL, n = 9), Controls (Con, n = 9), the resistance trained group climbing the ladder 3 times per exercise session (3LC, n = 9), and the resistance trained group climbing the ladder 6 times per exercise session (6LC, n = 9). # Significant difference vs. BL. * Significant difference vs. Con.

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

Group	Fmax (N)	EF (N×mm)
BL	59.8 ± 8.0	54.7 ± 7.6
Con	102.0 ± 10.1†	125.3 ± 20.4†
3LC	132.7 ± 13.7†*	211.4 ± 48.8†*
6LC	130.0 ± 22.8†*	194.4 ± 36.2†*

Bone strength of the tibia from the BL = Baseline Group (n = 9), Con = Control Group (n = 9), 3LC = Resistance Trained Group (n = 9) climbing the ladder 3 times per exercise session, and 6LC = Resistance Trained Group (n = 9) climbing the ladder 6 times per exercise session. Fmax = Maximum load to failure (in Newtons) and EF = Energy to Failure (area under the load-deformation curve in Newtons x millimeters). † Significant difference vs. BL. * Significant difference vs. Con

Baseline (◎ **Fig. 3**). Serum osteocalcin concentrations were not significantly different between 3LC and 6LC groups (◎ **Fig. 3**). The adjusted urinary deoxypyridinoline did not significantly differ between the Baseline (56.4 ± 22.1), Con (48.3 ± 11.3), 3LC (60.8 ± 12.9), and 6LC (57.1 ± 16.4) groups.

The 6 week growth period resulted in significant increases in bone strength parameters. The maximum force to failure and energy to failure were significantly greater for all groups (i.e. Con, 3LC, and 6LC) compared to Baseline (◎ **Table 3**). Specifically, the 6 week growth period yielded a significantly greater maximum force to failure (i.e. 70.6% increase) and energy to failure (i.e. 129.1% increase) from Con compared to BL groups. Incorporating resistance training during the growth period resulted in further increases in bone strength of the right tibia for the 3LC and 6LC groups compared to controls (◎ **Table 3**). The maximum force to failure was significantly greater for 3LC (i.e. 30.1% increase) and 6LC (i.e. 27.5% increase) compared to controls (◎ **Table 3**). Similarly, the energy to failure was significantly greater for 3LC (i.e. 68.7% increase) and 6LC (i.e. 55.2% increase) compared to controls (◎ **Table 3**). However, the maximal force and energy to failure were not significantly different between the 3LC and 6LC groups (◎ **Table 3**). Last, prior to assessing bone strength as indicated above, right tibial length was measured. The length of the right tibia was significantly greater for Con (43.6 ± 3.1 mm), 3LC (42.5 ± 2.6 mm), and 6LC (41.0 ± 1.1 mm) compared to BL (37.4 ± 2.8 mm), but there were no significant differences in tibial length between Con, 3LC, or 6LC groups.

Discussion

The Con, 3LC, and 6LC 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 increase in serum OC for both 3LC and 6LC compared to Con suggests that the osteogenic response may be attributable to an elevation in osteoblast activity, at least during the latter part of the training period. Further, both the 3LC and 6LC groups demonstrated augmented bone strength when compared to controls. While the BMD, bone strength, and serum OC were elevated for both 3LC and 6LC compared to Con, there was no significant difference between the 3LC and 6LC groups. Thus, the results support our hypothesis of an apparent bone formation threshold, where more work performed within a given exercise session was ineffective in stimulating additional increases in bone mineral density in young, growing male rats. During the growth period, the hormonal milieu provides a stimulus for bone modeling. Incorporating resistance training during this period provides an even greater stimulation for bone formation, thereby maximizing peak bone mass. In support, the elevation in bone mass or BMD when combining exercise during the growth period has previously been reported in both humans [1,5,14,16,23,27,28] and animals [6,7,10,13,18–20,24,29]. Thus, the current findings support the benefits of resistance training during the maturation period in rats culminating in even more bone accrual compared to growth alone. Further, our results also confirm previous reports in humans [4,5,17] and animals [6,7,24,29] pertaining to the exercise-induced elevation in osteoblast activity as the potential mechanism for the augmented BMD, supported by the significant increase in serum OC at the end of training for both 3LC and 6LC groups compared to corresponding controls.

Although the general findings of the current study were consistent with prior reports on the impact of exercise in stimulating a bone formation response, we submit that the importance of this investigation is threefold (in ascending order). The first pertains to the use of animals in examining the impact of resistance training during the growth period. While prior studies in humans support the elevation in bone mass or BMD when exercise is implemented during the growth period, there are a number of factors contributing to BMD in humans including: height, weight, lean mass, sex, and age, to name a few [23]. These endogenous determinants of BMD in human studies make the interpretation of the results challenging when attempting to understand the impact of exercise intervention strategies on bone accrual during the growth period. Further, a majority of the human studies employ cross sectional comparisons, which add to the complexity of the interpretation of these studies, since genetic predisposition and growth rates become additional confounding variables. While the use of an animal model helps to eliminate many of the confounding variables associated with human studies, most of the prior animal studies investigated the impact of treadmill exercise (i.e. weight bearing) on bone [10,11,19]. High-impact activities (e.g. jumping) or resistance exercises (e.g. strength training) have been advocated as the best putative methods to stimulate an osteogenic response and promote an elevation in bone mineral density. However, employing a strength training model in rats was a significant methodological drawback due to the challenge of getting animals to lift a heavy mass.

To overcome this obstacle, prior studies in rats designed to simulate resistance training involved electric shock as the motivation for animals to jump with weighted vests [18,29], mimicking leg squat exercise. In a different study, Robling et al. [21] immobilized the forelimb of rats and applied compressive force to the ulna using a motor-driven device to ensure equivalent mechanical loads between groups. However, this required the animals to be anesthetized during the loading procedure. While both these prior animal studies help to eliminate the confounding variables encountered when extrapolating the results to humans, they also introduce other factors such as the independent effects of electric shock upon the bone and the use of anesthetic drugs, which can negatively impact blood flow. In contrast, Notomi et al. [20] introduced a different model of resistance training for animals that involved climbing a wire meshed tower. Hornberger and Farrar [9] later verified the efficacy of a similar model (i.e., ladder climbing task, 80° incline) for mimicking resistance training in humans, as confirmed by muscle hypertrophy in the Flexor Hallucis Longus of rats. In prior studies, we have employed a modified version of this ladder climbing task (90° incline) with use of a rat model for the investigation of the impact of resistance exercise on bone mineral density. More importantly, all of our studies (including the current report) were examined in conscious rats and in the absence of electric shock to motivate the animals.

The second notable finding of the current study involves the quality of the work performed rather than the quantity of work executed per training bout. In a prior study, we compared 2 resistance trained groups where one group of growing animals carried 30% BM the last week of the exercise program yet performed the same amount of work as another exercised group of rats carrying 150% BM the last week of the exercise program [24]. While the total volume of work was equivalent between exercised groups throughout the strength training regimen, only the resistance trained group carrying 150% BM demonstrated elevations in BMD [24]. Of interest, the amount of work performed by the 30% BM group in our prior study [24] was greater than the 3LC group in the current report. Despite the higher volume of work executed by the 30% BM group in our prior report, no elevation in BMD was observed whereas the 3LC group in this study performed less work at a higher relative intensity and demonstrated significant increases in BMD. This supports the contention that the exercise intensity (rather than the amount of work) needs to be strong enough to create a fluid flow within the lacunar-canalicular network to stimulate bone formation [2]. The comparison of the current results with our prior study also supports the work of Rubin and Lanyon [22] who demonstrated that sporadic bone loading was more effective in eliciting an osteogenic response rather than prolonged repetitive activity. Creating the fluid flow and triggering a bone formation response via infrequent bone loading is apparently accomplished with more weight appended to the animal's tail (i.e. carrying 150% BM with less repetitions compared to carrying 30% BM with more repetitions) rather than the total amount of work performed. Nevertheless, the minimal amount of resistance exercise required to maximize bone accrual remains to be elucidated.

The third and most important finding pertains to the purpose of the study and our initial hypothesis of the existence of a maximal exercise threshold for stimulating bone formation. In prior studies we have attempted to provide an additional stimulus for bone formation by interrupting the exercise bouts as proposed by Turner and Robling [25]. They hypothesized that mechanosensors within the bone can reset after a bout of exercise [25]. In this regard, partitioning the exercise into multiple bouts throughout a training day would provide greater stimulation for bone formation [25]. Despite numerous attempts to support the hypothesis submitted by Turner and Robling [25], we failed to observe additional training-induced elevations in BMD when interrupting the resistance training bouts within a training day [6,7,13]. In contrast to Turner and Robling [25], we observed equivalent training-induced elevations in bone mineral density that was independent of the resistance exercise regimen, i.e. interrupted vs. continuous bouts of exercise [6,7,13]. This led us to speculate that a maximal threshold had been reached attributable to the hormonal milieu during the growth process in combination with resistance training [13]. In essence, the bone had reached some type of maximal capacity where any additional osteogenic stimulation, via interrupted exercise bouts, was ineffective. This would suggest that during the growth period, high volumes of resistance training are not required to maximally stimulate a bone formation response. Thus, the results of the current study support the existence of an exercise threshold during the growth period where additional work per training bout was ineffective for promoting even more bone formation.

While training-induced elevations in BMD are noteworthy, the most important factor in the prevention of fractures is bone strength. We acknowledge that interpretations of 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. In the current report, when compared to control animals, we observed a training-induced average increase of 29% and 62% in the maximal load to failure and energy to failure, respectively. Therefore, our results were consistent with prior animal reports [6,7,10,13,25,26] demonstrating that relatively small elevations in bone mineral density culminates in large increases in bone strength.

Finally, we acknowledge several limitations in the interpretation of our results. First, a rat may not be the best animal model to extrapolate the results to humans since the epiphyseal plates in rats do not close. As such, rats would continually favor a bone formation response thereby limiting an extrapolation of the results to mature humans with use of adult animals. Thus, we chose to examine the growth period in rats which would be comparable to growing humans. This minimizes the problems associated with extrapolating the results in adult rats (where the epiphyseal plates do not close) to adult humans (where the epiphyseal plates close). Next, our control animals were not exposed to any activity, giving rise to the dramatic differences in BMD between groups. Last, there are limitations in the use of the DXA for the assessment of BMD. Specifically, the DXA expresses bone mineral density in grams per area attributable to the 2-dimensional image, rather than a true volumetric density as attained via quantitative computed tomography (QCT). Further, QCT can determine the bone mineral content of cortical and trabecular bone, whereas a DXA is unable to discriminate between bone types. As it pertains to our DXA measurements of tibial BMD, we also recognize that a larger bone could yield a higher BMD as assessed via DXA [3]. This could potentially favor the 3LC group given the greater body mass compared to the 6LC group. However, the length of the tibia was not significantly different between the 3LC and 6LC groups (as well as the Con group). Given the architecture of the rat tibia we could not accurately assess the diameter or width. Although tibial length is a crude measurement, this would indirectly suggest that despite differences in body mass, the approximate bone size between the 3LC and 6LC groups were similar, thereby minimizing the errors associated with areal measurements of BMD. In support, we failed to observe any differences in bone strength between the resistance trained groups. We acknowledge that a QCT would have provided greater interpretation of our data compared to DXA measurements. Notwithstanding our use of the DXA, we submit that the body mass differences between the 3LC and 6LC groups had minimal impact upon the outcome and our conclusions. Despite all of these limitations noted above, to the extent that our findings in animals can be applied to humans, our results support the existence of an exercise threshold where more exercise will not result in further increases in bone mineral density during the growth period. We recognize that specific exercise intervention strategies (i.e. type and intensity) need to be further elucidated, especially in children.

In summary, using conscious animals and a mode of exercise that mimics resistance training, we provide evidence that during the growth period, an exercise threshold exists whereby more work was not effective for stimulating additional bone formation. This was supported by the equivalent elevations in BMD despite the significant difference in work performed by the 6LC group compared to the 3LC group. The effectiveness of both resistance training programs in stimulating bone formation were further supported by elevations in: serum OC and bone mechanical properties as assessed from 3-point bending tests. While the amount of exercise performed by the 3LC group was just as effective in stimulating BMD as the amount of exercise performed by the 6LC group, it is unknown if even less exercise (than the 3LC group) can still elicit bone formation. Thus, we acknowledge that further investigations are warranted in growing animals to determine the minimal amount of exercise required for maximal stimulation of BMD.

References

1 *Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, Carlson J, Seeman E.* Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. J Bone Min Res 1998; 13: 1814–1821
2 *Burger EH, Klein-Nulend J.* Mechanotransduction in bone – role of the lacuno-canalicular network. FASEB J 1999; 13 (Suppl): S101–S112
3 *Carter DR, Bouxsein ML, Marcus R.* New approaches for interpreting projected bone densitometry data. J Bone Min Res 1992; 7: 137–145
4 *Danz AM, Zittermann A, Schiedermaier U, Klein K, Hotzel D, Schonau E.* The effect of a specific strength-development exercise on bone mineral density in perimenopausal and postmenopausal women. J Women Health 1998; 7: 701–709
5 *Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T, Hayashi K, Tokuyama K, Suzuki M.* Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. J Bone Min Res 1997; 12: 656–662
6 *Godfrey JK, Kayser BD, Gomez GV, Bennett J, Jaque SV, Sumida KD.* Interrupted resistance training and BMD in growing rats. Int J Sports Med 2009; 30: 579–584
7 *Goettsch BM, Smith MZ, O'Brien JA, Gomez GV, Jaque SV, Sumida KD.* Interrupted vs. uninterrupted training on BMD during growth. Int J Sports Med 2008; 29: 980–986
8 *Harriss DJ, Atkinson G.* International Journal of Sports Medicine – Ethical Standards in Sport and Exercise Science Research. Int J Sports Med 2009; 30: 701–702
9 *Hornberger TA, Farrar RP.* Physiological hypertrophy of the FHL muscle following 8 weeks progressive resistance exercise in the rat. Can J Appl Physiol 2004; 29: 16–31
10 *Huang TH, Lin SC, Chang FL, Hsieh SS, Liu SH, Yang RS.* Effects of different exercise modes on mineralization, structure, and biochemical properties in growing bone. J Appl Physiol 2003; 95: 300–307
11 *Iwamoto J, Yeh JK, Aloia JF.* Effect of deconditioning on cortical and cancellous bone growth in the exercised trained young rats. J Bone Min Res 2000; 15: 1842–1849
12 *Johnston CC, Hui SL, Wiske P, Norton JA, Epstein S.* Bone mass at maturity and subsequent rates of loss as determinants of osteoporosis. In: DeLuca HF (ed). Osteoporosis: Recent Advances in Pathogenesis and Treatment. University Park Press, Baltimore, MD; 1981; 285–291
13 *Kayser BD, Godfrey JK, Cunningham R, Pierce RA, Jaque SV, Sumida KD.* Equal BMD after daily or triweekly exercise in growing rats. Int J Sports Med 2010; 31: 44–50
14 *Lehtonen-Veromaa M, Mottonen T, Nuotio I, Heinonen OJ, Viikari J.* Influence of physical activity on ultrasound and dual-energy X-ray absorptiometry bone measurements in peripubertal girls: a cross-sectional study. Calcif Tissue Int 2000; 66: 248–254
15 *Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.* Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–275
16 *MacKelvie KJ, Petit MA, Khan KM, Beck TJ, McKay HA.* Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. Bone 2004; 34: 755–764
17 *Menkes A, Mazel S, Redmond RA, Koffler K, Libanati CR, Gundberg CM, Zizic TM, Hagberg JM, Pratley RE, Hurley BF.* Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. J Appl Physiol 1993; 74: 2478–2484
18 *Notomi T, Lee SJ, Okimoto N, Okazaki Y, Takamoto T, Nakamura T, Suzuki M.* Effects of resistance exercise training on mass, strength, and turnover of bone in growing rats. Eur J Appl Physiol 2000; 82: 268–274
19 *Notomi T, Okazaki Y, Okimoto N, Saitoh S, Nakamura T, Suzuki M.* A comparison of resistance and aerobic training for mass, strength, and turnover of bone in growing rats. Eur J Appl Physiol 2000; 83: 469–474
20 *Notomi T, Okimoto N, Okazaki Y, Tanaka Y, Nakamura T, Suzuki M.* Effects of tower climbing exercise on bone mass, strength, and turnover in growing rats. J Bone Min Res 2001; 16: 166–174
21 *Robling AG, Burr DB, Turner CH.* Recovery periods restore mechanosensitivity to dynamically loaded bone. J Exp Biol 2001; 204: 3389–3399
22 *Rubin CT, Lanyon LE.* Regulation of bone formation by applied dynamic loads. J Bone Joint Surg Am 1984; 66: 397–402
23 *Scerpella TA, Davenport M, Morganti CM, Kanaley JA, Johnson LM.* Dose related association of impact activity and bone mineral density in pre-pubertal girls. Calcif Tissue Int 2003; 72: 24–31
24 *Smith MZ, Goettsch BM, O'Brien JA, Van Ramshorst RD, Jaque SV, Sumida KD.* Resistance training and bone mineral density during growth. Int J Sports Med 2008; 29: 316–321
25 *Turner CH, Robling AG.* Designing exercise regimens to increase bone strength. Exerc Sport Sci Rev 2003; 31: 45–50

26 *Umemura Y, Sogo N, Honda A.* Effects of intervals between jumps or bouts on osteogenic response to loading. J Appl Physiol 2002; 93: 1345–1348
27 *Van Lagendonck L, Claessens AL, Vlietinck R, Derom C, Beunen G.* Influence of weight-bearing exercises on bone acquisition in prepubertal monozygotic female twins: a randomized controlled prospective study. Calcif Tissue Int 2003; 72: 666–674
28 *Welten DC, Kemper HCG, Post BG, Van Mechelen W, Twisk J, Lips P, Teule GJ.* Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Miner Res 1994; 9: 1089–1096
29 *Westerlind KC, Fluckey JD, Gordon SE, Kraemer WM, Farrell PA, Turner RT.* Effect of resistance exercise training on cortical and cancellous bone in mature male rats. J Appl Physiol 1998; 84: 459–464