Shorter, more frequent mechanical loading sessions enhance bone mass

ALEXANDER G. ROBLING, FELICIA M. HINANT, DAVID B. BURR, and CHARLES H. TURNER

Department of Anatomy and Cell Biology; Department of Orthopaedic Surgery; Biomechanics and Biomaterials Research Center; Indiana University School of Medicine, Indianapolis, IN

ABSTRACT

ROBLING, A. G., F. M. HINANT, D. B. BURR, and C. H. TURNER. Shorter, more frequent mechanical loading sessions enhance bone mass. *Med. Sci. Sports Exerc.*, Vol. 34, No. 2, pp. 196–202, 2002. **Purpose:** The beneficial effects of exercise on bone mass and strength can be attributed to the sensitivity of bone cells to mechanical stimuli. However, bone cells lose mechanosensitivity soon after they are stimulated. We investigated whether the osteogenic response to a simulated high-impact exercise program lasting 4 months could be enhanced by dividing the daily protocol into brief sessions of loading, separated by recovery periods. **Methods:** The right forelimbs of adult rats were subjected to 360 load cycles·d^{-1}, 3 d·wk^{-1}, for 16 wk. On each loading day, one group received all 360 cycles in a single, uninterrupted bout (360 × 1); the other group received 4 bouts of 90 cycles/bout (90 × 4), with each bout separated by 3 h. After sacrifice, bone mineral content (BMC), and areal bone mineral density (aBMD) were measured in the loaded (right) and nonloaded control (left) ulnae using DXA. Volumetric BMD (vBMD) and cross-sectional area (CSA) were measured at midshaft and the olecranon by using pQCT. Maximum and minimum second moments of area (I_{MAX} and I_{MIN}) were measured from the midshaft tomographs. **Results:** After 16 wk of loading, BMC, aBMD, vBMD, midshaft CSA, I_{MAX}, and I_{MIN} were significantly greater in right (loaded) ulnae compared with left (nonloaded) ulnae in the two loaded groups. When the daily loading regimen was broken into four sessions per day (90×4), BMC, aBMD, midshaft CSA, and I_{MIN} improved significantly over the loading schedule that applied the daily stimulus in a single, uninterrupted session (360×1). **Conclusion:** Human exercise programs aimed at maintaining or improving bone mass might achieve greater success if the daily exercise regime is broken down into smaller sessions separated by recovery periods.
Key Words: MECHANICAL LOADING, BONE ADAPTATION, RECOVERY, EXERCISE, OSTEOPOROSIS, BMD

The crucial role of physical activity in the acquisition and maintenance of bone mass is becoming widely accepted (11,14,27). The beneficial effects of exercise on bone mass and mechanical competence can be attributed to bone tissue's sensitivity to physical forces created in the skeleton during exercise. Bone cells respond to tissue deformation, or its consequences (e.g., fluid flow), by adapting the structure to more adequately withstand future deformations (6). This adaptive process entails adding bone (either with or without prior resorption) to appropriate skeletal surfaces (7,8). However, the type of exercise modulates the anabolic response; high-impact exercises (e.g., volleyball, gymnastics) are more effective than low-impact exercises (e.g., cycling, swimming) in promoting bone gain (2,23,24). Data from animal experiments and mathematical modeling suggest that the osteogenic effects of high-impact exercise in humans are probably related to the greater strain rates associated with those activities (15,16,26). Another important factor governing the anabolic response to exercise is skeletal age. Although exercise during the adult years can retard the natural bone loss associated with aging, a much greater improvement in bone mass (and fracture resistance later in life) is achieved if vigorous exercise is engaged in during the childhood and adolescent years, when peak bone mass can still be affected (10,12,13,17).

Although the effects of exercise on bone health are becoming clear, little is known about how exercise protocols can be optimized to further promote bone mass accumulation and maintenance. A major factor to consider in designing exercise programs aimed at maintaining or improving bone mass is the sensitivity of the resident bone cell populations to mechanical stimuli. Animal loading experiments have demonstrated that bone cells desensitize soon after a loading session is initiated. The osteogenic effects of exercise could therefore reach a saturation point (where the cells are essentially unresponsive to further stimulation), perhaps after 50–100 repetitions of loading (22,26,28). These data indicate that extended exercise sessions are no more beneficial to bone mass than shorter sessions because the maximal osteogenic response is probably achieved within the first few minutes.

In a previous communication, we showed that partitioning a daily mechanical stimulus (360 repetitions of load per day) into smaller loading bouts, separated by recovery periods, enhanced bone formation over that elicited from the same stimulus applied in a single, uninterrupted loading bout (20). Presumably, the bone cells in the single, longer bout had lost sensitivity early in the session, so that load repetitions occurring toward the end of the bout were ignored. By making the loading sessions shorter and providing recovery periods between sessions (during which mechano-

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sensitivity could be restored), bone formation improved by as much as 90%. A follow-up experiment showed that approximately 8 h of load-free recovery restores full mechanosensitivity to previously stimulated bone cells (21). Mechanical loading sessions initiated before full recovery was achieved resulted in bone formation rates that were proportional to the recovery time, indicating that even modest recovery periods can restore some degree of sensitivity.

Although our previous studies have shed light on the dynamics of bone cell mechanosensitivity, they were limited in that the duration of the loading protocol lasted from 1 to 2 wk. In light of strain-feedback models of bone adaptation (6), it was unclear whether the improvement in bone formation using short, separated bouts would be maintained if the loading protocols were implemented over a longer time period. Using a noninvasive loading model that applies well-controlled forces to the rat ulna, we sought to determine whether 4 months of loading using short loading bouts, interspersed with recovery periods, is more beneficial than single, longer daily sessions. We hypothesized that after 16 wk of loading, bone mass and structural properties of ulnae loaded for 90 cycles, 4 times·d^{-1} would be greater than in ulnae loaded for 360 uninterrupted cycles·d^{-1}.

MATERIALS AND METHODS

Forty-four virgin female Sprague-Dawley (12 wk old) rats were purchased for the experiment from Harlan Sprague-Dawley (Indianapolis, IN). The rats were housed two per cage at Indiana University's Laboratory Animal Resource Center for 15 wk before the experiment began (acclimation period) and were provided standard rat chow and water *ad libitum* during the acclimation and experimental periods. Body mass measurements were collected periodically during the acclimation period and 3 times/wk during the experimental (loading) period. Under ether-induced anesthesia, the right ulna of rats in the loading groups was subjected to axially applied compressive loads, using a nonsurgical loading preparation that transmits mechanical force to the ulna through the olecranon and flexed carpus (Fig. 1A) (25). The natural curvature of the ulnar diaphysis translates the axial load into a bending moment in the middiaphysis that produces tension in the lateral cortex and compression in the medial cortex. Force was applied to the ulnae by an open loop, stepper motor-driven spring linkage with an in-line load cell. All procedures performed in this experiment were conducted in AAALAC-approved facilities and were in accordance with ACSM, NIH, and Indiana University Animal Care and Use Committee guidelines.

Experimental design. Ten days before the start of the loading period, the rats were divided randomly into two loaded groups (N = 13/group) and two control groups (N = 9/group). The right ulnae of animals in the two loaded groups were subjected to 360 load cycles·d^{-1}, 3 d·wk^{-1} for 16 consecutive weeks. Load was applied as a haversine waveform at a frequency of 2 Hz and peak load magnitude of 17 N, which elicits a compressive strain of approximately 3600 $\mu\varepsilon$ on the medial surface of the ulnar midshaft (9). The

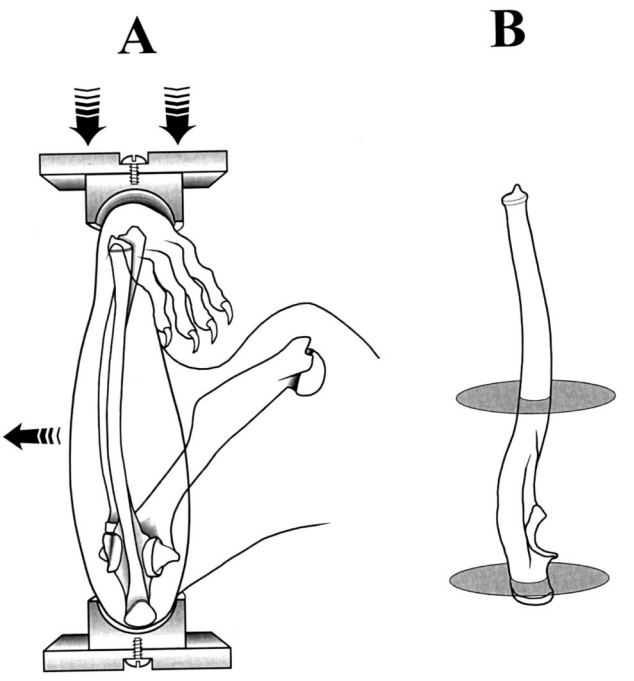

FIGURE 1—A, Caudal view of the rat forelimb *in situ* during loading. The right distal forelimb is held between upper and lower aluminum cups (shown in hemisection), which are fixed to the loading platens. When force is applied to the upper platen (*large arrows*), the preexisting mediolateral curvature of the ulnar translates the axial load into a bending moment (*small arrow*), which is maximal near the midshaft. B, After sacrifice, the entire ulna was scanned using DXA (not shown), and the midshaft and olecranon (*gray sectioning planes*) were scanned using pQCT. Panel A reproduced from reference (19) with permission from publisher.

two loaded groups differed from each another only in the timed delivery of the 360 load cycles received throughout each load day. One group was administered all 360 cycles in a single, uninterrupted session (360×1), which lasted 3 min. The other loaded group was administered the 360 cycles in four discrete bouts of 90 cycles/bout (90×4), with 3 h of recovery inserted between each of the brief (45-s long) loading bouts. We have shown previously, using the rat tibia bending model, that the 90×4 schedule enhances bone formation markedly over that produced by the 360×1 schedule after 1 wk of loading (20). All rats were allowed normal cage activity between bouts. The two control groups comprised a baseline control (BLC) group, which was sacrificed on the first loading day, and an age-matched control (AMC) group, which was sacrificed on the same day that the loaded groups were sacrificed (16 wk after baseline sacrifice). Neither control group was subjected to loading or anesthesia. In the loaded groups, the left ulnae were not loaded and served as internal controls for the loaded limb. After sacrifice, the right and left ulnae were dissected free of the articulating bones, cleaned of soft tissues, fixed in 10% neutral buffered formalin for 48 h, then transferred to 70% ethanol for storage.

DXA. Each right-left ulna pair was scanned side-by-side on the bed of a Hologic QDR-1000 x-ray densitometer (Hologic Inc., Waltham, MA) equipped with Hologic version 6.20C software. The bones were positioned with the lateral

surface of the diaphysis facing down and were scanned at 0.127-mm resolution. Upon completion of each scan, mutually exclusive region of interest (ROI) boxes were drawn around the right and left bone, from which bone area (BA; cm^2), bone mineral content (BMC; mg), and areal bone mineral density (aBMD; $g \cdot cm^{-2}$) measurements were collected. Two bones from the study, chosen at random, were scanned 10 times to assess reproducibility. The bone was removed from the scanner bed and repositioned between each repeat scan. From the 10 repeated measures, the coefficient of variation (CV) was calculated. The CVs for BMC and aBMD were 1.5% and 1.0%, respectively.

pQCT. Each ulna was placed in a plastic tube filled with 70% ethanol and centered in the gantry of a Norland Stratec XCT Research SA+ pQCT (Stratec Electronics, Pforzheim, Germany). Two cross-sectional levels were scanned on each ulna—one at the midshaft and one through the olecranon process, using 0.46 mm collimation (4×10^5 counts$\cdot s^{-1}$) and 0.08 mm voxel size (Fig. 1B). The slice through the olecranon was taken 3.5 mm distal to the proximal tip of the bone and included the cortical shell and secondary spongiosa of the proximal metaphysis. For each section, the x-ray source was rotated through 180° of projection (1 block). The scans were imported into BonAlyse version 1.3 software (BonAlyse Ltd., Jyväskyla, Finland) for *post hoc* pQCT analyses. From the midshaft slices, total cross sectional area (CSA—area within periosteum; mm^2), cortical volumetric BMD (vBMD; $mg \cdot cm^{-3}$), and maximum and minimum second moments of area (I_{MAX} and I_{MIN}; mm^4) were calculated in BonAlyse. The second moment of area (I) reflects a structure's resistance to bending by considering both cross-sectional area and material distribution (geometry). Beams (or long bone shafts) with the material distributed farther from the plane of bending will exhibit greater resistance to bending (I) than beams with material distributed closer to the plane of bending. This geometry-related increase in bending rigidity can occur even with reduced cross sectional area if the material is appropriately distributed. I is calculated by dividing the section into a series of small areas (pixels) and multiplying each area (dA) by its squared distance (y) from the neutral plane. This procedure is integrated over the entire cross section:

$$I = \int y^2 dA$$

This function is repeated about all possible neutral planes; the largest value is returned as I_{MAX}, and the smallest value is returned as I_{MIN}.

Analysis of bone envelopes at the midshaft slice was restricted to cortical bone, because trabecular bone is not normally present in the central diaphysis. From the olecranon slices, CSA and total vBMD were calculated in BonAlyse. Two additional measurements were conducted on the proximal slices to investigate trabecular bone adaptation in the proximal metaphysis. The peel mode in the Stratec software was used to separate cortical from trabecular bone, and vBMD was calculated for each bone envelope separately. Cortical bone was separated from trabecular bone using a density threshold of 900 $mg \cdot cm^{-3}$. Two bones from the study, chosen at random, were scanned 10 times to assess reproducibility. CVs for the pQCT measurements were as follows: CSA midshaft = 4.4%, cortical vBMD midshaft = 1.8%, I_{MAX} midshaft = 2.0%, I_{MIN} midshaft = 3.8%, CSA proximal = 4.0%, and total vBMD proximal = 1.9%.

Statistical analyses. Differences between right (loaded limb) versus left (control limb) values for all DXA and pQCT measurements were tested for significance using paired *t*-tests. Percent differences between right (R) and left (L) limbs were calculated as follows: $(R - L)/L \cdot 100$. One-way analysis of variance (ANOVA) was performed on control limb and percent difference values to detect differences among the four experimental groups. Significant ANOVAs were followed by Fisher's PLSD *post hoc* tests to determine significant differences between individual experimental groups. For the *t*-tests, ANOVA, and *post hoc* tests, $\alpha = 0.05$.

RESULTS

Two of the rats in the 360×1 group died toward the end of the experimental period, one from anesthesia-related complications and the other from unknown causes. One of the baseline control animals died during the acclimation period from unknown causes, and one animal from the age-matched control group was excluded from the DXA analysis and from the pQCT analysis of the olecranon because of tissue damage to the proximal ulna during processing. Over the 16-wk loading period, mean body mass increased slightly in each group (6–12%) but exhibited substantial fluctuation, even in the age-matched control group (Fig. 2). The age-matched control group gained significantly more weight than the 90×4 group (Fig. 2, inset), suggesting an effect of multiple ether exposures on body weight.

BMC in the left (control) ulnae was significantly different among groups ($P = 0.016$). *Post hoc* tests revealed significant differences between the baseline control group and the remaining three groups only; none of the 16-wk animals were significantly different from one another. Thus, there appears to be an aging effect but no systemic loading effect on BMC in the control limb. Paired *t*-tests revealed no significant differences between right and left limbs for BMC in either control group, but both loading groups exhibited significantly ($P < 0.001$) greater BMC in the loaded limb compared with the control limb (Table 1). Although both loaded groups showed significant loading effects, the percent difference between right and left ulnar BMC in the 90×4 group (11.7%) was 70% greater ($P = 0.001$) than the right versus left difference in the 360×1 group (6.9%; Fig. 3).

Areal BMD (aBMD) in the control ulna was not significantly different among groups ($P = 0.68$). Paired *t*-tests revealed no significant differences between right and left limbs for aBMD in either control group, but both loading groups exhibited significantly ($P < 0.001$) greater aBMD in the loaded limb when compared with the control limb (Table

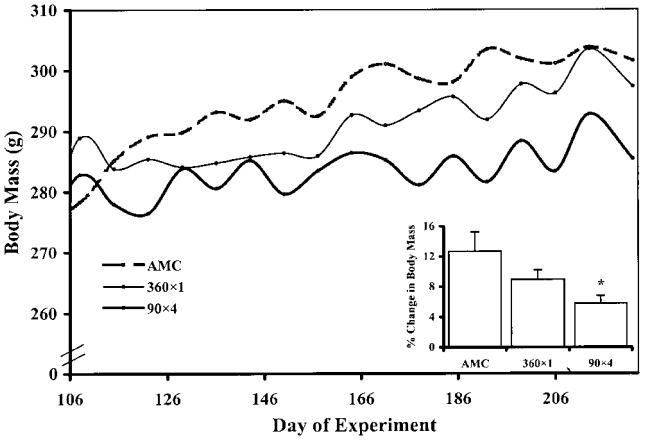

FIGURE 2—Mean body weight (± SEM) plotted by experimental group during the loading period. Twelve-week-old rats were received on day 1. After 96 d of acclimation (not shown), the rats were assigned to one of four groups so that each group had approximately the same mean and error term for body mass. Rats were assigned to the baseline control group (killed on day 106; not shown), the age-matched control group (AMC; no loading or anesthesia), the single load bout/day group (360×1), or the four load bouts/day group (90×4). Loading began 10 d after group assignment (day 106) and continued 3 d·wk^{-1} until day 215. The animals were sacrificed on day 222. During the 16-wk loading period, body mass fluctuated substantially, even in the age-matched control group. Inset: Percent change in body mass from the day of group assignment (body mass on day 96) to the average of the final three weeks of the experiment (mean body mass from days 201 to 222). * Significantly different from AMC at $\alpha = 0.05$.

1). The percent difference between right and left ulnar aBMD in the 90×4 group (8.6%) was approximately 60% greater ($P = 0.012$) than the right versus left difference in the 360×1 group (5.4%; Fig. 3).

Peripheral QCT measurements collected from the ulnar midshaft of control (left) limbs yielded significant differences among groups for vBMD and I_{MAX}. *Post hoc* pairwise comparisons among groups performed on control limb vBMD and I_{MAX} revealed significant differences ($P < 0.05$) between the baseline control group and each of the remaining three groups. The three groups sacrificed at 16 wk were not significantly different from one another. Paired *t*-tests showed significantly ($P < 0.001$) greater CSA, vBMD, I_{MAX}, and I_{MIN} in the loaded arm compared with the control arm in both loaded groups (Table 2). With the exception of I_{MIN} in the baseline control group and I_{MAX} in the age-matched control group, paired *t*-test *P*-values were not significant for any of the variables in the two control groups.

TABLE 1. DXA whole bone measurements of right and left ulnae at baseline and after 16 wk of loading or normal activity.

Group (Side)	*N*	BMC[a] (mg)		aBMD[b] (mg·cm^{-2})	
Baseline control	8				
Left		1081.4 (22.3)	NS	1706.9 (16.0)	NS
Right		1069.4 (18.9)		1698.8 (18.0)	
Age-matched control	8				
Left		1152.9 (16.7)	NS	1713.0 (18.4)	NS
Right		1140.6 (13.8)		1729.1 (19.9)	
360 × 1	11				
Left (control)		1184.2 (24.3)	*	1734.6 (19.3)	*
Right (loaded)		1264.5 (19.8)		1826.7 (8.5)	
90 × 4	13				
Left (control)		1183.8 (23.4)	*	1723.5 (12.8)	*
Right (loaded)		1320.4 (20.3)		1871.2 (15.5)	

[a] Bone mineral content.
[b] areal bone mineral density (BMC/bone area).
* Paired *t*-test significant at $\alpha = 0.05$.
NS, paired *t*-test not significant ($P > 0.05$).

The percent difference between right and left midshaft ulna cross-sectional area was significantly greater in the loaded groups compared with the control groups (Fig. 4). Differences were also detected between loading groups—the 90×4 group exhibited 37% greater ($P = 0.012$) right versus left difference in CSA than the 360×1 group (Fig. 4). Percent difference (right vs left) for vBMD was significantly greater in the loaded groups compared with the control groups, but no significant differences between the two loaded groups were detected (Fig. 5). Percent difference in I_{MAX} and I_{MIN} was also significantly greater among the loaded groups compared with the control groups, with the exception of the 360×1 versus baseline control *post hoc* comparison for I_{MAX} (Fig. 6). Between loaded groups, I_{MIN}

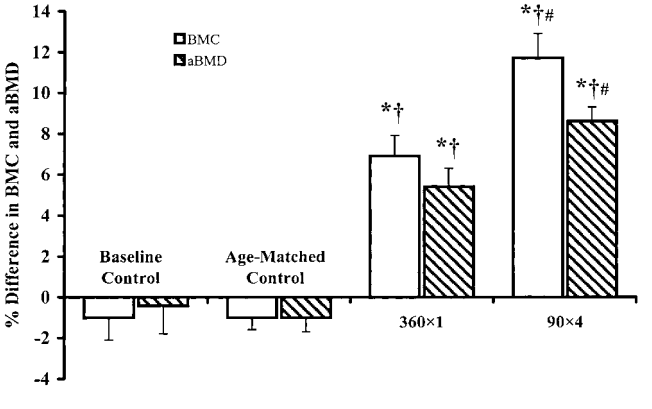

FIGURE 3—Mean percent difference (± SEM) between right (loaded in 360×1 and 90×4 groups) and left (control) BMC (*solid bars*) and areal BMD (aBMD; *hatched bars*) by experimental group. Data were collected using DXA. * Significantly different from baseline control group at $\alpha = 0.05$; † significantly different from age-matched control group at $\alpha = 0.05$; # significantly different from 360×1 group at $\alpha = 0.05$. See Table 1 for significance of right vs left comparisons within each group.

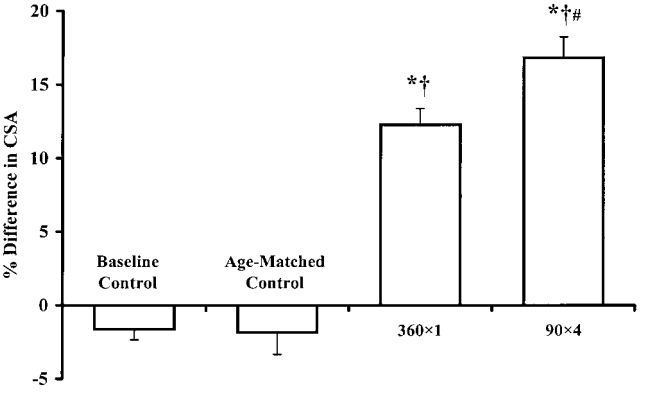

FIGURE 4—Mean percent difference (± SEM) between right (loaded in 360×1 and 90×4 groups) and left (control) cross-sectional area at the midshaft ulna, by experimental group. Data were collected using pQCT. * Significantly different from baseline control group at $\alpha = 0.05$; † significantly different from age-matched control group at $\alpha = 0.05$; # significantly different from 360×1 group at $\alpha = 0.05$. See Table 2 for significance of right vs left comparisons within each group.

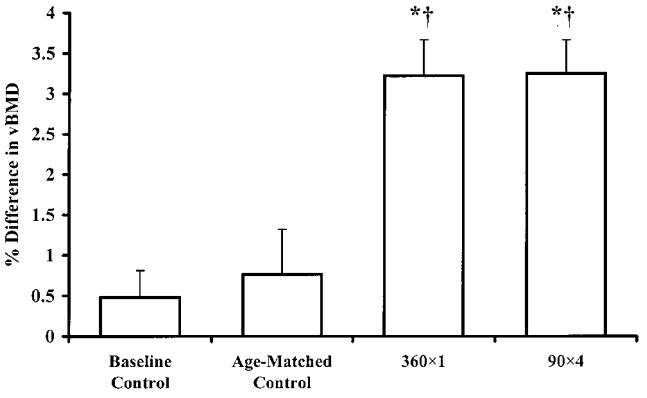

FIGURE 5—Mean percent difference (± SEM) between right (loaded in 360×1 and 90×4 groups) and left (control) volumetric BMD values at the midshaft ulna, by experimental group. Data were collected using pQCT. * Significantly different from baseline control group at $\alpha = 0.05$; † significantly different from age-matched control group at $\alpha = 0.05$. See Table 2 for significance of right vs left comparisons within each group.

(but not I_{MAX}) was significantly greater in the 90×4 group (46% greater; $P < 0.001$) compared with the 360×1 group (Fig. 6).

Measurements at the olecranon of the left ulnae yielded significant differences among groups in total vBMD and cortical vBMD; however, no differences in left limb CSA or trabecular bone vBMD were detected. *Post hoc* pairwise comparisons among groups were performed on total and cortical vBMD, and revealed significant differences ($P < 0.05$) between the baseline control group and each of the remaining three groups. The three groups sacrificed at 16 wk were not significantly different from one another. Paired *t*-tests detected no significant differences between right and left values for any of the olecranon measurements, with the exception of total vBMD in the 90×4 group (Table 2).

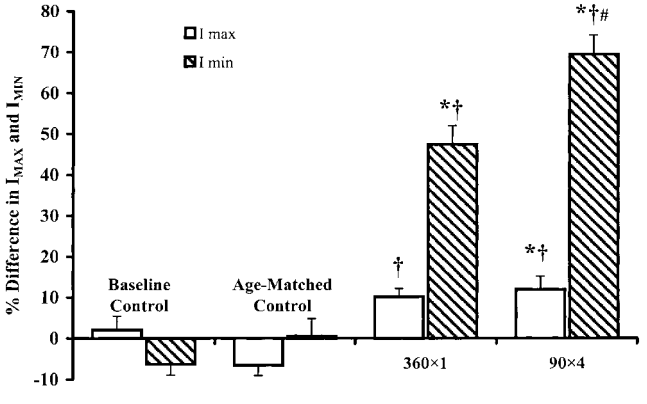

FIGURE 6—Mean percent difference (± SEM) between right (loaded in 360×1 and 90×4 groups) and left (control) maximum (*solid bars*) and minimum (*hatched bars*) second moments of area at the midshaft ulna, by experimental group. Data were collected using pQCT. * Significantly different from baseline control group at $\alpha = 0.05$; † significantly different from age-matched control group at $\alpha = 0.05$; # significantly different from 360×1 group at $\alpha = 0.05$. See Table 2 for significance of right vs left comparisons within each group.

TABLE 2. pQCT measurements of bone size, density, and geometry at the ulnar midshaft and olecranon at baseline and after 16 wk of loading or normal activity.

Group	Midshaft Slice				Proximal Slice			
	CSA[a] (mm^2)	Cort. vBMD[b] ($mg \cdot cm^{-3}$)	I_{MAX}[c] (mm^4)	I_{MIN}[d] (mm^4)	CSA[a] (mm^2)	Total vBMD[e] ($mg \cdot cm^{-3}$)	Cort. vBMD[b] ($mg \cdot cm^{-3}$)	Trab. vBMD[f] ($mg \cdot cm^{-3}$)
Baseline control								
Left	2.60 (0.04) } NS	1188.0 (4.2) } NS	1.12 (0.05) } NS	0.21 (0.01) } *	5.26 (0.11) } NS	1032.0 (11.7) } NS	1136.2 (12.6) } NS	645.6 (12.7) } NS
Right	2.56 (0.04) }	1193.7 (5.6) }	1.14 (0.04) }	0.19 (0.01) }	5.14 (0.07) }	1036.5 (8.2) }	1134.1 (7.0) }	643.6 (9.7) }
Age-matched control								
Left	2.72 (0.04) } NS	1246.6 (4.9) } NS	1.24 (0.03) } *	0.23 (0.01) } NS	5.01 (0.13) } NS	1085.0 (13.6) } NS	1194.5 (4.1) } NS	652.3 (11.4) } NS
Right	2.67 (0.05) }	1256.0 (6.4) }	1.16 (0.04) }	0.23 (0.01) }	4.99 (0.14) }	1090.4 (14.3) }	1194.9 (8.0) }	663.5 (13.3) }
360 × 1								
Left (control)	2.73 (0.04) } *	1235.0 (4.9) } *	1.30 (0.04) } *	0.23 (0.01) } *	5.41 (0.11) } NS	1083.6 (13.6) } NS	1190.5 (7.0) } NS	659.9 (9.4) } NS
Right (loaded)	3.06 (0.03) }	1274.5 (3.0) }	1.43 (0.05) }	0.33 (0.01) }	5.34 (0.08) }	1082.0 (12.6) }	1189.3 (7.3) }	662.2 (8.9) }
90 × 4								
Left (control)	2.72 (0.03) } *	1244.0 (3.9) } *	1.29 (0.05) } *	0.23 (0.01) } *	5.17 (0.09) } NS	1102.0 (7.7) } *	1203.9 (4.6) } NS	681.2 (11.9) } NS
Right (loaded)	3.18 (0.03) }	1284.4 (5.4) }	1.44 (0.05) }	0.39 (0.01) }	5.23 (0.08) }	1085.7 (8.7) }	1201.6 (5.8) }	657.0 (13.0) }

[a] Cross-sectional area of bone plus marrow; [b] volumetric bone mineral density of the cortical shell; [c] maximum second moment of area; [d] minimum second moment of area; [e] volumetric bone mineral density from the entire section; [f] volumetric bone mineral density of trabecular bone.
* Paired *t*-test significant at $\alpha = 0.05$; NS, paired *t*-test not significant ($P > 0.05$).

DISCUSSION

Our objective was to determine whether the beneficial osteogenic effects of a daily high-impact exercise protocol employing multiple short bouts would be preserved after 4 months of training. We found that ulnae from the 90×4 group had significantly greater BMC, aBMD, and minimum second moment of area at midshaft, compared with ulnae from the 360×1 group. These findings suggest that long-term (several months in duration) exercise protocols targeting bone health might result in greater returns in bone mass and structural properties if the daily exercise is partitioned into shorter, discrete bouts, rather than a single longer bout.

Both I_{MAX} and I_{MIN} were enhanced significantly by loading, but the load-induced increase in I_{MIN} over controls was 5–6 times greater than the load-induced increase in I_{MAX} over controls. The principal axes at the rat ulnar midshaft correspond roughly to the anatomical axes, with the major axis (plane along which I_{MAX} exists) oriented in the cranial–caudal direction and the minor axis (plane along which I_{MIN} exists) oriented in the medial–lateral direction. Previously characterized strain patterns at the midshaft during external loading (and during normal ambulation *in vivo*) show that bending occurs in the medial–lateral direction (25). Thus, the greatest change in strains during loading was produced on the medial and lateral surfaces of the midshaft, which is where the majority of the new bone formation occurred as a result of loading. The preferential localization of new bone to the medial and lateral surfaces explains why such large increases in I_{MIN} were found in the loaded groups, particularly in the 90×4 group.

Cortical vBMD at midshaft was significantly increased in the two loaded groups, indicating that loading increased mineralization of the tissue. We did not, however, detect a scheduling difference in vBMD between the two loaded groups, which suggests that mineralization was not affected by the timed delivery of load cycles.

The group differences observed in final body mass corresponded to the number of ether exposures during the loading period, a result we have reported previously (20). Despite the lower final body mass in the 90×4 group, bone mass and structural properties of the right ulna were greatest in this group. The differences in final body mass only strengthen our conclusions regarding the osteogenic response to different loading schedules; if whole bone (BMC, aBMD) and midshaft bending moment (I_{MAX}, I_{MIN}) values are standardized by final body mass, the differences between the 90×4 and 360×1 groups become even greater (from 12 to 50% greater than in unstandardized comparisons). Thus, differences in body mass do not appear to confound the results of this experiment.

Loading failed to produce any significant increase in bone density or mass at the olecranon. The 90×4 group exhibited significantly lower total vBMD in the loaded ulna when compared with the contralateral control ulna, but inspection of Table 2 reveals that this result was produced by an abnormally high control limb value rather than a suppressed value in the loaded bone. The lack of a positive-loading effect at the olecranon might be attributable to the small strains produced at that location during loading. Using a mean cortical bone cross sectional area of 4.2 mm^2 collected from the proximal pQCT scans (data not shown) and a previously calculated elastic modulus for rat cortical bone of 29.4 GPa (1), we estimate that strains at the olecranon during peak (17 N) loads reached only 140 $\mu\epsilon$. Considering the much larger strains occurring at the midshaft during peak loads (~3600 $\mu\epsilon$) (9), it appears possible that strain values at the olecranon were not great enough to exceed the threshold necessary to elicit an osteogenic response.

The Centers for Disease Control and the American College of Sports Medicine have made recommendations for all adults to "accumulate 30 minutes or more of moderate-intensity physical activity on most, preferably all, days of the week" (18). These guidelines were developed to promote exercise among the sedentary population, with the goal of improving general health and lowering risks for many diseases, including heart disease, osteoporosis, cancer, hypertension, and diabetes mellitus (among others). The temporal manner in which the 30 $min \cdot d^{-1}$ of exercise should be "accumulated" is not yet clear, but it may depend on the physiologic system being targeted for improvement. DeBusk et al. (4) showed that maximal oxygen uptake ($\dot{V}O_{2max}$) increased significantly more in men who were put on a daily exercise program involving a single, 30-min session each day, when compared with men who were put on a program of similar intensity involving three 10-min sessions each day. Conversely, Ebisu (5) found that high-density lipoprotein cholesterol levels increased significantly in men who ran three times per day, compared with men who ran the same total distance each day but did so in a single bout. Our data suggest that short periods of physical activity, conducted several times each day, might improve bone mass over that achieved from a single, sustained period of daily physical activity.

These data should be considered in light of several limitations of the experiment. We only tested one multiple-bout exercise schedule (90×4). Although we have shown previously in short-term experiments (1 wk of loading) that the osteogenic response to loading varies according to the number of bouts per day (20), it is unclear whether a different multi-bout schedule (e.g., 180×2 or 60×6) would be more or less beneficial than the 90×4 schedule after 4 months. Second, the loads used in this experiment elicited strains that were in excess of those measured in humans during vigorous exercises (3). It is unclear whether proportional benefits would occur at lower strains. Finally, we did find significant right versus left differences in I_{MAX} in the age-matched control group and in I_{MIN} for the baseline control group. In both of these comparisons, right ulna values were significantly lower than left ulna values despite the fact that these animals were not loaded or handled beyond measuring body mass. These two results are difficult to explain. However, in light of the fact that the remaining midshaft and whole-bone measurements showed no significant side-to-side differences in the control groups, it is unlikely that

these two observations suggest lateral dominance (left-handedness) in the rats used.

In conclusion, when 360 load repetitions are administered to the rat ulna 3 times·wk^{-1} for 16 wk, the anabolic response is much greater if the repetitions are divided into four smaller bouts of 90 repetitions/bout, separated by 3-h recovery periods, than if they are applied in a single, uninterrupted bout. These findings support other experimental evidence showing that bone cell mechanosensitivity declines quickly after initiation of a loading bout and suggest that by scheduling bone loading (exercise) sessions during times when the cells are more sensitive to mechanical stimuli, the osteogenic response can be improved. These concepts, applied to human exercise programs, hold potential for improving peak bone mass in the growing skeleton and/or preventing excessive bone loss in the aging skeleton.

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Address for correspondence: Alexander G. Robling, Ph.D., Department of Anatomy & Cell Biology, Indiana University School of Medicine, 635 Barnhill Dr., MS 5045, Indianapolis, IN 46202; E-mail: arobling@anatomy.iupui.edu.

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