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Bone-loading response varies with strain magnitude and cycle number

D. M. CULLEN,[1] R. T. SMITH,[2] AND M. P. AKHTER[1]
[1]*Osteoporosis Research Center, Creighton University, Omaha, Nebraska 68131; and*
[2]*North Carolina Wesleyan College, Rocky Mount, North Carolina 27804*

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Cullen, D. M., R. T. Smith, and M. P. Akhter. Bone-loading response varies with strain magnitude and cycle number. *J Appl Physiol* 91: 1971–1976, 2001.—Mechanical loading stimulates bone formation and regulates bone size, shape, and strength. It is recognized that strain magnitude, strain rate, and frequency are variables that explain bone stimulation. Early loading studies have shown that a low number (36) of cycles/day (cyc) induced maximal bone formation when strains were high (2,000 με) (Rubin CT and Lanyon LE. *J Bone Joint Surg Am* 66: 397–402, 1984). This study examines whether cycle number directly affects the bone response to loading and whether cycle number for activation of formation varies with load magnitude at low frequency. The adult rat tibiae were loaded in four-point bending at 25 (−800 με) or 30 N (−1,000 με) for 0, 40, 120, or 400 cyc at 2 Hz for 3 wk. Differences in periosteal and endocortical formation were examined by histomorphometry. Loading did not stimulate bone formation at 40 cyc. Compared with control tibiae, tibiae loaded at −800 με showed 2.8-fold greater periosteal bone formation rate at 400 cyc but no differences in endocortical formation. Tibiae loaded at −1,000 με and 120 or 400 cyc had 8- to 10-fold greater periosteal formation rate, 2- to 3-fold greater formation surface, and 1-fold greater endocortical formation surface than control. As applied load or strain magnitude decreased, the number of cyc required for activation of formation increased. We conclude that, at constant frequency, the number of cyc required to activate formation is dependent on strain and that, as number of cyc increases, the bone response increases.

tibia; adult rat; strain; histology; mechanical loading

THE SIZE, SHAPE, AND STRENGTH OF BONE are regulated in part by the mechanical forces applied to bone during daily physical activities. These forces are created during movement by muscle contractions and by impact with external objects such as the ground in walking or a ball in tennis. Bending, compression, tension, torque, and shear forces all cause bone deformation, which is quantified as strain (change in length/original length). The complex movement patterns associated with exercise result in complex strain patterns that vary in magnitude, rate, and frequency throughout the bone.

External loading devices designed for controlled force application are used to create well-defined strain patterns to examine specific strain variables (1, 22). Strain characteristics that are known to alter bone metabolism include strain magnitude (extent of deformation), frequency (number of strain cycles per second), and strain rate (change in magnitude per second or acceleration or deceleration of deformation) (5, 12, 24). Previous studies with loading models have reported that bone formation is linear with an increase in strain magnitude above 1,000 με (22, 29). When load and number of cycles/day (cyc) are constant, bone formation increases as frequency increases from 0 to 2 Hz (30), and, when frequency increases from 1 to 30 Hz, the strain threshold for bone maintenance decreases from 1,200 to 100 με (18). Strain rate, which reflects strain magnitude and cycle frequency, has been suggested to be one of the most important variables that determines bone response (16, 30).

Although, intuitively, the number of cyc should be an important variable for mechanical regulation of bone, an early loading study showed no effect of increasing the number of cyc from 36 to 1,800 cyc at 2,000 με and low frequency (0.5 Hz) (23). It is possible that the high strain magnitudes in that study overwhelmed the effect of variation in cyc and that the activation of maximal bone response was achieved at low cyc. In a theoretical model for mechanical regulation of bone, Whalen et al. (32) proposed that the effect of cycle number increased as strain magnitude decreased. At a high frequency (30 Hz), 108,000 cyc have been shown to maintain bone with forces as low as 100 με (18). However, no one has shown a relationship between cyc and loads at low frequency.

In the present study, we hypothesized that, as cyc increases, the bone response to loading increases and that the cyc required for activation varies with load magnitude. This study used the rat tibia four-point bending device to examine the effects of variation in cyc (40–400) and strain rate or magnitude (800–1,000 με) applied at a constant frequency on cortical bone formation.

MATERIALS AND METHODS

The effects of external mechanical loading cycles were studied in the tibiae of Sprague-Dawley (SASCO, Omaha, NE) female retired breeder rats (6 mo old and 331 ± 28 g).

Address for reprint requests and other correspondence: D. M. Cullen, Osteoporosis Research Center, Creighton Univ., 601 North 30th St. #5766, Omaha, NE 68131 (E-mail: dcullen@creighton.edu).

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The rats were individually housed in wire cages (20 × 24 × 18 cm) and provided food and water ad libitum. All procedures were approved by the University's Institutional Care and Use Committee.

Seventy rats were randomized by body weight to a control (nonloaded) group or six treatment groups (right leg loaded). External mechanical loads were applied to the lower right leg with a rat tibia four-point bending device (1, 20, 21). The upper pads in the loading device were 10.5 mm apart and centered between the lower pads, which were 22 mm apart. The maximal bending region was between 3.5 and 14 ± 0.5 mm proximal to the tibia fibular junction (20). An external force of 25 or 30 N was applied in a sinusoidal pattern at 2 Hz for 3 days/wk for 3 wk to create lateral periosteal compressive strains of 800 and 1,000 με and endocortical strains of 600 and 800 με, respectively. Loads were applied at each force at 40, 120, or 400 cyc.

All rats were administered an intraperitoneal Calcein (8 mg/kg; Sigma Chemical, St. Louis, MO) injection 10 and 3 days before death. Rats were anesthetized and killed by intracardiac injection (0.1 ml FatalPlus, Vortech Pharmaceuticals, Dearborn, MI). The right tibiae were collected from all rats and the left tibiae from one loaded group (nonloaded leg from 25 N and 40 cyc). The left (nonloaded) leg was collected as a control for the systemic effects of ether and handling. Bones were fixed in 70% ethanol, block stained in Villanueva stain (31), dehydrated in ethanol and acetone, and embedded in methyl methacrylate (2). Cross sections were cut 5–7 mm proximal to the tibia-fibular junction at 120-μm thickness on a low-speed diamond wheel saw (model 2680, South Bay Technology, Temple City, CA) and ground to 90-μm thickness for mounting. Two sections from each tibia were blind coded and analyzed, and their data were averaged.

Periosteal and endocortical surfaces were digitally traced with a microscope, camera lucida, graphics pad, and the BIOQUANT semi-automated image analysis system II (R&M Biometrics, Nashville, TN). Measurements included cortical area, woven bone area, periosteal and endocortical perimeter, double and single calcein-labeled perimeter, and woven bone perimeter. Woven bone was identified by irregular diffuse labeling patterns. Interlabel width (IrL.Th) was directly measured at equal intervals between all double calcein labels. The length of each unique type of surface [single calcein labeled (sLS), double calcein labeled (dLS), and woven bone bearing (WoS)] was reported as a percent of the total bone surface (BS). Formation surface (FS) was the sum of the three unique forming surfaces [(sLS/2 + dLS + WoS)/BS × 100]. Mineral apposition rate (MAR) was calculated at all dLS sites as the distance between labels divided by interlabel time (Ir.LWi/7 days). Surface-based bone formation rate (BFR) was calculated as MAR × FS/BS (17). When woven bone was present, then total BFR was the sum of lamellar BFR [(0.5 × sLS + dLS)/BS × MAR] and woven BFR (woven bone area)/21 days.

Strain (in με) on the lateral tibial surface during four-point bending was calculated from an equation based on cross-sectional moment of inertia, beam-bending theory, and previous in vivo strain gauge measurement (1). Cross sections were traced at ×20, and the moment of inertia about the anterior-posterior axis was computed with SECTION (Biomechanics Lab, Creighton Univ., Omaha, NE) on a VAX Station 2000 computer. Medial periosteal strains were calculated as 40% greater than lateral strains based on finite element mapping of strain distribution, moment of inertia, and load-application angle (1). The loaded leg estimated strain after 3 wk of loading.

Two control groups (right nonloaded control and nonloaded left from 25 N and 40 cyc) were used to *1*) control for the stress of animal handling and ether exposure and *2*) double the size for statistical analysis. Consistent with previous studies (21, 25, 28), no differences between groups were found by Student's *t*-test ($P > 0.09$, power = 0.5), and the data were pooled to form a single control group ($n = 19$). Differences between loaded legs because of load magnitude and cycle number were tested by two-factor ANOVA ($P < 0.05$). Differences because of cycle number within loads were tested by Newman-Keuls post hoc tests using the pooled control group ($P < 0.05$). As a secondary analysis, multiple regression analysis was used to determine the relationship of bone response to cycle number and strain magnitude.

RESULTS

During the course of the study, three rats died, leaving nine rats in each of the 400-cyc groups and in the control group. Animal weights did not vary significantly during the study, and the tibial cross-sectional area and moment of inertia were not different among groups (data not shown). Estimated from regression equations using moment of inertia (1), the periosteal lateral strains (compressive mean ± SD) in the loaded legs averaged −807 ± 140 με in the 25 N group and −1,029 ± 148 με in the 30 N group. Medial (tensile) strains were estimated to be 40% larger with 1,130 με at 25 N and 1,440 με at 30 N. Endocortical strains averaged 25% lower than the periosteal strains with strains of 600 to 1,080 με at 25 and 30 N, respectively. The lateral surface strain rate averaged −3,200 με/s at 25 N and −4,000 με/s at 30 N. In adult rats, sham loading without bending does not create a periosteal response to 36 cyc at forces below 35 N (21, 29). Although it is possible that, at the higher repetitions, soft tissue compression may have been a factor, no tissue swelling or injury was noted after loading or at the time of collection in the current study.

Load magnitude. Periosteal bone formation was greater at 30 than at 25 N applied force (Fig. 1). In the loaded leg at each cyc (40, 120, 400), FS was more than twofold greater at 30 than at 25 N applied load ($P < 0.03$). Mineral apposition rate was 60% higher at 30 than at 25 N. Woven BS was fivefold greater at 30 than at 25 N for 400 cyc ($P = 0.002$) and tended to be greater for 120 cyc ($P = 0.08$) but not different at 40 cyc (Fig. 2). The net effect of loading at 30 N was at least a threefold greater total BFR than at 25 N for 120 and 400 cyc of applied load ($P < 0.003$), but there was no difference in formation at 40 cyc (Fig. 1).

At the endocortical surface, the only significant difference because of load magnitude was greater woven BS seen at 400 cyc for 30 N compared with 25 N ($P = 0.02$; Fig. 2). However, compared with 25 N, FS tended to be higher at 30 N and 400 cyc ($P = 0.09$); there were no differences in MAR, and formation rate tended to be greater at 30 N and 120 cyc ($P = 0.06$; Table 1).

Cycle number. At the lowest force (25 N) periosteal FS was greater at 400 cyc than at 40 cyc ($P = 0.008$; Fig. 1). Periosteal woven bone was absent at 0 and 40 cyc and represented up to 3% of the surface at 120 and 400 cyc (Fig. 2). At 25 N, total BFR was greater at 400

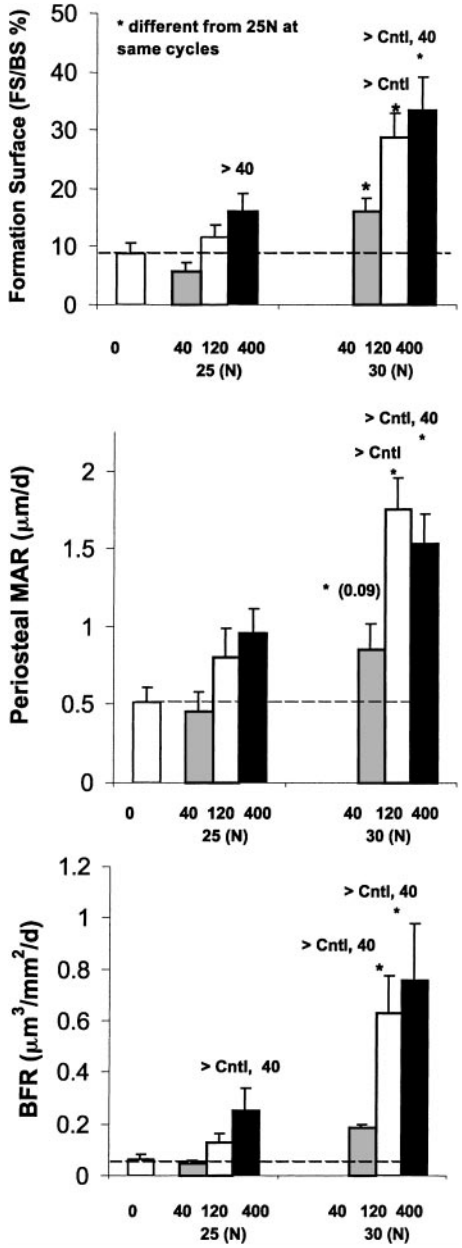

Fig. 1. These graphs represent periosteal formation surface (FS; *top*), mineral apposition rate (MAR; *middle*), and total bone formation rate (BFR; *bottom*). The dashed line is the control level. Formation associated with cycle number [40, 120, and 400 cycles/day (cyc)] was compared within each load (25 or 30 N) and with control (cntl; 0 cyc) with differences noted ($P < 0.05$). At 25 N, 400 cyc increased FS and BFR, whereas, at 30 N, 120 and 400 cyc increased FS, MAR, and BFR. Differences because of load at the same cycle number are seen for 120 and 400 cyc. Values are means ± SE. BS, bone surface. *Signifcantly different from 25 N at same cyc ($P < 0.05$).

cyc than at 0 (control) or 40 cyc ($P < 0.03$). At the highest force (30 N), periosteal FS and MAR were greater at 120 and 400 cyc than at 0 cyc and greater at 400 cyc than at 40 cyc ($P < 0.01$). All cycle levels showed some periosteal woven bone formation, but it was significantly greater at 400 than at 0 or 40 cyc ($P < 0.02$). At 30 N, total BFR was greater at 120 and 400 cyc than at 0 or 40 cyc ($P < 0.005$; Fig. 1).

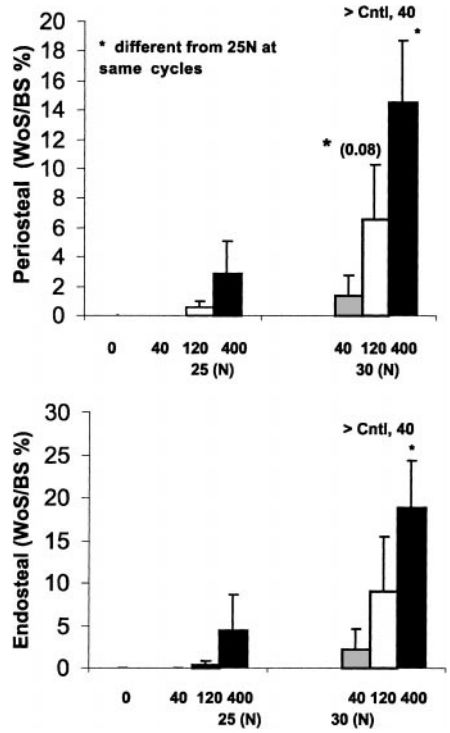

Fig. 2. These graphs represent periosteal (*top*) and endocortical woven BS (*bottom*) associated with cycle number (40, 120, and 400 cyc) compared within each load (25 or 30 N) and to control (0 cyc). Woven bone was seen at 25 and 30 N but was significantly elevated only at 30 N and 400 cyc. Differences because of load at the same cycle number were seen for 400 cyc. Values are means ± SE. WoS, woven surface. *Significantly different from 25 N at same cyc ($P < 0.05$).

For the periosteal surface, regression analysis showed that cyc accounted for 25% of the variation in periosteal FS in the 25 N load group and 19% of the variation in the 30 N group. Individually, cycle number and strain magnitude were significant predictors of formation ($P < 0.005$), and, when combined, they accounted for 37% of the variation in FS ($P < 0.001$). BFR was more variable, such that cycle number and strain

Table 1. *Tibial endocortical bone formation*

Force, N	Applied Cycle Number: 40 cyc ($n = 10$)	120 cyc ($n = 10$)	400 cyc ($n = 9$)	Control ($n = 19$)
		FS/BS, %		
25	14.0 ± 14	26.7 ± 20.9	25.00 ± 26.31	20.1 ± 16.8
30	20.8 ± 15.3	44.7 ± 33.0*†	50.1 ± 25.1*†	
		MAR, μm/day		
25	1.34 ± 0.24	1.50 ± 0.44	1.41 ± 0.05	1.33 ± 0.38
30	1.36 ± 0.49	1.52 ± 0.27	1.54 ± 0.32	
		BFR, μm/day		
25	0.25 ± 0.22	0.45 ± 0.39	0.50 ± 0.36	0.36 ± 0.24
30	0.34 ± 0.30	0.80 ± 0.62*†	0.77 ± 0.39*†	

Values are means ± SD. FS, formation surface; BS, bone surface; MAR, mineral apposition rate; BFR, bone formation rate; cyc, cycles/day. *Significantly different at same load vs. control ($P < 0.002$). †Significantly different at same load vs. 40 cyc ($P < 0.01$).

magnitude combined accounted for only 29% of the variation ($P < 0.001$).

There were no endocortical loading effects seen at 25 N for any cyc or at 30 N and 40 cyc (Table 1). Mineral apposition rate did not vary with cyc and was not different from control. At 30 N, applied load, endocortical FS, and BFR were greater with 120 or 400 cyc than with 0 or 40 cyc ($P < 0.05$). Woven bone, measured on the endocortical surface as nonlamellar bone formation, was greater at 30 N and 400 cyc than at 0 or 40 cyc ($P < 0.004$). Although uncommon, endocortical woven bone is seen occasionally in control legs and at low loads in our laboratory. It is normally associated with high bone formation and in regions of rapid MAR.

DISCUSSION

The bone response to external mechanical loading increased in this study with increases in number of applied cyc and load magnitude. From previous studies, we know that the loading response in the rat tibia four-point bending device is periosteal modeling or formation with no evidence of resorption (4, 9). In this study, a greater number of cyc was required to stimulate formation at low force than at high force with 400 cyc required at 25 N and 120 cyc at 30 N. There was a pattern of increased FS with increased cyc, which was confirmed by regression analysis. As predicted from previous work, for the effective cyc (i.e., 120 and 400), the formation response was greater at 30 N than at 25 N.

The strain magnitudes during loading in this study were low compared with our laboratory's previous studies (10). The primary variables in this and our laboratory's other studies have been peak strain magnitude and cycle number; because all of our laboratory's studies have used a sinusoidal load application at 2 Hz, average strain rates were four times maximal strains. The strains around the circumference of the tibia vary in magnitude and direction, depending on location relative to the neutral bending axis. Our laboratory's previous studies have shown that the formation response depends on strain magnitude when cyc is constant at 36 cyc and 2 Hz (10, 20, 21).

Two independent studies with external loading have demonstrated a strain threshold for activation of bone formation based on regression analysis. In the turkey compression model, the threshold for bone density change was 1,000 με at 100 cyc and 1 Hz (2,000 με/s) (22). In the rat four-point bending model, the threshold for BFR was −1,050 με at 36 cyc and 2 Hz (4,200 με/s) (29). Although the threshold may be 1,000 με, our laboratory's previous studies at 36 cyc suggest that detectable effects on bone may not occur below strain ranges of −1,200 to 1,600 με (4,800 με/s) (10, 20, 21). Compressive strains from 1,200 to 1,500 με on the lateral surface increased MAR and woven bone but not FS length. In the same studies, tensile strains from 1,650 to 2,150 με on the medial surface increased both formation rate and surface lengths with 36 cyc. In the current study, strains at 25 N were 50% lower but, with 400 cyc, showed an increase in BFR. Strains at 30 N in this study were 33% lower with no response at 40 cyc but a strong response at 120 and 400 cyc. Taken together, our laboratory's studies suggest that to increase periosteal BFR at least 400 cyc are required when forces create strain ranges of −800 to 1,100 με (3,200 με/s), 120 cyc for −1,000 to 1,400 με (4,000 με/s), and 36 cyc for −1,200 to 1,600 με (4,800 με/s). This apparent variation in bone activation with cycle number is consistent with high-frequency loading at 30 Hz for 108,000 cyc in which the strain threshold was only 100 με (6,000 με/s) (18). Although, in our study, cycle number accounted for 19–25% of the variation in formation, a greater range of forces and cyc are needed to confirm the apparent relationship between minimal effective strain and cyc and to establish the relationships proposed by Whalen et al. (32).

In contrast to Rubin and Lanyon's earlier work (23), we found that, at submaximal loads, increasing cyc above 40 cyc effectively increased the bone response. These results are consistent with Whalen's model for bone adaptation where the impact of cyc increases as strain magnitude decreases (32). If applicable to exercise models, then physical activities that create less than 1,000 με may be effective for increasing bone formation if performed at an appropriate frequency for a sufficient number of repetitions.

The maximal strains created during exercise are relatively low in physically active adults. In a human strain gauge study, Burr et al. (6) found that compressive and tensile maximal tibial strains ranged from 400 με during walking to <1,000 με with running at the measured sites. This is consistent with earlier reports (13). Zigzag running up- and downhill created the highest strains, which reached 1,226 με in compression and 2,000 με in shear. Although these regional surface strains approach the strain activation thresholds from low-frequency animal loading studies (22), the conclusions from loading models must be cautiously interpreted for humans because these models create unique strain distribution patterns (29).

In human adults, although exercise may be an excellent method for maintaining bone mass, it appears to be a very difficult method for increasing bone mass (3, 11). Cross-sectional studies show that years of intense athletic training with high force generation for countless repetitions is associated with high bone mass. The greatest bone mass is seen in athletes competing in sports that require high power generation, such as gymnastics and power lifting (8, 27). The bone response to a given load depends on *1*) strain magnitude that is based on previous bone adaptations and *2*) the interaction of strain magnitude, frequency, rate, distribution, and repetitions. In animal loading models, bone adapts within months to new loads showing transient increases in bone formation resulting in small regional increases in bone area (9, 19). Given the 1–2% detection limit for dual-energy X-ray absorptiometry and limitations on measurement sites relative to loading sites, it is understandable that long-term, high-power activities with maximal force generation, such as

jumping and weight lifting, are prescribed in studies to detect significant bone effects of exercise.

When cycle number is extremely high and intense loads are applied several hundred times a day for several weeks, as occurs in military training, an almost pathological response has been measured with up to 11% bone gain in 14 wk (14). More traditional responses to exercise have been reported as a 2.2% increase in tibial BMD after 15 wk of basic training (7) or, in gymnasts, as a 2.8% increase in BMD at the spine and a 1.6% increase at the femoral neck after 8 mo of training (26) or, in men, as a 2% increase in BMD at the spine (not significant) and a 3.8% increase at the femoral neck after 16 wk of strength training (15). Most adults are not willing or able to commit to repetitive high-intensity exercise, and forceful activities may not be safe for individuals at risk of osteoporotic fracture. However, animal studies have shown that walking 20 min/day (~1,200 cyc) can activate bone formation in very sedentary animals (19) and that very low force applied for 20 min/day at 30 Hz (36,000 cyc) can maintain bone mass (24). Those studies, along with the current data, suggest that high forces are not required if cyc are high and the other strain components (frequency, rate) are appropriate.

This study has shown that bone adaptation to loading is dependent on strain magnitude (rate) and the number of cyc at low frequency. The data from this study, combined with others, supports the hypothesis that, as applied force and strain increases, the number of cyc required to initiate bone formation decreases. Future studies are needed to confirm our results at lower forces and higher cyc. The more difficult task will be to design and test exercise prescriptions of low to moderate intensity and high cyc that would be appropriate for older adults and beneficial to bone.

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