The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli

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ABSTRACT **It is generally believed that mechanical signals must be large in order to be anabolic to bone tissue. Recent evidence indicates, however, that extremely low-magnitude (<10 microstrain) mechanical signals readily stimulate bone formation if induced at a high frequency. We examined the ability of extremely low-magnitude, high-frequency mechanical signals to restore anabolic bone cell activity inhibited by disuse. Adult female rats were randomly assigned to six groups: baseline control, age-matched control, mechanically stimulated for 10 min/day, disuse (hind limb suspension), disuse interrupted by 10 min/day of weight bearing, and disuse interrupted by 10 min/day of mechanical stimulation. After a 28 day protocol, bone formation rates (BFR) in the proximal tibia of mechanically stimulated rats increased compared with age-matched control (+97%). Disuse alone reduced BFR (−92%), a suppression only slightly curbed when disuse was interrupted by 10 min of weight bearing (−61%). In contrast, disuse interrupted by 10 min per day of low-level mechanical intervention normalized BFR to values seen in age-matched controls. This work indicates that this noninvasive, extremely low-level stimulus may provide an effective biomechanical intervention for the bone loss that plagues long-term space flight, bed rest, or immobilization caused by paralysis.—Rubin, C., Xu, G., Judex, S. The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli. *FASEB J.* 15, 2225–2229 (2001)**

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A PRINCIPAL GOAL of the National Aeronautics and Space Administration is to safely institute long-term human exploration of space. Whether this occurs in the near future by habitation of the International Space Station or within the next few decades by settling a permanent manned Moon base and embarking on a mission to Mars, it is clear that a better understanding of the ability of humans to tolerate extended exposure to microgravity must be developed. The National Research Council's Space Studies Board has stated that the principal physiological hurdle to humans' extended presence in space is the osteopenia that parallels reduced gravity (1, 2). The extent of the loss is extremely high despite prescribed daily exercise regimes designed to maintain physical fitness. In flights lasting 4–6 months, astronauts can lose bone mineral density in the lower appendicular skeleton at a rate approaching 1.6% per month (3, 4). Although there are no adequate long-term data to suggest that this high rate of erosion would necessarily continue, it must be considered that over a 2.5 year return trip to Mars, *half* of an astronaut's bone density could vanish from specific skeletal sites and thus severely jeopardize his/her health and well-being. Whereas this rapid bone loss potentiates renal lithiasis during flight (5), the most significant consequences—fractures in the skeleton—may be realized only upon return to planetary gravitational fields (6, 7). Given that removal of gravity is a central etiologic factor in this bone loss, it is presumed that reintroduction of specific mechanical factors may prevent the osteoporosis. Unfortunately, the skeletal benefits of lengthy bouts of strenuous exercise to combat microgravity-induced osteopenia remain unclear (8), and such a countermeasure is certain to erode valuable crew time.

Recent studies indicate that the anabolic potential of mechanical strain is strongly frequency dependent; whereas 1 Hz loads must exceed 1000 microstrain ($\mu\varepsilon$) to stimulate cortical bone formation (9), loads applied at 30 Hz mechanical necessitate strains on the order of 50 $\mu\varepsilon$ to achieve the same result (10), even though these signals are 2% of the peak strains that occur in bone during vigorous functional activity. In trabecular bone, strain signals can be as low as 5 $\mu\epsilon$ and still be strongly anabolic (11).

These high frequencies, which are anabolic to bone, similar to the contractile spectra of muscle (12), dominate the bone's strain history (13). This led to the hypothesis that these low-level mechanical signals are key determinants of bone mass and morphology. Thus, the inherent reductions in muscle dynamics that paral-

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lel the aging process, bed rest, microgravity, or paralysis will suppress osteoblastic activity and permit a net loss of bone tissue. Reintroducing these signals, therefore, would serve as a 'surrogate' to compensate for the removal of musculoskeletal forces, and thus represent an ideal countermeasure to the osteopenia that parallels disuse.

MATERIALS AND METHODS

Experimental design

Adult female Sprague-Dawley rats (6- to 8-month-old retired breeders, Charles River Laboratory, Wilmington, MA) were assigned to baseline controls (BLC, n=15), long-term (age matched) normal weight-bearing controls (LTC, n=30), normal weight-bearing animals subject to 10 min·day^{-1} 90 Hz mechanical stimulation at 0.25 g peak to peak (MS, n=21), animals subject to 24 h·day^{-1} disuse via hind limb suspension (Dis, n=11), animals subject to disuse interrupted by 10 min·day^{-1} of normal weight bearing (Dis+WB, n=7), and disuse interrupted by 10 min·day^{-1} of 90 Hz stimulation at 0.25 g (Dis+MS, n=19). All protocols ran for 28 days; animals were weighed at the beginning and end of the study. Baseline control rats were killed 1 day before the protocols commenced. Rats were individually housed at 24°C with free access to food and water. To measure indices of bone formation, all rats (except baseline controls) were given injections of demeclocycline [25 mg·kg^{-1}, intraperitoneal (i.p.)] before the beginning of the study and calcein (15 mg·kg^{-1}, i.p.) on day 18 of the protocol. Rats were killed by carbon dioxide inhalation, and right and left tibiae harvested. All procedures were reviewed and approved by the Animal Care Committee of SUNY Stony Brook, and met all guidelines for the health and welfare of the animals.

Suspension model and daily loading of animals

All disuse animals were subject to hind limb suspension for 28 days according to the Morey-Holton tail suspension model of disuse osteopenia (14). For those animals receiving mechanical intervention (MS, Dis+MS), this stimulus was provided by a platform that oscillated at 90 Hz, giving rise to a vertical accelerations of 0.25 g (9.8 m·s^{-2}=1 g = Earth's gravitational field). When a human stands on a plate providing a 0.25 g mechanical stimulus, the vibration is barely perceptible. The apparatus uses a small, low-force (18N) but highly linear moving coil actuator (15). During the mechanical stimulation, each rat was placed in regular plastic cage where it was allowed to move freely. Once a day, 5 days/wk, each animal in a loading protocol was subjected to 10 min·day^{-1} of a 0.25 g, 90 Hz mechanical load. The disuse plus weight-bearing animals were placed on an inactive platform for 10 min·day^{-1}.

Histomorphometry

The proximal tibia (right) was embedded in methyl-methacrylate (Fisher Scientific, Fair Lawn, NJ) using a three-step protocol (16). After trimming the plastic blocks, 50 μm-thick frontal sections from the central tibia were cut on a diamond wire saw (Well Wire Saws, Model 3241, Germany). Sections were mounted on an epifluorescent microscope (×10). Trabecular bone of the proximal tibial metaphysis was evaluated over an area enclosed by two lines 800 μm and 2000 μm distal of the growth plate. Twenty-four adjacent squares, each displaying 1.6 mm^2, were captured by a video camera interfaced with a digitizing pad (CalComp, Anaheim, CA) and a PC. Fluorescent labels and bone surfaces were traced and morphometry software (OsteoMetrics, Atlanta, GA) was used to determine bone histomorphometric indices. Trabecular bone formation rate, with bone volume as referent (BFR·BV^{-1}), mineralizing surface (MS·BS^{-1}), mineral apposition rate (MAR), and bone area (BV) were determined as described previously (17). All histomorphometric evaluations were performed without knowledge of which experimental group the bones came from.

Statistics

T tests were used to assess the anabolic potential of the mechanical signal (differences in histomorphometric indices between LTC and MS). A single-factor analysis of variance, followed by a Tukey post hoc test, was used to compare histomorphometric indices between BLC, LTC, MS, Dis, Dis+WB, and Dis+MS groups. Changes in body mass between day 0 and day 28 were evaluated via paired t tests within groups. Data analysis was performed using the statistical software package SPSS for Windows 9.0. The significance level was 0.05 and all data are presented as mean ± SD.

RESULTS

There were no significant changes in body mass in any of the groups during the course of the study. Over a 28 day period, 10 min/day of the 90 Hz, 0.25 g mechanical stimulation increased BFR·BV^{-1} by 97% (P<0.001) and MS·BS^{-1} by 76% (P<0.001), but not MAR (2%), vs. long-term controls (**Figs. 1–3**, **Table 1**). In contrast, tail suspension suppressed BFR·BV^{-1} by 72% (P<0.02), MS·BS^{-1} by 52% (P<0.04), and MAR by 45% (P<0.03) vs. the long-term controls. Tail suspension interrupted each day by 10 min of normal weight

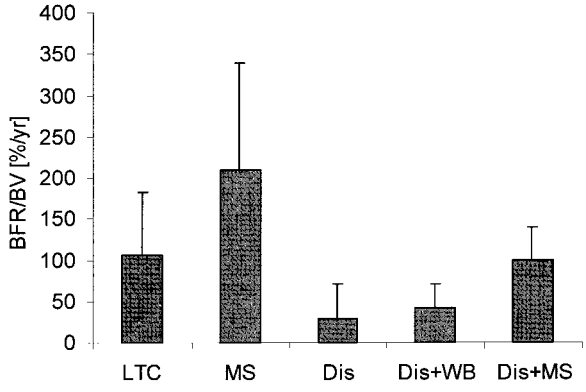

Figure 1. Proximal tibial bone formation rate per bone volume (BFR·BV^{-1}; mean±SD) after the 28 day protocol. Ten min·day^{-1} of mechanical stimulation (MS) significantly increased bone formation rates vs. long-term control animals (LTC), whereas BFR of rats subjected to 24 h·day^{-1} of disuse (Dis) or disuse interrupted by 10 min·day^{-1} of weight bearing (Dis+WB) were significantly smaller than BFR of both long-term controls and animals in which disuse was interrupted by 10 min·day^{-1} of mechanical stimulation (Dis+MS). DIS+MS values were not significantly different from LTC.

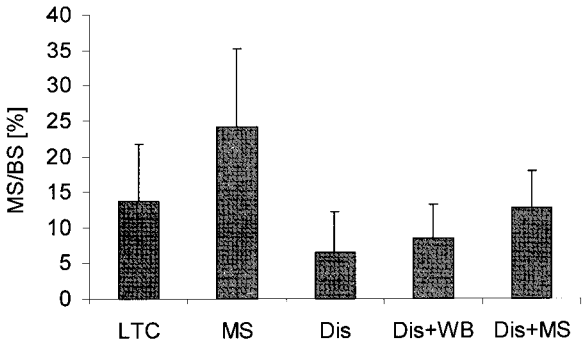

Figure 2. Mineralizing surface over bone surface (mean±SD) of long-term controls (LTC) and after 28 days of 10 min·day^{-1} mechanical stimulation (MS), 24 h·day^{-1} tail suspension (DIS), disuse interrupted by 10 min·day^{-1} of weight bearing (Dis+WB), and disuse interrupted by 10 min·day^{-1} of mechanical stimulation (Dis+MS). Data indicate that increased bone formation rates were achieved primarily by an increase in mineralizing surfaces. Similar to bone formation rates, the percentage of mineralizing surface was significantly greater in MS rats and significantly smaller in DIS rats when compared with LTC rats.

bearing failed to re-establish the growth patterns suppressed by disuse relative to control: BFR·BV^{-1} dropped by −61% ($P<0.03$), MS·BS^{-1} dropped by 38% ($P<0.04$), whereas MAR was not significantly different (−30%) from disuse. In contrast, disuse interrupted each day by 10 min of the 90 Hz loading maintained bone remodeling dynamics at control values: BFR·BV^{-1} was 7%, MS·BS^{-1} was 6%, and MAR was 1% below control values ($P>>0.05$.). Activity levels of the suspended rats allowed to freely ambulate for 10 min·day^{-1} were similar to those of normal rats during the 10 min of weight bearing. Trabecular bone area (BV/TV) was similar among all groups (Table 1).

DISCUSSION

The human body undergoes roughly 50 changes in a weightless environment, 8–10 of which also occur in aging bodies here on Earth. Of those, bone loss is recognized as perhaps the greatest physiological obstacle to an extended human presence in space (2). The majority of pharmacologic countermeasures for osteoporosis work by inhibiting bone resorption, whereas therapies that increase bone formation are highly desirable and unusual. Very few exist; those currently under investigation, such as parathyroid hormone, fluoride, and insulin-like growth factor I, have important and significant disadvantages. Data presented here demonstrate that noninvasive, low-level mechanical signals several orders of magnitude below those that cause damage to the bone tissue are strongly osteogenic even when applied for very short duration and effectively restore anabolic activity compromised by disuse.

The mechanism by which astronauts lose bone is poorly understood; it is unclear whether the decrease in bone mass is associated with increased bone resorption, decreased bone formation, or both (18). Limited osteoblastic activity in the skeleton of adult astronauts before flight does not exclude the possibility that microgravity causes an uncoupling between bone resorption and formation. Thus a defect in bone formation may be a principal cause for the net bone loss observed. In the ground-based model of microgravity

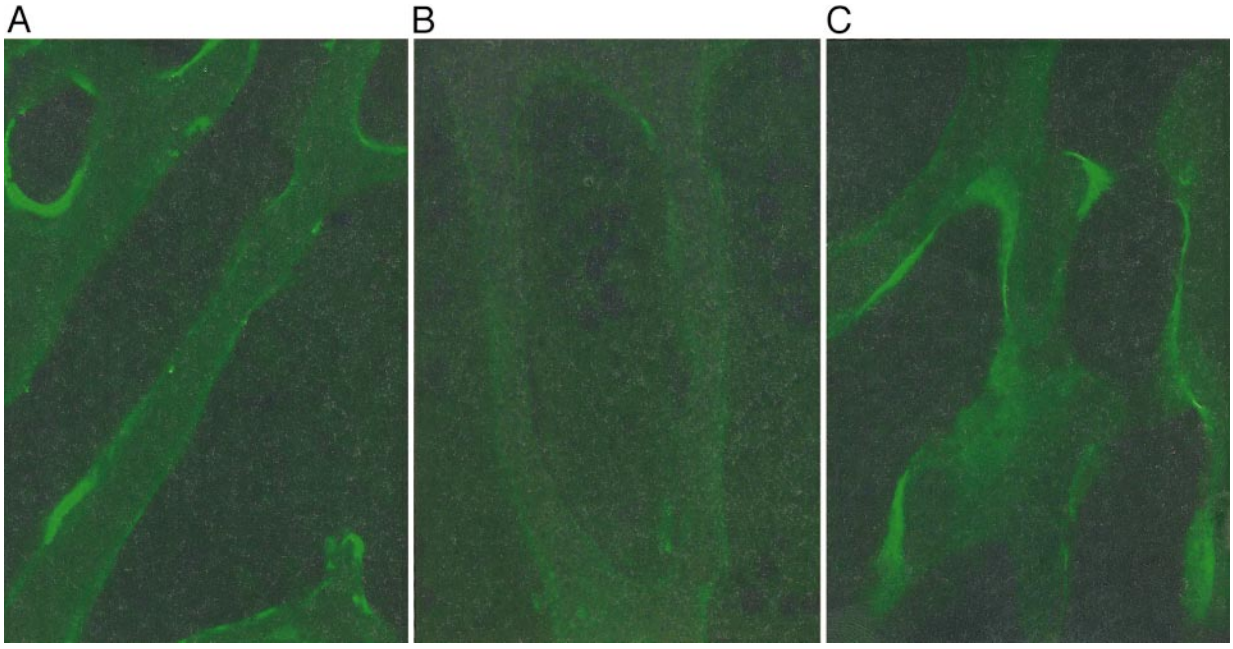

Figure 3. Examples of trabecular bone sections from *A)* control, *B)* disuse, and *C)* mechanically stimulated rats. A fluorescent filter was used to excite the bone matrix-deposited calcein labels administered 18 days into the study. The greater amount of bright green label in mechanically stimulated trabecular bone than bone from control and disuse rats indicates more mineralizing trabecular surfaces. Individual trabeculae were lightened for better visualization.

TABLE 1. *Tibial trabecular mineral apposition rates (MAR) and the percentage of bone volume with respect to tissue volumes ($BV \cdot TV^{-1}$) in different groups of rats (mean±SD)*[a]

	Baseline control	Long-term control	Stimulation	Disuse	Disuse + weight bearing	Disuse + stimulation
MAR [μm/day]	N/A	0.8 ± 0.4	0.8 ± 0.3	0.3 ± 0.3	0.6 ± 0.3	0.8 ± 0.3
$BV \cdot TV^{-1}$ [%]	19.1 ± 5.6	19.2 ± 8.5	22.0 ± 8.8	17.8 ± 4.9	19.4 ± 4.3	18.9 ± 8.3

[a] MAR of disuse rats was significantly lower ($P<0.001$) than that of the other groups except for MAR of rats in which disuse was interrupted by 10 min per day of weight bearing. There were no significant differences in normalized bone volume between groups.

used in this study, hind limb suspension significantly decreased bone formation, yet an increase in osteoclastic activity was not observed with this model in adult rats spanning a period of up to 5 wk (19). Consistent with this observation, we found similar tibial trabecular bone volumes in disuse and control rats, limiting our analyses to changes in bone formation. Whereas large, multinucleated osteoclasts can rapidly change the volume of a bone during the resorptive process (20), increases in lamellar bone formation in response to a subtle mechanical perturbation of the musculoskeletal system must accumulate over a longer period before changes in bone volume can be detected. Consequently, the similar bone volumes between mechanically stimulated rats and control rats were most likely due to the large initial bone mass of adult rats and the relatively short 4 wk experimental protocol. Furthermore, mechanical stimulation increased bone formation primarily by increasing the percentage of mineralizing surfaces, indicating that the low-level, high-frequency mechanical signals recruited additional osteoblasts rather than increasing the activity levels of existing osteoblasts.

Numerous mechanical parameters have been proposed, including strain magnitude (21), strain rate (22), strain energy density (23), and strain gradients (24), as controlling the adaptive response in bone. Perhaps the most accepted doctrine of 'form follows function' in the skeleton is that the peak strains induced by vigorous activity are the most potent influences. Thus, it has been presumed that the bone loss that parallels space flight, bed rest, or paralysis results from the absence of high strain signals that arise from impact loading. Reintroducing these large magnitude strain signals by vigorous exercise, however, had very limited success in impeding microgravity-related bone loss (25). In contrast, the studies reported here demonstrate that high-frequency but low-magnitude mechanical signals normalized bone formation to control values, despite combating 23 h and 50 min per day of a strong signal for resorption 10 min per day, whereas 10 min of normal weight bearing per day failed to curb the osteopenia stimulated by disuse.

The large amount of bone loss that accompanies space flight occurs even though astronauts are subjected to daily exercise regimes lasting up to 3 h. Although there are essentially no data on the amount of bone loss occurring in the absence of physical exercise, it is clear that current exercise regimes are ineffective and take up valuable crew time. Whether high-frequency, low-magnitude mechanical stimuli will prevent bone loss in conditions of microgravity will ultimately have to be answered by experiments performed in space. In this experiment, however, tail-suspended rats allowed to ambulate freely for 10 min per day and thus subject to some degree of high-frequency mechanical signals associated with standing and walking (13) failed to retain bone mass, a result in stark contrast to rats that were oscillated for the same amount of time. We conclude that the low-level signals generated by the musculature are effective only if the skeleton is subject to them for much longer periods (such as several hours) of standing or walking. Ironically, as small as the signals induced by the oscillating plate may be, in the realm of 20–100 Hz, where the musculature is active, these signals are relatively large. Nevertheless, the design of an effective countermeasure for the bone loss in space will require better understanding of the molecular mechanisms responsible for the bone loss and of the means by which biomechanical and/or biochemical interventions influence the bone cell kinetics.

Considering the anabolic potential of these high-frequency strains (11), it is important to establish to what degree they are intrinsic to the skeletal system. Strain within functionally loaded bones can be characterized as having an inverse power-law relationship between the magnitude of strain events and the frequency with which these events occur (13), making it reasonable to conclude that the bone tissue depends as much on the persistent, low-magnitude strains that arise through postural muscle activity throughout the day as on the relatively large, rarely occurring strain events induced by vigorous activity. Therefore, the bone wasting that occurs in space may arise not only from the diminished load bearing responsibility inherent to microgravity, but the sarcopenia that parallels it (26). FJ

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