AN ABSTRACT OF THE THESIS OF

Jacquelyn Camille Herron for the degree of Master's of Science in Exercise and Sport Science presented on July 26, 2007.

Title: The Effects of Whole Body Vibration on Bone Recovery Following Hindlimb Unloading of Adult Female Rats.

Abstract approved:

Gianni F. Maddalozzo

Introduction: A bone deficit is found to persist five years following a hip fracture and 10 years post tibial fracture in adults. We examined the effect of whole body vibration (Vib) as a means for enhancing whole body composition and bone recovery following hindlimb unloading (HU) of adult female rats.

Methods: Seven-month-old female Fischer 344 rats (two control and three experimental groups, n= 10 per group) were used in the study. The experimental groups were HU for 15 days, followed by a return to either weight bearing (HU + WB) or 10 min./day of whole body vibration (HU + Vib) for nine days. Body weight, food weight, whole body composition, and dynamic and static histomorphometric analyses of the proximal tibia metaphysis were measured. A t-test was used for comparisons during HU, while ANOVA followed by a post-hoc test was used with the recovery groups.

Results: Unloading resulted in lower body weight, fat mass, total bone area, total bone mineral content (BMC), and suppressed bone formation parameters in cancellous bone of the tibia. Following nine days of recovery, the HU + WB and HU + Vib groups had lower body weight and fat mass compared to the control group. The HU + Vib group had lower total bone area, while the HU + WB group

had a trend ($p < 0.10$) for lower total bone area compared to the control group. While there were no differences between recovery groups for BMC, the HU + Vib rats did have suppressed tibial Oc.Pm/B.Pm compared to the control group.

Conclusion: Whole body vibration may enhance bone recovery by suppressing bone resorption.

The Effects of Whole Body Vibration on Bone Recovery Following Hindlimb Unloading of Adult Female Rats

by
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APPROVED:

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Jacquelyn Camille Herron, Author

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TABLE OF CONTENTS

TABLE OF CONTENT (Continued)

LIST OF TABLES

DEDICATION

This is dedicated to my parents, who have always supported me in everything I do, instilled the value of education, and provided me with the resources to be successful.

The Effects of Whole Body Vibration on Bone Recovery Following Hindlimb Unloading of Adult Female Rats

INTRODUCTION

In the United States, 43.6 million people over the age of 50 have or are at risk for osteoporosis or low bone mass (~osteopenia). This figure is expected to rise to 52.4 million by 2010 and 61.4 million by 2020 due to our aging population (1). Osteoporosis contributes to 1.5 million fractures per year (2) at an annual direct medical care expenditure of $17.5 billion, in 2002 dollars (3). It is expected that 50% of women and 12.5% of men age 50 and older will suffer an osteoporotic fracture in their remaining lifetime (1).

Osteoporotic hip fractures are the most serious and costly of all fractures, given the 10-20% mortality rate within the first six months (2) and accounting for 63.1% of the total health care expenditure (~$37,000 per hip fracture, in 2002 dollars) (3). The average hospital stay with an osteoporotic fracture is 9.6 days (3). Traditional treatment methods, including bed rest (4), limb immobilization (5), localized stress shielding following arthroplasty (6), and restricted activity (7) further induce site-dependent bone loss. With a return to weight bearing, recovery of bone does not occur at the same rate as it is lost (8-15), and a regional bone deficit has been found to persist five years following a hip fracture (16) and 10 years post tibial fracture in adults of all ages (5,17,18). The resulting lower bone mineral density (BMD) increases the risk of a secondary fracture in the same

limb (19), with a 5% persistent BMD deficit increasing the risk of a fracture by 60% (20).

Hip fractures are highly debilitating, with 50% of sufferers unable to walk unassisted six months post surgery and an additional 25% requiring long-term care (2). Overall, quality of life is diminished due to limited mobility, fear of an additional fracture, depression, anxiety, and possible deformity and pain with vertebral fracture-induced kyphosis (stooped posture) (1). Finding feasible, therapeutic methods to promote a faster recovery of skeletal health is of utmost importance so individuals can walk unassisted, perform activities of daily living, improve health and quality of life, and attenuate the risks and costs associated with an osteoporotic fracture.

Weight bearing (WB) is essential for normal bone formation and turnover, as well as for maintaining bone mass (21). Recently, whole body vibration (Vib) training has been promoted as a mechanical stimulus that efficiently (10 minutes/day) and safely enhances bone mass in individuals with established low bone mass and at a high risk for a fracture (22,23). Vib has been shown to improve trabecular bone formation and architecture, bone volume, BMD, bone mineral content (BMC), bone strength, and the formation and mineralization rates in both humans and animals (24-28). While Vib has successfully prevented disuse osteoporosis in hindlimb unloaded adult rats (29,30), Vib has yet to be applied with reambulation to improve bone recovery following unloading. Given the previous findings, Vib may provide an ideal stimulus to improve bone recovery and allow individuals to lead independent, productive lives again.

Considering that 54% of 50-year-old women will experience an osteoporotic fracture during their remaining lifetime (31), there is a clear need to not only define the cellular mechanisms of recovery in the female skeleton, but also to determine whether mechanical stimuli can potentiate the recovery of bone mass by enhancing bone formation, architecture, and suppressing resorption. The purpose of this study was to determine the effects of Vib on adult female rat cancellous bone formation, architecture, cellular activity, and whole body composition during nine days of recovery following hindlimb unloading (HU). Specifically, we hypothesized that Vib would enhance the cancellous bone formation rate, architecture, osteoblast activity, BMC and BMD, and lower osteoclast activity and body fat as compared to weight bearing.

LITERATURE REVIEW

Bone loss due to unloading

The mechanical stimulus provided by WB is vital to the development and maintenance of robust, weight-bearing bone (32) able to resist fracture (33). Conversely, a reduction in WB of the lower extremities results in varying decremental losses of BMD, BMC, and bone strength (4,34-36). Unloading-induced osteoporosis is seen with limb immobilization (18), spaceflight (37), spinal cord injuries (38), and bed rest in humans (4) and HU of rodents (39,40). The rate of bone loss is dependent on the degree of normal mechanical stress and strain, with the normal weight bearing lower extremities undergoing a greater bone loss as compared to the non-weight bearing upper extremities (37). In addition, bones with higher peak bone mass undergo a greater loss than those with lower bone mass following a period of unloading (41,42). Also, bone loss is site-specific (40) and depends on the model employed (i.e. space flight vs. immobilization vs. hindlimb unloading) (36,41,43).

Metabolically more active cancellous bone undergoes the greatest initial loss (39,40), primarily due to decreased osteoblast number and suppressed bone formation (44,45), although increased osteoclastic activity and bone resorption may play a minor role (46). Cortical bone loss occurs later than cancellous bone, with cancellous bone loss being more pronounced after six months of space flight with human cosmonauts (37). Furthermore, periosteal, endocortical, and cancellous bone formation may be blocked or delayed with disuse, without a change in resorption, and is a primary cause for decreased cortical and cancellous

bone rodent models (44,45,47,48). This is mediated, in part, through resistance to insulin-like growth factor I (IGF-I) (49), a growth factor implicated in the regulation of bone formation and proliferation of osteoblasts (50,51). With endocortical bone formation suppressed and resorption unchanged, the ultimate result is an enlarged marrow cavity and osteopenia (52). Collectively, the incurred bone loss results in a decrease in mechanical bone strength (9,53-55), likely due to architectural changes including a decreased cross-sectional area of cortical bone, a decrease in trabecular thickness, number, connectivity, and the development of more rod-like trabeculi (39,56).

Implications with unloading-induced bone loss

Disuse osteoporosis increases the risk of a fracture. Indeed, spinal cord injury (SCI) patients who are non-ambulatory or have limited ambulation are two times more likely to suffer a fragility fracture (due to minor or no trauma) as compared to controls (57), with 34% of a SCI population having suffered a fracture after 21.1 ± 12.1 years (57,58). Furthermore, in skeletally mature adults of both genders, bone loss incurred by immobilization of a fracture is found to persist for five years in the hip (16) and 10 years in the tibia in adults of all ages (5,17,18). The lower bone mass increases the risk of a secondary fracture in the same limb (19), with a 5% persistent bone deficit increasing the risk of a fracture by 60% (20). Likewise, half of an astronaut's BMD at specific skeletal sites could be lost with an expected 2.5 year round trip to Mars, jeopardizing his/her health and well-being (29,59), and risking fracture (60).

Recovery of bone mass following unloading

Although the response to unloading has been studied extensively in both humans and animals, few studies have examined the effects of reloading. Theoretically upon reambulation, bone status should be restored (61). For example, with the re-establishment of normal weight bearing in both cosmonauts and HU rats, elevated mRNA expression of specific, bone-formation genes including TGF-β, type-1 collagen, and osteocalcin (62,63) leads to an increased bone formation rate that allows for the recovery of bone mass (64-66). These changes in gene expression and bone formation are likely the result of increased internal strain from the resumption of normal weight bearing, fluid flow sheer stress, and changing electrical potentials being sensed by mechanically-sensitive osteocytes (67-69). Osteocytes produce nitric oxide and prostaglandin E2, which regulate bone metabolism (67,70) and have direct communication with other osteocytes, osteoblasts, and bone lining cells (71,72).

While the bone formation rate increases with reloading, it may be below control values. For example, 14 days of reloading with space flight and HU rats resulted in lower osteoblast number as compared to the controls, while data on bone volume has provided mixed results (73,74), possibly due to subtle cellular differences, age and strain of the animals, weight gain/loss, food consumption by each group of rats, and/or the bone site examined (73). It is suggested that complete recovery of bone mass and strength may require more time, than the length of the unloading period, to be restored to control values (8-11). For instance, it has been extrapolated that 12 weeks of space flight may require five

years or more to restore calcium balance and BMC to normal (12-14). In addition, when rats were reloaded following 2-weeks of HU, extrapolation of a calcium linear regression curve indicated it would require 6-8 weeks to restore bone calcium to normal (15).

Mechanical loading to improve recovery

Greater than normal activity may be necessary to achieve full bone recovery (75). Weight bearing exercise acts as a greater than normal stimulus to bone by creating internal strains (24,33), fluid flow shear stress (67), increasing electric potentials (68), increasing IGF-I gene expression (51), and stimulating osteoblast formation and increased bone mass (24,33,75). As such, bone is formed along the lines of principal stress (76), thus enhancing the microarchitecture and quality of bone through increased trabeculi thickness, number, and connectivity (77) and enhanced bone strength (33). However, translating the bone strengthening potential of exercise to humans with osteoporosis is a relatively ineffective treatment (78), resulting in modest BMD gains of 1-2% per year (79). However, the gains in bone mass may be dependent on initial bone mass (42,78) and the type of exercise performed (75,80,81). Accordingly, those with the lowest initial bone mass have the most to gain from exercise (42,78).

High impact exercise, such as jumping, elicits a high strain magnitude and rate, which has generally been considered best for building bone (81,82). While jumping enhanced hip and spine bone mass in pre-pubescent children (83) this form of exercise may be difficult for the frail and elderly (81) and may increase the risk of developing osteoarthritis (84-86). Furthermore, resistance training,

which induces a high strain magnitude (87) and localized, circumferential strain gradients due to pressure differentials across a volume of tissue (88,89), has been effective for attenuating disuse bone atrophy in both humans and animals (90,91). On the other hand, possible limitations of performing resistance training include fatigue and limited physical ability (92,93). Given the limitations of high impact and resistance exercise, it is worthwhile to search for osteogenic exercise regimens that are effective, efficient, and easier to perform. One possible mechanism is by increasing the strain frequency (29,81).

Whole body vibration as a form of mechanical loading

It is commonly believed that high strain magnitudes and rates produced by vigorous activity have the greatest influence on bone adaptation (94-96). However, when examining the daily strain history in dog, sheep, and turkey models, non-uniform, large magnitude strains (>1000 microstrain (με)) (~walking, running) are infrequent, while low magnitude strains (<10 με) (~standing, sitting) occur thousands of times a day and are more spatially distributed across the bone cross section. The result is normal, well-adapted bones capable of supporting the animal and resisting failure (97). Furthermore, these small magnitude strains may be amplified by the lacunae walls, which stimulate stretch-activated ion channels and fluid flow shear stress of the mechanically sensitive osteocytes (69,98). Overall, the continual barrage of low magnitude but high frequency strains may be just as critical, if not more important as recently found (99), for signaling bone adaptation as the non-uniform, infrequent, high magnitude strains.

In the context of a recovery period, following three weeks of immobilization of young male rats, Kannus et al. (1996) found that normal weight bearing was not effective in restoring BMD and BMC to comparable levels of controls after an 11 week recovery period. On the other hand, both low and high intensity treadmill running were effective in restoring BMD and BMC. Subsequent deconditioning (normal weight bearing for 18 weeks) resulted in a loss of the beneficial effects. Not only does this suggest the need for greater than normal weight bearing activity during recovery of bone mass, but the activity must be continued, perhaps indefinitely, to maintain bone mass.

The low magnitude, high frequency strain produced by whole body vibration (Vib) improves bone mass in both humans and animals (29,30,100-105) via muscle (106-108) and acceleration-induced microstrains on bone (109,110). Vib enhances trabecular bone formation and architecture, bone volume, BMD, BMC, bone strength, and the formation and mineralization rates (24-28). At the same amplitude and duration, 90 Hz at a lower microstrain (0.74 με) was found to be more anabolic than 45 Hz at a higher microstrain (2.12 με), suggesting enhanced bone sensitivity at a higher frequency and lower strain magnitude (99). Furthermore, there appears to be no saturation effect with extremely low-level strains (111), which is seen with low frequency, high magnitude strains (112). Up to 90 Hz at 0.25g (peak-to-peak accelerations of 2.4525 m/s^2) applied for a 10 min./day prevented disuse bone loss in hindlimb suspended adult rats, while 10 min./day of normal weight bearing did not. To put it in perspective, 90 Hz delivers more frequent cycles of low magnitude strain during the 10 min. period

as compared to normal weight bearing alone, resulting in modifications to increase bone formation (29).

Most recently, short bouts of Vib increased the bone mass of young women with low BMD and a previous history of one fracture (22), which may be due to those with lower bone mass having enhanced sensitivity to anabolic signals (42). Moreover, Vib was recently useful for improving functional parameters (~muscle performance, balance/postural control, mobility) in those with limited physical ability (113-115) and for improving total volumetric tibial BMD in ambulant, disabled children (23), illustrating the low-risk potential of Vib training.

While Vib training has successfully prevented disuse osteoporosis in hindlimb suspended adult rats (29,30), to our knowledge Vib has yet to be applied with reambulation. Given the difficulty in recovering disuse bone loss in adults (16), the increased risk of osteoporotic fracture (20), and the increased risk of osteoarthritis associated with high impact and resistance exercise (84-86), relatively low-risk Vib may be best suited to increase bone mass in those with limited physical ability and/or possessing lower bone mass, such as following disuse.

MATERIALS AND METHODS

Experimental design

Seven-month-old female Fischer 344 rats (Harlan Laboratories, Indianapolis, IN) were used in the study. The animals were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Institutional Animal Care and Use Committee at Oregon State University (Corvallis, OR).

The animals were randomized by weight into five study groups (n = 10, per group). The study groups consisted of two weight bearing controls (Control 1 and Control 2) and three experimental groups (hindlimb unloaded (HU); HU + weight bearing (HU + WB); and HU + vibration (HU + Vib)). All three experimental groups were HU for 15 days. The HU group was sacrificed after 15 days, while the HU + WB and HU + Vib groups underwent a nine day recovery. Control 1 was sacrificed after the 15 day HU period, while Control 2 was sacrificed at the end of the recovery period.

The colony room was maintained on a 14:10 hour light-dark cycle, with the temperature maintained at 21–23°C. The control animals were housed individually in translucent durable polycarbonate cages (43 x 27 x 19 cm.) with bedding and an enclosed filter on the top cage and able to access food, water, and all areas of the cage. The HU animals were housed individually in metabolic cages (12 x 12.5 x 12 in.) and able to move on an x-y axis and rotate 360^{o} on their front paws to access food, water, and all areas of the cage (116). The animals were provided with water and a pellet formed commercial diet (Tekland rodent

chow, no. 8604 Harlan Tekland, Madison, WI, USA). The HU groups consumed food and water ad libitum. The control groups were pair-fed daily the mean amount of food consumed by the HU groups from the previous day (116). All rats were weighed prior to the HU protocol and prior to sacrifice. The attending veterinarian inspected the animals once a day for overall health, while the investigator inspected the HU animals twice a day (1-2.5 hours/day) and monitored their health by evaluating food and water consumption; activity level; shedding; porphyrin secretion around the eyes and nose; tail color as a measure of adequate blood flow; and, monitoring the tail for skin irritation. Each HU rodent was manually cleaned and dried once a day.

Hindlimb unloading model

The experimental groups were HU according to the Morey-Holton protocol (116). Fifteen days of HU was chosen because this is considered the earliest reliable time for detecting losses of cancellous bone, as seen in the tibia (15,39,117-120), femur (73,77), and vertebrae (15).

Recovery experimental model

The Control 2, HU + WB, and HU + Vib groups were used for the nine day recovery experiment. Nine days of recovery was chosen because a previous pilot study by a co-author (unpublished data) indicated muscle mass was approximately 50% recovered with nine days of weight bearing following 15 days of HU. Furthermore, recovery of muscle is important for inducing microstrains of bone and potentiating the effects of vibration (106-108). The HU + WB group underwent a recovery period consisting of 24 hrs./day of normal weight bearing

only, while the HU + Vib group underwent a recovery period consisting of 23 hrs. 50 min. /day of normal weight bearing plus 10 min./day of whole body vibration.

During the vibration protocol, the HU + WB and HU + Vib groups were carefully transported in their cages via a rolling cart to the room housing the Pneu-Vibe Pro™ (Pneumex, Sandpoint, ID) vibration platform. Each animal in the HU + Vib group was placed inside individual 13 x 28 x 15 cm compartments attached to the vibration platform. The rats were vibrated for 10 min./day at 90 Hz and .25 g (peak-to-peak accelerations of 2.4525 m/s^2). 10 min./day at 90 Hz and .25 g was chosen because this protocol was found to be effective for preventing bone loss in adult female rats (29) and for improving the bone mass in ambulant, disabled children (23). HU + WB animals remained in their cages during the vibration protocol, approximately 3 ft. from the vibration platform to expose the animals to the sound of the vibrating platform.

Fluorochrome labeling

Fluorochrome labels were used to determine actively mineralizing cancellous bone surfaces. The Control 1 and HU groups were injected subcutaneously with tetracycline HCl (15 mg/kg, Sigma Chemical Co., St. Louis, MO) at 15 days and with calcein (15 mg/kg, Sigma Chemical Co., St. Louis, MO) at nine days and two days prior to necropsy. The Control 2, HU + WB, and HU + Vib groups were injected subcutaneously with calcein at nine days, with tetracycline HCl at four days, and with calcein at one day prior to sacrifice. Calcein-to-calcein labels (seven days apart in the HU groups and eight days apart in the recovery groups, respectively) were used for dynamic histomorphometry analyses.

Whole body composition

Indirect measures of whole body composition, including total bone area, bone mineral content (BMC), bone mineral density (BMD), fat mass, percent body fat, and lean mass were determined by dual energy X-ray absorptiometry (DXA) small animal software (Model 4500-A, Hologic Inc., Waltham, MA) on the day of the necropsy. Least significant change for whole body BMD in our lab is 0.003 g/cm^2 at 95% confidence level. Prior to scanning, animals were lightly anesthetized with pentobarbital via the inter-peritoneal cavity (10mg/kg body mass).

Necropsy procedures

Following the whole body scan, each rat was deeply anesthetized with pentobarbital and euthanized by incision of the pneumothorax. The femora, tibiae, and L1-4 vertebrae were excised, cleaned of soft tissue, and stored as previously described (121). The proximal anterior crest of the right tibia was faced off with a razor blade to expose the bone marrow and provide a flat surface for sectioning. The right tibia was refrigerated at 4^oC in 70% ethanol for bone histological processing.

Bone histomorphometry

The proximal right tibia was dehydrated in graded ethanols and xylene and embedded undecalcified in modified methyl methacrylate (122). The bone samples were sectioned longitudinally with a Leica/Jung 2065 microtome at a thickness of 4 μm. The sections were affixed to slides pre-coated with 1% gelatin, covered with a thin plastic film, clamped, and placed in a 40-50^oC oven overnight

to dry. One section for the HU groups and two sections for the recovery groups were left unstained and used for assessing fluorochrome-based data under UV illumination (121). Another section was stained according to the Von Kossa method with a tetrachrome counterstain (Polysciences, Warrington, PA) and used for determining bone area and cellular endpoints.

Histomorphometric measurements were performed in cancellous bone tissue of the proximal tibia in a sample area beginning 0.5 mm distal to the growth plate to include secondary spongiosa only. Bone measurements were collected using Osteomeasure Analysis system (OsteoMetrics, Inc., Atlanta, GA) and reported in accordance with standard bone histomorphometry nomenclature (123). Fluorochrome-based indices of bone formation, including the mineralizing perimeter (percentage of cancellous bone perimeter with a double fluorochrome label (M.Pm)), the mineral apposition rate (distance between the fluorochrome labels in μm/time (MAR)), and the bone formation rate (mineralizing surface x mineral apposition rate (BFR)) (124) were measured at a magnification of X100. Additionally, microarchitectural endpoints, including trabecular thickness (Tb.Th), number (Tb.N), and spacing (Tb.Sp) (125) were calculated from measures of bone perimeter (B.Pm) and area (B.Ar). Osteoclast (Oc.Pm), osteoblast (Ob.Pm), and osteoid (O.Pm) perimeters, expressed as percentages of total cancellous bone perimeter, were measured in the stained sections at a magnification of X200. All measurements were performed blindly.

Statistical analyses

Data analysis was performed with the statistical software package SPSS for Windows (SPSS, Version 14.0; SPSS Inc., Chicago, IL). Levene's test of equality of error variance was first used to determine equality of variance. The Control 1 and HU groups were evaluated with an independent sample t-test, while analysis of variance (ANOVA) was used to evaluate an overall difference between the Control 2, HU + WB, and HU + Vib groups. If the ANOVA indicated significance between groups, a Tukey-Kramer post-hoc multiple comparison test was utilized to evaluate specific contrasts of interest. If Levene's indicated significant variance between the recovery groups, a Kruskal-Wallis nonparametric test was performed on the parameter of interest, followed by Tamhane's T2 post-hoc test. Alpha < 0.05 was used to determine statistical significance. Data are expressed as mean ± SE.

RESULTS

Hindlimb unloading

Whole body weight and food consumption data during HU are presented in Table 1. One HU animal was excluded from data analysis due to an underlying pathology that affected bone mass. Following 15 days of HU, whole body weight of the HU rats was 14.3% lower than the age-matched controls. However, the HU rat's consumed +11.6% more food than the control rats.

HU resulted in lower total bone area (-9.5%), BMC (-5.3%), fat mass (-71.7%), and percent body fat (-66.9%) compared to the age-matched control group. There was a trend ($p = 0.084$) for 3.7% higher BMD in the HU group than the control group. Differences were not detected for lean mass (Table 2).

Unloading resulted in a lower tibial cancellous bone M.Pm/B.Pm (-73.4%), MAR (-20.0%), and BFR per bone perimeter (-79.3%), bone area (-77.7%), and tissue area (-78.2%) referents. Differences were not detected for B.Ar/T.Ar, Tb.N, Tb.Th, and Tb.Sp. HU resulted in lower Ob.Pm/B.Pm (-47.5%) and lower O.Pm/B.Pm (-41.8%). However, a difference was not detected for Oc.Pm/B.Pm between the HU and age-matched control groups (Tables 3 and 4).

Recovery

Recovery data for whole body weight and food consumption is presented in Table 1. Following HU and nine days of recovery, the HU + WB and HU + Vib groups weighed less (-9.3% and -9.6%) than the age-matched control group. The HU + WB and HU + Vib groups consumed more food than the age-matched control group during both HU (+18.6% and +26.3%) and recovery (+29.7% and

+19.5%), respectively. From an observational perspective, the HU + Vib rats showed no visible signs of stress during vibration.

The HU + Vib group had lower total bone area (-6.8%), while the HU + WB group had a trend for lower total bone area (-4.7%) compared to the control group. While no differences were detected for BMC, the HU + Vib group had higher total BMD (+4.4%) compared to the HU + WB group. Fat mass and percent body fat were lower by 56.5% and 60.1% and 51.4% and 56.4%, respectively, in the HU + WB and HU + Vib groups compared to the control group. Differences were not detected for lean mass (Table 2).

No differences were detected between any of the recovery groups for tibial cancellous bone MAR; BFR per bone perimeter, bone area, and tissue area referents; Tb.N; Tb.Th; and, Tb.Sp. There were trends for differences between groups for M.Pm/B.Pm ($p = 0.058$), B.Ar ($p = 0.097$), and B.Ar/T.Ar ($p = 0.097$). The HU + Vib group had lower Oc.Pm/B.Pm (-48.5%) compared to the age-matched control group. Differences were not detected for Ob.Pm/B.Pm and O.Pm/B.Pm among any of the recovery groups (Tables 3 and 4).

Table 1. Effects of hindlimb unloading and recovery on whole body and food weight in 7-month old Fischer 344 female rats.

		Hindlimb Unloading				Recovery			
	Parameter	**Control 1** (n= 10)	**HU** (n= 9)	**t-test P ≤**		**Control 2** (n= 10)	**HU + WB** (n= 10)	**HU + Vib** (n= 10)	**ANOVA P ≤**
Whole body weight and food weight									
	Baseline Body Wt (g)	208.6 ± 3.8	208.3 ± 3.1	0.960		207.2 ± 3.9	210.5 ± 2.1	208.4 ± 3.1	0.748
	Necropsy Body Wt (g)	214.8 ± 3.3	184.0 ± 4.0	0.000		220.7 ± 4.3	200.2 ± 2.9[a]	199.6 ± 2.3[a]	0.000
	Food Wt/d during HU (g)	13.0 ± 0.3	14.7 ± 0.7	0.038		11.8 ± 0.3	14.0 ± 0.5[a]	14.9 ± 0.7[a]	0.002
	Food Wt/d during recovery (g)					12.8 ± 0.7	16.6 ± 0.5[a]	15.3 ± 0.3[a]	0.000
	HU= hindlimb unloading; HU + WB= HU + weight bearing; HU + Vib= HU + vibration								
	* Data are mean ± SE								
	[a] Sig. different from Control, P < 0.05								

Table 2. Effects of hindlimb unloading and recovery on whole body composition in 7-month old Fischer 344 female rats.

	Hindlimb Unloading			Recovery			
Parameter	**Control 1** (n= 10)	**HU** (n= 9)	**t-test P ≤**	**Control 2** (n= 10)	**HU + WB** (n= 10)	**HU + Vib** (n= 10)	**ANOVA P ≤**
Whole body composition							
total bone area (cm^2)	46.3 ± 0.5	41.9 ± 0.6	0.000	47.1 ± 0.8	44.9 ± 0.3[a*]	43.9 ± 0.6[a]	0.015
BMC (g)	7.5 ± 0.10	7.1 ± 0.07	0.003	7.6 ± 0.17	7.2 ± 0.08	7.3 ± 0.08	0.196
BMD (g/cm^2)	0.163 ± 0.002	0.169 ± 0.003	0.084	0.162 ± 0.002	0.160 ± 0.001	0.167 ± 0.002[b]	0.030
Fat mass (g)	34.6 ± 2.2	9.8 ± 1.2	0.000	40.9 ± 3.1	17.8 ± 1.4[a]	16.3 ± 0.9[a]	0.000
Percent body fat	15.7 ± 0.9	5.2 ± 0.6	0.000	17.9 ± 1.2	8.7 ± 0.7[a]	7.8 ± 0.4[a]	0.000
Lean mass (g)	177.9 ± 3.2	170.2 ± 4.0	0.144	178.9 ± 2.8	180.3 ± 2.9	183.3 ± 2.8	0.550

HU= hindlimb unloading; HU + WB= HU + weight bearing; HU + Vib= HU + vibration

BMC= Bone mineral content; BMD= Bone mineral content

* Data are mean ± SE

[a] Sig. different from Control, $P < 0.05$; [a*] $P < 0.1$

[b] Sig. different from HU + WB, $P < 0.05$

Table 3. Effects of hindlimb unloading and recovery on proximal tibia histomorphometry in 7-month old Fischer 344 female rats.

	Hindlimb Unloading			Recovery			
Parameter	**Control 1** (n= 10)	**HU** (n= 9)	**t-test P ≤**	**Control 2** (n= 10)	**HU + WB** (n= 10)	**HU + Vib** (n= 10)	**ANOVA P ≤**
Proximal tibia (uv sections)							
M.Pm/B.Pm (%)	10.9 ± 2.7	2.9 ± 1.0	0.017	15.9 ± 2.6	6.0 ± 2.0	11.2 ± 3.5	0.058
MAR (μm/day)	1.0 ± 0.1	0.8 ± 0.1	0.031	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.798
BFR/B.Pm (μm^2/μm/year)	45.3 ± 14.2	9.4 ± 3.0	0.032	64.5 ± 12.7	25.5 ± 9.5	50.8 ± 18.3	0.156
BFR/B.Ar (%/year)	115.1 ± 35.4	25.7 ± 8.7	0.032	153.6 ± 24.9	66.0 ± 23.8	108.9 ± 35.1	0.112
BFR/T.Ar (%/year)	34.8 ± 11.5	7.6 ± 2.5	0.043	46.6 ± 10.2	18.4 ± 7.3	40.7 ± 15.2	0.200

HU= hindlimb unloading; HU + WB = HU + weight bearing; HU + Vib= HU + vibration

M.Pm/B.Pm= mineralizing perimeter/bone perimeter; MAR= mineral apposition rate; BFR/B.Pm= bone formation rate/bone perimeter; BFR/B.Ar= bone formation rate/bone area; BFR/T.Ar= bone formation rate/tissue area

* Data are mean ± SE

[a] Sig. different from Control, $P < 0.05$

Table 4. Effects of hindlimb unloading and recovery on the proximal tibia architecture and cellular parameters in 7-month old Fischer 344 female rats.

	Hindlimb Unloading			Recovery			
Parameter	**Control 1** (n= 10)	**HU** (n= 9)	**t-test P ≤**	**Control 2** (n= 10)	**HU + WB** (n= 10)	**HU + Vib** (n= 10)	**ANOVA P ≤**
Proximal tibia (stained sections)							
T.Ar (mm^2)	3.23 ± 0.01	3.24 ± 0.00	0.343	3.24 ± 0.00	3.24 ± 0.00	3.24 ± 0.00	1.000
B.Ar (mm^2)	0.87 ± 0.05	0.88 ± 0.05	0.915	0.87 ± 0.08	0.77 ± 0.06	1.00 ± 0.07	0.097
B.Ar/T.Ar (%)	27.1 ± 1.6	27.2 ± 1.4	0.968	26.8 ±2.5	23.7 ± 1.9	30.7 ± 2.2	0.097
Tb.N (1/mm)	3.7 ± 0.1	3.8 ± 0.1	0.642	3.5 ± 0.1	3.5 ± 0.2	3.8 ± 0.1	0.339
Tb.Th (µm)	72.2 ± 2.1	70.9 ± 1.8	0.67	75.4 ± 4.4	66.5 ± 3.0	81.7 ± 5.2	0.123
Tb.Sp (µm)	200.0 ± 11.9	193.6 ± 9.4	0.684	216.1 ± 17.0	223.0 ± 15.3	186.7 ± 9.8	0.184
Oc.Pm/B.Pm (%)	4.8 ± 1.3	2.7 ± 0.7	0.212	6.8 ± 1.0	5.0 ± 1.8	3.5 ± 0.6[a]	0.038
Ob.Pm/B.Pm (%)	26.4 ± 3.2	13.9 ± 2.0	0.005	21.0 ± 3.7	18.0 ± 3.3	18.2 ± 3.0	0.776
O.Pm/B.Pm (%)	43.5 ± 4.5	25.3 ± 3.3	0.005	34.6 ± 5.7	31.9 ± 4.6	34.4 ± 4.6	0.918

HU= hindlimb unloading; HU + WB= HU + weight bearing; HU + Vib= HU + vibration

thickness; Tb.Sp= trabecular spacing; Oc.Pm./B.Pm= osteoclast perimeter/bone perimeter; Ob.Pm/B.Pm= osteoblast perimeter/bone perimeter; O.Pm/B.Pm= osteoid perimeter/bone perimeter

* Data are mean ± SE

[a] Sig. different from Control, $P < 0.05$

DISCUSSION

Hindlimb unloading of adult female rats resulted in lower whole body weight, fat mass, bone mass, and suppressed cancellous bone formation. Our findings suggest that Vib may enhance bone recovery by primarily suppressing bone resorption.

To our knowledge, this is the first study to apply high frequency (90 Hz), low magnitude (0.25g =2.4525 m/s^2 peak-to-peak accelerations) whole body vibration during a nine day recovery interval post-HU as a means to speed recovery. While there were no differences between the recovery groups for BMC or total bone area, the vibration group had higher total BMD as compared to the weight bearing group. Although the BMD results correlate with previous studies in disabled children (23) and young women with low bone mass (22), they must be interpreted with extreme caution, considering BMC, the true measure of bone mass, was not higher in the vibration group relative to the weight bearing group.

While no differences were observed in total bone area between the weight bearing and vibration groups, neither group achieved full recovery of this parameter as compared to the control group. Thus, more time may be needed to achieve full recovery of total bone area. Additionally, further investigation may be warranted, as vibration may have a significant impact on periosteal bone (24,89) and/or specific skeletal sites (27) that were not measured in this study.

Hindlimb unloading

Fifteen days of HU resulted in the following changes in whole body composition: lower body weight (-14.3%), fat mass (-71.7%) and percent body fat (-66.9%). The decreased body weight may be due to elevated stress levels (128,129); the diuretic and natriuretic effect of the hindlimb unloading-induced cephalad fluid shift (130); reduced energy absorption; and/or increased thermogenic activity of brown adipose tissue uncoupling proteins (131). The hindlimb unloaded rats appeared notably stressed during the first week (~lethargic, decreased food/water consumption). However, they quickly adjusted, and their activity levels appeared to be similar to the control groups during the second week. Overall, the significant loss in body mass may have negatively impacted bone mass, independent of the effects of loading status.

Despite pair-feeding to control for caloric intake, the HU rats consumed on average 11.6% more grams of food than the controls, which correlates with some studies (47,126) but contrasts with others (46). HU may have reduced estrogen levels, as previously found in six-month old female rats (47), which may have provided a stimulus for increased food consumption (127). However, it is possible we over-estimated food consumption for the HU rats, considering we did not weigh and account for any of the food that fell through the grid into the fecal collection tube.

The lower whole body BMC in the unloaded group is comparable to the results of other animal studies of similar duration (39,77,134). The trend for

higher total BMD in the HU group may be the result of lower total bone area (-9.5%), while BMC was lower by 5.3%. Given the significant differences in total bone area relative to BMC, BMD may not be a useful measure of bone mass when comparing the HU and control animals.

No changes in proximal tibia trabecular architecture were observed, which compares to a previous study utilizing the same animal model (46). However, other studies have found conflicting results for changes in architecture, which may be due to variations in study duration, techniques of analysis, gender, strain, hormonal status, and age of the animals (39,46,47,77).

Hindlimb unloading resulted in lower BFR, MAR, and M.Pm/B.Pm. During hindlimb unloading, cancellous bone formation may be blocked or delayed through resistance to insulin-like growth factor I (IGF-1) and inhibition of the IGF-1 signaling pathway (49) resulting in decreased proliferation of osteoblasts and osteoprogenitors (135,136). In addition, lower bone formation may be due to lower serum estradiol during hindlimb unloading (47).

Recovery

Weight bearing

Following a period of unloading, weight bearing alone may not be enough to fully recover bone mass and cancellous architecture (8-11), especially in skeletally mature adults where a bone deficit is found to persist five years following a hip fracture (16) and 10 years post tibial fracture (5,17,18). The

results of our study may confirm prior findings (8-11), as the HU + WB group was unable to achieve full recovery of body weight, fat mass, and total bone area.

While the HU + WB rats had improvements in body weight and fat mass, a deficit persisted compared to the age-matched controls, despite greater food consumption. Nine days of recovery may not have been enough time to achieve full recovery of these parameters, as 28 days was previously found to be sufficient for recovering body weight in six-month-old male rats (10).

While the WB group achieved full recovery of BMC, lower total BMD than the HU + Vib group and a trend for lower total bone area than the control group is similar to other studies in young rats following 28 days (77) and seven weeks (134) of reloading.

Vibration

Recent studies indicate that low magnitude, high frequency strains are anabolic towards bone (22,24,29,81,104) by inducing fluid flow shear stress which stimulates stretch-activated ion channels of mechanically-sensitive osteocytes (69,98,137). Mechanical stress is important for signaling bone adaptations to enhance microarchitecture (27,99) and consequently bone strength to resist fracture (27,138). A prior recovery study in young male rats found that 14 days of suspension followed by 28 days of recovery resulted in higher tibial B.Ar/T.Ar, trabecular thickness, and a trend for higher trabecular number in the treadmill group compared to the normal weight bearing group (77).

In our study, the vibration group was not different from the control group for bone formation parameters. The vibrated group had significantly suppressed Oc.Pm/B.Pm compared to the control group, which may mediate acute bone recovery. This finding is consistent with prior recovery studies involving 14-28 days of HU followed by normal loading and/or treadmill running (73,77) and also with 3-weeks of whole body vibration in female mice (111). Suppressed resorption may be mediated by strain-derived canalicular fluid flow shear stress (139), which up-regulates nitric oxide (NO) synthase production (28), stimulates NO release by osteocytes and osteoblasts (140,141), inhibits RANKL, stimulates OPG expression, and down-regulates osteoclastogenesis (142). Given our findings, it is quite plausible early bone recovery is initiated through osteoclast inhibition, and vibration may enhance this mechanism (28,137).

CONCLUSION

In summary, weight bearing during nine days of recovery was not sufficient to fully recover whole body composition and total bone area lost from hindlimb unloading of adult female rats. Indeed, the HU + WB rats had lower body weight, fat mass, and a trend for lower total bone area compared to the control group. The addition of Vib did not enhance body weight, fat mass, or total bone area to normal levels. However, the HU + Vib rats did have suppressed tibial Oc.Pm/B.Pm compared to the control group, suggesting that inhibition of resorption by Vib may play a role for enhancing acute bone recovery.

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