Bone 56 (2013) 461–473

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Previous exposure to simulated microgravity does not exacerbate bone loss during subsequent exposure in the proximal tibia of adult rats

Yasaman Shirazi-Fard [a,*], Rachel A. Anthony [a], Andrea T. Kwaczala [b], Stefan Judex [b], Susan A. Bloomfield [c], Harry A. Hogan [d,a]

[a] Department of Biomedical Engineering, Texas A&M University, College Station, TX 77843, USA
[b] Department of Biomedical Engineering, State University, Stony Brook, NY 11794, USA
[c] Department of Health & Kinesiology, Texas A&M University, College Station, TX 77843, USA
[d] Department of Mechanical Engineering, Texas A&M University, College Station, TX 77843, USA

ARTICLE INFO

Article history:
Received 29 March 2013
Revised 16 June 2013
Accepted 4 July 2013
Available online 17 July 2013

Edited by: David Burr

Keywords:
Disuse osteoporosis
Hindlimb unloading
Reambulation
Multiple unloading
Trabecular bone strength

ABSTRACT

Extended periods of inactivity cause severe bone loss and concomitant deterioration of the musculoskeletal system. Considerable research has been aimed at better understanding the mechanisms and consequences of bone loss due to unloading and the associated effects on strength and fracture risk. One factor that has not been studied extensively but is of great interest, particularly for human spaceflight, is how multiple or repeated exposures to unloading and reloading affect the skeleton. Space agencies worldwide anticipate increased usage of repeat-flier crewmembers, and major thrust of research has focused on better understanding of microgravity effects on loss of bone density at weightbearing skeletal sites; however there is limited data available on repeat microgravity exposure. The adult hindlimb unloaded (HU) rat model was used to determine how an initial unloading cycle will affect a subsequent exposure to disuse and recovery thereafter. Animals underwent 28 days of HU starting at 6 months of age followed by 56 days of recovery, and then another 28 days of HU with 56 days of recovery. *In vivo* longitudinal pQCT was used to quantify bone morphological changes, and *ex vivo* μCT was used to quantify trabecular microarchitecture and cortical shell geometry at the proximal tibia metaphysis (PTM). The mechanical properties of trabecular bone were examined by the reduced platen compression mechanical test. The hypothesis that the initial HU exposure will mitigate decrements in bone mass and density for the second HU exposure was supported as pre- to post-HU declines in total BMC, total vBMD, and cortical area by *in vivo* pQCT at the proximal tibia metaphysis were milder for the second HU (and not significant) compared to an age-matched single HU (3% *vs.* 6%, 2% *vs.* 6%, and 2% *vs.* 6%, respectively). In contrast, the hypothesis was not supported at the microarchitectural level as losses in BV/TV and Tb.Th. were similar during 2nd HU exposure and age-matched single HU. Recovery with respect to post-HU values and compared to aging controls for total BMC, vBMD and cortical area were slower in older animals exposed to single or double HU cycles compared to recovery of younger animals exposed to a single HU bout. Despite milder recovery at the older age, there was no difference between unloaded animals and controls at the end of second recovery period. Therefore, the data did not support the hypothesis that two cycles of HU exposure with recovery would have a net negative effect. Mechanical properties of trabecular bone were affected more severely than densitometric measures (35% loss in trabecular bone ultimate stress *vs.* 9% loss in trabecular vBMD), which can be attributed most prominently to reductions in trabecular bone density and tissue mineral density. Together, our data demonstrate that initial exposure to mechanical unloading does not exacerbate bone loss during a subsequent unloading period and two cycles of unloading followed by recovery do not have a cumulative net negative effect on total bone mineral content and density as measured by pQCT at the proximal tibia metaphysis.

Introduction

Extended periods of inactivity cause substantial bone loss and concomitant deterioration of the musculoskeletal system. Reduced mechanical loading during paralysis due to spinal cord injury, long-term spaceflight, or extended bed rest during convalescence causes extreme cases of bone loss. In addition, the natural aging process leads to declines in bone mass and also increases in fracture risk [1]. Considerable

* Corresponding author at: Department of Biomedical Engineering, 3120 TAMU, College Station, TX 77843, USA. Fax: +1 979 845 3081.
E-mail addresses: yasamanshirazi@gmail.com (Y. Shirazi-Fard), rachelanthony91@gmail.com (R.A. Anthony), andrea.kwaczala@gmail.com (A.T. Kwaczala), stefan.judex@stonybrook.edu (S. Judex), sbloom@hlkn.tamu.edu (S.A. Bloomfield), hhogan@tamu.edu (H.A. Hogan).

8756-3282/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2013.07.004

research has been aimed at better understanding the mechanisms and consequences of bone loss due to unloading and the associated effects on strength and fracture risk. One factor that has not been studied extensively, but is of great interest due to concerns with repeated spaceflights [2,3], is how multiple cycles of unloading and reloading affect weightbearing bone sites.

The rate of bone loss for crewmembers in microgravity is 10-fold more rapid than the rate of loss seen in elderly Caucasian females [2,4–6], and a significant increase in estimated fracture risk remains even 1 year after returning to Earth [7,8]. Furthermore, QCT results from crewmembers exhibit a discordant recovery dynamic at the proximal femur [7], where bone mineral content (BMC) recovered faster than bone mineral density (BMD). Most importantly, calculated bone strength indices derived from density and geometry recovered the slowest and remained well below pre-flight values after recovery period equivalent to twice the mission duration [7]. Newly published data [9] indicate that, based on Dual-energy X-ray absorptiometry (DXA) scans, pelvis bone mineral density (BMD) and bone mineral content, hip femoral neck BMD, trochanter BMD, and total hip BMD were unchanged from preflight for crewmembers who have had access to the advanced resistance exercise device (ARED) in more recent ISS missions. This is presumably due to improved resistance exercise protocols (exercise prescription and constant, greater absolute loads of up to 600 lb_f generated by ARED) coupled with adequate energy intake and vitamin D; however, it is unclear how these BMD data translate into structural adaptations or bone strength. According to this recent study, the fact that resorption remained elevated after flight provides further evidence that bone resorption exceeds formation despite apparent maintenance of bone mass using resistance exercise protocols. In addition, incomplete recovery of bone density and calcium balance in astronauts and cosmonauts even after up to 5 years post-flight [10–14] is a concern, especially for those who fly repeat missions on the ISS as the bone loss due to spaceflight is combined with age-related bone loss [2]. The average time between flights is approximately 3.8 years, and the shortest recovery time is roughly 4 times the typical mission duration [15]. DXA densitometry measures of bone mineral density (BMD) are commonly used clinically to assess skeletal integrity, but the two-dimensional DXA measures are insufficient for capturing the more complex three-dimensional changes in bone compartment-specific changes in mass, structure, and integrity that might provide an accurate predictor of bone strength and fracture risk [3,8,16–22].

Twenty eight days of unloading in skeletally mature rats induces changes in total vBMD, BMC and cortical area at the PTM that are similar to changes observed in humans' femoral neck and proximal femur after 4–6 months of spaceflight [6,16,23,24]. Consequently, the hindlimb rodent model provides an excellent platform for assessing skeletal strength following repeated exposures to simulated microgravity as it enables a controlled study and provides the only means to directly measure mechanical properties of bone. We have previously shown that changes in total vBMD and total BMC at the proximal tibia metaphysis (PTM) provide a better model for alterations in the human femoral neck with prolonged weightlessness [16] than do similar variables at the femoral neck (FN) for the adult rat model; however, no strength tests were done for the PTM. Therefore, the current study addresses the mechanical properties of trabecular bone at the PTM, and the response of the PTM region to two HU cycles followed by weightbearing recovery periods following each unloading period.

We are aware of only one other study that has looked at multiple disuse and recovery periods [25,26]. In this study, adult C57BL/6 male mice were exposed to 2 weeks of hindlimb unloading followed by 4 weeks of reambulation, where the unloading-recovery cycle was repeated once, twice, or thrice, and mid-diaphyseal and distal metaphyseal regions of the femur were tracked by *in vivo* μCT. When age is eliminated as a confounding variable, Gupta et al. [25] report that the magnitude of the response to unloading (reductions in trabecular BV/TV and thickness) diminished during subsequent exposures. Therefore, these data suggest that bone's mechanosensitivity is reduced with consecutive unloading/reambulation cycles. Despite this reduced responsivity with repeated unloading periods, Gupta et al. [25] also observed that multiple exposures to mechanical unloading are more detrimental than a single unloading exposure, as evidenced by cumulative net negative effects on trabecular morphology persisting after multiple unloading compared to baseline. However, this study did not include any direct measures of bone strength. The current study used two cycles of unloading but employed adult male rats rather than mice in order to be consistent with our previous studies [16,24] and to permit more extensive mechanical testing. *In vivo* longitudinal pQCT was used to quantify densitometric and morphological changes, *ex vivo* μCT was used to quantify trabecular microarchitecture, and reduced platen compression was used for mechanical testing of the trabecular bone at the proximal tibia metaphysis.

In our previous study [16], total BMC, trabecular vBMD and cortical area, but not total vBMD, at the PTM recovered to aging controls after weightbearing period (56 days) equivalent to twice the duration of unloading (28 days). Therefore, the present study tracked recovery over 56 days (twice the duration of unloading) of weightbearing activity after both periods of unloading. The primary hypothesis was that an initial HU exposure would mitigate the effects of a second HU exposure, in terms of changes during both the second HU and recovery thereafter. To test this hypothesis for HU effects requires comparing pre- to post-HU changes for the second HU to the changes for a single age-matched HU. Another important consideration was whether multiple unloading cycles would have a cumulative negative effect. Thus, we also hypothesized that two cycles of HU exposure plus recovery would still result in a net overall negative effect on bone properties. To test this hypothesis requires comparing the level, or magnitude, of variables after the recovery following the second HU to those after recovery following a single age-matched HU. In addition, we aimed to assess the effects of the first HU + recovery cycle on the recovery response following the second HU.

Materials and methods

Animals and experiment design

Adult male Sprague–Dawley rats were obtained (Harlan Laboratories Inc., Houston, TX) at 5.5 months of age and allowed to acclimate for 14 days prior to initiation of the study. All animals were singly housed in a temperature-controlled (23 ± 2 °C) room with a 12-hour light–dark cycle (10 PM–10 AM) in an AAALAC-accredited animal care facility and were provided standard rodent chow (Harlan Teklad 8604) and water *ad-libitum*, except where specified. Animal care and all experimental procedures described in this investigation were conducted in accordance with the Texas A&M University Institutional Animal Care and Use Committee rules and approvals.

At 6 months of age, rats were block assigned to groups normalized by body weight and total volumetric bone mineral density (vBMD) at the proximal tibia on day 0. Animals (n = 200) were assigned to three categories: baseline control (BC, n = 20, euthanized on study day 0), hindlimb unloaded (HU, n = 75), and age-matched control (AC, n = 105). The design is depicted schematically in Fig. 1, where “one month” refers to four weeks or 28 days. All HU animals underwent 1 month of hindlimb unloading from 6 to 7 months of age (2HU7) followed by 2 months (56 days) of recovery (2HU7 + R2). The 2-month recovery period was chosen based on our previous findings [16], where total BMC, trabecular vBMD, and cortical area at the PTM recovered to age-matched control (AC) values after two months of recovery, and total vBMD at PTM hit a plateau after the second month of recovery and remained constant during the 3rd recovery period. Animals were then exposed to a second hindlimb unloading from 9 to 10 months of age (2HU10), followed by 2 months of recovery (2HU10 + R2). To distinguish between effects of previous exposure *vs*. differences due to animal age at the start of HU, a subset of animals, denoted by 1HU