Bone 52 (2013) 433–443

Contents lists available at SciVerse ScienceDirect

Bone

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

Original Full Length Article

Discordant recovery of bone mass and mechanical properties during prolonged recovery from disuse

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ARTICLE INFO

Article history:
Received 30 June 2012
Revised 14 September 2012
Accepted 14 September 2012
Available online 24 September 2012

Edited by: David Fyhrie

Keywords:
Hindlimb unloading
Disuse osteoporosis
Prolonged recovery
Mechanical properties
Longitudinal in vivo pQCT
Simulated microgravity

ABSTRACT

Profound bone loss at weight bearing sites is a primary effect of long-duration spaceflight. Moreover, a significant increase in estimated fracture risk remains even 1 year after returning to Earth; hence, it is important to define how quickly bone integrity can recover following prolonged disuse. This study characterized the loss and recovery dynamics of bone following a period of rodent hindlimb unloading in three anatomic sites. We hypothesized that the rat femoral neck would exhibit a discordant recovery dynamic most similar to that observed in astronauts' proximal femur; that is, bone mineral content (absolute mass) at this site would recover faster and more completely than would bone density and cortical area, and they will all recover before bone strength does. We characterized loss and long-term recovery of densitometric properties at the femoral neck, proximal tibia metaphysis, and tibia diaphysis, and also mechanical properties at the femoral neck and tibia diaphysis for which mechanical testing is amenable. We assessed the relationship between calculated strength indices and measured mechanical properties.

Adult male Sprague–Dawley rats (6 months) were assigned to baseline, age-matched control (AC), and hindlimb unloaded (HU) groups. The HU group was unloaded for 28 days and then returned to normal cage activity for 84 days of weight bearing recovery (3 times the duration of HU). Fifteen animals were euthanized from each of the HU and AC groups on days 28, 56, 84, and 112 of the study. At baseline and then every 28 days in vivo longitudinal pQCT scans were taken at proximal tibia metaphysis (PTM) and tibia diaphysis (TD); ex vivo pQCT scans were taken later at the femoral neck (FN). TD and FN were tested to failure to measure mechanical properties.

The hypothesis that the femoral neck in rats will exhibit a discordant recovery dynamic most similar to that observed in astronauts' proximal femurs was not supported by our data. At the femoral neck, densitometric and geometric variables (total BMC, total vBMD, cancellous vBMD, and cortical area) recovered to age-matched control levels after a recovery period twice the duration of unloading. Contrary to our hypothesis, changes in densitometric variables at the PTM provided a better model for changes in the human femoral neck with prolonged weightlessness. Following 28 days of HU, PTM total BMC recovered to age-matched control levels after roughly two times the duration of unloading; however, total vBMD did not recover even after three recovery periods. Cortical thinning occurred at the PTM following HU likely due to inhibition of periosteal growth; cortical shell thickness did not recover even after three recovery periods. Calculated strength indices suggested a loss in strength at the tibial diaphysis, which was not confirmed with direct testing of mechanical properties. HU had no effect on maximum fracture force at mid-tibia diaphysis; however, femoral neck experienced a significant loss of maximum force due to unloading that fully recovered after 28 days. Estimated strength indices for the femoral neck suggested a recovery period of 56 days in contrast to the 28-day recovery that was observed with mechanical testing. However, the inaccuracy of strength indices vs. directly measured mechanical properties highlights the continued importance of ground based animal models and mechanical testing. Our results demonstrate that the PTM in the rat better matches loss and recovery dynamics observed in astronauts' proximal femur than does the rat FN, at least in terms of densitometric variables. More complete utility of the rat PTM as a model in this case, however, depends upon meaningful characterization of changes in mechanical properties as well.

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8756-3282/$ – see front matter
http://dx.doi.org/10.1016/j.bone.2012.09.021

Introduction

Profound bone loss at weight bearing sites occurs during spaceflight. Missions (4–6 months) to the Russian MIR and the International Space Station (ISS) resulted in integral bone mineral density (BMD) losses up to 1.5% per month at the lumbar spine and hip [1–3]. Furthermore, computed tomography (CT) measures following long-duration missions suggest that cancellous bone is much more susceptible to bone loss due to microgravity [2,4]. Crewmembers lost nearly 1/3 of the total expected lifetime loss for men in their femoral neck in only 4–6 months aboard ISS [5,6]. This rate of bone loss in microgravity is 10-fold more rapid than the rate of loss seen in elderly Caucasian females [7], the population group most predisposed to osteoporosis. Moreover, a significant increase in estimated fracture risk remains even 1 year after returning to Earth [2]. Newly published data [8] indicate that crewmembers in more recent ISS missions have maintained bone mass better, presumably due to improved resistance exercise protocols coupled with adequate energy intake and vitamin D; however, no predictors of bone geometry or strength were evaluated. According to this recent study, the fact that resorption remained elevated after flight may provide further evidence that bone strength must also be determined in astronauts, as remodeling seemed to continue despite maintenance of bone mass using resistance exercise protocols.

QCT scans on ISS crewmembers show that even after one year of recovery, estimates of bending and compression strength remain 15% and 20% below preflight values [3]. Furthermore, an important finding from the studies of Lang et al. is the existence of a “discordant recovery dynamic”, where bone mineral content (BMC) recovered faster than BMD and most importantly, calculated bone strength indices derived from density and geometry recovered the slowest and remained well below pre-flight values. Finite element models generated from QCT scans of 13 ISS crewmembers showed that subjects exposed to microgravity in long-duration space missions experience up to a 5.0% per month decrease in proximal femoral strength [9].

A number of studies demonstrate that bone density and calcium balance in astronauts and cosmonauts fail to completely recover when followed up to 5 years post-flight [4,10–13]. Recent studies on crewmembers continue to highlight concern with a lack of full recovery in many bone parameters, especially cancellous bone, following long duration missions on the ISS [5]. These changes, and the fact that strength measures lag behind densitometric properties during recovery, have unknown consequences and important implications for the skeletal health of crewmembers. The discordant recovery may cause a potential problem for crewmembers, because their normal, age-related rate of bone loss is superimposed upon the deficits they accrued while aboard the ISS, and this becomes more of a concern for crewmembers, especially those who make repeat missions to the ISS.

It is well known that DXA densitometry and BMD alone are generally insufficient for capturing the complex changes in bone mass, structure, and integrity and not an accurate predictor of fracture risk [9,14–19]. Therefore, it is essential to measure the mechanical properties of bone tissue, for example strength, directly. The hindlimb unloaded rat model is a well-established ground-based spaceflight analog [20–22]; and 28 days of unloading in skeletally mature rats effectively simulates changes observed in humans after 4–6 months of spaceflight. Only a few studies have used mature rats with adult skeletons in unloading models and have reported detrimental effects on mechanical properties due to HU [23–26]. Studies [27,28] show that cortical bone BMD is much less responsive to the durations of disuse, whereas cancellous bone loss persists regardless of the duration of disuse. However, these studies did not include a recovery component; therefore it is not clear how these predictors of bone strength recover in this rat model following unloading. Tou et al. [29] studied effects of 28 days unloading in 6-month old female Sprague–Dawley rats with 14 days of recovery reloading. They used DXA of the whole femur and whole tibia and also conducted 3-point mechanical tests of the femur. For BMC and areal BMD, they found 5–6% loss due to unloading for both the femur and the tibia but found no recovery during 14 days of reloading. For mechanical strength, neither the femur nor the tibia was significantly affected by unloading. A longer period of weight bearing recovery was observed in animals in the study performed by Allen et al. [30]. A significant loss in BMD (−7.6%) was observed during HU, which did not recover to within age-matched controls until after three recovery periods. These data suggest that recovery dynamics using a rodent ground-based model may accurately model those changes that occur in astronauts [2,3]. However, Allen et al. did not perform any mechanical testing. In the Vico et al. [31] study, animals were allowed to recover for duration twice as long as the period of unloading. Vico et al. observed losses of BMC in the tibia and the femur (8% and 6%, respectively). After a period of reambulation equal to twice the duration of suspension, the tibia had not entirely recovered and BMC and BMD were significantly decreased compared to control values. These data suggest that short duration disuse periods using rodent HU models are not sufficient to model the discordant recovery observed during long duration human ISS missions.

Our inability to directly measure mechanical properties in human astronauts disallows a direct comparison between changes in bone morphology and in mechanical strength following spaceflight. The present study used the hindlimb unloaded rat model to determine if the changes in mechanical properties of bone after long duration disuse and recovery match those observed in astronauts, which depend on non-invasive estimates of bone strength. In particular, this study characterized the loss and recovery dynamics of bone following a period of unloading in three anatomic sites. We hypothesized that the rat femoral neck will exhibit a discordant recovery dynamic most similar to that observed in astronauts' proximal femur, that is, bone mass at this site will recover faster and more completely than will bone density and cortical area, and they will all recover before and/or more than bone strength does. We also aimed to characterize loss and long-term recovery of mechanical properties at the femoral neck and tibia diaphysis for which mechanical testing is amenable and assessed the relationship between calculated strength indices and measured mechanical properties.

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 (10PM–10AM) in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility and were provided standard rodent chow (Harlan Teklad 8604) and water ad-libitum. 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.

Adult male Sprague–Dawley rats (6 months old) were assigned to groups based on body weight and total volumetric bone mineral density (vBMD) at the proximal tibia on day 0. Animals (n = 135) were assigned to three categories: baseline control (BC, n = 15, euthanized on study day 0), hindlimb unloaded (HU, n = 60), and age-matched control (AC, n = 60). All HU animals underwent 28 days of hindlimb suspension starting at 6 months of age (HU) followed by recovery periods of 28, 56, and 84 days. Groups (n = 15) of HU animals were euthanized every 28 days (days 28, 56, 84, and 112) and designated HU, HU + R1, HU + R2, and HU + R3, respectively. The notations R1, R2, and R3 indicate recovery periods one, two, and three times the period