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Moderate intensity resistive exercise improves metaphyseal cancellous bone recovery following an initial disuse period, but does not mitigate decrements during a subsequent disuse period in adult rats

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

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

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ABSTRACT

Spaceflight provides a unique environment for skeletal tissue causing decrements in structural and densitometric properties of bone. Previously, we used the adult hindlimb unloaded (HU) rat model to show that previous exposure to HU had minimal effects on bone structure after a second HU exposure followed by recovery. Furthermore, we found that the decrements during second HU exposure were milder than the initial HU cycle. In this study, we used a moderate intensity resistance exercise protocol as an anabolic stimulus during recovery to test the hypothesis that resistance exercise following an exposure to HU will significantly enhance recovery of densitometric, structural, and, more importantly, mechanical properties of trabecular and cortical bone. We also hypothesized that resistance exercise during recovery, and prior to the second unloading period, will mitigate the losses during the second exposure. The hypothesis that exercise during recovery following hindlimb unloading will improve bone quality was supported by our data, as total BMC, total vBMD, and cancellous bone formation at the proximal tibia metaphysis increased significantly during exercise period, and total BMC/vBMD exceeded age-matched control and non-exercised values significantly by the end of recovery. However, our results did not support the hypothesis that resistance exercise prior to a subsequent unloading period will mitigate the detrimental effects of the second exposure, as the losses during the second exposure in total BMC, total vBMD, and cortical area at the proximal tibia metaphysis for the exercised animals were similar to those of the non-exercised group. Therefore, exercise did not mitigate effects of the second HU exposure in terms of pre-to-post HU changes in these variables, but it did produce beneficial effects in a broader sense.

Introduction

Mechanical unloading has deleterious effects on the musculoskeletal system and, more specifically, induces significant decreases in trabecular bone mass and strength in humans that do not fully recover even years after returning to weightbearing [1–4]. In terms of spaceflight, findings from International Space Station (ISS) crewmembers continue to highlight concern with a lack of full recovery in many bone parameters, especially trabecular bone density, following long duration missions on the ISS [5]. Spaceflight data have documented that trabecular bone appears to be more affected by unloading compared to cortical or total (integral) bone parameters, and trabecular vBMD in the femoral neck experienced the most dramatic percent losses and never recovered even after extended durations (2.5–4 years) [5,6]. This raises concern not only for crewmembers, but also for individuals who are exposed to multiple bed rest periods or crewmembers that make repeated spaceflight missions.

Exercise offers a way to reduce or reverse bone loss as a result of spaceflight-, injury-, or illness-related disuse. Crewmembers on more recent ISS missions have enhanced maintenance of bone mass, presumably due to improved resistance exercise protocols coupled with better intake of vitamin D and energy [6,7]. Intensive and progressive resistance exercise prevents most of disuse-induced bone loss [8], and high impact and resistance exercise effectively increase bone mass [9–15]. Furthermore, high impact exercise [16] and jumping [9] are more effective than aerobic activities such as running or walking [17].

* 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), cmetzger@hlkn.tamu.edu (C.E. Metzger), 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).

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In skeletally mature rats, mechanical loads that are applied in an intermittent and dynamic pattern different from normal activity, as well as high intensity simulated resistance training exercise, lead to increases in bone mass [18,19], density [20], trabecular bone microarchitecture [21], and increased bone formation rate on the endocortical surface [22]. Therefore, given concerns with incomplete recovery, exercise may be a promising approach to investigate for adding bone on the endocortical surface and restoring trabecular morphology.

Previously, we used the adult hindlimb unloaded (HU) rat model to show that an initial exposure to HU had minimal effects on the bone parameters after a subsequent HU exposure followed by recovery, and that the decrements during second HU exposure were milder than during the initial disuse cycle [23]. For most variables, the detrimental effects of unloading were more pronounced for the initial exposure than for the second HU, and the differences were not due to age based on comparisons with an age-matched single HU group. In this study, we used a moderate intensity resistance exercise protocol as an anabolic stimulus during recovery to test the hypothesis that resistance exercise following the first exposure to unloading will significantly enhance recovery of densitometric, structural, and more importantly mechanical properties of trabecular and cortical bone. Furthermore, many studies have shown benefits of exercise persisting following cessation [24–27], though others have found this not to be true [28,29]. Therefore, we also hypothesized that resistance exercise during recovery and prior to a subsequent unloading period will mitigate the losses during the second exposure.

Materials and methods

Animals and experimental 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) and were provided standard rodent chow (Harlan Teklad 8604) and water ad-libitum, except where specified. Animal care and all experimental procedures were conducted in accordance with the Texas A&M University IACUC rules and approvals.

At 6 months of age, rats were randomly assigned to groups normalized by body weight and total volumetric bone mineral density (vBMD) at the proximal tibia metaphysis on day 0 (Fig. 1). Animals (n = 200) were assigned to three categories: baseline control (BC, n = 35, euthanized on study day 0), hindlimb unloaded (HU, n = 90), and age-matched control (AC, n = 75). As depicted schematically in Fig. 1, “one month” refers to 4 weeks, or 28 days. All HU animals underwent a month (28 days) of hindlimb unloading from 6 to 7 months old (2HU7) followed by 2 months (56 days) of recovery (2HU9), then a second hindlimb unloading exposure starting at 9 months of age and ending at 10 months (2HU10). The 2-month recovery period was chosen based on our previous findings [30], where total BMC, trabecular vBMD, and cortical area at the proximal tibia metaphysis (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. Subsets of the HU animals performed a resistance training exercises following the initial exposure to unloading during 2 months of recovery (2HU+EX9). Exercised animals were also exposed to a second hindlimb unloading from 9 to 10 months of age (2HU+EX10). To obtain *ex vivo* measures, subsets of animals (n = 15) from all HU (non-exercise) and AC groups were euthanized at 7 (2HU7 and AC7), 8 (2HU8 and AC8), 9 (2HU9 and AC9), and 10 (2HU10 and AC10) months of age. In addition, a group of exercised animals was euthanized at the end of the recovery period (2HU+EX9, n = 15) and after 2nd HU exposure (2HU+EX10, n = 15). To account for animal batch effects an extra group of age-matched control animals was euthanized at 10 months of age, making n = 30 for AC10. To balance the food intake, all HU and AC animals were pair-fed during the first week of each HU period to rule out effects of dietary changes and food intake (and hence body mass) on bone mass and content. Body weights were recorded weekly throughout the experiment.

Hindlimb unloading

Hindlimb unloading (HU) was achieved by the well-established tail suspension technique [20,23,30–32]. In this model, the animal is suspended by the tail through the use of a custom-made tail harness attached to the tail to remove weight-bearing loads from the hindlimbs of the animal, as described previously [30]. The height of the animal's hindquarters was adjusted to prevent any contact of the hindlimbs with the cage floor, resulting in approximately a 30° head-down tilt [33]. All animals were monitored twice daily for health, including assessment of tail integrity.

Resistance exercise

The exercise paradigm utilized in this study was a squat jumping protocol that closely resembles progressive overload resistance exercise training as performed by humans, incurring the integrated physiological response to exercise (e.g., increased sympathetic nervous system outflow, blood flow, and IGF-1 production) [34–37]. Additionally, unlike other frequently used bone-loading paradigms (i.e., ulna and tibia compression or 4-point bending of the tibia), the squat jumping model produces significant lower leg muscle hypertrophy and increased skeletal

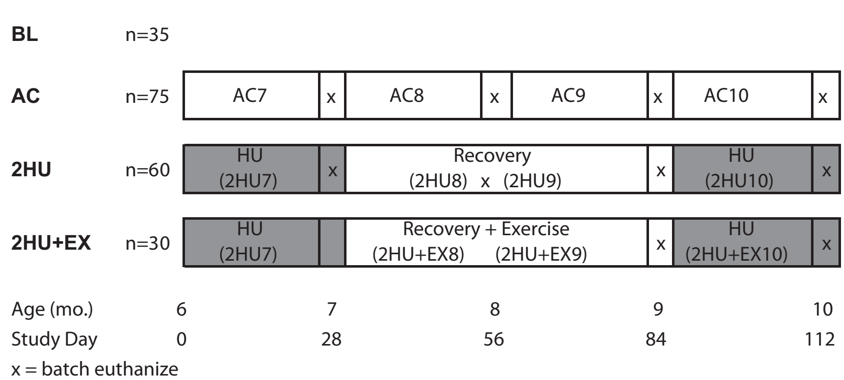

Fig. 1. Experimental design. All HU animals underwent 1 month (28 days) of hindlimb unloading from 6 to 7 months of age (2HU7) followed by 2 months (56 days) of normal ambulatory recovery (2HU9, n = 60) or with added resistance exercise (2HU+EX9, n = 30). Animals were then exposed to a second hindlimb unloading from 9 to 10 months of age (2HU10 or 2HU+EX10) and euthanized after the end of 2nd HU period (n = 15 each). To obtain *ex vivo* measures, subsets of animals (n = 15) from HU, HU+EX, and AC groups were euthanized at 7 (2HU7 and AC7), 8 (2HU8 and AC8), 9 (2HU9, 2HU+EX9, and AC9), and 10 (2HU10, 2HU+EX10, and AC10) months of age.