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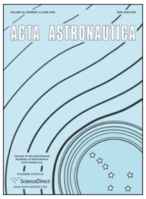

Reloading partly recovers bone mineral density and mechanical properties in hind limb unloaded rats

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ABSTRACT

Skeletal unloading results in decreased bone formation and bone mass. During long-term space flight, the decreased bone mass is impossible to fully recover. Therefore, it is necessary to develop the effective countermeasures to prevent spaceflight-induced bone loss. Hindlimb Unloading (HLU) simulates effects of weightlessness and is utilized extensively to examine the response of musculoskeletal systems to certain aspects of space flight. The purpose of this study is to investigate the effects of a 4-week HLU in rats and subsequent reloading on the bone mineral density (BMD) and mechanical properties of load-bearing bones.

After HLU for 4 weeks, the rats were then subjected to reloading for 1 week, 2 weeks and 3 weeks, and then the BMD of the femur, tibia and lumbar spine in rats were assessed by dual energy X-ray absorptiometry (DXA) every week. The mechanical properties of the femur were determined by three-point bending test. Dry bone and bone ash of femur were obtained through Oven-Drying method and were weighed respectively. Serum alkaline phosphatase (ALP) and serum calcium were examined through ELISA and Atomic Absorption Spectrometry.

The results showed that 4 weeks of HLU significantly decreased body weight of rats and reloading for 1 week, 2 weeks or 3 weeks did not recover the weight loss induced by HLU. However, after 2 weeks of reloading, BMD of femur and tibia of HLU rats partly recovered (+10.4%, +2.3%). After 3 weeks of reloading, the reduction of BMD, energy absorption, bone mass and mechanical properties of bone induced by HLU recovered to some extent. The changes in serum ALP and serum calcium induced by HLU were also recovered after reloading. Our results indicate that a short period of reloading could not completely recover bone after a period of unloading, thus some interventions such as mechanical vibration or pharmaceuticals are necessary to help bone recovery.

1. Introduction

Weightlessness during space flight results in significant physiologic challenges to those astronauts in long-term spaceflight for prolonged time. Previous studies have shown that space flight has devastating effects on musculoskeletal system [1]. On average, astronauts suffer from bone loss at the rate of 1–2% per month during space flight [2,3]. Bone loss induced by space flight may not be

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recovered entirely, even return to gravitational fields [1]. There are a number of potential countermeasures to prevent bone loss, such as mechanical stimulation [4], bisphosphonates [5], exercise [6], nutrition [7] and artificial gravity [8].

Due to the high cost and limitations in conducting experiments in space, it is necessary to develop ground-based models of microgravity. In the early days, Jaworski et al. [9] have employed an immobilization model to simulate the effect of disuse-induced bone loss, this immobilization model simulated unloading condition successfully, but at the cost of long experiment period. With time went on, more models simulating the effect of space flight were constructed, for example head down tilt bed rest were employed more in clinical trial to give evidence in the treatment and precaution of astronaut pathological changes [10,11]. Although these models have provided sufficient data for the study of simulated microgravity-induced bone loss, trial period and the availability of experiment objects have restricted their applications.

Hindlimb unloading (HLU) as an animal model to simulate the effects of space flight, have been well established in the ground-based experiments. It allows researchers to investigate the musculoskeletal response [12] and disuse atrophy in rodents [13]. Animals in HLU model experience a cephalic fluid shift. This becomes a relevant feature, as reduced skeletal perfusion, which may induce musculoskeletal changes during spaceflight [14]. Most importantly, HLU induces significant bone loss and muscle atrophy [15,16]. The Morey-Holton HLU method is widely accepted by the National Aeronautics and Space Administration (NASA) as a ground-based model for studying, with easily available experimental animals, shortened experimental cycle, and excellent reproducibility of results.

With an urgent need for the exploring of the outer space, numerous countermeasures had been evaluated to mitigate the musculoskeletal effects of unloading successfully, which include pharmacological interventions, kinetic and mechanical stimuli, and nutritional strategies [17]. As an alternative, the effect of reloading to space flight-induced physiological changes was studied in recent years. It is reported that reloading promotes recovery of muscle mass and elicits an early phase characterized by elevating the activation of nuclear factor κ B, 3-nitrotyrosine, p-HSP25, and p-Akt levels [18]. Basso et al. [19] studied the effect of a 2-week reloading to rat bone histomorphology and osteogenic cell population and activity, and found that bone volume could restore to normal level, but osteoblasts *in vivo* were only partially recovered. A recent study on bone recovery after prolonged disuse assessed densitometric properties and mechanical properties at femoral neck and tibia found that rats do not exhibit a discordant recovery dynamic similar to that observed in astronauts' proximal femora, and total BMC of proximal tibia metaphysis recovered to age-matched control levels after two times the duration of unloading, but not total vBMD even after three recovery periods [20].

Although there are quite a few number of researches on reloading, they vary from each other on experiment designs, research objectives, and technical aspects, which make it difficult to compare the results obtained from related studies. Besides, the opinions on reloading duration are still controversial, some researchers insist that a long-period of reloading is appropriate for study [20,21], while others think a relatively short period is also representative [18,19,22–25]. Lastly, as one of our point of interest, there is not much knowledge to evaluate the potential and feasibility of applying vibration and pharmaceuticals as countermeasures for space flight-induced bone loss, thus some backgrounds for further studies are unclear.

With time of space exploration and missions becoming longer and longer, long-term risks from multiple exposures to microgravity on crew's musculoskeletal health need to be defined and addressed in future. Therefore, the specific aim of this study was to ascertain whether the detrimental effects on the musculo-skeleton system induced by the loss of load-bearing can be mitigated through reloading. The findings will provide guidance and reference for astronauts' rehabilitation training after space flight or clinical disuse and aid in developing more effective countermeasures.

2. Materials and methods

2.1. Animal grouping and model construction

A total of 56 healthy adult male Sprague-Dawley (SD) rats with a mean body weight of 280 ± 10 g were obtained from the Laboratory Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi, China). The rats were 3 months old when obtained, and have been raised for 3 weeks before the experiment, with seven per cage, in animal lab of School of Life Sciences, Northwestern Polytechnical University. To begin with, 28 rats were picked randomly to construct the 4-week HLU model (S, $n=28$), with the rest 28 rats as control animals (C, $n=28$). After the period of HLU, control rats were randomly divided into 4 groups: (1) normal load-bearing control for 4 weeks (CR0, $n=7$); (2) normal load-bearing control for 5 weeks (CR1, $n=7$); (3) normal load-bearing control for 6 weeks (CR2, $n=7$); (4) normal load-bearing control for 7 weeks (CR3, $n=7$). Unloaded rats were also randomly divided into 4 groups: (5) HLU for 4 weeks continuously (SR0, $n=7$); (6) HLU for 4 weeks and reloaded for 1 week (SR1, $n=7$); (7) HLU for 4 weeks and reloaded for 2 weeks (SR2, $n=7$); (8) HLU for 4 weeks and reloaded for 3 weeks (SR3, $n=7$). A schematic diagram is given in Fig. 1 to show animal grouping.

Tail suspension (-30°) was employed to simulate the weightlessness condition according to Morey-Holton method [2]. Briefly, the laboratory rats were suspended with tails hanging at the top of cages, forelimbs stepping on the bottom of cages, and the hindlimbs hanging in the air. The angle between the horizontal plane and the body ordinate axis was 30°. The rats in the control group could move freely without tail suspension. All animals were fed on rat chow and water *ad libitum*. After the 4 weeks of tail suspension, reloading began immediately. Rats were put down to move freely for continuously 3 weeks [19,22], and without any other interventions or treatments. The rats were weighed every week during tail suspension and reloading. The experimental procedures followed the principle of