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Local vibration enhanced the efficacy of passive exercise on mitigating bone loss in hindlimb unloading rats

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A R T I C L E I N F O

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A B S T R A C T

Spaceflight induced bone loss is seriously affecting astronauts. Mechanical stimulation from exercise has been shown to restrain bone resorption as well as improve bone formation. Current exercise countermeasures in space cannot prevent it completely. Active exercise may convert to passive exercise in some ways because of the loss of gravity stimulus and inertia of exercise equipment. The aim of this study was to compare the efficacy of passive exercise or/and local vibration on counteracting the deterioration of the musculoskeletal system, including bone, muscle and tendons in tail-suspended rats. We hypothesized that local vibration could enhance the efficacy of passive exercise on countering bone loss. 40 Sprague Dawley rats were randomly distributed into five groups (n = 8, each): tail-suspension (TS), TS+35 Hz vibration (TSV), TS + passive exercise (TSP), TS + passive exercise coupled with 35 Hz vibration (TSPV) and control (CON). Passive exercise or/and local vibration was performed for 21 days. On day 0 and 21, bone mineral density (BMD) was observed by dual energy X-ray absorptiometry (DXA), and trabecular microstructure was evaluated by microcomputer tomography (μCT) analysis in vivo. Mechanical properties of tibia and tendon were determined by a mechanical testing system. Soleus and bone ash weight was tested by an electronic balance. Results showed that the passive exercise could not prevent the decrease of trabecular BMD, microstructure and bone ash weight induced by TS, whereas vibration and passive exercise coupled with local vibration (PV) could. Biomechanical properties of the tibia and tendon in TSPV group significantly increased compared with TS group. In summary, PV in this study was the best method in preventing weightlessness-induced bone loss. Consistent with our hypothesis, local vibration partly enhanced the effect of passive exercise. Furthermore, this study will be useful in improving countermeasure for astronauts, but also for the rehabilitation of disused or aged osteoporosis.

1. Introduction

Osteoporosis induced by spaceflight or bedridden diseases is seriously affecting people's health [1,2]. It is characterized by bone loss and decreased bone strength [3]. Long-term spaceflight has been demonstrated to cause precipitous declines (>10%) of bone mineral density (BMD) at the hip and spine of astronauts [4]. Bone fracture, associated with decreased BMD as well as degenerated bone microstructure, is the major consequence of osteoporosis [5]. The tail-suspended rat was a useful model to generate osteoporosis and simulate space conditions by reducing loadings on weight-bearing bones and causing redistribution of body fluid on the ground [6,7].

Physical exercise countermeasures presently adopted have been shown to play a positive effect on the treatment of osteoporosis, but they have only limited success [8,9]. For example, swimming, jumping, and vibration therapies were efficient in improving bone mass, bone strength

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and bone formation using HU model [10]. Resistive exercise has been proven to prevent bone loss at the tibial diaphysis and proximal femur during prolonged bed rest [11]. In contrast, exercise countermeasures were found useless in preventing the decrease of BMD and deteriorative trabecular microstructure [12]. Investigating the mechanism of how exercise works for the prevention of bone loss in space can help us to find more effective and harmless methods against osteoporosis.

Studies demonstrated that there was less energy consumption of exercise in space flight than on the ground when doing the same thing [13–15]. Lower oxygen consumption and diastolic blood pressure appeared when doing bicycle ergometer in the ISS than on the ground [13]. These results matched the limited success of exercise countermeasures. Gravitational load and muscle contraction force are two major mechanical forces on bone in normal human movement and exercise [16]. On the ground, the muscle contracts to overcome the resistance of body weight. The absence of gravitational load in space is like “an invisible hand” that helps astronauts work against the resistance of body weight. Thus, the muscle force and muscle stimulation on bone would dramatically be reduced during daily work and performing exercise countermeasures, which are similar to the passive exercise that external force helps to counteract the resistance. This might be why exercise trainings in space could not fully prevent weightlessness-induced osteoporosis [17]. Active exercise presently adopted in space may convert to passive exercise in some ways because of the loss of gravity stimulus and inertia of exercise equipment. As a result, the converting of exercise patterns must be considered during the seeking of an efficient countermeasure against bone loss.

In addition to exercise trainings, vibration has been used either alone as a countermeasure or coupled with other methods to enhance their effects [18]. Vibration combined with exercise training on improving the performance of the musculoskeletal system was more effective than either one of them [19]. Whole body vibration (WBV) was made to prevent the decrease in femoral strength and bone loss in tail-suspended or ovariectomized rats [20–23]. It could enhance trabecular bone formation, increase the expression of bone formation related gene, reduce the osteoclast activity, inhibit bone loss at the spine and femur, counteract muscle atrophy and enhance the strength of a muscle tendon [24–27]. However, some other studies showed that WBV did not preclude the increase in bone resorption and the effects of bed rest on bone [28,29]. WBV might damage the peripheral vessel of animals and make people feel uncomfortable [30,31]. What's more, the effects of vibration not only depended on the frequency but also the body posture [20]. Bone loss of astronauts and bedridden patients mainly happened in the lower limbs and spine [1,2]. We believe local vibration that can be applied in a wider spectrum of frequencies, and settings would be better for preventing bone loss [25].

To avoid the limitation of WBV we developed a device that can generate local vibration on rat's hindlimbs that had been confirmed beneficial in counteracting musculoskeletal degeneration in our previous study [18]. The main objective of this study was to investigate the efficacy of local vibration and passive exercise on bone loss, and additional bone-related muscle and tendon. Our hypothesis was that local vibration could enhance the efficacy of passive exercise on countering bone loss in tail-suspended rats.

2. Methods and materials

2.1. Experimental animals and animal care

40 female 8-week-old Sprague Dawley rats were recruited from the Experimental Animal Center of Beijing University and were adapted for 7 days. All animal treatments were conducted in accordance with the Regulation of Administration of Affairs Concerning Experimental Animals of State Science and Technology Commission of China and were approved by the Animal Care Committee of Beihang University.

All rats were housed in the same cages with rationed lab chow and enough water. The room was controlled at 25 ± 2 °C with a 12/12 h light/dark cycle. Animals were randomly divided into five groups ($n = 8$, each group): 1) tail-suspension (TS), 2) TS plus 35 Hz vibration (TSV), 3) TS plus passive exercise (TSP), 4) TS plus passive exercise plus 35 Hz vibration (TSPV) and 5) control (CON). On day 0 and on day 22 of the experiment, rats were anaesthetized by pentobarbital sodium (6 ml per kilogram of body weight). Their tibiae and femora were then scanned in vivo by dual energy X-ray absorptiometer (DXA) and microcomputer tomography (μCT). After the scan on day 22, the rats were sacrificed by narcotic overdose (1% Pentobarbital Sodium, 18 ml/kg, i.p.). Then rats' tibiae, femora, tendons were harvested without other soft tissues and preserved at −20 °C wrapped in a saline-soaked gauze bandage for mechanical test.

2.2. Hindlimb unloading

In TS, TSV, TSP and TSPV groups, rats' tails were suspended and hindlimbs were unloaded for 3 weeks according to Morey's methods [32]. Briefly speaking, the rat's tail was cleaned with 70% ethanol and sprayed with benzoin and rosin tinctures to increase the adhesiveness between tape and tail. When it was dried, the tail was suspended by two tail-parallel strips of adhesive tape attached. They were protected with three strips of Gauze bandage to prevent the tail peeling off the tape. Then the adhesive tape was tied to a fish-line swivel that hanged on a chain from the top of the cage (30 × 30 × 50 cm) allowed rats move freely on a x-y axis and rotate 360° (Fig. 1a). The tape and bandage were changed every 7 days considering the growing size of the tail. The body was maintained at approximately 30° angle from the cage floor to ensure that the feet did not touch the cage floor. The animal was able to reach food and water easily.

2.3. Passive exercise or/and vibration

Animals in TSV, TSP and TSPV were trained respectively in local vibration, passive exercise and passive exercise coupled with local vibration twice a day (at 8 a.m. and 5 p.m.) for 21 days. We developed a novel training device for passive exercise and local vibration on hindlimbs as showed in Fig. 1b. During training, the rat's body was maintained 30° angle in a fixed box. Its feet were both immobilized on the footplates of the device with medical adhesive tape. Passive exercise was performed by a lifting motor drove the footplates overcoming a 4 N load generated by the gravity of footplates that caused passive contraction of rat's hindlimbs. The hindlimbs were from fully extended to fully bended, then back to be fully extended as one bout. Each bout lasted 2 s with 8-s interval. 20 bouts were applied every time. Another motor connected to an eccentric bearing generated the vibration (35 Hz, 1 mm amplitude). It was applied for 200s every time. Passive exercise and local vibration were applied simultaneously in TSPV group for 200s.

2.4. DXA

On day 0 and day 22, the tibiae and femora were scanned and analyzed by DXA (Discovery QDR, USA) with regional high-resolution scanning mode for small animals. The change rate of BMD was calculated: $\triangle BMD = (BMD_{D22}\text{-}BMD_{D0})/BMD_{D0} \times 100\%$. BMD_{D0} and BMD_{D22} meant the values of BMD measured by DXA on day 0 and on day 22.

2.5. μCT

After DXA, μCT (SkyScan 1076, Belgium) was used to scan distal femora and proximal tibiae of rats. Scanning parameters were as follows: 70 kV voltage, 140 μA current, 1 mm aluminum filter, 18 μm pixel size, 180° rotation and 0.6° step. Reconstruction parameters were 1 smoothing, 8 ring artifacts and 30% beam hardening.

An 1898 μm-thick trabecular region (100 reconstruction images), 1898 μm distance from the growth plate, and a 949 μm-thick cortical