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Use of micro-computed tomography to evaluate the effects of exercise on preventing the degeneration of articular cartilage in tail-suspended rats

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ABSTRACT

Space flight has been shown to induce bone loss and muscle atrophy, which could initiate the degeneration of articular cartilage. Countermeasures to prevent bone loss and muscle atrophy have been explored, but few spaceflight or ground-based studies have focused on the effects on cartilage degeneration. In this study, we investigated the effects of exercise on articular cartilage deterioration in tail-suspended rats. Thirty-two female Sprague-Dawley rats were randomly divided into four groups ($n = 8$ in each): tail suspension (TS), tail suspension plus passive motion (TSP), tail suspension plus active exercise (TSA), and control (CON) groups. In the TS, TSP, and TSA groups, the rat hindlimbs were unloaded for 21 days by tail suspension. Next, the cartilage thickness and volume, and the attenuation coefficient of the distal femur were evaluated by micro-computed tomography (μCT). Histological analysis was used to assess the surface integrity of the cartilage, cartilage thickness, and chondrocytes. The results showed that: (1) the cartilage thickness on the distal femur was significantly lower in the TS and TSP groups compared with the CON and TSA groups; (2) the cartilage volume in the TS group was significantly lower compared with the CON, TSA, and TSP groups; and (3) histomorphology showed that the chondrocytes formed clusters where the degree of matrix staining was lower in the TS and TSP groups. There were no significant differences between any of these parameters in the CON and TSA groups. The cartilage thickness measurements obtained by μCT and histomorphology correlated well. In general, tail suspension could induce articular cartilage degeneration, but active exercise was effective in preventing this degeneration in tail-suspended rats.

1. Introduction

Long-term space flight has been shown to induce conditions such as bone loss and muscle atrophy (Baldwin, 1996; Fitts et al., 2000; Lang et al., 2004). In addition, studies have shown that unloading could have a negative impact on the articular cartilage in rats (Wang et al., 2010; Niu et al., 2012), although few studies have examined the impact of spaceflight on articular cartilage. This problem might compromise the performance of astronauts and increase the risk of injury in space (McCrory et al., 2002).

Articular cartilage is crucial for providing low-friction and resilient joint-bearing surfaces. Under physiological conditions, the knee cartilage can withstand hydrostatic pressure, as well as transmitting and dissipating forces across the joint from one subchondral bone to the other (Mow et al., 1993). Thus, cartilage can act as a shock absorber and protect the subchondral bone. Articular cartilage is a special tissue that has no blood supply, and thus the nutrients required to maintain its normal physiological function come mainly from the interstitial fluid. The pressure caused by weight-bearing and joint motion assists in the

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diffusion of interstitial fluid throughout the tissue (Suh, 1996; Bachrach et al., 1998), as well as protecting articular cartilage. Immobilization has adverse effects on articular cartilage such as cartilage thinning in rabbits (Sood, 1971), necrosis of the superficial chondrocytes and cartilage layer in rats (Jozsa et al., 1987), and decline in the biomechanical properties in dogs (Leroux et al., 2001; Jurvelin et al., 1989). Tail suspension in rats induces the degeneration of articular cartilage, including a significant reduction in cartilage thickness, as well as significant decreases in the uniaxial modulus and proteoglycan (PG) content (Wang et al., 2010; Niu et al., 2012). In humans, bed rest induces decreases in the articular cartilage thickness (Liphardt et al., 2009).

Exercise is often used to treat problems related to immobilization. Evidence from rabbit studies has shown that continuous passive movements of the knee significantly improves motion and the biological properties of articular cartilage (Knapik et al., 2013). Results obtained with rats have shown that exercise can prevent degenerative changes in femoral articular cartilage (Maldonado et al., 2013), as well as maintaining the range of motion and reducing the changes in joint components (Matsuzaki et al., 2013) caused by immobilization. Moreover, studies suggest that vibration training may be a potent countermeasure against the loss of cartilage thickness in humans during bed rest (Liphardt et al., 2009). The use of exercise regimens as a countermeasure is common for minimizing the risks to the musculoskeletal system induced by weightlessness (Oser and Damann, 1997; Baldwin et al., 1996; Schneider et al., 2003; Clément, 2005), and many studies have tested countermeasures to prevent bone loss and muscle atrophy under unloading (Lam et al., 2011; Swift et al., 2011; Li et al., 2012; Sun et al., 2013). However, few ground-based studies have focused on the effects on articular cartilage in humans or animals.

Micro-computed tomography (μCT) can facilitate three-dimensional (3D) and quantitative morphological analyses of hard tissues such as bone at micrometer-level voxel resolutions (scan resolution in vivo = 1–160 μm). In general, soft tissues are not detectable by μCT due to their low X-ray attenuation, and segmentation from other tissues is not possible in such images. Therefore, a contrast-enhanced technique using an iodic solution was developed to compensate for this poor radiopacity and to improve the X-ray imaging of soft tissues. Compared with the traditional method of histomorphology, equilibrium partitioning of an ionic contrast agent via μCT is a noninvasive imaging technique, and it has been used to assess the morphology of articular cartilage in rat models (Xie et al., 2009; Wang et al., 2012). In addition, PG is an important constituent of the cartilage as well as collagen, which attaches to the glycosaminoglycan (GAG) (Buckwalter and Mankin, 1997; Burstein et al., 2009; Silvast et al., 2009) chains, thereby conferring considerable compressive strength on cartilage (Burstein et al., 2009).

In this study, we investigated the effects of exercise on the deterioration of articular cartilage in tail-suspended rats. We employed a custom-made stepper device to allow tail-suspended rats to perform active and passive exercise. The effects of these two exercise modes on the prevention of cartilage deterioration induced by unloading were evaluated by μCT and histomorphology.

2. Materials and methods

2.1. Experimental animals and animal care

Female 8-week-old Sprague-Dawley rats were purchased from the Experimental Animal Center of Beijing University. After one week of adaptation to standard laboratory cages ($n = 2$, each cage), 32 animals ($n = 32$) were randomly selected and divided into four groups ($n = 8$ in each): tail suspension (TS), tail suspension plus passive motion (TSP), tail suspension plus active exercise (TSA), and control (CON) groups. TS, TSP, and TSA rats had their hindlimbs unloaded for 21 days via tail suspension (Morey-Holton and Globus, 2002). While tail-suspended, the TSP and TSA rats were individually subjected to either passive motion or active exercise using a custom-made TS-rat training device designed in our laboratory (Sun et al., 2013). All of the groups were subjected to the same nursery/housing conditions for 21 days in the animal facility at Beihang University, China, with 12:12 h dark:light cycles and food and water were provided ad libitum. Animal treatment and care were administered according to the Regulations for the Administration of Affairs Concerning Experimental Animals promulgated by Decree No. 2 of the State Science and Technology Commission of China and the Guiding Principles for the Care and Use of Animals approved by the Beijing Government. All of the protocols were approved by the Animal Care Committee of Beihang University, China.

2.2. Exercise training with a stepper device

A novel exercise stepper training device was used to provide active and passive motion training to TS-rats, as described previously (Sun et al., 2013). The rat's body was maintained with a head down tilt angle of 30° to ensure complete hindlimb unloading. During active exercise, the rat hindlimb was contracted by a pulse from an electrical arc stimulator to overcome a load of 0–4 N. When passive mode exercise was performed with the same stepper device, a lifting motor drove the pedal to overcome the load of 0–4 N. The conscious rats in the TSA and TSP groups were trained twice each day (at 8 a.m. and 4 p.m.) by stepper training for approximately 6 min per day with exactly 40 bouts.

2.3. Contrast agent concentration and scanning

At the end of the experiment (day 22), all of the rats were sacrificed and both femora were harvested. The femora were excised to clean the soft tissues, wrapped in gauze bandages soaked with phosphate-buffered saline (PBS), and then preserved at 4 °C. To prevent cartilage degeneration, the PBS used throughout this study contained 1% protease inhibitor (Xie et al., 2009). Next, the distal femur was immersed in 5 mL of PBS diluted with a contrast agent (compound meglumine diatrizoate injection, China) for 15 min at 37 °C, and transferred to the μCT (SkyScan1076, Aartselaar, Belgium) for scanning (contrast agent dilution: 30% compound meglumine diatrizoate injection, 70% PBS) (Wang et al., 2012). The distal femurs of the rats were scanned as described previously (Sun et al., 2011). Briefly, all of the scans used the following settings: 70 kV X-ray voltage, 143 μA current, 1 mm aluminum filter, 18 μm pixel size, 360° tomographic rotation, and a rotation step of 0.6°. The overall procedure of scanning, reconstruction, segmentation, and remodeling the articular cartilage is shown in Fig. 1. The transverse images of the distal femur were compiled by a construction program and then converted into sagittal images using Data Viewer (SkyScan, Aartselaar, Belgium), which was employed to view a stack of the images in 2D/3D at their original range and resolution. In order to accurately segment the contrast agent, articular cartilage, and bone, the cartilage contour was drawn manually according to the CT value. Semi-automatic contouring was applied every 3–6 slices. The 3D morphology of the entire articular cartilage was remodeled using Mimics software (Fig. 1E), and the volume and surface area were calculated. In addition, the cartilage thickness and attenuation coefficient (the attenuation coefficient of cartilage will increase due to the loss of GAG content, and PG attaches to the GAG chains) were calculated using CTAn (SkyScan, Aartselaar, Belgium), which was designed to allow SkyScan μCT