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● *Original Contribution*

ULTRASOUND EVALUATION OF SITE-SPECIFIC EFFECT OF SIMULATED MICROGRAVITY ON ARTICULAR CARTILAGE

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Abstract—Space flight induces acute changes in normal physiology in response to the microgravity environment. Articular cartilage is subjected to high loads under a ground reaction force on Earth. The objectives of this study were to investigate the site dependence of morphological and ultrasonic parameters of articular cartilage and to examine the site-specific responses of articular cartilage to simulated microgravity using ultrasound biomicroscopy (UBM). Six rats underwent tail suspension (simulated microgravity) for four weeks and six other rats were kept under normal Earth gravity as controls. Cartilage thickness, ultrasound roughness index (URI), integrated reflection coefficient (IRC) and integrated backscatter coefficient (IBC) of cartilage tissues, as well as histological degeneration were measured at the femoral head (FH), medial femoral condyle (MFC), lateral femoral condyle (LFC), patello-femoral groove (PFG) and patella (PAT). The results showed site dependence not significant in all UBM parameters except cartilage thickness ($p < 0.01$) in the control specimens. Only minor changes in articular cartilage were induced by 4-week tail suspension, although there were significant decreases in cartilage thickness at the MFC and PAT ($p < 0.05$) and a significant increase in URI at the PAT ($p < 0.01$). This study suggested that the 4-week simulated microgravity had only mild effects on femoral articular cartilage in the rat model. This information is useful for human spaceflight and clinical medicine in improving understanding of the effect of microgravity on articular cartilage. However, the effects of longer duration microgravity experience on articular cartilage need further investigation. (E-mail: rsguoxia@inet.polyu.edu.hk) © 2010 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Articular cartilage, Simulated microgravity, Ultrasound biomicroscopy, Site-dependence, Surface roughness.**

INTRODUCTION

Spaceflight induces acute changes in normal physiology as a result of changes in gravitational force. All physiologic systems are affected to some degree, particularly musculoskeletal and cardiovascular systems (Globus et al. 1986; Hargens and Watenpaugh 1996; Payne et al. 2007; Vico et al. 2000; White and Averner 2001; Williams et al. 2009). Muscle atrophy, osteoporosis and osteoarthritis (OA) are the main musculoskeletal diseases induced by microgravity (Smith and Matina 2002; Williams et al. 2009). Effects of microgravity on the cartilage tissues including the epiphyseal growth plate (Duke and Montufar-Solis 1999; Montufar-Solis et al. 1996) and intervertebral discs (Hutton et al. 2002; Yasuoka et al. 2007) have been reported. However, few spaceflight or ground-based studies have focused on the changes in the properties of articular cartilage.

It is well known that articular cartilage is a hyaline cartilage that covers the end of bone, serving as a mechanical, frictionless cushion during weight-bearing and joint locomotion. When the body stands or moves on Earth, articular cartilage in the hip and knee joints is especially subjected to high static and dynamic loading. Sensing mechanical stimuli, chondrocytes are important in the control of matrix turnover throughout the production of collagens, proteoglycans (PGs) and enzymes for cartilage metabolism. Under microgravity, the loads on articular cartilage disappear; thereby the changes in mechanical forces affect the components and structure of articular cartilage (Buschmann et al. 1999; Lippiello et al. 1985). Because the degeneration of articular cartilage is

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regarded as an indicator of OA (Ostergaard et al. 1997), monitoring the changes in articular cartilage is helpful to detect the occurrence of OA.

When musculoskeletal ultrasound was first applied in space, it showed potential for real-time monitoring of microgravity-induced changes in muscles, tendons and bone (Fincke et al. 2005). Because of the low thickness and anatomical location of articular cartilage, monitoring the tissue has been a challenge. Ultrasound biomicroscopy (UBM) imaging, or acoustic microscopy (Foster et al. 2000; Saijo and Chubachi 2000), was developed for clear imaging of small tissues such as intervertebral discs, articular cartilage and cornea (Cherin et al. 1998; Laasanen et al. 2005; Naish et al. 2003; Saarakkala et al. 2004, 2006; Skribek et al. 2008; Spriet et al. 2005; Wang et al. 2008a; Wang and Zheng 2009; Zheng et al. 2004). Earlier studies reported the suitability of ultrasound measurement of articular cartilage thickness (Joiner et al. 2001; Jurvelin et al. 1995) and the extraction of acoustic parameters such as reflection coefficient and backscatter coefficient for evaluating articular cartilage (Cherin et al. 1998; Laasanen et al. 2005; Saarakkala et al. 2006; Hattori et al. 2005). The surface roughness of articular cartilage was previously visualized using optical microscopy (Gardner and McGillivray 1971), and it was determined that surface roughness plays a role in lubrication (Mow and Hung 2001). A recently introduced parameter, ultrasound roughness index (URI), has been successfully used to assess surface irregularity of articular cartilage and become an index to evaluate degradation of articular cartilage (Laasanen et al. 2005; Saarakkala et al. 2004, 2006). Because acoustic properties are related to the structural and mechanical properties of materials, UBM may provide a sensitive, nondestructive tool for quantitative analysis of articular cartilage.

Because of the limitations of human and animal space flight experiments, the tail suspension (TS) rodent models have been used to investigate space flight–like effects on the body in a ground-based laboratory setting (Morey-Holton and Globus 2002). The TS rodent model is a ''microgravity model'' rather than a simple ''unloading model.'' Model testing of TS in rat was initiated in 1975 and the results were published in 1979 (Morey-Holton 1979). Technically, the angle formed between the torso of the animal and the floor of the cage is very important for the TS model. A 30° angle has been recommended as a standard TS model because it provided normal weight-bearing on the forelimbs, unloaded the hindlimbs (Globus et al. 1986) and induced a cephalad fluid shift (Hargens et al. 1984). Cosmos 2044 was the first spaceflight mission that included the TS model (30° angle of unloading) as a control group coinciding with the flight. Both space flight and TS animals showed that rats displayed similar atrophic changes in muscle and bone and similar responses in many systems (Booth and Grindeland 1994). Although some nonidentical physiological responses have also been reported (Tischler et al. 1993), to date, TS has been accepted as a rodent model to mimic space flight in ground-based studies.

Significant bone loss and reductions in bone formation resulted from four-week TS (Allen and Bloomfield 2003; Sakata et al. 1999; Lam and Qin 2008). To compare with bone loss, this study used the four-week TS rat model to evaluate changes in articular cartilage. The objectives of this study were to examine the responses of articular cartilage to simulated microgravity using UBM and to investigate the site dependence of thickness and acoustic parameters of articular cartilage. We hypothesized that the four-week TS-induced changes in the properties of articular cartilage would be detected by UBM and that the parameters would be site dependent.

MATERIALS AND METHODS

Animal care and experimental protocol

Ethics approval was obtained from the Animal Ethics Committee of the administering institution in Hong Kong before conducting the experiment. Twelve male Sprague-Dawley rats (5 months old, weight 368.5 to 418.0 g) were divided randomly into two groups: TS group ($n = 6$) and control group (C) ($n = 6$). The experimental animals were kept individually in metal cages and fed with standard rabbit diet and water *ad lib*. Each rat in the TS group was suspended with surgical tape around the length of the tail for four weeks, with the body in a head-down position (Fig. 1), and the angle formed between the torso of the rat and the floor of the cage was kept at 30° (Hargens et al. 1984). Rats in the control group were kept in the normal quadruped position under normal Earth gravity. On day 29, all animals were euthanized with an overdose of sodium pentobarbital (Euthanyl, MTC Pharmaceutical, Cambridge, Ontario, Canada). Hindlimbs were excised, wrapped in wet gauze soaked with physiological saline, and stored at –20°C until ultrasound examination. In this study, both the right and left femur were collected, so there were 12 femur specimens in each group.

UBM system

A high-resolution ultrasound imaging system specially developed for small animal research (Vevo 770, Visual-Sonics Inc., Toronto, Ontario, Canada) was used in this study. The high-frequency ultrasound probe (RMV-708) was composed of a focused transducer with a nominal central frequency of 55 MHz and a focal length of 4.5 mm. The axial resolution was approximately 30 μm. The transducer scanning was performed with a mechanical-sector scan in real time. Two-dimensional