Ultrasound in Med. & Biol., Vol. 42, No. 1, pp. 290–298, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2015.08.004

● *Original Contribution*

QUANTITATIVE ULTRASOUND ASSESSMENT OF CARTILAGE DEGENERATION IN OVARIECTOMIZED RATS WITH LOW ESTROGEN LEVELS

Qing Wang,*† Zhiwei Liu,* Yinong Wang,* Qingya Pan,* Qianjin Feng,*† Qinghua Huang,‡ and Wufan Chen*†

*Institute of Medical Information, School of Biomedical Engineering, Southern Medical University, Guangzhou, Guangdong, China; †Guangdong Provincial Key Laboratory of Medical Image Processing, School of Biomedical Engineering, Southern Medical University, Guangzhou, Guangdong, China; and ‡School of Electronic and Information Engineering, South China University of Technology, Guangzhou, Guangdong, China

(*Received* 6 *February* 2015; *revised* 3 *August* 2015; *in final form* 6 *August* 2015)

Abstract—The aim of this study was to assess quantitatively the site-specific degeneration of articular cartilage in ovariectomized rats with low estrogen levels using a high-frequency ultrasound system. Fourteen female Sprague–Dawley rats were randomly divided into two groups (n = 7 per group): a sham group in which only the perivarian fatty tissue was exteriorized and an ovariectomized group that underwent bilateral ovariectomy to create a menopause model with low estrogen levels. All animals were sacrificed at the end of the third week after ovariectomy. Hindlimbs were harvested. The articular cartilage from five anatomic sites (*i.e.*, femoral caput [FC], medial femoral condyle [MFC], lateral femoral condyle [LFC], medial tibial plateau [MTP] and lateral tibial plateau [LTP]) was examined with ultrasound. Four parameters were extracted from the ultrasound radiofrequency data: reflection coefficient of the cartilage surface (RC_1), reflection coefficient of the cartilage–bone interface (RC_2), ultrasound roughness index (URI) and thickness of the cartilage tissue. The results indicated significant ($p < 0.05$) site dependence for cartilage thickness, URI and RC_1 in the sham group. The 3-wk post-menopause ovariectomized rats exhibited significant increases ($p < 0.05$) in the URI at the LFC, MTP and LTP; significant decreases ($p < 0.05$) in RC_1 at the FC, LFC and MTP; and significant decreases ($p < 0.05$) in cartilage thickness at the MFC, LFC, MTP and LTP. These results of this study suggest that post-menopausal estrogen reduction induces morphologic and acoustic alterations in the articular cartilage of the hip and knee joints in ovariectomized rats. (E-mail: wq8740@smu.edu.cn or qianjinfeng08@gmail.com) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Cartilage degradation, High-frequency ultrasound, Ovariectomized rat, Estrogen, Post-menopause.**

INTRODUCTION

Articular cartilage is a special and important connective tissue located at the ends of articulating bones. Articular cartilage is often described as a special biphasic mixture composed of a solid matrix and an interstitial fluid. The matrix is a fibrous network of primarily type II collagen with electrolytes, macromolecules and proteoglycans (PGs) that are trapped within the matrix. These hydrophilic macromolecules can bind water in the tissue to make the articular cartilage more flexible (Mow and Huiskes 2005). Degradation of articular cartilage often occurs in the early stages of osteoarthritis (OA), a severe progressive joint disorder commonly diagnosed among the elderly (Felson et al. 1997).

Although OA affects both men and women, the prevalence of OA is higher in women than in men after the age of 50 (Birchfield 2001; Oliveria et al. 1995). Moreover, it has been reported that the annual rate of tibiofemoral cartilage loss is greater in women (Hanna et al. 2009). These phenomena might result from menopause-related changes in estrogen levels. Previous studies have reported on the relationship of estrogen level to articular cartilage and OA. Høegh-Andersen et al. (2004) suggested that OA symptoms appear with reductions in estrogen in ovariectomized (OVX) rats, which indicates an important role for estrogen level in the pathogenesis of OA. Additionally, Claassen et al. (2001) provided evidence that chondrocytes have estrogen receptors

Address correspondence to: Qing Wang and Qianjin Feng, Institute of Medical Information, School of Biomedical Engineering, Southern Medical University, Guangzhou, Guangdong, 510515, China. E-mail: wq8740@smu.edu.cn or qianjinfeng08@gmail.com

(ERα or ERβ); thus, articular cartilage could be a target of estrogen. Calvo et al. (2007) reported that osteoporosis induced by estrogen reduction increases the severity of cartilage damage caused by alterations in the subchondral bone. The degeneration of articular cartilage triggers the occurrence of OA. Currently, estrogen replacement therapy (ERT) is occasionally used to counter the deleterious effects of estrogen deficiency related to post-menopause diseases, including post-menopausal OA, although controversy exists regarding the actual benefits of ERT (Sniekers et al. 2008).

The quantitative detection of prior degeneration of the articular cartilage in post-menopausal women is difficult with current clinical imaging modalities. In contrast to other imaging techniques such as X-ray, magnetic resonance imaging (MRI) and computed tomography (CT), ultrasound enables high-resolution imaging and quantitative measurement of articular cartilage. The recently developed field of quantitative ultrasound imaging (QUI) is providing promising methods for assessing the structure and function of articular cartilage. Many acoustic parameters, such as the surface roughness index, ultrasound propagation velocity of the cartilage, attenuation coefficient, reflection coefficient and scattering coefficient, are typically used for quantitative evaluations of the properties of articular cartilage (Nieminen et al. 2009). Previous studies have reported that the characteristics of cartilage tissue can be assessed with high-frequency ultrasound. Saarakkala et al. (2004) quantified the integrity of the cartilage surface with an ultrasound reflection coefficient (*i.e.*, the spatial variation of the ultrasound reflection coefficient [SVR]) and an ultrasound roughness index (URI) and reported significant decreases in the SVR and increases in the URI of damaged cartilage tissues Wang et al. (2010a) reported that a 4-wk tail suspension rat model induced significant decreases in cartilage thickness in the medial femoral condyle and patella and a significant increase in the URI at the patella. Gelse et al. (2010) reported that integrated reflection coefficients of repaired cartilage tissue are significantly greater than those of healthy tissue. Niu et al. (2012) observed a strong correlation of acoustic parameters, for example, surface roughness index and reflection coefficient, with pathologic assessments of articular cartilage. These studies indicate that QUI has the potential to be a useful measurement tool for the quantitative analysis of articular cartilage.

In experimental studies, the OVX animal model has been used to explore the effects of estrogens and estrogen-like substances (Høegh-Andersen et al. 2004). Therefore, in the present study, bilateral ovariectomies were performed in rats to create an animal model of menopause. The aims of this study were to employ a high-frequency ultrasound system to quantitatively assess site-specific alterations in the morphologic and acoustic parameters of OVX rats with low estrogen levels and to acoustically characterize the articular cartilage of different anatomic sites. The hypothesis of the study is that in articular cartilage, post-menopausal estrogen reduction induces morphologic and acoustic alterations that trigger OA.

METHODS

Animal care and experimental protocol

Fourteen female Sprague–Dawley rats (10 mo old, weight: 344.8 ± 26.7 g) were randomly divided into two groups: a sham group and an OVX group. Each group consisted of seven rats. The experimental rats were individually maintained in metal cages and provided standard rat diet and water *ad libitum* in the Department of Comparative Medicine, Guangdong Medical Laboratory Animal Center, China. Ethical approval was obtained from the Animal Experimental Ethical Inspection Committee of Guangdong Medical Laboratory Animal Center before conducting the experiments. Experiments on rats were performed in accordance with the *Guidelines for the Care of Laboratory Animals* of the National Institutes of Health.

Rats in the OVX group underwent bilateral ovariectomy, whereas peri-ovarian fatty tissue was exteriorized in rats in the sham group. After surgery, animals were returned to their cages and fed according to the aforementioned feeding protocol. On day 21 after ovariectomy, all animals were euthanized with an overdose of sodium pentobarbital (P3761, Sigma, USA). The hindlimbs were excised, harvested and stored at −20°C until the ultrasound examination. Both femurs and tibias were collected. Figure 1 illustrates the anatomic sites examined with ultrasound.

Ultrasound system and experimental methods

The high-frequency ultrasound system consisted of a computer, an ultrasound pulser/receiver (Olympus 5900 PR, Panametrics-NDT, Waltham, MA, USA), a 50-MHz single-element transducer (Olympus, PI50-2-R0.75, Panametrics-NDT), a 12-bit A/D acquisition card (CompuScope 12400, Gage, ON, Canada), a 3-D translating stage with a control box (ETSN400, TianRuiZhongHai Instrument, Beijing, China) and self-developed control software (Version 1.0, National Engineering Research Center for Mobile Ultrasonic Detection Technology, South China University of Technology, Guangzhou, China). The transducer was fixed to the arm of the 3-D translating stage, and the accuracy of its positioning was adjusted *via* the control software.

Before ultrasound scanning, the sample to be measured was thawed in a saline solution. Next, the