Long-Term Study for the Effect of Ovariectomy on Rat Bone - Use of In-Vivo Micro-CT -

Dae Gon Woo, Chang Yong Ko, Tae Woo Lee, Han Sung Kim, and Beob Yi Lee

Abstract**—**In the present study, changes of morphology and mechanical characteristics in the lumbar vertebrae of the ovariectomised (OVX) rat were investigated. In previous researches, there were many studies about morphology like volume fraction and trabecular thickness based on Micro - Computed Tomography (Micro - CT). However, detecting and tracking long-term changes in the trabecular bone of the lumbar vertebrae for the OVX rat were few. For this study, one female Sprague-Dawley rat was used: an OVX rat. The 4th Lumbar of the OVX rat was subjected to in-vivo micro-CT. Detecting and tracking long-term changes could be investigated in the trabecular bone of the lumbar vertebrae for an OVX rat using in-vivo micro-CT. An OVX rat was scanned at week 0 (just before surgery), at week 4, at week 8, week 16, week 22 and week 56 after surgery. Finite element (FE) analysis was used to investigate mechanical characteristics of the lumbar vertebrae for an OVX rat. When the OVX rat (at week 56) was compared with the OVX rat (at week 0), volume fraction was decreased by 80% and effective modulus was decreased by 75%.

Keywords**—**OVX rats, Trabecular bone, In-vivo Micro-CT, FE analysis

I. Introduction

OSTEOPOROSIS, a disease characterized by the progressive loss of bone tissue, is one of the most common complications of aging [1]-[3]. After the age of 50, bone mineral density (BMD) decreases at a rate as high as 3% per year in postmenopausal women [4]-[5]. Osteoporotic vertebral fractures are a major health care problem in the world. Therefore there were numerous studies investigating the mechanical characteristics of vertebral bone [6]-[8]. Recently, several researchers investigated relations between osteoporosis and morphology of vertebral trabecular bone in rats. Laib et al. [9] evaluated the short- and long-term effects of estrogen deficiency on trabecular bone in proximal tibiae of ovariectomized (OVX) rats by using micro-computed tomography (micro-CT). Waarsing et al. [10] detected and tracked local changes for the tibiae of OVX rats. Cao et al. [11] and Hara et al. [12] investigated the effect of ovariectomy on rat bone by comparing changes of bone architectures in cortical bone between at week 0 (just before surgery) and at weeks 12 (after surgery). Jiang et al. [13] researched mandible bone loss in osteoporotic rats killed at 3 and 6 months following ovariectomy. In previous researches, there were many studies about morphology such as bone mineral density and trabecular microstructure (thinning or disconnection of trabeculae). They investigated changes of local bone architecture up to week 12, but did not analyze changes of mechanical characteristics for trabecular bone of the lumbar vertebra in OVX rats. In the present study, long-term changes, up to week 56, of not only morphologies but also mechanical characteristics in the lumbar vertebra of the OVX rat were investigated and analyzed by Finite Element (FE) models based on Micro-CT (Skyscan-1076, Skyscan, Belgium).

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II. Materials and Methods

A. Investigation for Changes of Morphologies

For this study, 14-weeks-old Sprague-Dawley rats were housed in a stainless steel cage in an air-conditioned environment (room temperature 23°±2°C, humidity 50%±10%). The period for day and night was exchanged every 12 hours. This study observed regulations of Animal Care Committee for animal experiments of Konkuk University in Korea. A high-resolution in-vivo micro-CT system was used to scan the 4th lumbar of one OVX rat (Fig. 1), resulting in reconstructed three dimensional (3D) models with 35×35×35μm cubic voxels [14]. The In-vivo micro-CT system consists of an animal bed, around which the X-ray source and the detector rotate. The machine is equipped with a 100 kV X-ray source with a spot size of 5 μm. One rat was scanned at week 0, before the rat was ovariectomised. The in-vivo micro-CT measurement was repeated for a long-term (at week 4, 8 16, 22 and 56). Body weight of the rat was measured once four weeks before scanning (Table I).

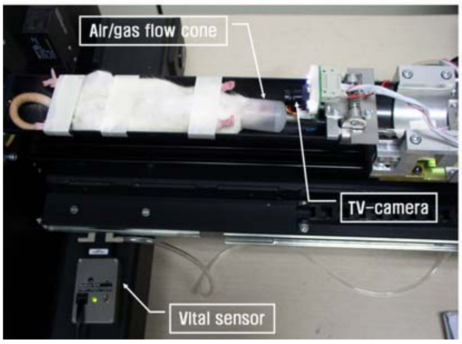


Fig. 1 OVX rat on in-vivo micro-CT

TABLE I
CHANGES OF BODY WEIGHT IN OVX RAT

	Week 0	Week 4	Week 8	Week 16	Week 22	Week 56
weight (g)	224	245	253	287	322	339

The OVX rat was dosed orally with purified diet without added calcium (AIN-93M, Dyets, USA) to accelerate osteoporosis in the rat. Changes of morphologies were detected and tracked in the trabecular bone of the lumbar vertebra for the OVX rat. In the 3D analysis, the tissue volume (TV, mm^3) and the trabecular bone volume (BV, mm^3) were measured by the direct method available on in-vivo micro-CT, and the trabecular bone volume fraction (BV/TV, %) was calculated. Trabecular thickness (Tb.Th, µm), trabecular separation (Tb.Sp, µm) and trabecular number (Tb.N, 1/mm) were measured directly on 3D image data. The plate-rod characteristic of the bone structure can be measured by using the structure model index (SMI). For an ideal plate, the SMI value is zero and three for rod structure.

In the present paper, to inhibit an increase in quantity of radiation, minimum scan time with 35µm resolution and the shutter was used. During scanning, breathing rate of the rat was considered to acquire scan images of good quality.

B. FE Analysis

The scanned images were converted to 3D voxel images by BIONIX 3.0 (CANTiBio Co., Korea) in Fig. 2. In the models, the pixels of micro-CT images were changed to hexahedron finite elements (voxels).

Simulated compression tests of 3D FE models were performed to investigate mechanical characteristics in the whole vertebral bone model (trabecular and cortical bone) of the 4th lumbar vertebra for an OVX rat. Displacement boundary conditions were applied to the specimens to simulate a uniaxial compression test. For the elastic characteristic of micro-FE analysis, effective elastic modulus was measured by applying a compressive displacement of 0.5% strain. All FE analyses were performed by using a commercial FE software (ABAQUS 6.4, HKS Inc, U.S.A).

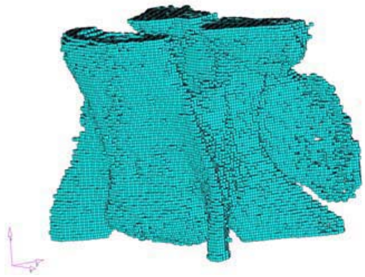


Fig. 2 3D FE model in the 4th lumbar of a rat(ISO view)

III. RESULTS

A. Parameters of Bone Structure

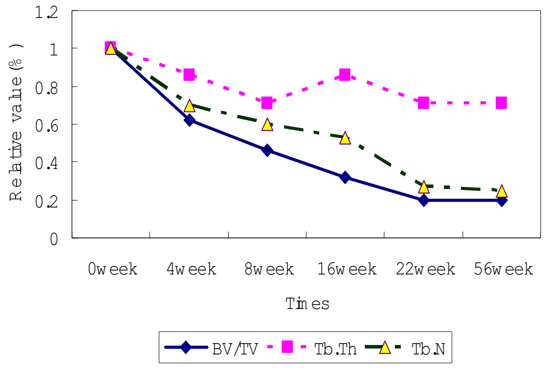
Structural parameters of trabecular bone were investigated and analyzed by using in-vivo micro-CT (Table II). As shown in Fig. 3, values of BV/TV and Tb.N were significantly lower and Tb.Sp value was significantly higher in 4th Lumbar vertebra of the rat at week 16 after ovariectomy than those at week 0, 4, 8 after ovariectomy. Tb.Th decreased until week 8 after ovariectomy and significantly increased at week 16. Subsequently, Tb.Th decreased again at week 22, but there was a little change from week 22 to week 56. SMI gradually increased from week 0 (just before OVX) to week 22 and decreased at week 56. Other results except SMI were presented as relative values, normalized by those at week 0. As a result, changes in the trabecular bone of OVX rat were tracked and analyzed. Detailed examination of the matched data sets showed thinning and eventual loss of metaphyseal trabeculae (Fig. 4). Ovariectomy induced a dramatic loss of both epiphyseal and metaphyseal trabecular bone of the rat.

B. Simulated Compression Tests

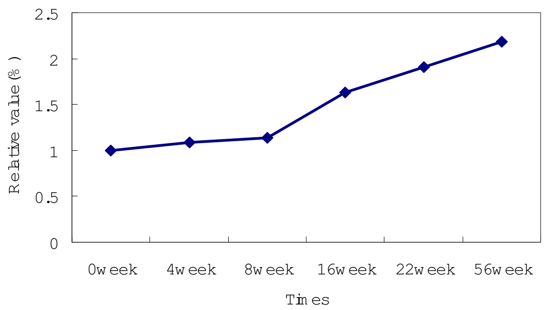
In the simulated compression tests of whole vertebral bone models, effective elastic modulus significantly decreased between week 0 and week 8 (Figs. 4-5). For detecting and tracking long-term changes of effective elastic modulus, there was not a little difference between week 16 and week 56.

TABLE II
STRUCTURE PARAMETER OF THE L4 IN OVX RAT

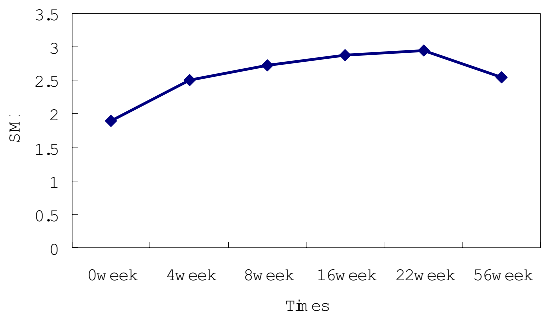
	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (mm^{-1})	SMI
Week 0	30.94	0.14	0.22	2.28	1.89
4	19.32	0.12	0.24	1.59	2.50
8	14.28	0.10	0.25	1.37	2.73
16	9.87	0.12	0.39	0.85	2.88
22	6.5	0.10	0.42	0.65	2.95
56	6.1	0.10	0.48	0.58	2.55



(a) BV/TV, Tb.Th and Tb.N



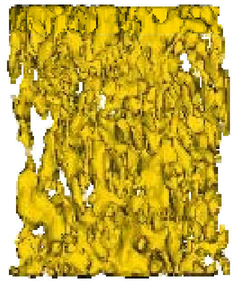
(b) Tb.Sp



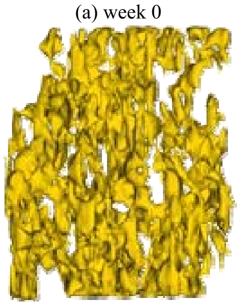
(c) SMI

Fig. 3 Changes in structure indices after OVX

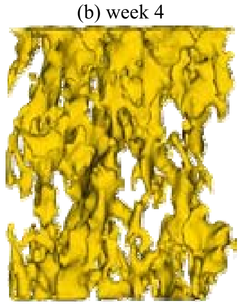
(a) week 0



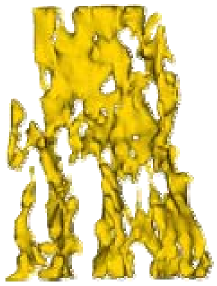
(b) week 4



(c) week 8



(d) week 16



(e) week 22

(d) week 56

Fig. 4 Examples of thinning and eventual loss of trabeculae in OVX rat at week 0, week 4, week8, week 16, week 22 and week 56

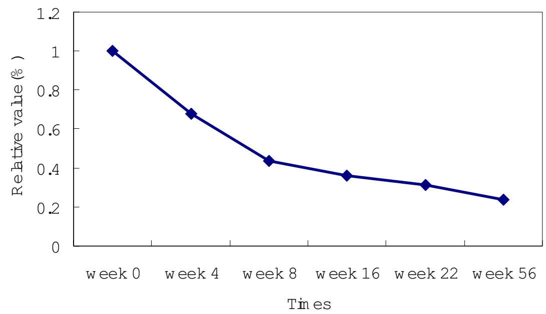


Fig. 5 Changes of effective elastic modulus after OVX

IV. Discussion and Conclusions

In the present study, changes of physical properties (morphology and mechanical characteristics) in the lumbar vertebra of the OVX rat were investigated and analyzed by a high-resolution in-vivo micro-CT system and FE analysis. Detecting and tracking changes of morphologies in the lumbar vertebrae of an OVX rat were performed. This paper shows that it is possible to detect and track changes of not only bone architectures but also mechanical characteristics in living animals. From 3D image data based on in-vivo micro-CT image, the present study could also detect the resorption of some trabeculae and the slow increase in thickness of the trabeculae at week 16 after ovariectomy. This supports the result of Waarsing et al. [10]. Estrogen depletion caused by ovariectomy induces dramatic trabecular bone loss and changes in the trabecular architecture up to week 56. The thickness of the trabeculae decreased from at week 0 to at week 8 and increased at week 16, but decreased again in the following weeks to week 56. SMI gradually increased from week 0 to week 22 but decreased at week 56. This result shows that such research for effect of ovariectomy on rat bone needs long-term study up to week 56.

The present FE method, based on in-vivo micro-CT, make it possible to perform longitudinal studies in small animals (rats), with accurately measuring mechanical characteristics of the bone. Furthermore, for an identical rat, detecting and tracking long-term changes of mechanical and morphological characteristics could sacrifice rats at a minimum. The method suggested in this study will greatly contribute to experimental studies in small animals concerning orthopaedic or pharmacological intervention and transgenic rat models.

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