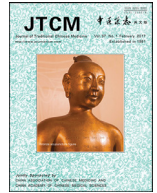




Contents lists available at ScienceDirect

## Journal of Traditional Chinese Medicine

journal homepage: [www.elsevier.com/locate/jtcm](http://www.elsevier.com/locate/jtcm)  
<http://www.journaltcm.com>Effect of acupuncture at acupoint of Shenshu (BL 23) on the bones of ovariectomized rats<sup>☆</sup>Atsushi Takeda<sup>a,b,\*</sup>, Teruhiko Koike<sup>c</sup>, Shigeru Urata<sup>b</sup>, Torao Ishida<sup>d</sup>, Yoshiharu Oshida<sup>c</sup><sup>a</sup> Graduate School of Medicine, Nagoya University, Showa-ku 466-8550, Japan<sup>b</sup> Department of Acupuncture Medicine, Suzuka University of Medical Science, Suzuka 510-0293, Japan<sup>c</sup> Research Center of Health, Physical Fitness and Sports, Nagoya University, Furou-cho Chikusa-ku Nagoya 464-8601, Japan<sup>d</sup> Institute of Traditional Chinese Medicine, Suzuka University of Medical Science, Suzuka 510-0226, Japan

## ARTICLE INFO

## Article history:

Received 11 November 2016

Revised 10 February 2017

Accepted 8 April 2017

Available online xxx

## Keywords:

Acupuncture

Point BL 23 (Shenshu)

Estrogens

Osteoporosis

Ovariectomy

## ABSTRACT

**Objective:** To investigate the effect of acupuncture at acupoint of Shenshu (BL 23) in ovariectomized rats and sham-operated rats.**Methods:** Female Sprague–Dawley rats were divided into the following four groups ( $n = 12$  each): sham operation only (Sham), sham operation with acupuncture (Sham + ACP), ovariectomy only (OVX), and ovariectomy with acupuncture (OVX + ACP). Operations were performed at the 9th week of age. Acupuncture of Shenshu (BL 23) was started at the 11th week of age and conducted 3 times per week until the 26th week of age.**Results:** Among ovariectomized rats, the acupuncture-treated rats had significantly lower body weights and cross-linked N-telopeptide of type 1 collagen levels in the urine after 3 weeks of acupuncture treatment, higher bone mineral density of the lumbar vertebra after 7 weeks, and smaller trabecular separation of the sixth lumbar vertebra, measured by micro-computed tomography, after 17 weeks than control rats. These effects were not observed in sham-operated rats. Blood concentrations of estradiol were significantly higher in the OVX + ACP group than in the untreated group. Acupuncture treatment increased plasma testosterone levels in sham-operated rats, while this increase was not observed in OVX rats.**Conclusion:** Acupuncture of Shenshu (BL 23) prevents bone loss and structural changes by suppressing bone resorption.

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## 1. Introduction

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone architecture, leading to an increased risk of bone fracture. Fractures in elderly patients reduce their ability to perform activities of daily living, and osteoporosis adversely affects the prognosis of fracture healing [1,2]. The prevalence of osteoporosis and osteoporosis-related fractures is increasing in an ageing society, and particularly in postmenopausal women. A bisphosphonate and a selective estrogen receptor modulator are widely used for the treatment of osteoporosis [3–5].

Acupuncture, a form of Traditional Chinese Medicine (TCM), has also been used for the treatment of osteoporosis. The validity of acupuncture treatment for osteoporosis has been reported using an ovariectomized rat as an animal model for postmenopausal osteoporosis. [6,7] Zhang et al. reported that acupuncture administered to an ovariectomized rat decreased levels of deoxyypyridinoline (Dpd), a bone resorption marker, in the urine, [6] and increased levels of osteocalcin, a bone formation marker, [7] which suggests that acupuncture can suppress bone resorption and enhance bone formation. However, the effects and underlying mechanisms are not fully understood. We therefore conducted experiments examining the effect of acupuncture at acupoint of Shenshu (BL 23) on bone mineral density, urinary N-terminal telopeptide (NTx), a bone resorption marker, as well as plasma estradiol and testosterone levels. The effect of acupuncture was evaluated in both ovariectomized and sham-operated rats. Additionally, the trabecular bone structure was analyzed using micro-computed tomography (micro-CT).

<sup>☆</sup> Peer review under responsibility of Traditional Chinese Medicine Periodical Press.

\* Corresponding author at: Department of Acupuncture Medicine, Suzuka University of Medical Science, Suzuka 510-0293, Japan

E-mail addresses: [flc@pearl.ocn.jp](mailto:flc@pearl.ocn.jp), [Japan.flc@pearl.ocn.jp](mailto:Japan.flc@pearl.ocn.jp) (A. Takeda).

<https://doi.org/10.1016/j.jtcm.2018.02.004>

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Please cite this article as: A. Takeda et al., Effect of acupuncture at acupoint of Shenshu (BL 23) on the bones of ovariectomized rats, Journal of Traditional Chinese Medicine (2018), <https://doi.org/10.1016/j.jtcm.2018.02.004>

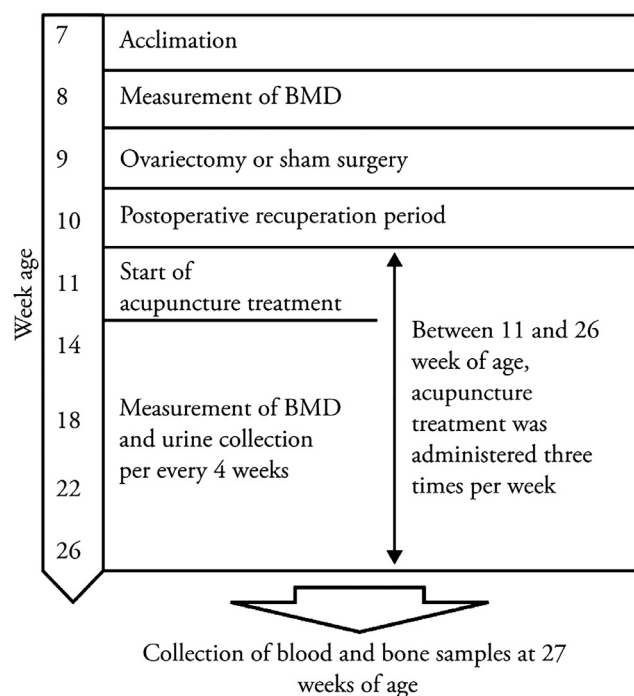


Fig. 1. Study design.  
BMD: bone mineral density.

## 2. Materials and methods

### 2.1. Animals

The forty-eight 7-week-old female Sprague–Dawley rats weighing ( $192 \pm 9$ ) g were purchased from CLEA Japan, Inc. (Tokyo, Japan) and housed in plastic cages in a room kept at ( $22 \pm 2$ ) °C with  $55\% \pm 10\%$  relative humidity and a 12-h light/dark cycle. The animals were acclimated to their environment for 1 week and had ad libitum access to tap water and a standard rodent diet, which contains 340.3 kcal/100 g. To prevent the effect of isolation stress (i.e., reduced body weight and food intake), the rats were housed in groups of three rats per cage. [8] All animals in a single cage were assigned to the same treatment group.

### 2.2. Study design

Acupuncture treatment was administered three times per week for 15 weeks. Fig. 1 depicts the study design. Forty-eight rats were divided into the following four groups: sham operation only (Sham), sham operation with acupuncture (Sham + ACP), ovariectomy only (OVX), and ovariectomy with acupuncture (OVX + ACP). One rat in the OVX + ACP group died at the 22nd week of age and was excluded from the study. A bilateral ovariectomy or a sham operation was performed at the 9th week of age under pentobarbital anesthesia (50 mg/kg). After the postoperative recovery period, at week 1, rats received acupuncture treatment starting at the 11th week of age and continuing until the 26th week of age. Blood and bone samples were collected at week 27.

### 2.3. Acupuncture treatment

Acupuncture was administered to the Sham + ACP group and the OVX + ACP group three times per week. Beginning at 11 weeks of age, the acupuncture was conducted for 15 weeks. The Shenshu (BL 23) acupoint was described in the classical Traditional Chinese Medicine (TCM) writings of *Su Wen*. The equivalent Shenshu (BL

23) site in rats is located 1 cm lateral to the spine on each side at the height of the tip of the spinous process of the second lumbar vertebra. Rats were fixed only initially by a positioner so that the acupuncture needle could be inserted. The acupuncture was administered to the bilateral acupuncture points for 10 min using a stainless-steel needle, 30 mm in length and 0.3 mm in diameter (Carbo Deluxe Needles; Carbo Medical Supplies, Toronto, Canada). During the acupuncture treatment, rats were unrestricted in their cages. Fifteen seconds of fixation by the positioner was applied in the Sham and OVX groups, and they were then allowed unrestricted movement for 10 min. All acupuncture treatment processes were conducted without anesthesia, and the same person administered these treatments.

### 2.4. Bone densitometry

Bone mass density (BMD) was measured by the dual-energy X-ray absorptiometry method (DEXA) with a DEXA-based bone densitometry system (DCS-600R; Hitachi Aloka Medical, Ltd., Tokyo, Japan) under pentobarbital anesthesia at the 14th, 18th, 22nd, and 26th week of age. The method of Kasai *et al.* [9] was modified to measure the regions of interest (ROIs) in rats. Bone mass densities of the lumbar spine, lower body, right hind leg, right femur, and right tibia were measured.

### 2.5. Analysis of urine cross-linked N-telopeptide of type 1 collagen (NTx)

A 24-h urine sample was collected using metabolic cages at the 14th, 18th, 22nd, and 26th week of age. Urine samples were tested for NTx, a marker of bone resorption, using a quantitative enzyme-linked immunosorbent assay (ELISA) kit (Osteomark™ NTx; Inverness Medical Innovations Inc., Waltham, MA, USA).

### 2.6. Analysis of blood estradiol and testosterone levels

At the 27th week of age, animals were anaesthetized with pentobarbital (50 mg/kg), and intracardiac blood samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Blood plasma was harvested by centrifugation at  $1000 \times g$  for 15 min. Samples were at  $-80$  °C stored until analysis. Commercially available ELISA kits were used to measure the plasma concentrations of estradiol (Estradiol EIA Kit; Cayman Chemical Company, Ann Arbor, MI, USA) and testosterone (Testosterone EIA Kit; Cayman Chemical Company, Ann Arbor, MI, USA).

### 2.7. Morphological analysis

At the 27th week of age, the sixth lumbar vertebra was used for trabecular bone structure analysis. The sixth lumbar vertebra was scanned in the range between 500 and 600  $\mu\text{m}$ , from the caudal side toward the rostral side, with a voxel size of 15  $\mu\text{m}$ , by using a bench-top micro-CT system (inspeXio SMX-90CT; Shimadzu Corp., Kyoto, Japan). The trabecular volume/total volume (BV/TV) ratio, which is the volume ratio of the bone marrow and bone; trabecular thickness (Tb.Th), which is the width of trabecular bone; trabecular number (Tb.N), which is the number of trabeculae per unit length (1 mm); and trabecular separation (Tb.Sp), which is the distance between trabeculae, were analyzed with TRI/3D-BON software (Ratoc System Engineering Co., Ltd., Tokyo, Japan).

### 2.8. Statistical analysis

All data are presented as the mean  $\pm$  standard error (SE). Data were analyzed with the analysis of variance (ANOVA) test for multiple comparisons, followed by Tukey's test; the Tukey–Kramer

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