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Assessment of enhancement of peak bone gain by isoflavone enriched standardized soy extract in female rats

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ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form
24 January 2014
Accepted 27 January 2014
Available online 24 February 2014

Keywords:
Bone histomorphometry
Estrogen deficiency
Osteoporosis
Fracture risk
Soy isoflavones

ABSTRACT

In a longitudinally designed study, we tested whether an isoflavone enriched soy extract (SE) stimulated peak bone gain in rats during growth and maturity so as to confer better bone conserving effect after ovariectomy with concurrent treatment discontinuation. Weaned female rats were given SE or vehicle for 12-weeks and bone parameters were recorded (baseline). One group was then ovariectomized (OVx) and the other group sham operated. Vehicle group after OVx was given 17β-estradiol (E2) or continued with vehicle (OVx + vehicle). SE group after OVx was given vehicle (SEV). After 12-weeks, all groups were killed (endpoint). At baseline, SE group had greater cortical bone parameters over control. At endpoint, SEV group displayed significant bone conservation which was comparable to OVx + E2 group. Data suggest that SE enhanced peak bone accrual that supported skeletal preservation post-OVx on a par with E2 supplementation, not withstanding SE withdrawal at OVx.

1. Introduction

Bone mass after menopause is critically dependent upon the bone quantity attained during young adulthood when peak bone mass is accrued. Peak bone mass has a vital role in bone health. Several longitudinal studies recording the bone mass and strength through childhood and adolescence, and mathematical models suggest that modifying peak bone mass has direct consequence on skeletal fragility in old age. Peak bone mass is a combination of multiple factors, with genetic impact being paramount, although several environmental and nutritional factors of varying importance control bone gains during childhood and adolescence (Bonjour, Chevalley, Rizzoli, & Ferrari, 2007; Matkovic, Fontana, Tominac, Goel, & Chesnut, 1990; Orwoll, Belknap, & Klein, 2001).

Interest in soybeans and soy-based products has strongly increased in the last decades due to their reported nutritional and health-promoting effects (Fengjuan et al., 2013; Marazza et al., 2013; Muguruma et al., 2012). Women from East Asian countries have about 50% reduced chance of experiencing a hip fracture compared to their Caucasian counterparts (Kanis, 1994; Kwok et al., 2012; Seeman, 1998). One explanation is that lifelong habitual consumption of soy-based food exerts a protective effect in postmenopausal women, either by

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http://dx.doi.org/10.1016/j.jff.2014.01.029
1756-4646/

increasing peak bone mass or by decreasing the rate of bone loss after menopause or both. A study to investigate the effect of soy isoflavones intake on the maintenance of peak bone mass in a cohort of 132 women aged 30–40 years who were followed up for 3 years revealed that soy intake had a significant effect on the maintenance of spinal BMD (Ho, Chan, Yi, Wong, & Leung, 2001). Although these studies show potential beneficial skeletal effects of soy isoflavones in peri- or postmenopausal women, there are no known publications addressing the question, whether soy extract supplementation enriched in isoflavones has a functional effect on peak bone mass assessed by its ability to resist osteopenia after menopause. Accordingly, we sought to determine the causal effect of a standardized soy extract on skeletal outcome in a longitudinal study in which female rats at weaning were treated with either vehicle or soy extract by oral gavage for 12 weeks, followed by concurrent induction of estrogen (E2) deficiency by ovariectomy (OVx) and withdrawal of the treatment with soy extract for another 12 weeks.

2. Materials and methods

2.1. Reagents and chemicals

Calcein, tetracycline and E2 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fragments of type 1 collagen (CTx) ELISA kits was purchased from Immunodiagnostic Systems Ltd (Tyne & Wear, Boldon, UK).

2.2. Composition of SE extract

Soybean extract (Soy Life™ capsule, 750 mg each and product number 000005) standardized to contain 3% isoflavone was purchased from Holland and Barrett (London, UK) and referred as SE hereafter. Isoflavone compositions in SE were daidzein and daidzin (1.6%), glycitin and glycitein (~1.0%) and, genistein and genistin (0.33%). Soy saponin content in SE was 3.0%. The powdered extract present in the capsule was taken out, weighed and saved at room temperature in a desiccator for further use as described below. According to the United States Department of Agriculture Database, isoflavone content of raw soybean mature seed is 0.034% (Bhagwat, Haytowitz, & Holden, 2008). Therefore, SE with 3% isoflavone content is equivalent to 8800 g of raw soybean seed.

2.3. Experimental design

OVx rat exhibits most of the characteristics of human postmenopausal osteoporosis and has been recommended by the World Health Organization as a preferred preclinical animal model for the testing of therapeutic agents for anti-osteoporosis (Lelovas, Xanthos, Thoma, Lyritis, & Dontas, 2008). Accordingly, we used female rats in our study. Fifty recently weaned (21–22d old) female Sprague Dawley rats (25–30 g) were purchased from the Laboratory Animal Facility Division of CSIR-Central Drug Research Institute (CDRI) for the study. The animal experimental protocol in this study was approved by the Institutional Animal Ethical Committee (IAEC) at CDRI and the study was conducted in compliance with the standards and guideline mentioned by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The CPCSEA registration number of the IAEC of CDRI is 34/1999 and approval number of the study protocol is IAEC/2012/136/Renew 04 dated August 08, 2012 with a validity of 1 year.

Animals were treated with either vehicle or SE (50- and 100 mg/kg p.o. that were equivalent to 1.5- and 3.0 mg/kg isoflavones) by gavage for 12 weeks, followed by concurrent induction of E2 deficiency by OVx and withdrawal of SE treatment for another 12 weeks (Fig. 1). Vehicle treated rats that were continued with vehicle post-OVx have been referred as OVx + vehicle group. Skeletal effects were evaluated by static histomorphometry (bone microarchitecture using μCT), dynamic histomorphometry (accrual of bone and mineral in vivo), biomechanical strength and bone resorption marker, urinary levels of C-terminal crosslink of type 1collagen (CTx) by ELISA. To estimate the bone conserving effect of SE withdrawal in OVx rats (henceforth SEV group), 17-β estradiol (E2) was used as a reference hormone (OVx + E2 group).

2.4. μCT scan and analysis

In vivo μCT scans of femur were obtained after 12 weeks treatment (baseline) after anesthetizing rats with ketamin (90 mg/kg) and xylazine (10 mg/kg) during the scan, which lasted about 20 min. We followed our previously described protocol (Sharan et al., 2011). Briefly, from in vivo scan, hundred projections were acquired at 360° angular range with special mode width adjusted to full. Reconstruction was carried out using a modified Feldkamp algorithm using the Sky Scan Nrecon software. The X-ray source was set at 70 kV and 100 mA, with a pixel size of 18 μm. A hundred projections were acquired over an angular range of 180°. The image slices were reconstructed using the cone-beam reconstruction software version 2.6 based on the Feldkamp algorithm (Skyscan). For cortical bone analysis, 350 serial image slides were discarded from growth plate to exclude the trabecular region, and 200 consecutive image slides were selected and quantification was done using CTAn software. Various cortical parameters (2D) were analyzed by following previously published protocols (Sharan et al., 2011).

Ex vivo μCT scanning of femur and L5 vertebrae was carried out using the Sky Scan 1076 μCT scanner (Aartselaar, Belgium) as described (Khan et al., 2012). The bone samples were scanned in batches of three at a nominal resolution (pixels) of 18 μm. Reconstruction and analysis of the scanned images are similar to that mentioned above.

2.5. Three-point bending and vertebral compression tests

After ex vivo μCT scans, femurs were subjected to three-point bending test using TK-252C (Muromachi Kikai Co. Ltd., Tokyo, Japan) following our previously published protocol (Sharan et al., 2010). All femur specimens were tested with a load applied at a constant rate. This test predominantly measured breaking force.

Following endplate removal, the fifth lumbar vertebrae (L5) from each rat was isolated for compression testing using TK-252C. We followed our previously published protocol