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Serum equol, bone mineral density and biomechanical bone strength differ among four mouse strains

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Abstract

The extent of conversion of daidzein to its metabolite, equol, by intestinal microflora may be a critical step that determines if a diet rich in daidzein protects against the deterioration of bone after estrogen withdrawal. The objective was to determine the extent that daidzein is converted to equol. In addition, bone mineral content (BMC), bone mineral density (BMD) and strength of femurs and lumbar vertebrae (LV) in four mouse strains were measured. Mice were ovariectomized and fed control diet (AIN93G) with or without daidzein (200 mg daidzein/kg diet) for 3 weeks, after which serum, femurs and LV were collected. Serum daidzein and equol were elevated in all mice fed daidzein. Among mice fed daidzein, the CD-1 and Swiss–Webster (SW) mice had higher ($P<.001$) serum equol than C57BL/6 (C57) and C3H mice. Differences due to mouse strain were observed for all bone outcomes. C57 mice had lower femur BMC ($P<.001$), BMD ($P<.001$) and peak load at femur midpoint ($P<.001$) and neck ($P<.001$) than other mouse strains. C57 mice also had a lower femur midpoint yield load ($P<.001$) and resilience ($P<.001$) than C3H mice. C57 mice had a lower LV1–4 BMC ($P<.001$) and BMD ($P<.001$) compared with all mouse strains and peak load of LV3 was lower than CD-1 and SW mice. Differences in serum equol, BMD and bone strength properties should be considered when selecting a mouse strain for investigating whether dietary strategies that include isoflavones preserve bone tissue after ovariectomy.

Keywords: Daidzein; Equol; Bone biomechanics; Bone mineral density; Ovariectomized mice

1. Introduction

Several human and animal feeding trials have demonstrated that soy isoflavones prevent or attenuate the loss of bone mineral density (BMD) that occurs after estrogen withdrawal [1–7]. However, some studies have reported that isoflavones do not protect against bone loss [8–12] or have positive effects at only specific sites of the skeleton [13–17]. These incongruent findings among some studies may be because not all humans, and perhaps rodents, can metabolize the isoflavone daidzein to its metabolite equol.

Equol is the final metabolite resulting from the metabolism of daidzein in the intestine. Due to its high estrogenic activity, it may be the predominant mediator of beneficial effects of daidzein on bone [18]. Not all humans metabolize daidzein to equol. It is reported that approximately 30–50% of adult humans who consumed soy on a regular basis did not excrete equol [18]. The reasons why some individuals are "equol producers" while others are not are unclear. It may be due to differences in the type and amount of bacteria that is found in the gut and/or may have a genetic basis [18]. There are several studies that support the hypothesis that equol is an important mediator of biological effects on bone tissue. For example, a study in postmenopausal women demonstrated that women who metabolized daidzein to equol experienced preservation of lumbar spine BMD, unlike women who did not produce equol [19]. A study in ovariecomtized rats demonstrated that daidzein was more effective than genistein at protecting against the deterioration of BMD and biomechanical bone strength but conversion to equol was not measured [7]. Thus, there is evidence that the benefits of soy may be related to daidzein and its ability to be converted to equol. A recent study in ovariectomized mice provides the first evidence that equol can preserve BMD of the whole femur, proximal femur, as well as lumbar spine and whole-body BMD [20].

Ovariectomized rodents are commonly used as animal models for evaluating the effectiveness of dietary interventions to prevent and/or manage postmenopausal osteoporosis

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[6,14,16,21]. The ovariectomized mouse model is often used because mice are widely available, responsive to isoflavone treatment [14,16,22] and have similar bone metabolism to humans [23]. Furthermore, feeding purified isoflavones or mixtures of isoflavones can be expensive, as feeding trials are generally a minimum of 8–12 weeks to allow for sufficient alterations in bone metabolism due to ovariectomy. Thus, from a practical standpoint, the mouse is an ideal model as they consume markedly less food than rats. In addition, when selecting a mouse model to test the effectiveness of soy or isoflavone interventions on bone, it is important to know if a specific mouse strain can metabolize daidzein to equol, and if so, to what extent equol is produced. To our knowledge, no study has compared serum equol levels after daidzein feeding in different strains of mice.

Several studies have shown that specific bone parameters such as BMD vary among different strains of mice simply due to genetic differences [24–27]. In this study, two inbred strains (C57BL/6 [C57] and C3H) and two outbred strains (CD-1 and Swiss–Webster [SW]) of mice were investigated. The C57 and C3H strains were chosen as they are frequently used in bone studies because of their contrasting differences in femur BMD and similarities at the lumbar vertebrae 5–6 (LV5–6) [24]. C3H mice have greater femur BMD and femur strength than C57 mice [24–28]. The similarity in BMD and strength of the LV between the C57 and C3H mice may be due to differences in the distribution of cortical and trabecular bone. The skeleton of C3H mice is abundant in cortical bone, but they are deficient in trabecular bone, which is the predominant type of bone in the LV [27]. The outbred strains studied, SW and CD-1 mice, were chosen because they are widely used for studying dietary interventions [29–34].

The primary objective of this study was to determine the extent to which daidzein is converted to equol in four strains of ovariectomized mice (C57, C3H, CD-1 and SW). By design, a 3-week feeding period was used because it is a sufficient duration to observe differences in serum equol after feeding daidzein. Because the findings regarding serum equol are intended to be used in designing future studies of the effects of soy isoflavones on bone metabolism, a secondary objective was to elucidate the differences and similarities of bone outcomes such as bone mineral content (BMC), BMD and biomechanical strength properties of these four strains of mice. Differences in these bone outcomes were not expected due to the short duration of feeding daidzein, and previous studies have reported the effects of long-term soy isoflavones feeding (i.e., >8 weeks) on bone in various strains of rodents [2–4,7,14,16].

2. Methods

2.1. Animals and diets

Four different strains of female, 8-week-old ovariectomized mice (C57, C3H, CD-1 and SW; $n=32$ mice/strain) were obtained from Charles River Canada (Montreal, Quebec, Canada) and housed four per cage. Ovariectomy was performed at Charles River, Canada. Mice were housed in standard clean environmental conditions with a 12-h light/dark cycle throughout the study. Animal care and procedures were conducted according to the guidelines and regulations set out by the Faculty Advisory Committee on Animal Services, University of Toronto, Toronto, Canada, and the Canadian Council on Animal Care [35].

Mice were randomized to receive control diet (AIN93G) [36] (Dyets, Bethlehem, PA) or control diet containing purified daidzein at a level of 200 mg daidzein/kg diet (98% purity, catalogue D-7802, Sigma-Aldrich, Canada, Oakville, ON). Mice were fed either control or daidzein diet 2 weeks after ovariectomy and remained on the diet for a total of 3 weeks. A feeding period of 3 weeks was used to ensure that mice had adapted to the daidzein-containing diet. It was not the intention of this study to determine if daidzein had effects on bone outcomes over this short feeding period as a markedly longer intervention period (≥ 8 weeks) is required to observe changes in BMD or biomechanical strength properties due to isoflavone feeding [14,16].

Every 2–3 days, mice were provided with fresh food, and food intakes were recorded. Food intake per mouse was determined by dividing the total food intake per cage by four. Mice had free access to distilled water throughout the study. At the end of the feeding trial, mice were asphyxiated with CO_2 and killed by cervical dislocation. Whole blood was collected by cardiac puncture immediately prior to cervical dislocation and serum was obtained by centrifugation at 10,000 rpm for 15 min and stored at $-70^{\circ}C$ until later analyses.

2.2. Body weights and uterine weights

Body weights were measured once weekly. At necropsy, uteri were carefully removed and cleaned of fat tissue. Uterine weights were measured to monitor for any estrogenic effects of the daidzein intervention.

2.3. Serum daidzein and equol

Serum concentrations of daidzein and equol were determined in duplicate using commercially available time-resolved fluoroimmunoassay kits (Labmaster Diagnostics, Turku, Finland). Serum samples of mice fed daidzein were diluted 1:2 with assay buffer [14]. Fluorescence was measured at 620 nm using a microplate reader (Fusion Universal Microplate Analyzer, Packard Bell). Sample concentrations were interpolated from the standard curve using Graph Pad software (GraphPad Prism 3.0, Version 3.02, San Diego, CA).

2.4. Femur and LV1–4 BMC and BMD

Femurs and LV1–4 were removed at time of necropsy, cleaned of soft tissue and stored at $-70^{\circ}C$ until analyses were performed. For determination of BMC and BMD,