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Journal of Nutritional Biochemistry 16 (2005) 743–749

Journal of Nutritional Biochemistry

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

Wendy E. Ward*, Susie Kim, Daphne Chan, Debbie Fonseca

Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 3E2

Received 23 February 2005; received in revised form 3 April 2005; accepted 8 April 2005

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

* Corresponding author. Tel.: +1 416 946 7366; fax: +1 416 978 5882.
E-mail address: wendy.ward@utoronto.ca (W.E. Ward).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.04.002

[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,

individual femurs and intact vertebra (LV1–4) were placed on a plastic tray and scanned in air by PIXImus dual energy X-ray absorptiometry (GE Medical Systems, Mississauga, Ontario, Canada) as previously described [14].

2.5. Biomechanical strength testing of femurs and LV3

Biomechanical strength properties of right femurs and LV3 were determined using a material testing system (Model 4442 Universal Testing System, Instron, Canton, MA) and a specialized software program (Instron Series IX Automated Materials Tester-Version 8.15.00, Instron). Bones were rehydrated in physiological saline (9 g NaCl/L) for 4 h at room temperature prior to testing as previously described [14].

2.5.1. Three-point bending at the femur midpoint

Three-point bending was performed on the right femurs to determine the elastic and plastic properties contributing to femur strength (yield load, resilience, peak load, toughness and ultimate stiffness) [14]. Yield load is a measure of the elastic limit of the femur and was determined as the point in which the slope of the load–deformation curve deviates from being a straight line. *Resilience* is a measure of the amount of energy that the femur absorbs until the yield point is reached; *peak load* is a measure of the maximum force that the femur withstands before fracture; toughness is a measure of the work energy that is required to fracture the femur; and *ultimate stiffness* is a measure of the extrinsic rigidity of the femur. This test was carried out at the femur midpoint, which predominately contains cortical bone. The femur midpoint was determined using digital calipers (Cedarlane Laboratories, Hornby ON). The posterior sides of the femur were placed on two base supports of a bending jig separated by 6 mm, with the midpoint directly under the crosshead. The crosshead was lowered at a constant speed of 6 mm/min until fracture occurred. The tips of the bending jig are rounded to minimize shear forces during the test.

2.5.2. Femur neck fracture

Femur neck fractures were performed on the right femurs after the three-point bending test was completed as previously described [14], and the elastic and plastic properties were determined. Individual femurs were placed in a customized holder, and the crosshead was lowered at a constant speed of 6 mm/min, such that direct force was applied directly onto the proximal femur head until the femur neck fractured.

2.5.3. Compression testing of LV3

LV3 from each mouse was isolated for compression testing [14]. This test predominately measures the strength of trabecular bone. Soft tissue and spinous processes were removed prior to testing. Individual LV3 were placed in the center of a smooth stainless steel plate, and a compression force was applied to the vertebra by lowering a second suspended stainless steel plate at a constant rate of 2 mm/min. Visual inspection during each test verified that a vertebra remained in a stable position. Compression force was applied, and peak load of compression was determined to be the first peak on the load–deformation curve.

2.6. Statistical analyses

Data were analyzed by two-way ANOVA using mouse strain and diet as main effects as well as testing for interactions (Mouse Strain×Diet). Where statistical interactions were observed, Student–Newman Keul's test was used for comparison of multiple means (SigmaStat, Jandel, San Rafael, CA). Differences were considered significant if $P<.05$.

3. Results

3.1. Food intakes and body weight

Both mouse strain ($P<.001$) and diet ($P=.024$) had a significant effect on average daily food intake, however, the Mouse Strain×Diet interaction was not significant (Table 1). C57 mice had a lower average daily food intake than all other mouse strains, and mice fed daidzein had a higher average daily food intake than mice not fed daidzein (Table 1). Initial body weights differed due to mouse strain ($P<.001$) but did not differ due to diet (Table 1). C57 mice had lower initial body weight than all other mouse strains (Table 1). In addition, C3H mice had lower initial body weight than SW and CD-1 mice. Both mouse strain ($P<.001$) and diet ($P=.009$) had a significant effect on

Table 1
Average daily food intake, initial and final body weights and uterine weights

	Diet	C57	C3H	CD-1	SW	Mouse strain	Diet	Mouse Strain×Diet
Food intake (g)	C	2.92±0.04	3.36±0.06	3.38±0.07	3.29±0.08	<.001	.024	NS
	D	3.10±0.07	3.38±0.06	3.49±0.07	3.30±0.06			
Initial body weight (g)	C	23.1±0.3	24.7±0.3	27.9±0.3	26.8±0.5	<.001	NS	NS
	D	22.6±0.3	24.1±0.3	28.3±0.6	25.9±0.5			
Final body weight (g)	C	26.3±0.4	30.1±0.6	30.8±1.0	29.8±0.8	<.001	.009	NS
	D	27.6±0.8	29.9±0.5	33.3±0.8	31.6±0.6			
Uterine weight (mg/kg body weight)	C	798.3±85.0	973.0±89.2	798.6±68.0	741.9±45.2	NS	NS	NS
	D	990.9±106.1	804.7±87.5	730.4±74.6	701.9±97.3			

Values are expressed as mean ± S.E.M. C = control diet; D = daidzein diet (200 mg daidzein/kg diet).

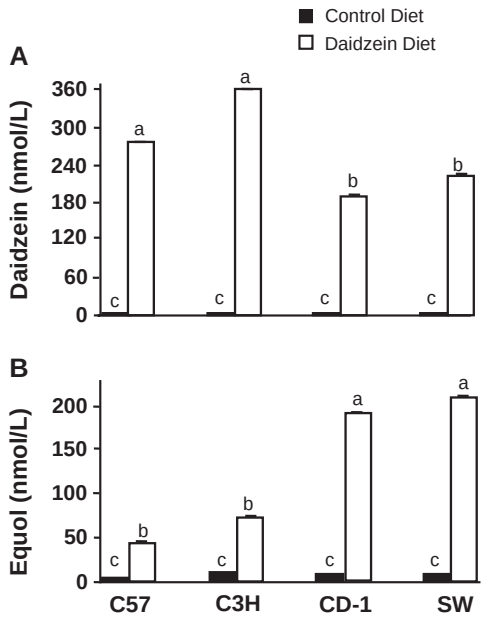

Fig. 1. Serum concentrations of (A) daidzein and (B) equol. Values are expressed as mean ± S.E.M. Bars with different letters, $P < .05$.

final body weight but the Mouse Strain×Diet interaction was not significant (Table 1). Final body weight was lower among C57 mice compared with all other mouse strains, and mice fed daidzein diet had greater final body weights than mice not fed daidzein (Table 1).

3.2. Serum daidzein and equol

All mice fed daidzein had higher ($P < .001$) serum daidzein than mice not fed daidzein, and there was a significant difference ($P = .002$) due to mouse strain (Fig. 1A). The Mouse Strain×Diet interaction was significant ($P = .001$), as C57 and C3H mice fed daidzein had higher serum daidzein than CD-1 and SW mice fed daidzein (Fig. 1A). Mice fed daidzein also had higher ($P < .001$) serum equol than mice not fed daidzein. There was also a significant effect due to mouse strain ($P < .001$) (Fig. 1B). The Mouse Strain×Diet interaction was significant ($P < .001$), as SW and CD-1 mice fed daidzein had significantly higher serum equol than C57 and C3H mice fed daidzein (Fig. 1B).

3.3. Femur BMC, BMD and biomechanical strength properties at the femur midpoint and femur neck

Femur BMC ($P < .001$) and BMD ($P < .001$) were significantly different due to mouse strain but not diet (Table 2). C57 mice had a lower BMC and BMD than all other mouse strains, and C3H mice had a higher BMD than CD-1 mice (Table 2). At the femur midpoint, mouse strain

Table 2
Whole femur BMC and BMD and biomechanical strength properties at the femur midpoint and femur neck

	Diet	C57	C3H	CD-1	SW	Mouse strain	Diet	Mouse Strain×Diet
Whole femur								
BMC (mg)	C	25.2±0.4	31.0±0.6	32.1±0.6	29.2±1.1	<.001	NS	NS
	D	25.4±0.4	30.2±0.5	32.4±0.8	31.9±1.3			
BMD (mg/cm^2)	C	52.2±0.3	64.7±0.5	61.9±0.6	61.7±1.3	<.001	NS	NS
	D	52.4±0.5	64.9±0.7	62.7±0.6	64.5±1.5			
Femur midpoint								
Yield load (N)	C	10.0±0.4	19.6±0.5	12.4±0.4	6.1±1.3	<.001	<.019	NS
	D	10.3±0.5	19.9±0.5	14.9±0.9	6.7±1.2			
Resilience (×10^{-4} J)	C	3.8±0.4	6.7±0.1	3.9±0.6	2.0±0.4	<.001	NS	NS
	D	3.3±0.6	7.3±0.1	4.8±0.1	4.1±0.5			
Peak load (N)	C	18.0±0.4	27.4±0.5	23.3±0.7	20.3±0.7	<.001	NS	NS
	D	17.8±0.4	28.1±0.6	25.6±0.9	20.8±0.6			
Toughness (×10^{-3} J)	C	8.7±1.1	6.4±0.7	7.5±0.6	9.3±0.7	<.001	.011	NS
	D	11.9±1.6	7.5±0.8	8.2±0.8	10.5±0.5			
Ultimate stiffness (N/mm)	C	132.8±5.2	223.0±9.0	181.9±6.5	149.2±7.4	<.001	NS	NS
	D	136.2±4.6	234.7±5.2	188.1±8.6	147.2±6.3			
Femur neck								
Yield load (N)	C	6.6±0.9	10.6±1.4	11.5±1.7	6.3±0.9	.002	NS	NS
	D	7.5±0.7	7.2±0.9	10.0±0.8	7.6±0.8			
Resilience (×10^{-4} J)	C	4.0± 1.0	4.5±0.6	5.0±0.9	3.1±0.7	NS	NS	NS
	D	3.8±2.2	3.3±0.9	6.2±1.2	3.2±0.6			
Peak load (N)	C	11.9±1.2	16.2±1.1	17.6±0.9	15.2±1.0	<.001	NS	NS
	D	11.7±0.8	14.1±1.2	18.2±0.8	15.5±0.9			
Toughness (×10^{-3} J)	C	3.4±1.0	2.8±0.5	2.9±0.5	2.5±0.4	NS	NS	NS
	D	2.4±0.4	2.1±0.3	3.9±0.6	2.1±0.3			
Ultimate stiffness (N/mm)	C	77.1±5.7	112.6±8.9	122.6±9.8	112.5±18.7	<.001	NS	NS
	D	88.8±6.1	103.6±5.9	104.2±5.1	108.8±5.9			

Values are expressed as mean ± S.E.M. C = control diet; D = daidzein diet (200 mg daidzein/kg diet).

Table 3
BMC and BMD of LV1–4 and peak load of LV3

	Diet	C57	C3H	CD-1	SW	Mouse strain	Diet	Mouse Strain×Diet
BMC (mg)	C	37.5±0.8	46.8±5.2	48.3±1.4	45.6±2.2	<.001	NS	NS
	D	36.5±0.8	46.0±1.3	49.1±1.3	48.4±1.5			
BMD (mg/cm^2)	C	56.1±0.6	61.0±0.7	66.3±1.0	63.9±1.8	<.001	NS	NS
	D	55.9±0.8	60.1±1.0	65.3±1.0	64.6±1.6			
Peak load (N)	C	29±1	35±3	44±5	47±4	<.001	NS	NS
	D	32±2	34±2	46±5	45±3			

Values are expressed as mean ± S.E.M. C = control diet; D = daidzein diet (200 mg daidzein/kg diet).

had a significant effect on all outcomes measured, including yield load (P<.001), resilience (P<.001), peak load (P<.001), toughness (P<.001) and ultimate stiffness (P<.001) (Table 2). C3H mice had a higher yield load than all other mouse strains while CD-1 mice had a higher yield load than the C57 and SW mice (Table 2). All mouse strains had a higher peak load than the C57 mice (Table 2). The C3H and CD-1 mice had a higher peak load than the SW mice (Table 2). C3H mice had a higher resilience than C57 and SW mice (Table 2). Both the C57 and SW mice had a greater toughness than the C3H and CD-1 mice. The C3H mice had a greater ultimate stiffness than all other mouse strains while CD-1 mice had a greater ultimate stiffness than C57 and SW mice, and SW mice had a greater ultimate stiffness than C57 mice. Diet had a significant effect on both yield load (P=.019) and toughness (P=.011) but there was no Mouse Strain×Diet interaction (Table 2). Mice fed daidzein diet had higher yield load and toughness than mice not fed daidzein (Table 2). At the femur neck, mouse strain had a significant effect on yield load (P=.002), peak load (P<.001) and ultimate stiffness (P<.001). C57 and SW mice had a lower yield load than CD-1 mice (Table 2). C57 mice had a lower peak load and ultimate stiffness than all other mouse strains (Table 2). Diet did not have a significant effect on any of the biomechanical strength properties measured at the femur neck (Table 2).

3.4. LV1–4 BMC, BMD and peak load

Mouse strain had a significant effect on BMC (P<.001) and BMD (P<.001) of LV1–4 and peak load of LV3 (P<.001) while diet had no effect on any of these outcomes (Table 3). C57 mice had a lower LV1–4 BMC and BMD than all other mouse strains (Table 3). C3H mice had a lower LV1–4 BMD than CD-1 and SW mice (Table 3). C57 and C3H mice had a lower peak load of LV3 than CD-1 and SW mice (Table 3).

3.5. Uterine weights

Neither mouse strain nor diet had a significant effect on uterine weights among groups (Table 1).

4. Discussion

Because equol, the metabolite of daidzein, may have a greater biological effect on bone than isoflavones such as daidzein, selecting a mouse strain that metabolizes a notable quantity of daidzein to equol is ideal. The findings of this study suggest that both CD-1 and SW strains may be the more appropriate strain of mouse, compared with C57 and C3H mice, for studying if soy- or isoflavone-containing diets prevent or attenuate bone loss after ovariectomy. CD-1 and SW mice had serum equol levels that were 19 and 21 times, respectively, higher than their respective controls not fed daidzein. In contrast, the C57 and C3H mice experienced a much smaller increase in serum equol, with serum levels that were 5 and 7 times, respectively, higher than mice not fed daidzein. Of particular interest was the finding that CD-1 and SW mice had 2.5–4 and 2.8–4.2 times, respectively, higher levels of serum equol than the C57 and C3H strains. As might be expected, the CD-1 and SW strains that had the highest levels of equol also had the lowest levels of daidzein. It is possible that a mouse strain that produces a maximal amount of equol would result in the greatest protection against deterioration of bone after ovariectomy. Indeed, Setchell et al. [18] remarks that some of the conflicting data with respect to effects of soy or isoflavone interventions on bone may, in fact, be due in part to differences in the extent of the conversion of daidzein to equol in humans and animals.

In a previous study from our laboratory, C57 mice were used to determine whether daidzein, at either a low (100 mg daidzein/kg diet) or high (200 mg daidzein/kg diet) dose of daidzein prevented a loss of BMD and biomechanical strength at multiple sites: femur midpoint, femur neck and LV [14]. The bone outcomes were not as anticipated. Although the high-daidzein diet, but not the low-daidzein diet, preserved femur BMC and BMD to the level of shams, the detrimental effect of ovariectomy on LV outcomes such as BMC, BMD and peak load of LV3 were not attenuated [14]. It is possible that, with a different mouse strain such as the CD-1 or SW strain, more protective effects on bone outcomes would have been observed due to a higher conversion of daidzein to equol. To date, only one study has reported the direct effects of equol on bone metabolism in vivo [20]. In this study, equol was administered as a continuous, subcutaneous infusion and was shown to preserve BMD at various sites in ovariectomized ddY mice [20].

In addition to considering serum equol levels after feeding daidzein, the bone characteristics including BMD

and biomechanical bone strength properties of a mouse strain need to be considered. Our study has also shown, in general, that the two outbred strains, CD-1 and SW mice, also have femurs with greater BMD and biomechanical strength properties compared with C57 mice. With respect to LV, C57 and C3H had similar vertebral strength that was lower than the two outbred strains. Unlike some previous reports, BMD of LV were higher among C3H mice compared with C57 mice, but both mouse strains had a lower BMD of LV than CD-1 and SW mice.

The differences in bone outcomes among mouse strains are likely not simply due to the fact that these strains of mice, by nature, have contrasting body weights. For example, BMD is calculated as BMC divided by the area of the bone, and thus BMD measurement is proportionate to bone size. An unexpected finding was the higher food intake among mice fed daidzein diet, and it is likely that this phenomenon explains the higher body weight at necropsy among mice fed daidzein compared with mice fed control diet. Differences in bone outcomes may also be due to innate differences in rates of bone formation and bone resorption and calcium metabolism [28,37,38]. For example, C57 and C3H mice have been shown to have significantly different rates of bone formation, possibly due to differences in the number of osteoblasts [37]. Other mechanisms to explain differences between C57 and C3H mice include differences in intestinal calcium absorption and bone volume [28,38].

As expected due to the short feeding time of 3 weeks, differences in uterine weights were not observed. In addition, it was also expected that differences in bone outcomes due to daidzein diet would be minimal because of the short study duration. In designing this study, it was not our objective to evaluate the effects of daidzein on bone outcomes as a 3-week feeding intervention is too short to observe effects on bone outcomes. Rather, the primary objective was to observe whether the conversion of daidzein to equol differed among different strains of mice. While daidzein diet resulted in a greater yield load and toughness at the femur midpoint compared with mice fed control diet devoid of daidzein, no interaction of mouse strain and diet was observed. To observe differences in bone outcomes due to dietary interventions, studies in ovariectomized rodents are usually a minimum of 8 weeks in duration [14,16].

In summary, these findings demonstrate that mice, like humans, can metabolize daidzein differently, with significant differences in the levels of equol produced, depending on the mouse strain that is chosen. Most notably, the C57 and C3H strains have a markedly lower production of equol than the CD-1 and SW strains when fed same level of purified daidzein. Thus, these findings are important to consider when selecting a mouse model for studying the effects of a dietary intervention that includes soy, a mixture of isoflavones or purified daidzein, as it is prudent to ensure the level of equol production that occurs in a specific mouse strain. Mouse strain should also be an important consideration when interpreting the results of studies that have used rodents to test the effects of soy or isoflavones on bone.

Acknowledgments

The authors are grateful to the Natural Sciences and Engineering Research Council (NSERC) of Canada for a Discovery Grant to W. Ward and a summer studentship to S. Kim.

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