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Research Article

Soy isoflavones and fatty acids: Effects on bone tissue postovariectomy in mice

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Osteoporosis is a silent disease that leads to fragility fractures that can diminish quality of life and contribute to death. With no ideal drug treatment available to manage osteoporosis, soy isoflavones (ISO), and omega-3 long chain PUFAs in fish oil (FO) may be integral in a dietary strategy that prevents bone loss. The overall objective of this study was to determine if combining ISO with omega-3 long chain PUFAs resulted in greater protection against the loss of bone mineral and skeletal weakening in ovariectomized mice. Ovariectomized CD-1 mice were randomized to control diet or a diet containing ISO alone (250 mg of genistein + 250 mg of daidzein/kg diet), FO alone (7% menhaden oil), or ISO + FO. Each dietary intervention prevented the loss of bone mineral density (BMD) in the femur and preserved femur strength, but only FO, either alone or combined with ISO, resulted in a higher BMD of lumbar vertebra (LV). Most notably, FO + ISO resulted in a higher peak load of LV4, indicating that vertebra were more resistant to fracture. Whether a dietary strategy providing FO in combination with ISO attenuates bone loss in postmenopausal women awaits investigation.

Keywords: Biomechanical bone strength / Bone mineral density / Fish oil / Isoflavones / Mice

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1 Introduction

A recent government report, "Bone Health and Osteoporosis" estimates that by 2020, one in two Americans over age 50 will be at risk for fractures from osteoporosis or low bone mass [1]. Similar estimates exist for other developed countries [2–6]. The report also states that skeletal health "appears to be in jeopardy, and left unchecked it is only going to get worse as the population ages" [1]. The overwhelming statistics identifying unacceptable morbidity and mortality due to poor bone health emphasize the urgent need to develop interventions to combat osteoporosis. Treatment as well as prevention strategies are urgently needed to slow the rise in osteoporosis and its related fragility fractures.

Dietary strategies that incorporate multiple novel food components may provide protection against the deterioration of bone tissue after cessation of estrogen production. Some but not all feeding studies in humans and animals have demonstrated that soy isoflavones (ISO) prevent or slow the loss of bone mineral that occurs in the absence of ovary-produced estrogen [7–11]. As summarized by Weaver and Cheong [8], the variable results among studies may be due to differences in the stage of the life cycle of subjects, differences in dose administered, interactions of ISOs with other dietary components (either synergistic or opposing effects), lack of dietary control, and whether subjects produce equol or not. Equol is a metabolite that results from the metabolism of daidzein in the intestine, and may have greater biological activity than ISO such as daidzein or genistein due to its higher estrogenic activity [12]. While mechanisms of ISO were initially shown to occur *via* estrogenic activity, it is now known that ISO may also act through non-estrogenic mechanisms.

Consumption of fish oil (FO), rich in omega-3 long chain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has also been shown to favorably modulate bone metabolism, possibly *via* modulating inflammatory mediators including prostaglandin E_2, IL-1β, IL-6, and TNF-α [13–15] and/or directly affect intestinal calcium absorption [16]. One study has reported that feeding a diet containing soy ISO and FO to young (2 months old) ovariectomized rats protected against loss of tibia bone mineral content (BMC) but not bone mineral density

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Abbreviations: BMC, bone mineral content; **BMD,** bone mineral density; **DHA,** docosahexaenoic acid; **EPA,** eicosapentaenoic acid; **FO,** fish oil; **ISO,** isoflavones; **LV,** lumbar vertebra

(BMD), and reduced serum pyridinoline crosslinks, a biochemical marker of osteoclastic acitivity [17]. The effect at other skeletal sites such as lumbar spine and effects on bone quality, including resistance to fracture are uncertain.

This study was designed to investigate if soy ISO, specifically genistein and daidzein, in combination with omega-3 long chain PUFAs present in FO, protected against the loss of BMD and weakening of bones to a greater extent than ISO or FO alone in the ovariectomized mouse model.

2 Materials and methods

2.1 Animals and diets

All procedures were carried out in accordance to the policies set out by the Canadian Council on Animal Care [18] and were approved by the Animal Ethics Committee at the University of Toronto, Toronto, Canada.

Sixty CD-1 mice were obtained from Charles River Canada at 6 months of age ($n = 12$ sham (SH) mice, $n = 48$ ovariectomized mice). Mice were housed four *per* cage and allowed to adapt to their new environment for 1 wk before being randomized into one of the four interventions: ovariectomy alone (OVX), ISO, FO, or ISO + FO. SH mice were fed modified AIN93G diet (containing 10% safflower oil/kg diet). The dietary interventions were the following: ISO group received control diet supplemented with 250 mg of genistein + 250 mg of daidzein/kg of diet; FO group received control diet with 7% menhaden oil + 3% safflower oil; and the ISO + FO group received control diet with 250 mg of genistein + 250 mg of daidzein/kg of diet and 7% menhaden oil + 3% safflower oil. All diets were supplied by Dyets (Bethlehem, PA), contained 10% fat, and were isocaloric. Purified ISO were purchased from Sigma Chemical (Mississauga, ON). The fatty acid composition is similar to that previously published [17] in which safflower oil was used as the oil in the control diet, and was blended with menhaden oil to make the FO diet or the isoflavone + FO diet. The composition of the oils and ISO used in the diets are provided in Table 1. Mice were pair-fed against the SH group to maintain similar body weights among groups as hyperphagia often results after ovariectomy. Mice were provided with fresh food every 2 – 3 days. Distilled water was provided on demand for the duration of the study. Mice were fed these diets for a total of 12 wk while being housed in standard clean environmental conditions with a 12-h light/dark cycle during the intervention period. Body weights were measured on a weekly basis using a digital portable scale (Model XP-1500, Denver Instrument). At the end of the 12 wk intervention, mice were euthanized with CO_2 and blood was collected *via* cardiac puncture. Because of the circadian variation of serum biochemical markers, all mice were killed in the morning. Uteri were weighed. Femurs and lumbar vertebrae (LV1 – LV4) were excised, cleaned of soft tissue, and stored at $-70^{\circ}C$.

Table 1. Fatty acid content of experimental diets (all study diets contained 10% fat. The control diet contained 10% safflower oil, and the FO diet contained 7% menhaden + 3% safflower oil)

Component		Control diet[a)] (mg/kg diet)	FO diet[a)] (mg/kg diet)
Myristic acid	14:0	0.1	6.3
Palmitic acid	16:0	6.9	14.0
Palmitoleic acid	16:1n-7	0.2	8.8
Stearic acid	18:0	2.9	2.8
Oleic acid	18:1n-9	12.2	11.6
Linoleic acid	18:2n-6	78.0	24.4
α-Linolenic acid	18:3n-3	0.1	1.1
Stearidonic acid	18:4n-3	–	2.5
Arachadonic acid	20:4n-6	–	0.6
EPA	20:5n-3	–	10.8
Erucic acid	22:1n-9	–	0.3
Docosapentaenoic acid	22:5n-3	–	1.6
DHA	22:6n-3	–	6.3

a) Daidzein and genistein were added directly to either the control diet or FO diet to make the isoflavone diet and isoflavone + FO diet, respectively. For the isoflavone diet, 250 mg of daidzein and 250 mg of genistein were added to *per* kg of control diet. For the isoflavone + FO diet, 250 mg of daidzein and 250 mg of genistein were added to *per* kg of FO diet. The purity for both the daidzein and genistein was a minimum of 98% (Sigma Chemical).

2.2 Bone mineral density (BMD) of femur and LV1 – LV4

Whole left femurs and intact spines (LV1 – LV4) were placed on a plastic tray and scanned in air for determination of BMD using PIXImus dual energy X-ray absorptiometry (DEXA) (LUNAR Corporation, GE Medical Systems, Mississauga, Ontario, Canada) and a specialized software program (Lunar Software Version 1.46) [11].

2.3 Biomechanical strength testing of femurs and LV4

Biomechanical strength testing was performed at the femur midpoint and LV4 as previously described [11]. Femurs and LV4 were soaked in physiological saline (9 g NaCl/L) for 3 h at room temperature prior to testing. Three point bending at the femur midpoint and compression testing of LV4 were performed 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).

2.3.1 Three point bending at the femur midpoint

Femur weight was measured by electronic scale, and femur dimensions (length and width in two directions) were measured using an electronic precision caliper (Cedarlane Laboratories, Hornby, ON). The width at the midpoint of the

femur, the site of testing, was measured in two directions to represent the mediolateral width (width) and anterioposterior width (depth). Femurs were positioned such that the posterior side was placed on two base supports of the bending jig separated by 6 mm and oriented so that the midpoint was directly under the crosshead. The crosshead was then lowered at a constant speed of 2 mm/min, applying force to the femur midpoint until fracture occurred. The tips of the bending jig are rounded to reduce shear forces during the test. From the load–displacement curve that is generated, the elastic and plastic properties of femurs are determined. The following biomechanical strength properties were determined: yield load, resilience, ultimate stiffness, peak load, and toughness. Yield load primarily represents the contribution of mineral to bone strength while peak load primarily represents the contribution of matrix to bone strength. Resilience is the energy the femur absorbs until the yield load is reached, while toughness is the energy the femur absorbs until the peak load (*i.e.*, fracture) is reached. Ultimate stiffness is the extrinsic rigidity of the bone tissue, and relates to the mineral content of the femur.

2.3.2 Compression testing of LV4

LV4 was isolated from the LV1–LV4 and positioned at the center of a smooth stainless steel plate. Height, width, and depth measurements were determined for LV4 using electronic precision calipers. A second stainless steel plate was lowered onto LV4 at a constant speed of 2 mm/min until compression of LV4 was achieved. Peak load was identified as the first peak of the load–displacement curve.

2.4 Serum osteocalcin and serum collagen crosslinks

Blood was centrifuged at 10 000 rpm for 15 min and serum was stored at −70°C. Intact serum osteocalcin was measured using a commercially available enzyme-linked immunoassay specific for mice (Mouse osteocalcin, Biotechnologies, Stoughton, MA). This assay is a sandwich assay in which a polyclonal antibody directed against the N-terminus is bound to the polystyrene wells in the 96-well plate. Serum samples are added to the wells followed by an overnight incubation with biotinylated antibody specific for the C-terminus. Serum collagen crosslinks were measured using a commercially available enzyme-linked immunoassay specific for mice (RatLaps, Nordic Bioscience Diagnostics, Denmark) [19, 20]. This assay quantifies free C-terminal telopeptides of type 1 collagen.

2.5 Statistical analysis

All statistical analysis was carried out using Sigma Stat (Jandel Scientific, Version 2.0 San Rafael, CA). One way analysis of variance (ANOVA) was used to compare treatment groups and Student–Newman–Keuls multiple comparison tests were used to determine differences between treatment groups. Statistical significance was determined at $p < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

3 Results

3.1 Femur outcomes after 12 wk of intervention: Weight, dimensions, BMD, and biomechanical strength properties

There were no significant differences in femur weight or dimensions among groups (Table 2). ISO, FO, and ISO + FO groups had higher ($p < 0.05$) whole femur BMD than OVX group, and a similar BMD to the SH group (Table 2). Femur yield load of the FO group was greater ($p < 0.05$) than the OVX group and was similar to the SH, ISO, or

Table 2. Femur dimensions, BMD and biomechanical strength properties after 12 wk of intervention (values are expressed as mean ± SEM; within a row, values with different superscripts are significantly different (p <0.05))

	SH	OVX	ISO	FO	ISO + FO
Whole femur					
Weight (mg)	130 ± 10	140 ± 10	140 ± 10	130 ± 10	140 ± 10
Length (mm)	17.04 ± 0.14	17.27 ± 0.21	17.33 ± 0.15	17.28 ± 0.10	17.64 ± 0.16
Depth[a)] (mm)	1.42 ± 0.03	1.45 ± 0.04	1.52 ± 0.03	1.51 ± 0.04	1.50 ± 0.04
Width[b)] (mm)	1.76 ± 0.04	1.76 ± 0.06	1.78 ± 0.05	1.83 ± 0.03	1.82 ± 0.03
BMD (mg/cm^2)	69.53 ± 0.92^a	65.30 ± 1.07^b	71.35 ± 2.03^a	74.39 ± 1.56^a	74.62 ± 1.34^a
Femur midpoint					
Yield load (N)	34.86 ± 1.82^{a,b}	32.33 ± 1.08^b	34.52 ± 1.32^{a,b}	39.29 ± 1.52^a	36.56 ± 0.82^{a,b}
Resilience (J × 10^{-4})	3.22 ± 0.34	5.20 ± 1.15	3.46 ± 0.12	4.68 ± 1.15	3.38 ± 0.30
Peak load (*N*)	47.80 ± 1.62^{a,b}	43.97 ± 1.39^b	51.61 ± 2.32^a	53.34 ± 1.27^a	52.59 ± 1.05^a
Toughness (JE × 10^{-3})	14.34 ± 1.79	19.51 ± 2.60	23.05 ± 3.86	19.54 ± 1.72	21.25 ± 3.24
Stiffness (N/mm)	239.11 ± 13.29^a	190.74 ± 13.36^b	271.10 ± 21.05^a	285.44 ± 17.84^a	268.40 ± 13.11^a

a) Depth refers to the anteroposterior width at the midpoint of the femur.
b) Width refers to the mediolateral width at the midpoint of the femur.

Table 3. Dimensions of LV4 after 12 wk of intervention (values are expressed as mean ± SEM)

	SH	OVX	ISO	FO	ISO + FO
Weight (mg)	70 ± 10	90 ± 10	90 ± 10	90 ± 10	80 ± 10
Length (mm)	4.15 ± 0.11	4.06 ± 0.20	4.39 ± 0.13	4.38 ± 0.13	4.55 ± 0.20
Depth[a)] (mm)	3.61 ± 0.10	3.47 ± 0.10	3.53 ± 0.09	3.64 ± 0.06	3.62 ± 0.09
Width[b)] (mm)	3.38 ± 0.07	3.28 ± 0.06	3.52 ± 0.08	3.41 ± 0.07	3.48 ± 0.10

a) Depth refers to the anteroposterior width of LV4.
b) Width refers to the mediolateral width of LV4.

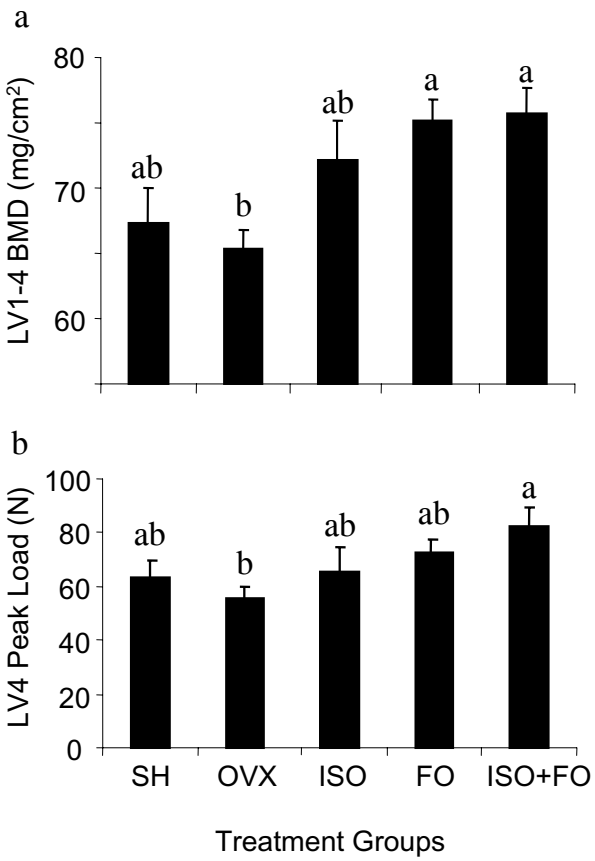

Figure 1. (a) BMD (mg/cm²) of LV1 – LV4 and (b) peak load (*N*) of LV4 after 12 wk of intervention. Data are expressed as mean ± SEM. Bars with different letters, $p < 0.05$.

ISO + FO groups (Table 2). Femur peak load and ultimate stiffness of the ISO, FO, and ISO + FO groups were significantly higher ($p < 0.05$) than the OVX group and similar to the SH group (Table 2). Resilience and toughness did not differ among groups (Table 2).

3.2 Lumbar vertebrae outcomes after 12 wk of intervention: BMD of LV1 – LV4, and weight, dimensions, and peak load of LV4

The weight and dimensions of LV4 did not differ among groups (Table 3). Mice fed FO or FO + ISO diet had significantly higher ($p < 0.05$) BMD of LV1 – LV4 than the OVX group but did not differ from the SH and ISO groups (Fig. 1). Peak load of LV4 for the ISO + FO group was significantly higher ($p < 0.05$) than the OVX group but did not differ significantly ($p > 0.05$) from the other treatment groups or SH group (Fig. 1).

3.3 Contribution of whole femur BMC to femur biomechanical strength properties after 12 wk of intervention

The BMC of the whole femur was significantly and positively correlated with yield load ($r = 0.348$, $p = 0.010$), ultimate stiffness ($r = 0.354$, $p = 0.006$), and peak load ($r = 0.430$, $p < 0.001$) (Fig. 2).

3.4 Serum osteocalcin and serum collagen crosslinks after 12 wk of intervention

Biochemical markers of bone turnover, serum osteocalcin, and serum collagen crosslinks were not significantly different among groups (Table 4).

3.5 Uterine weights after 12 wk of intervention

The SH group had heavier ($p < 0.05$) uterine weights than all groups that had been ovariectomized (Fig. 3). There were no significant differences in uterine weight among ovariectomized groups (OVX, ISO, FO, ISO + FO) (Fig. 3).

4 Discussion

The availability of ISO and omega-3 fatty acids in the diet has and continues to increase due to the wide variety of omega-3 enriched foods and soy protein-based foods throughout the world. Findings from the present study demonstrated that after 12 wk of intervention, peak load of LV4 was highest with the combination of ISO and FO, suggesting that trabecular bone, compared to cortical bone, was most responsive to the combination of ISO and FO. Other bone outcomes such as biomechanical strength properties at the femur midpoint, and femur and LV BMD did not differ among intervention groups, demonstrating that the combination of soy ISO and FO was manifested only for peak

Table 4. Serum osteocalcin and serum collagen crosslinks after 12 wk of intervention (values are expressed as mean ± SEM)

	SH	OVX	ISO	FO	ISO + FO
Osteocalcin (ng/mL)	12.17 ± 1.78	8.00 ± 1.38	13.00 ± 2.89	13.25 ± 2.80	10.50 ± 2.70
Collagen crosslaps (ng/mL)	33.65 ± 4.36	30.17 ± 7.71	54.40 ± 10.99	51.66 ± 7.89	34.14 ± 3.19

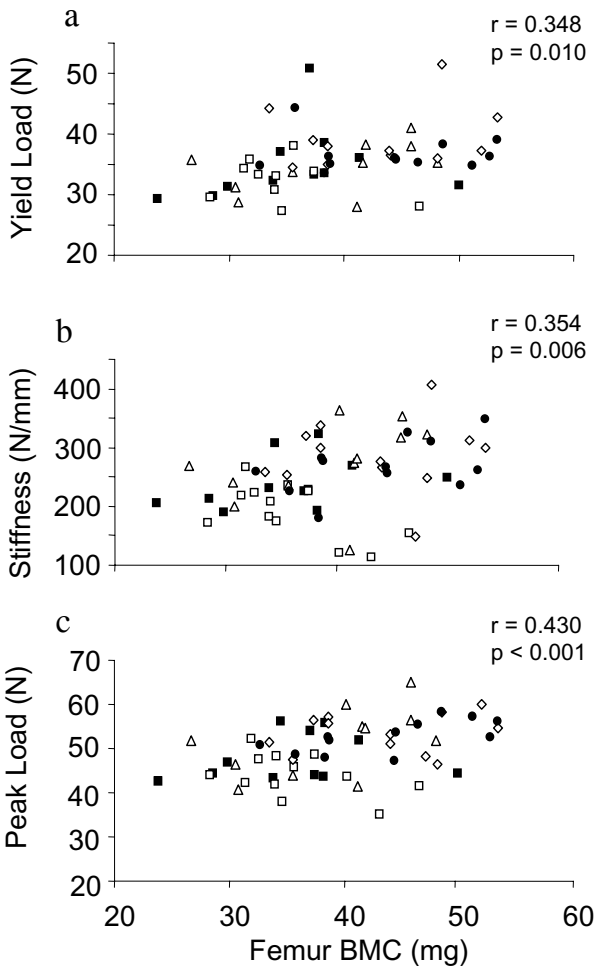

Figure 2. Relationship between whole femur BMC and (a) yield load, (b) stiffness, (c) peak load after 12 wk of intervention. Symbols used: ■ SH, □ OVX, △ ISO, ◇ FO, ● ISO + FO.

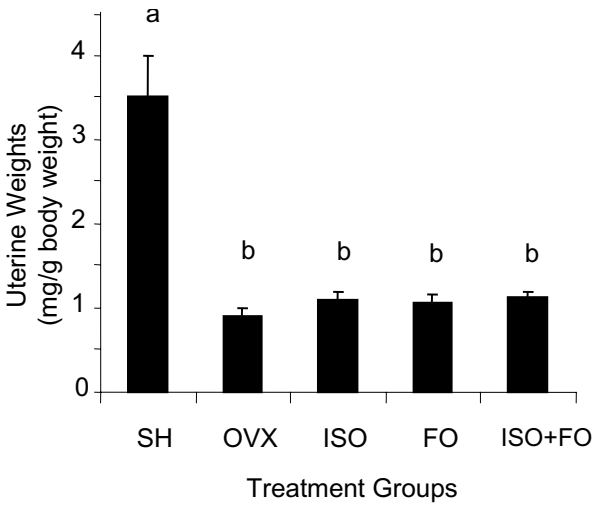

Figure 3. Uterine weights expressed *per* body weight (mg/g) after 12 wk of intervention. Data are expressed as mean ± SEM. Bars with different letters, $p < 0.05$.

load of LV4 in this mouse model after 12 wk of intervention.

Of interest is the fact that the ISO alone resulted in higher femur BMD, that was similar to estrogen-replete mice (SH group), compared to the OVX group, as studies using ovariectomized rodents have reported mixed findings with feeding ISO on preservation of BMD or attenuation of ovariectomy-induced bone loss [7–11]. In contrast to femur BMD, ISO alone did not preserve BMD of the LV. Similar findings were previously reported in ovariectomized rats fed isoflavone extract; ISO did not attenuate the decline in LV BMD [9]. The total isoflavone dose studied in the present study (500 mg of genistein + daidzein/kg diet) was slightly higher than the isoflavone level expected in a 20% soy protein diet for rodents (~400 mg of genistein + daidzein/kg diet) but did contain a similar ratio of genistein and daidzein (~1:1) that is present in soy protein isolate. A previous study from our laboratory reported that feeding 200 mg of daidzein/kg diet to C57BL/6 mice did not attenuate bone abnormalities that occurs postovariectomy [11]. Combining the data from this former and the present study, it is possible that ISO attenuated the decline in bone health due to (i) the higher dose of ISO (500 mg/kg diet *vs.* 200 mg/kg diet); (ii) greater conversion of daidzein to equol due to differences in strain; or (iii) due to the presence of genistein in addition to daidzein.

A study that used high levels of daidzein, equivalent to levels achieved only through supplements and approximately four times higher than the present study, reported preservation of trabecular and cortical bone in femurs [21]. Studies that have fed markedly lower levels of daidzein report mixed findings, possibly due to differences in dose administered [11, 22]. One study that fed 100 or 200 mg of daidzein/kg diet showed no benefit to BMD and bone strength in ovariectomized C57BL/6 mice while a study in ovariectomized rats showed that daidzein preserved both femur and spine BMD, and was similar to SH group [22]. This study also compared daidzein and genistein, at equal

doses, and showed that genistein did not protect against the loss of BMD [22], suggesting that conversion of daidzein to its metabolite, equol, may be important to observe benefits to bone. Because equol is more estrogenic than the ISO daidzein and genistein, it may modulate bone metabolism to a greater extent than its precursor, daidzein. A study in ovariectomized mice demonstrated that equol can attenuate the deterioration of bone tissue postovariectomy [23]. Moreover, a study in ovariectomized rats that directly compared the effects of genistein *versus* daidzein demonstrated that daidzein had greater modulatory effects on bone metabolism compared to genistein [22]. This finding suggested that metabolism of daidzein to equol may be critical for the observed benefits. While it is known that conversion of daidzein to equol is strain-specific, CD-1 mice used in the present study convert more daidzein to equol when compared to inbred strains of mice such as C57BL/6 mice [24].

Genistein and daidzein were provided together in the diet rather than in isolation to more closely mimic the isoflavone composition in soy protein isolate. Based on various studies reporting effects of daidzein or genistein in vitro, ISO have been shown to act through both estrogen-like and nonestrogen-receptor mediated mechanisms. ISO modulate markers of osteoblastic differentiation such as alkaline phosphatase, collagen synthesis and bone nodule formation, possibly *via* increased production of bone morphogenetic protein-2, an important regulator of osteogenesis [25]. ISO, specifically genistein, decreases osteoclast number [26, 27] and modulates osteoclastogenesis by regulating mRNA expression of receptor activator of NF-κB ligand (RANKL) and osteoprotegrin [28]. ISO can also modulate expression and/or activity of inflammatory mediators such as prostaglandin E_2 that can stimulate bone resorption and decrease bone formation.

Unlike intervention with ISO alone, FO alone attenuated the loss of BMD in the lumbar spine, and like ISO alone resulted in a similar femur BMD and femur peak load compared to SH group. There is evidence that the deterioration of bone tissue and loss of mineral that occurs after estrogen withdrawal is at least partially mediated by an inflammatory response. For example, TNF-α knockout mice do not experience the rapid loss of BMD that usually occurs postovariectomy [29, 30]. It has been hypothesized that the decline in estrogen after ovariectomy and menopause results in an increase in T cell activity, stimulating production and release of TNF-α. In turn, TNF-α enhances stromal cell production of RANKL and macrophage colony-stimulating factor, key stimuli of osteoclastogenesis [29, 30]. FO intervention may attenuate the inflammatory response after estrogen withdrawal. Feeding DHA and EPA to healthy mice or IL-10 knockout mice which develop intestinal inflammation associated with bone abnormalities [31] was shown to suppress the activation of T cells [32, 33]. Other studies report that rats fed FO at a level similar to the present study, have lower prostaglandin E_2 [14]. FO may also favorably modulate bone metabolism by enhancing intestinal Ca absorption and reducing urinary Ca excretion [16].

In the sole animal study reporting the combined effect of ISO and FO, the combination of ISO and FO protected against loss of femur BMC and reduced serum pyridinoline crosslinks, a marker of osteoclastic acitivity, in ovariectomized rats [17]. Similar to the reported study [17], the present study reports benefits to femur bone mineral, however, no differences in collagen crosslinks, a serum marker of bone resorption, were observed; other studies have also reported no change in bone resorption markers with isoflavone feeding [34 – 37]. It is important to note that the particular bone resorption assay used measures free collagen crosslinks (as opposed to both free and protein bound crosslinks) and thus may not be a true reflection of the extent of osteoclastic activity. This may also explain why there was no difference in collagen crosslinks among the SH and ovariectomy groups. The study by Watkins *et al.* [17] and the present study are similar in that both fed a diet containing about 10% fat, approximately 7% menhaden oil and 3% safflower oil, but ISO were present in the soy protein (20% protein) as a mixture of glycones and aglycones [17] rather than in the form of purified ISO used in the present study. In theory it is possible that ISO have differing effects when present in soy as soy contains many other components, and the interaction of ISO with these components likely modulates their biological activity. However, the only study to have directly compared the effects of ISO in soy protein *versus* isolated ISO demonstrated that whether or not the ISO were present with soy protein had no effect on measures of calcium metabolism or bone [38]. This study also reported that there was no effect on calcium absorption but that soy protein decreased excretion of urinary calcium, regardless of the level of isoflavone [38].

Linear regression analyses revealed that femur BMC is significantly and positively associated with yield load, peak load, and stiffness measured at the femur midpoint, however, these associations are somewhat weak, providing evidence that factors other than bone mineral are important determinants of the strength of individual bones. This finding is in agreement with literature that has shown that bone geometry and structural properties must also be considered when assessing fracture risk [39, 40].

ISO are postulated to act as selective estrogen receptor mediators (SERMs) [12], and as such may have estrogenic effects in some tissues without exerting estrogenic effects in other tissues. For this reason, and to determine if ISO had potential adverse effects on uterus, an estrogen-sensitive tissue, uterine weights were measured. Previous studies from our lab in which lower doses of purified ISO (*i.e.*, 100 or 200 mg of daidzein/kg diet) were fed for 12 wk to ovariectomized mice resulted in no change in uterus weights [11]. Although the level of ISO in the present study was approximately 2.5 – 5 times higher than this earlier study, uterus weight was unaffected. Another study also reported

no effect on uterus weight at markedly higher dose of daidzein than the present study [21].

In conclusion, the findings from this study demonstrate that the combination of FO and ISO resulted in stronger LV that are more resistant to compression fracture. Further studies are needed to investigate the potential benefits of combining soy ISO and PUFAs on bone mass and biomechanical strength to determine the appropriate doses and timing of the interventions that will provide the maximum protection to the skeleton. It will be important to expand this research to study the effect of soy protein itself, rather than isolated purified ISO, in combination with FO. Inclusion of a high Ca group is also worthy of investigation as Ca absorption and/or excretion may be modulated by ISO and long chain omega-3 PUFAs [16, 38, 41].

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