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ORIGINAL CONTRIBUTION

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Effects of soy isoflavone consumption on bone structure and milk mineral concentration in a rat model of lactation-associated bone loss

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Abstract *Background* Like menopause, during complete lactation, circulating estrogen concentrations are markedly reduced, resulting in amplified bone resorption. *Aim of study* To investigate the effects of soy isoflavones, common dietary components used to mitigate the bone loss of menopause, on the bone loss associated with lactation. *Methods* Lactating rats were randomized to one of four diets supplemented with different levels of soy isoflavones (0, 2, 4, 8 mg aglycone isoflavone/g protein). Milk was collected from all dams between days 12 and 15 of lactation and was analyzed for calcium, phosphorus and genistein concentrations. Serum and bones from half of the animals from each diet group were taken at weaning and from the remaining half at 4 weeks post-weaning. Bones underwent histomorphometric analysis and serum was used for genistein determinations. *Results* Serum genistein and milk concentrations reflected dietary isoflavone dose. Isoflavone intake had no effect on any of the bone changes associated with lactation or recovery. Milk calcium and mineral concentrations were unaffected by dietary isoflavones. *Conclusions* Consumption of soy isoflavones, in levels that can be readily attained through soy foods, have neither protective effects on bone nor deleterious effects on milk quality or quantity during lactation.

Key words bone loss – isoflavones – lactation – rat model – genistein

Introduction

Estrogen plays a key role in the maintenance of the adult skeleton [14, 34]. It acts as a potent anabolic steroid concerning bone mass (via receptors, ER-α and ER-β on bone cells) by directly suppressing bone resorption [9]. Indirectly, estrogen is also thought to exert skeletal effects through enhancement of intestinal calcium absorption by way of its trophic effect on 1,25-dihydroxyvitamin D and perhaps independently [9, 26].

Like menopause, during complete lactation, circulating estrogen concentrations are markedly reduced, resulting in amplified bone resorption [7, 15, 23, 34]. This breakdown of existing bone provides a ready-source of minerals for milk production but also results in significant bone loss as approximately 3–7% of the maternal skeleton is depleted in only 6 months [7]. Interestingly, this lactation-associated bone loss appears to be independent of lifestyle factors generally thought to be protective, such as high calcium intakes or increased physical activity [6, 18]. Once estrogen returns to normal with some

level of weaning, parathyroid hormone (PTH) increases without a concomitant increase in activation of vitamin D to 1,25-dihydroxyvitamin D [6]. This unusual combination promotes bone formation and decreased renal calcium excretion without a change in intestinal calcium absorption [16, 17, 35]. While complete recovery generally occurs within 6–8 months post-weaning [15], this unique hormonal milieu may offer a potential for bone gain beyond that which was lost. Since soy isoflavones have shown promise in conserving and, perhaps, building bone during menopause [5, 37], could they have the same effects during lactation, another estrogen-deficient state?

Soy isoflavones, often referred to as phytoestrogens, are weak estrogens and have long been promoted as a natural therapy to mitigate some of the negative effects of ovarian estrogen depletion such as bone loss [33]. The efficacy of soy as a prophylactic for this bone loss has been demonstrated in many studies involving peri- and postmenopausal women [5, 36]. Recently two meta-analysis were conducted to clarify the effects of soy isoflavone intake on bone turnover and loss during menopause. In the first meta-analysis, data from 10 randomized controlled trials involving 608 menopausal women showed that soy isoflavones significantly attenuates bone loss of the spine [19]. In the second meta-analysis, data from nine randomized controlled trials on 432 subjects showed that soy isoflavones significantly increase bone formation and decrease bone resorption [20]. Interestingly, one of the first studies published on soy and bone actually showed that soy isoflavone consumption increase (i.e. not just diminish loss) bone mineral density in postmenopausal women [30].

There is a potential concern regarding isoflavone consumption unique to lactation. Theoretically, administering soy isoflavones during lactation could result in reduced mineral availability for milk production. Findings from a study of lactating mice found that estrogen treatment decreased milk calcium concentrations compared with controls [41].

The objective of the study presented herein was to determine the skeletal effects of consuming graded doses of soy isoflavone extracts during lactation and post-weaning using a rat model. We hypothesized that since the more potent, competing, endogenous circulating estrogens are reduced, isoflavone consumption during lactation will attenuate the associated bone loss and may contribute to greater bone gains with weaning. The second objective of this study was to determine the effects of soy isoflavones on milk mineral content and adequacy in supporting litter growth.

Materials and methods

Animal breeding and experimental design

All animal facilities and protocols were approved by the University of Missouri-Columbia Animal Care and Use Committee. Forty-eight (6 per group) female first-time breeder Wistar-origin rats (Odell-MacDonald colony, University of Missouri) were randomly-assigned to receive one of four dietary treatments for three weeks (lactation phase) or 7 weeks (recovery phase).

At ~100 days of age, animals were housed and bred in wire hanging cages at room temperature (70–75°F) with a 12-h (0700–1900 h) light and dark cycle and allowed free access to food and water. Animals were fed Formula 5008 rat chow (Bourne Feed, Columbia, MO) prior to and during breeding. Body weights were measured before breeding, and then weekly throughout the study period. Following 2 weeks in the breeding cages, pregnant females were transferred to individual polycarbon shoebox cages supplied with soft wood bedding and allowed free access to water and a casein-based isoflavone-free control diet. Since the Formula 5008 rat chow contains soy protein and, consequently, isoflavones, the control diet served as a "wash-out" diet that was continued until one day after parturition.

Post-partum female rats were then randomly-placed on one of four isocaloric, nutritionally-complete, casein-based, modified AIN-93G experimental diets [32] with and without added soy isoflavone extract (NovaSoy, Archer Daniels Midland, Decatur, IL): Control = 0, Low = 2; Med = 4; or High = 8 mg total aglycone isoflavones/g protein. These doses were based on the range of total aglycone isoflavone concentrations found naturally in soy protein (~0.11–4 mg/g protein). Two of the doses fell within this range and highest dose was twofold greater than the upper natural concentration (Table 1) [29]. Experimental diets were continued throughout the 3-week lactation phase for all rats and intake was measured weekly.

Female rats that successfully gave birth to six or more pups and began lactating were not disturbed for at least 3 days, except to supply treatment diets. After postpartum day five, litters were culled to eight pups, if necessary. Thus, lactating rats nursed six to eight pups each. Dams were allowed to lactate for 21 days, after which the litter was weighed and removed. At the end of lactation (weaning status group), animals were killed by exsanguination via cardiac puncture following an intramuscular injection of an anesthetic cocktail (ketamine and xylazine); blood was collected for determination of serum genistein and estradiol. Right and left tibias were harvested for mineral content analysis and bone histology/histomorphometry.

Table 1 Diet formulations

Ingredient	Control	Low (g/kg)	Medium (g/kg)	High (g/kg)
NovaSoy[a]	–	1.668	3.336	6.672
Casein	200	200	200	200
Corn starch	75	75	75	75
Dyetrose[b]	54.5	52.8	51.2	47.8
Sucrose	500	500	500	500
Cellulose	50	50	50	50
Safflower oil	20	20	20	20
Corn oil	50	50	50	50
Mineral Mix AIN93G[c]	35	35	35	35
Vitamin Mix AIN93[c]	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
DL-methionine	3	3	3	3

[a]Donated by Archer Daniels Midland Company, Decatur, IL. 40% total aglycone isoflavones with ratio of genistin: diadzin:glycitin of 1.3:1:0.3
[b]Purchased from Dyets Inc, Bethlehem, PA. A glucose polymer
[c]Purchased from Harlan Teklad, Madison, WI

Remaining animals were housed individually and maintained on experimental diets for 28 days post-weaning, which has been shown to be an adequate length of time to return to baseline bone mass [4] At the end of the recovery phase (post-weaning status group), animals were killed and tissues were extracted as described above.

Milk collection

Between days 12–15 of lactation, female rats were removed from their pups between 0800 and 1000, approximately 2 h prior to milk collection. Oxytocin (2 IU) was administered interperitoneally to promote milk let-down. Rats were lightly anesthetized with inhaled isofluorane for approximately 20 min, during which time milk was collected using a vacuum system and gentle manipulation [13, 43]. After recuperation, animals were returned to their pups.

Serum and milk analysis

Genistein (primary soy isoflavone) concentrations in serum and milk were measured in triplicate by high performance liquid chromatography (HPLC) with CoulArray detection as previously described [29]. Serum estradiol was measured using a commercially-available radioimmunoassay kit (ultra-sensitive estradiol RIA DSA-4800, Diagnostic Systems Laboratories, Webster, TX, intra-assay CV = 9.7%).

Milk and bone mineral analysis

Aliquots of milk samples were wet-ashed using nitric and perchloric acid in preparation for mineral analysis. Left tibias underwent dry ashing after extraction of lipid with hexane and diethyl ether as previously described [28]. Ashed milk and bone samples were then analyzed for calcium in duplicate by atomic absorption spectrophotometry [28]. Bone and milk phosphorus were measured in duplicate by reduction of molybdovanadate using a colorimetric method [28].

Bone histology and histomorphetry

The right tibia was excised and soft tissue was removed. The bone was trimmed at the distal end of the shaft and placed in Millonig's fixative. Following dehydration, the undecalcified specimens were embedded in partially polymerized methymethacrylate. Ten sections at 5–8 μm were cut from each tibia on a Reichert-Jung 2050 Supercut microtome (Cambridge Instuments, Heidelberg, Germany). Sections were mounted, and the plastic removed by a 24-h xylene soak. The sections were stained with a modified Masson [22]. Two stained sections per proximal tibia specimen were quantified using a bone histomorphometry system (OsteoMeasure System, Atlanta, GA). Cancellous bone measurements were taken from an approximately 6 mm^2 area in the central region beginning 1 mm distal from the growth plate as previously reported [27]. Histomorphometric parameters measured were as follows: trabecular bone volume, marrow volume per tissue volume, mineralized volume per tissue volume, bone surface per bone volume, trabecular thickness, trabecular plate separation, and trabecular density; all units and nomenclature comply with current recommendations [25].

Statistical analyses

One-way (by diet groups) or two-way (diet by status) analysis of variance (ANOVA) were used to determine differences among diet groups and/or between weaning vs. post-weaning status, respectively (SAS 8.2 statistical software package, SAS Institute Inc., Cary, NC). Data are shown in tables and figures as means ± SE. Status (weaning vs. post-weaning) means were considered significantly different at $P < 0.05$. Diet group means were also considered significantly different at $P < 0.05$ as determined by Tukey's multiple comparisons procedure. Serum estradiol and serum and milk genistein data were log transformed before the ANOVA.

Results

Animal weights and feed Intake

Dam ages at breeding (~106d; P value for diet effects = 0.702; P value for phase effects = 0.722, based

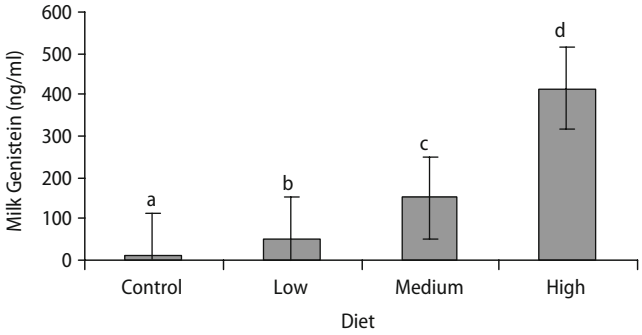


Fig. 1 Milk genistein concentrations from dams fed one of four diets supplemented with different levels of soy isoflavones during lactation. Data are expressed as mean ± SEM (n = 10–12 per group). [a–d]Means with unlike superscripts are significantly different, $P < 0.05$ based on one-way ANOVA. Control, 0; Low, 2; Med, 4; or High, 8 mg total aglycone isoflavones/g protein

on two-way ANOVA) and final body weights (~280 g; P value for diet effects = 0.244; P value for phase effects = 0.767, based on two-way ANOVA) at weaning and post-weaning were similar among all groups. Likewise, litter weights at weaning were not significantly different among groups (~250 g; P value for diet effects = 0.426; P value for phase effects = 0.111, based on two-way ANOVA).

The addition of isoflavones to the diet in the amounts used in this experiment did not influence feed intakes of dams during either the lactation or the recovery phase (P value for diet effects = 0.987, based on two-way ANOVA). As expected, however, animals consumed more feed during the lactation phase than during the recovery phase (~169 g vs. 125 g/wk, respectively; P value for phase effects < 0.0001, based on two-way ANOVA).

Milk isoflavone and mineral content

The concentration of genistein in milk harvested from all animals at days 12–15 of lactation reflected dietary isoflavone intakes and were significantly different among all groups (Fig. 1). Milk calcium and phosphorus concentrations were remarkably comparable regardless of the isoflavone content of the diet (Table 2).

Serum genistein and estradiol

Results of the serum analysis are shown in Table 3. Weaning animals had significantly greater serum genistein concentrations compared with the post-weaning animals. Like the milk, serum genistien concentrations reflected dietary isoflavone intakes. All isoflavone diet groups had significantly greater serum genistein concentrations than the control groups; while the High group was significantly greater than the Low group, but not the Medium. There was a small interaction effect of lactational status on diet (P = 0.047). Serum estradiol concentrations were not significantly different among diet groups or between weaning and post-weaning animals.

Table 2 Milk mineral concentrations of rats fed one of four diets supplemented with different levels of soy isoflavones during lactation[a]

Diet Groups[b]	Calcium (g/l)	Phosphorus (g/l)
Control[c]	3.30 ± 0.37	2.69 ± 0.34
Low	3.44 ± 0.22	2.59 ± 0.10
Medium	3.22 ± 0.25	2.53 ± 0.11
High	3.59 ± 0.69	2.67 ± 0.31
P vlaue	0.535	0.318

[a]Data are expressed as means ± SEM (n = 8–12 per group)
[b]Milk was collected from dams between days 12–15 of lactation
[c]Control, 0; Low. 2; Med, 4; or High, 8 mg total aglycone isoflavones/g protein

Bone mineral content and histomorphometric findings

Mean tibia dry fat-free, ash and relative ash (ash/dry) weights did not differ between status or among diet groups (data not shown). The bone histomorphometric data in Table 4 confirm the typical pattern of

Table 3 Serum total genistein and estradiol concentrations of rats fed one of four diets supplemented with different levels of soy isoflavones during lactation and recovery*

Diet groups by status	Total genistein* (ng/ml)	Estradiol (pg/ml)
Weaning[†]		
Control**	5.9 ± 3.63[a]	18.7 ± 3.59
Low	182.3 ± 44.76[c]	10.4 ± 1.82
Medium	295.7 ± 12.68[cd]	11.0 ± 1.74
High	1164.1 ± 272.99[d]	22.2 ± 2.09
Post-Weaning[‡]		
Control	3.1 ± 2.29[a]	15.8 ± 3.90
Low	25.2 ± 9.22[b]	14.5 ± 4.79
Medium	54.5 ± 7.14[bc]	11.6 ± 2.09
High	115.1 ± 24.59[c]	12.7 ± 0.97
Main effects	Two-way ANOVA (P value)	
Status	<0.0001	0.364
Diet	<0.0001	0.094
Status × Diet	0.047	0.142

*Data are expressed as means ± SE (n = 5–6 per group)
**Control, 0; Low, 2; Med, 4; or High, 8 mg total aglycone isoflavones/g protein
[†]Weaning, animals were allowed to lactate for 21 days and then tissues were harvested
[‡]Post-weaning, animals were allowed to lactate for 21 days, the pups weaned, and maintained for four weeks post-weaning (recovery) before tissue harvest
[a–d]Means with unlike superscripts within a column are significantly different. $P < 0.0001$, based on one-way ANOVA

Table 4 Histomorphometric measures of proximal tibia bone volume and trabeculae of rats fed one of four diets supplemented with different levels of soy isoflavones during lactation and recovery*

Diet group by status	Trabecular bone volume (% tissue volume)	Marrow volume (% tissue volume)	Mineralized volume (% tissue volume)	Bone surface per bone volume (mm^2/mm^3)	Trabecular thickness (μm)	Trabecular plate separation (μm)	Trabecular density (#/mm)
Weaning†							
Control**	12.17 ± 3.49[a]	87.83 ± 5.26[a]	12.17 ± 5.26[a]	53.33 ± 12.59[a]	39.21 ± 7.83[a]	280.35 ± 30.59	2.98 ± 0.94
Low	16.83 ± 3.49[a]	83.17 ± 3.49[a]	16.83 ± 3.49[a]	47.65 ± 3.08[a]	42.59 ± 4.93[a]	228.37 ± 36.98	3.94 ± 0.51
Medium	15.95 ± 4.34[a]	84.06 ± 4.34[a]	15.94 ± 4.34[a]	49.44 ± 4.73[a]	41.06 ± 3.45[a]	257.64 ± 42.33	3.88 ± 0.94
High	13.24 ± 3.84[a]	86.76 ± 3.84[a]	16.24 ± 10.10[a]	53.37 ± 4.61[a]	37.73 ± 3.28[a]	228.43 ± 23.18	3.48 ± 0.86
Post-weaning‡							
Control	20.02 ± 7.65[b]	80.00 ± 7.70[b]	20.02 ± 7.66[b]	38.28 ± 6.70[b]	53.69 ± 9.13[b]	253.61 ± 58.99	3.64 ± 1.15
Low	17.92 ± 5.64[b]	82.33 ± 5.19[b]	17.92 ± 5.64[b]	38.09 ± 4.33[b]	51.72 ± 4.59[b]	256.50 ± 41.61	3.45 ± 1.02
Medium	21.51 ± 7.51[b]	77.75 ± 7.09[b]	21.51 ± 7.51[b]	37.68 ± 5.83[b]	52.63 ± 6.26[b]	208.74 ± 23.07	4.31 ± 1.39
High	21.71 ± 6.23[b]	78.29 ± 6.23[b]	21.71 ± 6.22[b]	38.96 ± 5.81[b]	52.94 ± 8.79[b]	173.98 ± 30.01	4.05 ± 0.60
Main effects	Two-way ANOVA (*P* value)						
Status	0.001	0.001	0.006	<0.0001	<0.0001	0.346	0.214
Diet	0.766	0.655	0.692	0.510	0.899	0.366	0.261
Status × Diet	0.366	0.319	0.585	0.719	0.390	0.678	0.373

*Data are expressed as means ± SE (n = 5–6 per group)
**Control, 0; Low, 2; Med, 4; or High, 8 mg total aglycone isoflavones/g protein
†Weaning, animals were allowed to lactate for 21 days and then tissues were harvested
‡Post-weaning, animals were allowed to lactate for 21 days, the pups weaned, and maintained for four weeks post-weaning (recovery) before tissue harvest
[a–b]Means with unlike superscripts within a column are significantly different. $P < 0.05$, based on one-way ANOVA

lactation-associated bone loss and are in agreement with other animal models of lactation [38, 40, 41]. Trabecular bone volume, mineralized volume and trabecular thickness were greater in the post weaning status animals compared with the weaning status animals, but there were no differences among diet groups. Marrow and bone surface per bone volume were greater in the weaning status animals when compared to post-weaning animals, but there were no differences among diet groups. There were no differences in trabecular plate separation or trabecular density between status or among diet groups.

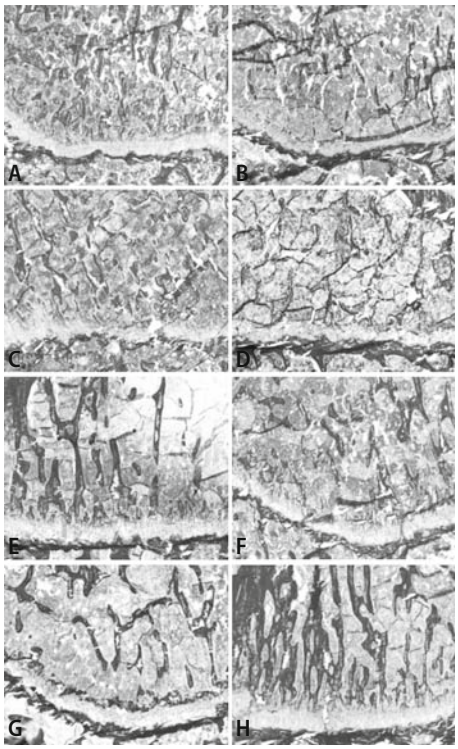
Figure 2 shows representative histologic sections from animals of each diet group within both the weaning and post-weaning status groups. While both growth plate volume and thickness were greater in the post weaning status animals, there were no significant differences for either of these measures attributed to isoflavone content of the diet.

Discussion

Throughout the last decade, myriad soy products have been developed and promoted because of the mounting evidence of the possible health advantages of soy. In a 2008 survey, 85% of US consumers rated soy products as "healthy", up from 59% in 1997 [1]. Consumers now recognize soyfoods and beverages for amelioration of menopausal symptoms (10%), protein content (17%), generally "being good for you" (19%), reducing the risk of some cancers (23%), heart health (31%), and weight management (31%) [1].

In response to this growing awareness of soy and human health, foodstuffs such as soy nut snacks, soy protein shakes, textured-vegetable protein foods (e.g. "veggie burgers"), and various nutritional supplements with added isoflavone extracts, are recent additions to the grocery and drug store shelves. Moreover, much of these products have been aggressively marketed to and used by health-conscientious women of all ages [10, 33]. Thirty-two percent of Americans consume soy products at least once a month and the number of consumers who have tried soymilk in particular has more than doubled to 40% of all consumers. Consumption of other soy products, such as edamame which tripled since 2000, is also on the rise [1].

While research supports the inclusion of soy products in the typical adult diet as protective agents against the development of chronic disease including postmenopausal osteoporosis, the effects of postpartum consumption of soy isoflavones on bone mineralization and milk production had not been addressed. This is the first report to investigate the effects of soy isoflavones, common dietary components, on the bones of and milk produced by an intact rat model of lactation. Findings from this study show that while isoflavone consumption had few significant effects on the bone changes of lactation and recovery, there were also no deleterious effects on the quality and quantity of milk.



Diet group By Status	*Growth Plate Volume* (% Tissue Volume)	*Growth Plate Thickness* (μm)
Weaning†		
A. Control	19.66 ± 1.54[a]	71.92 ± 6.78[a]
B. Low	20.19 ± 2.09[a]	78.36 ± 7.53[a]
C. Medium	20.18 ± 3.52[a]	74.32 ± 8.49[a]
D. High	17.58 ± 1.63[a]	66.64 ± 3.84[a]
Post-Weaning‡		
E. Control	23.07 ± 1.75[b]	86.05 ± 7.60[b]
F. Low	25.35 ± 1.61[b]	96.03 ± 5.75[b]
G. Medium	25.73 ± 2.15[b]	86.34 ± 8.15[b]
H. High	23.10 ± 0.96[b]	92.32 ± 9.46[b]
	Two-Way ANOVA (p value)	
Main Effect		
Status	0.0008	0.0038
Diet	0.4960	0.6900
Status X Diet	0.9234	0.8464

Fig. 2 Light micrographs of representative histologic sections from rats fed one of four diets supplemented with different levels of soy isoflavones during lactation and recovery. Sections were stained with modified Masson Marrow. Data are expressed as means ± SEM (n = 5–6 per group). †Weaning, animals were allowed to lactate for 21 days and then tissues were harvested. ‡Post-weaning, animals were allowed to lactate for 21 days, the pups weaned, and maintained for four weeks post-weaning (recovery) before tissue harvest. [a–b]Means with unlike superscripts within a column are significantly different. $P < 0.0001$, based on one-way ANOVA

Only three other published reports looked at the skeletal effects of either exogeneous estrogen or phytoestrogens during lactation. All observed beneficial outcomes, yet only one used a stand-alone model of lactation. Vanhouten and Wysolmerski demonstrated that estradiol-treated lactating mice had significantly greater bone mineral density, as measured by DXA, of the lumbar spine, femur and whole body sites than vehicle-treated animals [41]. Furthermore, histomorphometry confirmed that estrogen treatment effectively prevented the rise in bone resorption seen in lactation.

In the other two published lactation- estrogen/phytoestrogen studies, Garner et.al. [12] showed that treating ovariectomized, lactating rats with estradiol resulted in a significant reduction of the loss of bone ash; while Anderson and coworkers showed that mean femur ash weights were significantly greater in ovariectomized, lactating animals consuming a diet supplemented with genistein (primary soy isoflavone) compared with those treated with conjugated estrogens or vehicle [2].

The disparate results between Anderson's study, the only other study to look at isoflavones and bone during lactation, and the one described herein are most likely explained by the differences in the model or doses of isoflavone used. In Anderson's study, the animal model was that of lactation superimposed on ovariectomy, eliminating most of the endogenous estrogen available to compete for the estrogen receptor with circulating isoflavones, thus potentially heightening isoflavone's effects. The type and dose of isoflavones used in each study also differed. Our study used a mixture of soy isoflavones (50% genistein, 40% daidzein, 10% glycitein), while Anderson's study used a pure genistein preparation.

Genistein is considered to be the most biologically potent soy isoflavone [24, 33, 42]. Assuming a mean intake of ~23 g of diet per day (20% protein content), with 50% of the isoflavone extract as genistein, the calculated dosage range used in our study is approximately 4.5, 9 and 18 mg/day of genistein for the Low, Med, and High isoflavone diet groups, respectively. In contrast Anderson's dosages were much lower at 0.5, 1.6, and 5.0 mg genistein/day. Importantly, only the animals treated with low dose of genistein (0.5 mg/day) responded with attenuations in the bone loss associated with estrogen withdrawal, demonstrating biphasic effects of the agent [2].

This biphasticity of genistein has been reported by others and may be attributable to an estrogen receptor saturation effect [33]. At low concentrations, genistein may occupy available estrogen receptors to exert estrogen-like effects, while at higher concentrations, genistein in excess of that required to occupy available receptor sites may exert other effects that are not anabolic to bone.

Our study doses were based on the isoflavone concentrations found naturally in soy protein; the

results, therefore, document the consequences of isoflavone intakes possible through the consumption of soy foods commonly found in the US marketplace. In addition to examining soy isoflavones' effects on lactation-associated bone loss and recovery, we were also interested in their effects on bone growth and milk production.

In humans, estrogen is crucial for epiphyseal fusion; and although rats complete epiphyseal closure much later in life, they serve as a useful model for exploring the bone response to estrogen or phytoestrogen treatment [21]. Our growth plate data are in agreement with others showing lactation causes a decrease in the thickness of the growth plate which is consistent with a decrease in endochondral growth [31]. Although estrogen treatment is known to inhibit chondrocyte proliferation [39], phytoestrogens in the form of isoflavones and in the doses used in this study did not affect endonchondral growth during lactation just as they had no effect on bone turnover.

This study is the first to demonstrate that milk calcium and phosphorus concentrations and litter weight (surrogate for milk production) are not affected by soy isoflavone consumption. Two of the three investigations discussed above on exogeneous estrogen treatment during lactation have shown some negative impact on milk production. Garner and associates found that litters of ovariectomized lactating rats receiving a low calcium diet and treated with estrogen weighed significantly less than controls [12]; while VanHouten and Wysolmerski found that intact lactating rats administered estrogen had decreased milk calcium compared to untreated animals [41].

Our results also confirm the observations of others that consumption of large doses of isoflavones by lactating animals yields significant concentrations of isoflavones in milk [8, 11]. There is evidence that exposure to isoflavones very early in life may confer health benefits, such as protection from breast cancer, into adulthood and beyond that experienced when exposure was limited to later in life [3]. Conversely, there are also laboratory studies of young, developing animals that have demonstrated soy's potential for numerous adverse effects such as permanent changes in morphogenesis and dysfunctional reproductive behaviors [33]. The findings herein suggest that consumption of phytoestrogens during lactation in doses comparable to those that can be achieved through consumption of soy foods not only results in measurable concentrations of isoflavones in milk, but it also does not appear to have detrimental effects on the adequacy or mineral content of milk.

The major limitation of this study is that did not provide isoflavones in a dose comparable to the only other study of isoflavone and lactation-associated bone loss which did find benefits [2]. However, unlike the Anderson study, the purpose of the one described herein, was to test the bone effects of isoflavones in amounts that could be obtained from dietary sources. Our results indicate more research is warranted. In particular, isoflavone dosing studies are essential to elucidate the effective dose required to have positive effects on bone during lactation, while still retaining milk quality and quantity.

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