Interaction of Sesame Seed and Tamoxifen on Tumor Growth and Bone Health in Athymic Mice

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Some premenopausal breast cancer patients use phytoestrogen-rich soy and flaxseed to alleviate side effects induced by drugs such as tamoxifen (TAM). Lignan-rich flaxseed protects against breast cancer and increases the effectiveness of TAM. This study determined the interactive effects of lignan-rich sesame seed (SS) and TAM on estrogen-responsive MCF-7 breast tumor growth and bone health in ovariectomized athymic mice under premenopausal-simulated conditions. Ovariectomized mice with an estrogen implant and established MCF-7 tumors were treated for 8 weeks as follows: (i) positive control fed basal diet (BD), (ii) SS group fed BD supplemented with 10% ground SS, (iii) TAM group with TAM implant fed BD, (iv) SS + TAM group with TAM implant fed BD supplemented with 10% SS, and (v) negative control fed BD with no estrogen implant. Palpable tumor data, adjusted for body weight, showed that SS does not inhibit MCF-7 tumor growth and tends to negate the tumor inhibitory effect of TAM by increasing cell proliferation and reducing apoptosis. SS alone and combined with TAM enhanced femur biomechanical strength but caused no differences in bone mineral content or bone mineral density in either the femur or lumbar vertebrae. SS is not protective and interacts adversely with TAM in MCF-7 breast tumors but induces beneficial effects on bone both alone and when combined with TAM. Exp Biol Med 232:754–761, 2007

Key words: sesame seed; lignans; athymic mice; breast cancer; bone

Introduction

Lignans are a class of phytoestrogens that have been suggested to reduce the risk of breast cancer (1–5) and are found in exceptionally high amounts in flaxseed (FS) (6, 7) and sesame seed (SS) (6, 8, 9). Lignans such as secoisolariciresinol diglycoside and sesamin are metabolized by microflora in the colons of humans and animals into the mammalian lignans enterodiol and enterolactone (6–10). Enterodiol and enterolactone are of interest because of their structural similarity to the hormone 17β-estradiol (E2), suggesting that they have weak estrogenic and antiestrogenic properties and hence a potential role in hormone-related diseases.

FS and the mammalian lignans have been shown to alter estrogen metabolism, bioavailability, and action (11), as well as exert both estrogen agonistic and antagonistic effects depending on endogenous estrogen levels (12, 13). However, numerous studies have demonstrated that FS, secoisolariciresinol diglycoside, and the mammalian lignans are protective against breast cancer in premenopausal- and postmenopausal-simulated experimental models (14–19). FS and the mammalian lignans also exert no adverse effects on other estrogen-sensitive tissues such as the bone (20) and uterus (14, 20), suggesting that the lignans are protective against breast cancer while not increasing the risk of other hormone-related diseases such as endometrial cancer and osteoporosis. In addition, FS and the mammalian lignans can increase the effectiveness of tamoxifen (TAM) (14, 15), which is important in some patients who use phytoestrogens as a complementary therapy to alleviate menopausal-like symptoms that are induced by TAM use (21). It is unknown, however, if other lignan-rich foods such as SS could produce similar effects.

SS contains up to 2.5 times more plant lignans than FS (6, 8, 9, 22); however, the lignan profile of SS differs from that in FS. While the lignans in FS occur predominantly as secoisolariciresinol diglycoside, SS consists mainly of sesamin, sesamolin, and sesaminol.

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In postmenopausal women, consumption of 25 g SS/day for 4 weeks has been shown to result in similar increases in urinary mammalian lignan excretion as FS (8), suggesting that SS may exert similar anticancer effects as FS if the effects of FS are due to the mammalian lignans. However, studies investigating the role of SS in tumorigenesis are limited, and none have explored its potential effects on various estrogen-sensitive tissues under various levels of circulating estrogen or its possible interaction with chemotherapeutic drugs such as TAM.

Considering our recent findings that SS is a lignan-rich food source that can produce mammalian lignans at the same concentrations as FS (8) and that lignan-rich FS protects against breast cancer and enhances the tumor inhibitory effect of TAM without exerting adverse effects in other tissues (11, 14, 16, 17, 19), the objective of this study was to determine under high circulating estrogen levels, the effect of dietary SS alone and in combination with TAM on tumor growth and bone health in ovariectomized athymic mice with established estrogen-responsive MCF-7 breast tumors.

Materials and Methods

Cell Line and Cell Culture. MCF-7 estrogen-responsive human breast cancer cells (American Type Culture Collection, Manassas, VA) were grown in Dulbecco minimum essential medium/F12 supplemented with 10% fetal bovine serum and 1% antibiotic antimycotic solution. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air and were given fresh medium every 2–3 days. For injection, the MCF-7 cells (7.9×10^6 cells/ml) were trypsinized and resuspended in serum-free medium with 1:1 Matrigel (VWR International, Mississauga, Canada) and kept on ice. A trypan blue exclusion assay was used to confirm cell viability (>85%) after cell injections.

Animals and Diets. The care and use of the animals was in accordance with the *Guide to the Care and Use of Experimental Animals* (23), and the experimental protocol was approved by the University of Toronto Animal Care Committee. Ovariectomized female athymic mice (BALB/c nu/nu; 4–5 weeks old; mean body wt 17.3 ± 0.13 g) were purchased from Charles River Canada (St-Constant, Canada), housed four per cage, and maintained within a pathogen-free isolation facility with 12:12-hr light:dark cycle at 22°–24°C and 50% humidity.

The basal diet (BD) was AIN-93G formulation (24) modified to have a high fat content (20% corn oil) at the expense of cornstarch. The SS diet consisted of BD supplemented with 10% freshly ground SS corrected for the contribution of SS to fat, dietary fiber, available carbohydrate, and protein components, resulting in diets that were isocaloric. SS was from Grain Process Enterprises Ltd. (Scarborough, Canada). The diets were prepared by Dyets Inc. (Bethlehem, PA) and sterilized by ^{60}Co radiation by Steris Isomedix Services (Whitby, Canada). Fresh diet was provided every 2–3 days, and mice were fed *ad libitum*.

Experimental Design. Mice were acclimated for 7 days on the BD and then anaesthetized with isoflurane (3%) dissolved in oxygen, implanted subcutaneously with a sterilized E2 pellet (1.7 mg; 60-day release, producing a blood level of 3–4 n*M*/l; Innovative Research of America, Sarasota, FL) in the interscapular region, and injected with MCF-7 cells (395,000 cells in a 50-µl suspension) into four sites of mammary fat pads as previously described (14).

Starting 2 weeks after cancer cell injection, tumors were palpated weekly using digital precision calipers (Cedarlane Laboratories Ltd., Hornby, Canada). The tumor surface area was calculated using the formula (length/2 × width/2) × π. At week 6 the existing E2 pellets were removed from all mice and replaced with new E2 pellets to simulate a premenopausal situation. The mice were divided into the following dietary treatment groups ($n = 9$–10 mice/group) such that their tumor sizes and body weights were similar: (i) positive (POS) control fed BD, (ii) SS group fed BD supplemented with 10% dietary SS, (iii) TAM group fed BD and subcutaneously implanted with a TAM pellet (5 mg; 60-day-release, producing a blood level of 3–4 ng/ml; Innovative Research of America), (iv) SS + TAM group fed the SS diet and implanted with a TAM pellet implant, and (v) negative (NEG) control group with no E2 implant fed BD. Food intake, body weights, and palpable tumor areas were monitored weekly. At the end of the 8-week treatment (week 14), the mice were sacrificed by CO_2 asphyxiation. At necropsy, body, tumor, and uterus weights were recorded. Femurs and lumbar vertebrae (LV) 1–4 were removed, cleaned of soft tissue, and stored at –20°C until further analyses.

Ki-67 Labeling Index (LI) and Apoptosis. Ki-67 LI was used as a marker of cell proliferation and was determined by immunohistochemistry as previously described (14). DNA fragmentation was demonstrated using an *in situ* terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling assay in the ApopTag Detection Kit (Intergen, Purchase, NY) according to the manufacturer's protocol. All slides were read blindly under a light microscope at ×400 magnification. The Ki-67 LI was calculated as the percentage of positive cells divided by total cells (600-1100) counted from 4–11 fields. The number of apoptotic cancer cells was counted and expressed as apoptotic cell number/mm^2. All assays were conducted blind to the treatment groups.

Bone Mineral Content (BMC) and Bone Mineral Density (BMD) of Whole Femur and LV1–LV3. Left femurs and intact vertebrae (LV1–LV3) were placed on a plastic tray and scanned in air at room temperature by PIXImus dual-energy x-ray absorptiometry (Lunar software version 1.46; General Electric Medical Systems, Madison, WI) to determine whole femur and LV BMC and BMD (25).

Femur and LV4 Dimensions. The right femurs and LV4 were soaked in phosphate-buffered saline for 4 hrs at room temperature immediately before biomechanical test-

Table 1. Effect of 10% Dietary SS, TAM, and Their Combination (SS + TAM) on Body Weights, Food Intake, and Relative Uterus Weights[a]

Treatment[b]	Total food intake, g/mouse (8 weeks)	Body weight, g		Uterus weight, mg/g body weight
		Week 6	Week 14	
POS	118.9 ± 4.9[cd]	15.3 ± 0.5	14.8 ± 0.4[d]	4.98 ± 0.33
SS	137.2 ± 2.7[c]	15.3 ± 0.5	19.7 ± 0.5[c]	4.59 ± 0.70
TAM	97.5 ± 7.6[d]	14.6 ± 0.8	13.2 ± 0.7[d]	4.67 ± 0.58
SS + TAM	121.3 ± 6.1[cd]	14.6 ± 0.8	18.8 ± 0.6[c]	4.05 ± 0.43

[a] Data are means ± SEM; $n = 6$–10 mice/group.
[b] TAM implant = 5 mg, 60-day release.
[cd] Different letters within the same column indicate significant difference at $P < 0.05$ by one-way ANOVA followed by Student-Newman Keul's test.

ing. Femur weights, lengths, and widths at the midpoint (both mediolateral and anteroposterior widths were measured) and LV4 weights, heights, and widths were measured using digital precision calipers as previously described (25).

Biomechanical Strength Testing of Femurs and LV4. Biomechanical strength properties of right femurs and LV4 were determined using a materials testing system (4442 Universal Testing System; Instron Corp., Canton, MA) and a specialized software program (Instron Series IX Automated Materials Tester-Version 8.15.00; Instron Corp.). Three-point bending was performed at the femur midpoint to determine the structural properties of a skeletal site rich in cortical bone as previously described (25). The posterior surfaces of the right femurs were placed on two 1-mm wide base supports with a jig span width of 5 mm and a crosshead speed of 2 mm/min. The biomechanical strength properties determined were yield load, a measure of the elastic limit of the femur, which was determined as the point at which the slope of the load-deformation curve deviates from being a straight line; resilience, a measure of the amount of energy that the femur absorbs until the yield point is reached; ultimate stiffness, a measure of the extrinsic rigidity of the femur; peak load, a measure of the maximum force that the femur withstands before fracture; and toughness, a measure of the work energy that is required to fracture the femur. To determine the peak load of LV4, compression testing was performed as previously described (25). The peak load of LV4 was determined by placing individual vertebra in the center of a stainless steel disk and applying a compression force to the vertebra by lowering a second suspended stainless steel plate at a constant rate of 2 mm/min.

Statistical Analysis. To determine the difference in palpable tumor growth among treatment groups over treatment time, analysis of variance (ANOVA) with general linear model–repeated measures procedure was used followed by post hoc Tukey test (SPSS, Inc., Chicago, IL). Because the NEG control group was included in the study to verify that the MCF-7 tumors would regress in the absence of estrogen, thus proving that the mouse model was working, this group was included only in the statistical analysis to determine differences in palpable tumor growth. Differences in tumor area between the pre- and post-treatments within the same group were assessed by Student's t test (SigmaStat version 2.0; Jandel Scientific, San Rafael, CA). One-way ANOVA followed by post hoc Student-Newman Keul's test was used to determine differences among groups in food intake, body weights, relative uterus weights, tumor cell Ki-67 LI and apoptosis, BMD and BMC of left femur and lumbar LV1–LV3, and biomechanical strength testing of right femur and LV4. To examine the relationship between total food intake and change in tumor size; final body weight and change in tumor size; and final tumor weight and BMD, peak load, and uterus weight, linear regression analyses were performed (Sigma Stat; Prism 3.0; GraphPad Software Inc., San Diego, CA). The significance level was set at $P < 0.05$.

Results

Food Intake, Body Weights, and Uterus Weights. SS and SS + TAM induced the highest total food intake during treatment compared with all other groups; however, significant difference was reached only between SS and TAM whereby TAM resulted in significantly lower food intake than SS ($P < 0.01$) (Table 1). TAM consequently resulted in a significantly lower body weight than SS ($P < 0.001$) as well as the SS + TAM and NEG control groups ($P < 0.001$). Although the POS control group did not differ significantly in food intake compared with the other treatment groups, it had significantly lower body weight compared with both SS and SS + TAM groups ($P < 0.001$).

Tumor Growth. Over the 7-week treatment period tumors in the POS control group continued to grow, resulting in a palpable tumor area that was 237% larger than pretreatment size ($P < 0.0001$; Fig. 1), while tumors in the NEG control group regressed by 73% ($P < 0.0001$), confirming that the tumor model was working. Palpable tumor size in the TAM group was intermediate between the POS and NEG control groups and was 48% larger than pretreatment tumor size ($P < 0.01$). SS stimulated the

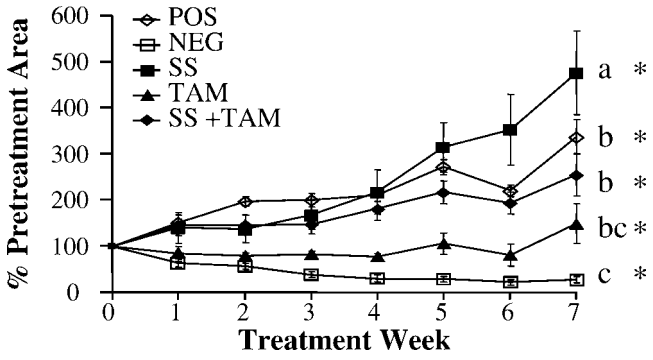

Figure 1. Effect of 10% dietary SS and TAM alone and in combination (SS + TAM) on the growth of MCF-7 breast tumors in female ovariectomized athymic mice. TAM implant was 5 mg, 60-day release. Tumor areas were measured weekly and are expressed as the percentage of pretreatment area. Different letters (a–c) indicate significant difference at $P < 0.05$ among groups by ANOVA with general linear model–repeated measures procedure followed by Tukey test. $n = 22$–31 tumors/group, 6–10 mice/group. *Significantly different than pretreatment tumor size by paired t test ($P < 0.05$).

pretreatment size by 376% ($P < 0.001$) and beyond that of all groups including the POS control group ($P < 0.001$). When combined with TAM, SS negated the tumor inhibitory effect of TAM, resulting in tumors 154% larger than pretreatment size ($P < 0.0001$) and significantly larger than those in the NEG control ($P < 0.001$) suggesting that SS interferes with the tumor inhibitory effect of TAM.

Because there were significant differences in food intake and body weights, linear regression analyses were performed to determine if they partly contributed to the changes in palpable tumor area over treatment time. A significant positive relationship was found between change in tumor area and total food intake ($r = 0.453$, $P < 0.0001$) and change in body weight ($r = 0.205$, $P = 0.040$). Therefore, the palpable tumor data were adjusted to correct for differences in body weight (Fig. 2). The SS group no

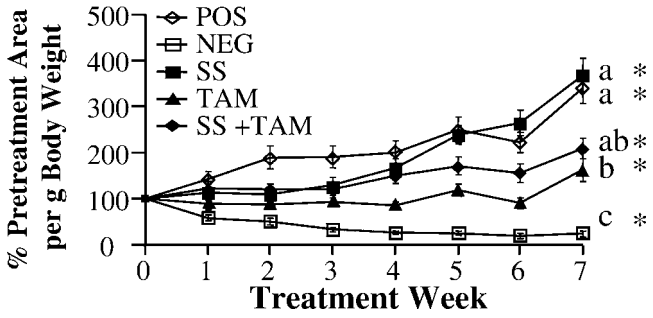

Figure 2. Effect of 10% dietary SS and TAM alone and in combination (SS + TAM) on the body weight–adjusted growth of MCF-7 breast tumors in female ovariectomized athymic mice. TAM implant was 5 mg, 60-day release. Tumor areas were measured weekly and are expressed as tumor area (mm^2) per gram of body weight. Different letters (a–c) indicate significant difference at $P < 0.05$ among groups by ANOVA with general linear model–repeated measures procedure followed by Tukey test. $n = 22$–31 tumors/group, 6–10 mice/group. *Significantly different than pretreatment tumor size by paired t test ($P < 0.05$).

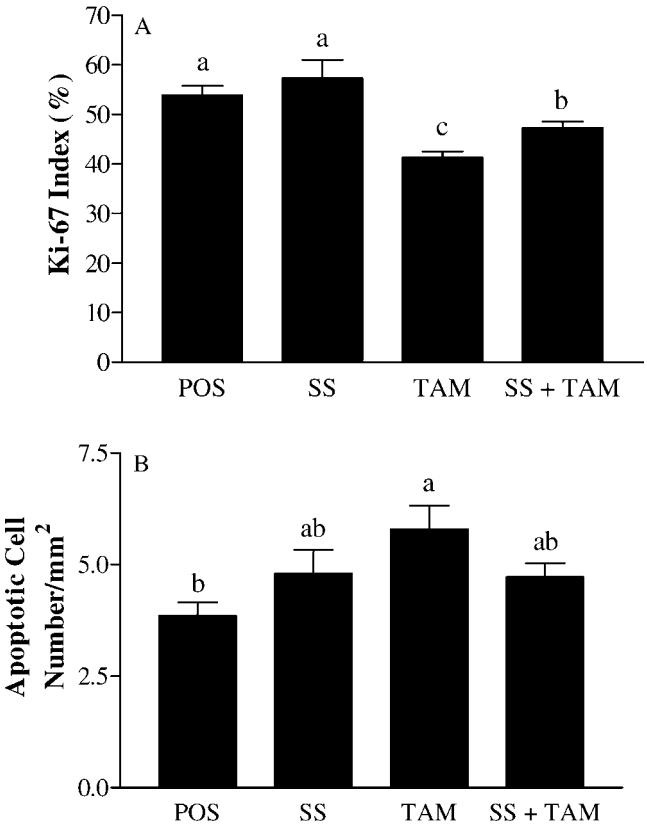

Figure 3. Effect of 10% dietary SS and TAM alone and in combination (SS + TAM) on MCF-7 (A) tumor cell proliferation and (B) apoptosis in female ovariectomized athymic mice. TAM implant was 5 mg, 60-day release. Different letters (a and b) indicate significant difference at $P < 0.05$ among groups by one-way ANOVA followed by Student-Newman Keul's test. Data are means ± SEM. $n = 7$–11 tumors/group, 5–7 mice/group.

longer differed from the POS control group, while TAM significantly inhibited tumor growth compared with the POS control ($P < 0.001$). When combined, SS still tended to negate the tumor inhibitory effect of TAM, resulting in tumors similar in size to the POS control.

Ki-67 LI and Apoptosis. Cell proliferation, expressed as Ki-67 LI, was the lowest in the TAM group ($P < 0.05$), followed by the SS + TAM group ($P < 0.01$ compared with the POS control; $P < 0.001$ compared with SS); the SS group did not differ from the POS control group (Fig. 3A). Tumor cell apoptosis was higher in the TAM group compared with the POS control group ($P < 0.05$), while both SS and SS + TAM groups had intermediate values (Fig. 3B).

BMC and BMD of Whole Femurs, Femur Dimensions, and Biomechanical Strength. Femur BMD and BMC did not differ among groups (Table 2), but anteroposterior width was significantly higher in the SS and SS + TAM groups compared with the POS control ($P < 0.01$) and TAM ($P < 0.05$) groups. There were no differences in length, mediolateral width, and weight among groups. Toughness was significantly higher in the SS + TAM group compared with the POS control ($P < 0.01$) and

Table 2. Effect of 10% Dietary SS, TAM, and Their Combination (SS + TAM) on Whole Femur Bone Mass and Dimensions and Biomechanical Strength Properties at Femur Midpoint and Femur Neck[ab]

	POS	SS	TAM	SS + TAM
Whole femur				
BMC, mg	28.48 ± 0.49	33.26 ± 1.19	28.65 ± 1.51	32.78 ± 1.11
BMD, mg/cm^2	90.06 ± 2.98	93.26 ± 2.47	89.63 ± 2.97	94.52 ± 2.30
Area, cm^2	0.32 ± 0.01	0.36 ± 0.01	0.32 ± 0.01	0.35 ± 0.01
Length, mm	13.84 ± 0.09	14.34 ± 0.07	14.11 ± 0.24	14.32 ± 0.17
Anteroposterior width, mm	1.06 ± 0.01[d]	1.14 ± 0.02[c]	1.08 ± 0.02[d]	1.14 ± 0.02[c]
Mediolateral width, mm	1.37 ± 0.02	1.42 ± 0.03	1.38 ± 0.03	1.40 ± 0.03
Weight, mg	54.02 ± 0.69	60.68 ± 1.14	55.2 ± 2.62	59.59 ± 2.12
Femur midpoint				
Yield load, N	21.32 ± 0.66	23.90 ± 1.31	22.41 ± 0.63	24.69 ± 1.88
Resilience, J × 10^{-4}	1.12 ± 0.13	1.10 ± 0.11	1.00 ± 0.04	1.12 ± 0.12
Ultimate stiffness, N/mm	229.60 ± 18.76[d]	298.20 ± 15.34[c]	268.00 ± 11.81[cd]	297.70 ± 17.23[c]
Peak load, N	25.94 ± 0.78[d]	32.79 ± 1.87[c]	25.88 ± 0.93[d]	33.70 ± 1.93[c]
Toughness, J × 10^{-3}	1.98 ± 0.19[d]	3.34 ± 0.42[cd]	2.12 ± 0.40[d]	4.03 ± 0.46[c]

[a] Data are means ± SEM; $n = 4$–9 mice/group.
[b] TAM implant = 5 mg, 60-day release.
[cd] Different letters within the same row indicate significant difference at $P < 0.05$ by one-way ANOVA followed by Student-Newman Keul's test.

TAM ($P < 0.05$) groups, while SS had an intermediate effect. There were no significant differences in yield load or resilience among groups.

BMC and BMD of LV1–LV3 and Dimensions and Peak Load of LV4. BMC of LV1–LV3 tended to be higher ($P = 0.098$) in the SS and SS + TAM groups compared with the TAM and POS control groups, but these differences did not reach statistical significance (Table 3). BMD did not differ among groups. There were no significant differences in height, anteroposterior width, mediolateral width, weight, and peak load among groups.

Relationship with Tumor Weight. Linear regression analysis showed no relationship between the final MCF-7 tumor weight and whole-femur BMD ($r = 0.154$, $P = 0.471$), femur midpoint peak load ($r = 0.177$, $P = 0.387$), or uterus weight ($r = 0.055$, $P = 0.786$).

Discussion

This study demonstrated for the first time that, under high circulating estrogen levels simulating a premenopausal condition, SS is not protective on MCF-7 tumors and negates the tumor inhibitory effect of TAM in ovariectomized athymic nude mice. Interestingly, SS alone or combined with TAM enhanced bone strength at the femur midpoint but was not stimulatory in the uterus.

The initial observation that SS stimulates tumor growth beyond the POS control group suggests that SS is a tumor-promoting food source. However, SS alone and in combination with TAM induced significantly higher food intake than the TAM group and significantly higher body weight than both the POS control and TAM groups. The differences in food intake and body weight may be due to the hypophagic effects of estrogen and TAM rather than the hyperphagic effects of SS since estrogen and TAM decrease food intake and body weight (26–29), while more past studies (8, 9, 22, 30–33) have not shown the same effects after the ingestion of SS or its purified lignans. However, we cannot completely rule out the possibility that there are certain unknown SS components that may have contributed to the effect. Because subsequent regression analyses revealed a significant positive relationship among food intake, body weight, and tumor size, the palpable tumor data

Table 3. Effect of 10% Dietary SS, TAM, and Their Combination (SS + TAM) LV1–LV4 Bone Mass, Dimensions, and Peak Load[ab]

	POS	SS	TAM	SS + TAM
BMC, mg	21.33 ± 0.74	23.20 ± 0.57	20.72 ± 1.13	22.98 ± 0.71
BMD, mg/cm^2	66.08 ± 1.28	67.96 ± 1.82	65.50 ± 2.84	67.88 ± 1.36
Height, mm	2.06 ± 0.08	2.14 ± 0.07	2.12 ± 0.03	2.18 ± 0.08
Anteroposterior width, mm	2.94 ± 0.09	3.04 ± 0.08	3.09 ± 0.07	2.99 ± 0.08
Mediolateral width, mm	2.46 ± 0.04	2.45 ± 0.03	2.52 ± 0.06	2.50 ± 0.04
Weight, mg	20.32 ± 0.87	21.36 ± 0.77	20.30 ± 0.53	21.51 ± 0.72
Peak load, N	43.36 ± 9.78	39.63 ± 3.92	37.29 ± 3.07	46.41 ± 7.69

[a] Data are means ± SEM; $n = 6$–10 mice/group. No significant differences among groups.
[b] TAM implant = 5 mg, 60-day release.

were adjusted for differences in body weight. This then showed that SS no longer exhibited a tumor stimulatory effect, although it still tended to negate the tumor inhibitory effect of TAM.

A previous study showed in the same breast cancer mouse model that dietary 10% FS inhibits MCF-7 tumor growth and enhances the tumor inhibitory effect of TAM under premenopausal conditions (14). Hence, the present study also used SS at 10% to mimic 10% FS (14). Nevertheless, further investigation is required to determine whether other doses of SS would produce similar effects.

The exact mechanisms by which SS negates the tumor inhibitory effect of TAM are unclear, but SS resulted in larger tumors caused by an increase in tumor cell proliferation and a decrease in apoptosis. The effects of the lignans, their metabolites, and other compounds in SS on the estrogen receptor (ER) have yet to be elucidated, and their interactions with TAM on the ER may be multifaceted. The interaction of SS and TAM may also be affected by other factors such as the relative concentrations of each compound and the endogenous estrogen in tumor tissue and in the circulating blood. Nevertheless, because the high mammalian lignan-producing FS has been shown to inhibit MCF-7 tumor growth (14), while SS did not, the results of this study suggest that not all high mammalian lignan-producing foods are protective against breast cancer. The bioavailability of lignans in SS appears to be similar to that in FS since consumption of both seeds produces similar amounts of mammalian lignans, as indicated by their urinary levels in humans and rats (8, 9). Hence, in addition to lignans, other SS components, including linoleic acid, oleic acid, γ-tocopherol, phytosterols, and other phytochemicals (34, 35), may play an important role as well in the modulation of breast cancer. The present findings may have important implications in the clinical setting since many women undergoing TAM therapy may also consume phytoestrogen-rich foods as complementary therapy and to decrease the menopausal-like symptoms induced by TAM (21).

TAM is a first-line adjuvant therapy for estrogen-responsive breast cancer (36), but resistance to this drug can occur in 30%–40% of patients within 5 years of treatment initiation (36–38). However, TAM continues to be the antiestrogen treatment of choice for premenopausal patients because it effectively reduces mortality by 24%–39% (39). The tumor inhibitory effect of TAM observed in the present study is consistent with previous literature that demonstrated tumor inhibitory action under high circulating estrogen levels (14, 39, 40). In addition, the reduction of tumor cell proliferation and the increase in apoptosis induced by TAM are consistent with previous work that used the same experimental breast cancer mouse model and pellet doses of TAM and estrogen (14). The suggested mechanisms of TAM action include both ER-mediated and nonmediated mechanisms such as modulation of growth factors and signaling proteins, as previously described (38, 41, 42).

Regarding bone health, SS exerted favorable effects at the femur midpoint, a skeletal site containing predominately cortical bone, as shown by the higher peak load, ultimate stiffness, and toughness. However, femur BMD in the SS group did not differ from the other treatment groups, suggesting that the greater bone strength was not due to differences in the quantity of mineral in the femur. SS lignans or their metabolites may induce beneficial effects on bone *via* binding to the bone ER and inducing transcription of estrogen-responsive genes, which promote bone strength perhaps through production of bone matrix proteins. However, further investigation is required to determine the binding potential of the SS lignans, sesamin, sesamolin, and sesaminol, to the ER. Demethylpiperitol is a sesamin metabolite that has previously been shown to have weak affinities for the ER at 10 μ*M* in MCF-7 cells (43); however, its effect on transcription is not known and warrants further investigation. Other potential mechanisms of SS action may also include modulation of estrogen metabolism, bioavailability, and action in the body. For example, in postmenopausal women fed 50 g of SS powder daily for 5 weeks, lower levels of dehydroepiandrosterone sulfate but higher levels of serum sex hormone–binding globulin and 2-hydroxyestrone were observed compared with control subjects, suggesting that SS may possess antiestrogenic action in tissue (33). However, no biochemical markers of bone metabolism were measured due to the small size of the mice, which resulted in an insufficient amount of collected blood samples for analysis.

Previous studies have not examined the effect of SS or its lignans on bone metabolism in premenopausal women or using a model system that mimics the premenopausal condition. Because differences in body weight have been shown to affect bone strength in premenopausal women (44), it is possible that the beneficial effects induced by SS in the present study may be related to their higher body weights compared with the positive control and TAM groups (33% and 50% higher, respectively). Further study is required to elucidate the mechanisms by which SS acts on bone tissue.

The combination of SS with TAM resulted in a significantly greater ultimate stiffness, yield load, and toughness at the femur midpoint compared with the TAM and POS control groups, suggesting that TAM does not negate the beneficial effect of SS on bone. The effect of TAM on bone in premenopausal woman is antagonistic and induces bone loss (45, 46). The discrepancy between this mouse model and previous clinical studies is not known. While it is recognized that athymic mice are deficient in T lymphocytes, which are partially responsible for inducing bone loss upon estrogen withdrawal, athymic mice do experience bone loss in estrogen deficient states (47). Because the present study was conducted under high estrogen levels, resistance to bone loss is not a factor when considering T-cell deficiency. The null effects of TAM on bone health in the present study may be due to higher

circulating estrogen levels used (3–4 nmole/l E2 produced by 1.7 mg of E2 pellet; Ref. 14), which are at the upper physiologic level in premenopausal women and may have been enough to counteract the potential antagonistic effect of TAM on bone.

Like the breast and bone, the uterus is an estrogen-sensitive tissue in which compounds such as TAM can induce cell proliferation, subsequent growth (48), and potentially increased risk for the development of endometrial cancer. No differences in uterine weights were observed among groups, suggesting that SS does not act estrogenically or antiestrogenically in the uterus with endogenous estrogen production and may not increase endometrial cancer risk. Similar to the bone, uterine weight did not relate significantly to tumor weights, suggesting that the treatments acted differently in these tissues.

In conclusion, SS alone is not protective or stimulatory in established MCF-7 tumors but negates the tumor inhibitory effect of TAM in ovariectomized athymic mice under premenopausal conditions. In addition, SS alone and in combination with TAM are beneficial to bone health and exert no adverse effect on uterus weight, which is a safety marker. Although beneficial effects on bone were observed when SS was combined with TAM, its clinical application in a breast cancer setting may not be practical since negative interactions were observed on tumor growth. TAM is also prescribed as a preventive therapy to women who are at high risk for developing breast cancer. Future studies should be conducted to determine the potential role of SS in breast cancer prevention in high-risk populations who use TAM, since the mechanisms of action responsible for the development of breast cancer may differ from those responsible for cancer progression. The potential interactive effect of SS and TAM on bone health should be investigated in a chemopreventive experimental model because SS could potentially inhibit the TAM-induced bone loss experienced under premenopausal conditions, without interfering with the protective effect of TAM on breast cancer risk. The precise mechanisms of action, including the role of the ER signaling pathway, and examination of ER target genes in tumors, bone, and uterus should be established in the future.

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1. Adlercreutz H, Mazur W. Phyto-estrogens and Western diseases. Ann Med 29:95–120, 1997.
2. Dai Q, Franke A, Jin F, Shu X, Hebert J, Custer L, Cheng J, Gao Y, Zheng W. Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. Cancer Epidemiol Biomarkers Prev 11:815–821, 2002.
3. Linseisen J, Piller R, Hermann S, Chang-Claude J. Dietary phytoestrogen intake and premenopausal breast cancer risk in a German case-control study. Int J Cancer 110:284–290, 2004.
4. McCann S, Muti P, Vitp D, Edge S, Trefisan M, Freudenheim J. Dietary lignan intakes and risk of pre- and postmenopausal breast cancer. Int J Cancer 111:440–443, 2004.
5. Pietinen P, Stumpf K, Mannisto S, Kataja V, Uusitupa M, Adlercreutz H. Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. Cancer Epidemiol Biomarkers Prev 10:339–344, 2001.
6. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutr Cancer 54:184–201, 2006.
7. Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignan production from various foods. Nutr Cancer 16:43–52, 1991.
8. Coulman KD, Liu Z, Hum WQ, Michaelides J, Thompson LU. Whole sesame seed is a rich source of mammalian lignan precursors as whole flaxseed. Nutr Cancer 52:156–165, 2005.
9. Liu Z, Saarinen NM, Thompson LU. Sesamin is one of the major precursors of mammalian lignans in sesame seed (Sesamum indicum) as observed in vitro and in rats. J Nutr 136:906–912, 2006.
10. Rowland I, Faughnan M, Hoey L, Wahala K, Williamson G, Cassidy A. Bioavailability of phyto-oestrogens. Br J Nutr 89(Suppl 1):S45–S58, 2003.
11. Power KA, Thompson LU. Flaxseed and lignans: Effects on breast cancer. In: Awad AB, Bradford PG, Eds. Nutrition and Cancer Prevention. Boca Raton, FL: Taylor & Francis Group, pp385, 2006.
12. Phipps WR, Martini MC, Lampe JW, Slavin JL, Kurzer MS. Effect of flax seed ingestion on the menstrual cycle. J Clin Endocrinol Metab 77: 1215–1219, 1993.
13. Wilcox G, Wahglvist ML, Burger HG, Medley G. Oestrogenic effects of plant foods in postmenopausal women. Br Med J 301:905–906, 1990.
14. Chen J, Hui E, Ip T, Thompson LU. Dietary flaxseed enhances the inhibitory effect of tamoxifen on the growth of estrogen dependent human breast cancer (MCF-7) in nude mice. Clin Cancer Res 10:7703–7711, 2004.
15. Chen J, Thompson LU. Lignans and tamoxifen, alone or in combination, reduce human breast cancer cell adhesion, invasion and migration in vitro. Breast Cancer Res Treat 80:163–170, 2003.
16. Chen J, Wang L, Thompson LU. Flaxseed and its components reduce metastasis after surgical excision of solid human breast tumor in nude mice. Cancer Lett 234:168–175, 2006.
17. Dabrosin C, Chen J, Wang L, Thompson LU. Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts. Cancer Lett 185:31–37, 2002.
18. Power KA, Saarinen NM, Chen JM, Thompson LU. Mammalian lignans enterolactone and enterodiol, alone and in combination with the isoflavone genistein, do not promote the growth of MCF-7 xenografts in ovariectomized athymic nude mice. Int J Cancer 118:1316–1320, 2006.
19. Bergman Jungeström M, Thompson LU, Dabrosin C. Flaxseed and its lignans inhibit estradiol-induced growth, angiogenesis and secretion of VEGF in human breast cancer xenografts in vivo. Clin Cancer Res 13: 1061–1067, 2007.
20. Power KA, Ward WE, Chen JM, Saarinen NM, Thompson LU. Genistein alone and in combination with the mammalian lignans enterolactone and enterodiol induce estrogenic effects on bone and uterus in a postmenopausal breast cancer mouse model. Bone 39:117–124, 2006.
21. Duffy C, Cyr M. Phytoestrogens: potential benefits and implications for breast cancer survivors. J Womens Health 12:617–631, 2003.
22. Penalvo JL, Heinonen SM, Aura AM, Adlercreutz H. Dietary sesamin is converted to enterolactone in humans. J Nutr 135:1056–1062, 2005.
23. Olfert ED, Cross BM, McWilliam AA, Eds. Guide to the Care and Use of Experimental Animals, (2nd ed.). Ottawa: Canadian Council on Animal Care, Vol 1, 1993.
24. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad

hoc writing committee on the reformation of the AIN-76A rodent diet. J Nutr 123:1939–1951, 1993.
25. Fonseca D, Ward WE. Daidzein together with high calcium preserve bone mass and biomechanical strength at multiple sites in ovariectomized mice. Bone 35:489–497, 2004.
26. Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. Philos Trans R Soc Lond B Biol Sci 361:1251–1263, 2006.
27. Geary N, Asarian L, Korach KS, Pfaff DW, Ogawa S. Deficits in estrogen-dependent control of feeding, weight gain, and cholecystokinin satiation in ER-alpha null mice. Endocrinology 142:4751–4757, 2001.
28. Jordan VC, Phelps E, Lindgren JU. Effects of anti-estrogens on bone in castrated and intake female rats. Breast Cancer Res Treat 10:31–35, 1987.
29. Wallen JW, Belanger MP, Wittnich C. Sex hormones and the selective estrogen receptor modulator tamoxifen modulate weekly body weights and food intakes in adolescent and adult rats. J Nutr 131:2351–2357, 2001.
30. Hirose N, Doi F, Ueki T, Akazawa K, Chijiiwa K, Sugano M, Akimoto K, Shimizu S, Yamada H. Suppressive effect of sesamin against 7, 12-dimethylbenz[a]-anthracene induced rat mammary carcinogenesis. Anticancer Res 12:1259–1265, 1992.
31. Sheng H, Hirose Y, Hata K, Zheng O, Kuno T, Asano N, Yamada Y, Hara A, Osawa T, Mori H. Modifying effect of dietary sesaminol glucosides on the formation of azoxymethane-induced premalignant lesions of rat colon. Cancer Lett 246:63–68, 2007.
32. Sirato-Yasumoto S, Katsuta M, Okuyama Y, Takahashi Y, Ide T. Effect of sesame seed rich in sesamin and sesamolin on fatty acid oxidation in rat liver. J Agric Food Chem 49:2647–2651, 2001.
33. Wu WH, Kang YP, Wang NH, Jou HJ, Wang TA. Sesame ingestion affects sex hormones, antioxidant status, and blood lipids in postmenopausal women. J Nutr 136:1270–1275, 2006.
34. Phillips KM, Ruggio DM, Ashraf-Khorassani M. Phytosterol composition of nuts and seeds commonly consumed in the United States. J Agric Food Chem 53:9436–9445, 2005.
35. USDA: Nutrient Data Laboratory. Available at: www.nal.usda.gov/fnic/foodcomp/search. Accessed March 20, 2007.
36. Jordan VC. Selective estrogen receptor modulation: concept and consequences in cancer. Cancer Cell 5:207–213, 2004.
37. Gottardis MM, Jordan VC. Development of tamoxifen-stimulated growth of MCF-7 tumors in athymic mice after long-term antiestrogen administration. Cancer Res 48:5183–5187, 1988.
38. MacGregor JI, Jordan VC. Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev 50:151–196, 1998.
39. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365: 1687–1717, 2005.
40. Osborne KC, Hobbs K, Clark GM. Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res 45:584–590, 1985.
41. Lewis JS, Jordan VC. Selective estrogen receptor modulators (SERMs): mechanisms of anticarcinogenesis and drug resistance. Mutat Res 591: 247–263, 2005.
42. Mandlekar S, Kong AN. Mechanisms of tamoxifen-induced apoptosis. Apoptosis 6:469–477, 2001.
43. Nakano D, Kwak CJ, Fujii K, Ikemura K, Satake A, Ohkita M, Takaoka M, Ono Y, Nakai M, Tomimori N, Kiso Y, Matsumura Y. Sesamin metabolites induce an endothelial nitric oxide-dependent vasorelaxation through their antioxidative property-independent mechanisms: possible involvement of the metabolites in the antihypertensive effect of sesamin. J Pharmacol Exp Ther 318:328–335, 2006.
44. Khan A. Management of low bone mineral density in premenopausal women. J Obstet Gynaecol Can 27:345–349, 2005.
45. Mackey JR, Joy AA. Skeletal health in postmenopausal survivors of early breast cancer. Int J Cancer 114:1010–1015, 2005.
46. Vehmanen L, Elomaa I, Blomqvist C, Saarto T. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. J Clin Oncol 24:675–680, 2006.
47. Lee SK, Kadono Y, Okada F, Jacquin C. Koczon-Jaremko B, Gronowicz G, Adams DJ, Aquila HL, Choi Y, Lorenzo JA. T lymphocyte-deficient mice lose trabecular bone mass with ovariectomy. J Bone Miner Res 21:1704–1712, 2006.
48. Reed CA, Berndtson AK, Nephew KP. Dose-dependent effects of 4-hydroxytamoxifen, the active metabolite of tamoxifen, on estrogen receptor-alpha expression in the rat uterus. Anticancer Drugs 16:559–567, 2005.