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Whole Sesame Seed Is as Rich a Source of Mammalian Lignan Precursors as Whole Flaxseed

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Abstract: *The mammalian lignans enterolactone and enterodiol, which are produced by the microflora in the colon of humans and animals from precursors in foods, have been suggested to have potential anticancer effects. This study determined the production of mammalian lignans from precursors in food bars containing 25 g unground whole flaxseed (FB), sesame seed (SB), or their combination (FSB; 12.5 g each). In a randomized crossover study, healthy postmenopausal women supplemented their diets with the bars for 4 wk each separated by 4-wk washout periods, and urinary mammalian lignan excretion was measured at baseline and after 4 wk as a marker of mammalian lignan production. Results showed an increase with all treatments (65.1–81.0 μmol/day*; $P < 0.0001$*), which did not differ among treatments. Lignan excretion with the whole flaxseed was similar to results of other studies using ground flaxseed. An unidentified lignan metabolite was detected after consumption of SB and FSB but not of FB. Thus, we demonstrated for the first time that 1) precursors from unground whole flaxseed and sesame seed are converted by the bacterial flora in the colon to mammalian lignans and 2) sesame seed, alone and in combination with flaxseed, produces mammalian lignans equivalent to those obtained from flaxseed alone.*

Introduction

The mammalian lignans enterolactone (EL) and enterodiol (ED) are produced by the action of bacterial flora in the colon of humans and animals on precursors in foods (Fig. 1) (1,2). Although the plant lignans secoisolariciresinol (SECO) diglucoside (SDG) and matairesinol are originally thought to be the only precursors of mammalian lignans (1,2), recent studies have shown that pinoresinol, lariciresinol, syringaresinol, arctigenin, and 7-hydroxymatairesinol can also be converted to these compounds (3,4). Once formed, the mammalian lignans are absorbed, circulated enterohepatically, and in part excreted in the urine (5). Hence, consumption of foods rich in the mammalian lignan precursors has been shown to greatly increase urinary ED and EL concentrations, and urinary lignan excretion has been used as a marker of the intake levels of lignan-rich foods (6–12).

Mammalian lignans are of interest because they have been suggested to have anticancer effects. Because of their chemical structural similarity to estrogen and their ability to bind to estrogen receptors α and β (13), they are thought to have weak estrogenic/antiestrogenic properties and thus can reduce the risk of hormone-related diseases such as breast cancer. Some epidemiological studies showed a negative relationship between breast cancer risk and the plasma levels, urinary excretion, or intake of lignans (14–18), although some studies also showed no relationship (19–21). Treatment with the mammalian lignan precursors SDG (22–25) and hydroxymatairesinol (4) or with the mammalian lignan EL (26) reduced mammary tumor development in carcinogen-treated rats. SDG also reduced the metastasis of estrogen receptor-negative human tumors (MDA-MB-435) (27,28) and of melanoma cells (29). In in vitro studies, mammalian lignans inhibited the activity of enzymes involved in hormone biosynthesis (aromatase, 7β-hydroxysteroid dehydrogenase, and 5α-reductase) (30–33) and the growth-stimulating effect of estrogen (34). They have antioxidant (35,36) and antiangiogenesis (37) properties and can reduce the adhesion, migration, and invasion of MDA-MB-435 cells, steps that are involved in the metastasis process (38).

Flaxseed has been reported to be one of the richest sources of mammalian lignan precursors, particularly SDG, with levels 100–800 times higher than that of other plant foods (39,40). Therefore, it has been used as a food model to determine the anticancer effects of lignans, the results of which have recently been reviewed (41). It reduced mammary tumor development when given at the preinitiation, promotion, or late promotion stages of carcinogenesis in carcinogen-treated rats (23,24,41,42). It also reduced the tumor growth and metastasis of estrogen receptor-negative human

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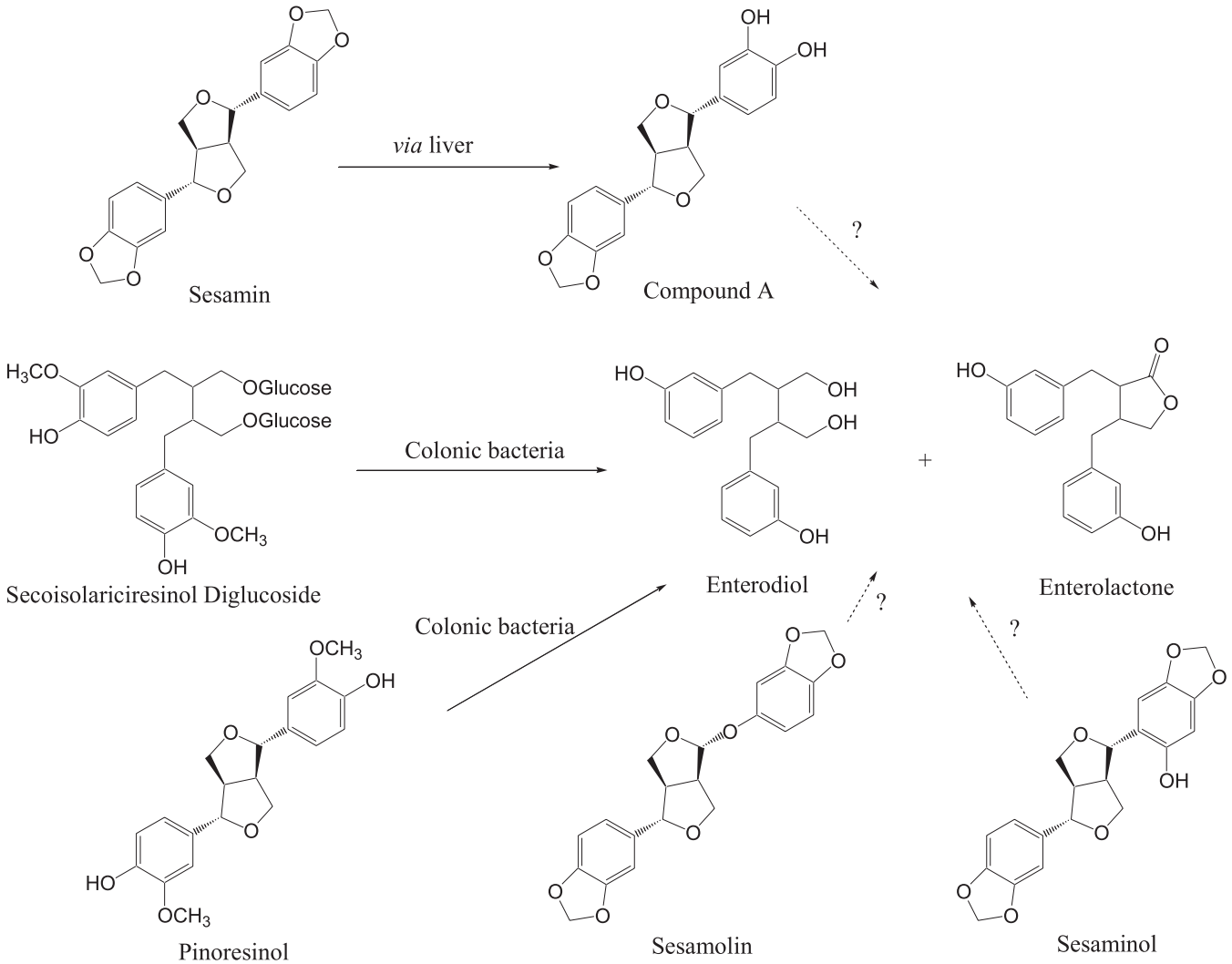

Figure 1. Metabolic conversion of plant lignans to mammalian lignans.

breast cancer cells (MDA-MB-435) (27,28,43,44) and the tumor growth of estrogen receptor-positive human breast cancer cells (MCF-7) in athymic mice (45). Reduced cell proliferation and erbB2 expression and increased cell apoptosis were also observed in the tumors of breast cancer patients who consumed flaxseed (46). Because the effect of SDG at levels found in flaxseed was similar to that of flaxseed, the anticancer effects of flaxseed have been attributed largely to the mammalian lignans produced from its SDG and other precursors (23–25,27,28,41).

Sesame seed is an oilseed that contains high concentrations of the plant lignans sesamin (SES), sesamolin (SMN), and sesaminol (Fig. 1) (47). It is unknown whether these lignans are converted to ED and EL because they are primarily fat soluble in contrast to the water-soluble SDG in flaxseed.

Many studies on sesame seed and its lignans have concentrated on their antioxidant (48–50) and blood pressure- (51–53) and serum lipid-lowering effects (50,54–58). Only limited studies have been conducted on their potential anticancer effects. Dietary SES has been shown to reduce the number of mammary tumors in carcinogen-induced rats (59). In in vitro studies, SES, sesaminol, and SMN inhibited the growth of human lymphoid leukemia Molt 4B cells by inducing apoptosis (60–62). Sesamol, a sesaminol derivative produced from sesame oil refining or heat treatment, also induced growth arrest and apoptosis in MCF-7 and T47D breast cancer cells (63). In epidemiological studies, sesame oil-containing lignans were associated with decreased risk of stomach cancer (64).

The previous studies on flaxseed and sesame seed have been conducted using the ground seed or pure lignans, and it is unknown whether the production of mammalian lignans in unground whole seed found in many commercially available food products would be the same as that in the ground seed. There is a concern that the hard seed coat, particularly of flaxseed, prevents the release and metabolism of the seed components if they are eaten unground, thereby lessening their physiological effects. There is also a lack of human studies examining both the production of mammalian lignans and the physiological effects of ground or unground sesame seed. The combination of flaxseed and sesame seed has also

not been studied. Because the two seeds contain different types of lignans, which may complement each other, they may have better health benefits when consumed together than when taken alone. Thus, the objective of this study was to examine, in postmenopausal women, the production of mammalian lignans from precursors in food bars containing 25 g unground flaxseed, sesame seed, or their combination. If they are produced, the whole seeds in bar formulation can be used in future studies to further demonstrate in clinical studies the potential anticancer effects of flaxseed and sesame seed alone or in combination.

Subjects and Methods

Subjects

Postmenopausal women were chosen as subjects for this study because many breast cancer patients are postmenopausal and may also consume foods rich in phytoestrogens such as lignans as potential alternative or complementary treatments for menopausal symptoms, osteoporosis, and cancer (65). Sixteen healthy, postmenopausal women (mean age 66.4 ± 1.7 yr; 14.4 ± 2.0 yr since menopause) volunteered in this study and nobody dropped out. At the commencement of the study (first baseline measurement), the mean weight and body mass index of the women were 73.1 ± 3.1 kg and 28.6 ± 1.5 kg/m^2, respectively. Both remained constant throughout the duration of the study. Subjects were included if they were omnivorous nonsmokers with stable body weights. Subjects were excluded if they were taking or had ever taken hormone replacement therapy, antibiotics within the last 6 mo, and/or lipid-lowering drugs 4 wk prior to beginning the study. The consumption of food containing flaxseed, sesame seed, or soy and the use of herbal supplements were discontinued 4 wk prior to the commencement of the study. Basic vitamin and mineral supplements, if taken regularly before commencing the study, were allowed to continue for the duration of the study. All subjects provided written informed consent, and the study protocol was reviewed and approved by the University of Toronto Human Ethics Committee.

Study Design

In a randomized crossover design, subjects supplemented their normal diets with one of three treatment food bars—flaxseed bar (FB), sesame seed bar (SB), or flaxseed+sesame seed bar (FSB)—for a period of 4 wk with a 4-wk washout period between each treatment. Subjects were randomized to the order in which they received FB, SB, and FSB.

Similar to our previous study on flaxseed (11), 2 days prior to the beginning of the study (pre-study Day 1), subjects consumed a standard low-fiber, low-lignan dinner. On the morning of the day prior to the beginning of the study (pre-study Day 2), subjects collected a 24-h urine sample (first urine of day discarded) through to the first urine of Day 1 of the study period while continuing to consume standard low-fiber meals. The first treatment bar was then consumed at breakfast on study Day 1 and every day at breakfast after that up until Day 28. On the evening prior to the last treatment bar consumption (Day 27), subjects again consumed a standard dinner and began collecting a 24-h urine sample starting on the morning of Day 28 through to Day 1 of the next 4-wk period as previously described for pre-study Day 2 to study Day 1. Subjects ate their normal diets throughout the rest of the study days (Day 1–26); however, they were required to record 3 days of food intake (one weekend, two weekdays) during each treatment and washout periods. Subjects avoided other flaxseed-, sesame seed-, or soy-containing foods and herbal supplements throughout the course of the study. Macronutrient intakes from the diet records were calculated and averaged using the NutriWatch nutrient analysis program (version 6.1.22E Delphi 1, based on the 1997 Canadian Nutrient File; Elizabeth Warwick, PEI, Cornwall, Canada).

Food Bar Formulation and Analysis

Each bar contained 25 g flaxseed (Pizzey's Milling, Angusville, MB, Canada), 25 g sesame seed (Grain Process Enterprises, Ltd., Scarborough, ON, Canada), or 12.5 g flaxseed and 12.5 g sesame seed plus 6.7 g high-fructose corn syrup and 5.6 g sugar to allow formulation into bar form and provide flavor. The ingredients were blended, portioned into bar format, and heated for 8 min at 190°C in a conventional oven. The bars were then cooled at room temperature for at least 60 min, vacuum-sealed in polyethylene film, and stored at –20°C until distributed to subjects. Subjects kept the bars in the freezer until the day of consumption.

Protein, carbohydrate, fat, and total, insoluble, and soluble dietary fiber contents of food bars were analyzed according to the standard methods of the Association of Official Analytical Chemists (66). The plant lignans (SDG, matairesinol, lariciresinol, pinoresinol, sesaminol, SES, and SMN) in the treatment bars were analyzed by capillary gas chromatography-mass spectrometry (GC-MS) using a modification of other methods (7,39,67). Briefly, the samples were ground and defatted with hexane, and the lignans from the defatted ground samples were extracted with 70% MeOH, hydrolyzed with NaOH, and then neutralized with acetic acid. The lignan-rich fraction was eluted using C18 reversed-phase octadecylsilane bonded silica (C18) extraction columns (Scientific Products and Equipment, Ltd., Concord, ON), dried, and redissolved in sodium acetate buffer. After overnight enzyme hydrolysis with β-glucuronidase (*Helix pomatia*; Sigma Chemical Co., St. Louis, MO), samples were again passed through a C18 column, dried, and redissolved in MeOH. The internal standards 5α-androstane-3β,17β-diol and stigmasterol (Steraloids, Inc., Wilton, NH) were added, MeOH was evaporated under nitrogen, and the lignans were derivatized (Tri-Sil Reagent; Pierce, Rockford, IL), dissolved in hexane, and analyzed by GC-MS (GC 6890 Series

II, MS 5973; Hewlett-Packard, Avondale, PA) as we have previously described (7,8,39).

Because sesame lignans are primarily present in oil, their levels were also determined in the oil extracted from the seed by hexane. After removal of hexane from the extract by rotary evaporator, the pure oil (0.04 g) was redissolved in hexane (1 ml). To an aliquot (40 µl) was then added 40 µl of internal standard (1,000 µg/ml of 4′-OH-flavanone) and 120 µl methanol. After vortex mixing and filtration through a 0.45-µm pore-size filter, an aliquot (10 µl) of the filtrate was injected into a high-performance liquid chromatograph (Waters 2690 separation system; Milford, WA) coupled to a Waters 996 photodiode array detector equipped with a Waters symmetry C18 column (4.6 × 75 mm, 3.5 µm). Elution was with a gradient solvent system containing solvent A (water) and solvent B (acetonitrile) programmed at 20% B to 50% B in 15 min, 50% B to 100% B in 15 min, and then 20% B in 5 min running at a flow rate of 1 ml/min. The total lignan in the sample was the sum of the levels present in the defatted sample and the extracted oil.

Urinary Lignan Analysis

The concentrations of lignans [for example, ED, EL, and SECO (aglycone of SDG)] were measured in the urine by a GC-MS procedure, which is routinely used in our laboratory as previously described (7,8,11,39).

Statistical Analyses

All statistical analyses were performed using PROC MIXED, version 8.1 (SAS Institute, Cary, NC). Urinary lignans were corrected for order, treatment, and order × treatment. Least-squares means was performed to detect if treatments resulted in any significant change between baseline and follow-up measurements. Tukey-adjusted pairwise comparisons were performed to detect any differences between changes in treatments. The acceptable level of significance was $P < 0.05$.

Results

Food Bar Composition

The composition of the food bars is presented in Table 1. SB provided 193 kJ more than FB due to the higher fat content of sesame seed. FB contained 4 g more dietary fiber than SB. As expected, FB had very high concentrations of SDG, whereas SB had very high concentrations of SES, SMN, and sesaminol. Both FB and SB had small amounts of matairesinol, pinoresinol, and lariciresinol, which, like SDG, are known to be converted by the colonic microflora to ED and EL (3). The total lignan concentration was higher in SB than FB. FSB had lignan concentrations intermediate to those of FB and SB.

Macronutrient Intake

Table 2 shows significant change in intake of energy, protein, dietary fiber, and fat in the SB group, in protein and dietary fiber in the FB group, and in dietary fiber in the FSB group. However, the change did not differ significantly among treatment groups. The increase in total fiber intake (6.7, 3.3, and 2.9 g) in the FB, SB, and FSB groups, respectively, can be attributed primarily to the fiber content of the food bar supplements.

Urinary Lignans

There was a large variability in the urinary lignans of subjects in response to the treatment bars (Fig. 2). However, the urinary excretion of SECO, ED, EL, ED+EL, and total lignans (SECO+ED+EL) generally increased with all treatment bars (µmol/day, by 65.1 with FB, 81.0 with SB, and 66.4 with FSB; $P < 0.0001$), although the increase in all uri-

Table 1. Composition of Treatment Bars With 25 g Seed[a]

Macronutrient	FB	SB	FSB
Total energy (kJ)	685	878	776
Protein (g, % energy)	5.2 (12.7)	6.0 (11.4)	5.6 (12.1)
Fat (g, % energy)	11.1 (60.7)	15.9 (68.2)	13.4 (64.7)
Available CHO (g, % energy)	10.9 (26.6)	10.7 (20.4)	10.8 (23.2)
Total dietary fiber (g)	7.0	3.1	4.7
Insoluble fiber	4.4	3.0	4.2
Soluble fiber	2.6	0.1	0.5
SDG (µmol)	200.05	0.04	100.04
Matairesinol (µmol)	0.64	0.08	0.36
Pinoresinol (µmol)	2.47	5.58	4.02
Lariciresinol (µmol)	1.75	0.21	0.98
Sesamin (µmol)	0	378.88	189.44
Sesamolin (µmol)	0	90.44	45.22
Sesaminol (µmol)	0	69.37	34.69

a: Abbreviations are as follows: FB, flaxseed bar; SB, sesame seed bar; FSB, flaxseed + sesame seed bar; CHO, carbohydrate; and SDG, secoisolariciresinol diglycoside.

Table 2. Macronutrient Intake of Subjects[a]

Treatment	Energy (kJ)	Protein (g)	CHO (g)	Dietary Fiber (g)	Fat (g)
Baseline	7,056 ± 318	71.2 ± 2.8	225.2 ± 9.7	19.5 ± 1.4	56.0 ± 5.3
FB	7,629 ± 347	81.8 ± 4.7	232.9 ± 10.5	26.2 ± 1.7	64.4 ± 6.1
Change	573 ± 331	10.6 ± 3.9[b]	7.7 ± 10.3	6.7 ± 1.2[c]	8.4 ± 5.0
Baseline	6,868 ± 314	70.5 ± 3.5	228.6 ± 11.1	19.9 ± 1.6	51.9 ± 5.6
SB	7,909 ± 414	79.9 ± 4.2	237.5 ± 11.9	23.2 ± 1.7	73.5 ± 5.8
Change	1,041 ± 367[d]	9.4 ± 3.9[b]	8.9 ± 8.6	3.3 ± 1.4[d]	21.6 ± 6.3[c]
Baseline	7,378 ± 372	74.3 ± 3.3	229.0 ± 7.7	21.0 ± 1.4	62.6 ± 8.3
FSB	7,603 ± 368	76.8 ± 4.3	232.6 ± 14.0	23.9 ± 1.6	65.6 ± 5.0
Change	225 ± 470	2.5 ± 3.7	3.6 ± 13.5	2.9 ± 1.2[b]	3.0 ± 7.9

a: Abbreviations are as follows: CHO, total carbohydrate; FB, flaxseed bar; SB, sesame seed bar; and FSB, flaxseed + sesame seed bar. Values are expressed as mean ± SE. Change values in the same column are not significantly different at $P > 0.05$.

b: Change from baseline significant at $P < 0.05$.

c: Change from baseline significant at $P < 0.001$.

d: Change from baseline significant at $P < 0.01$.

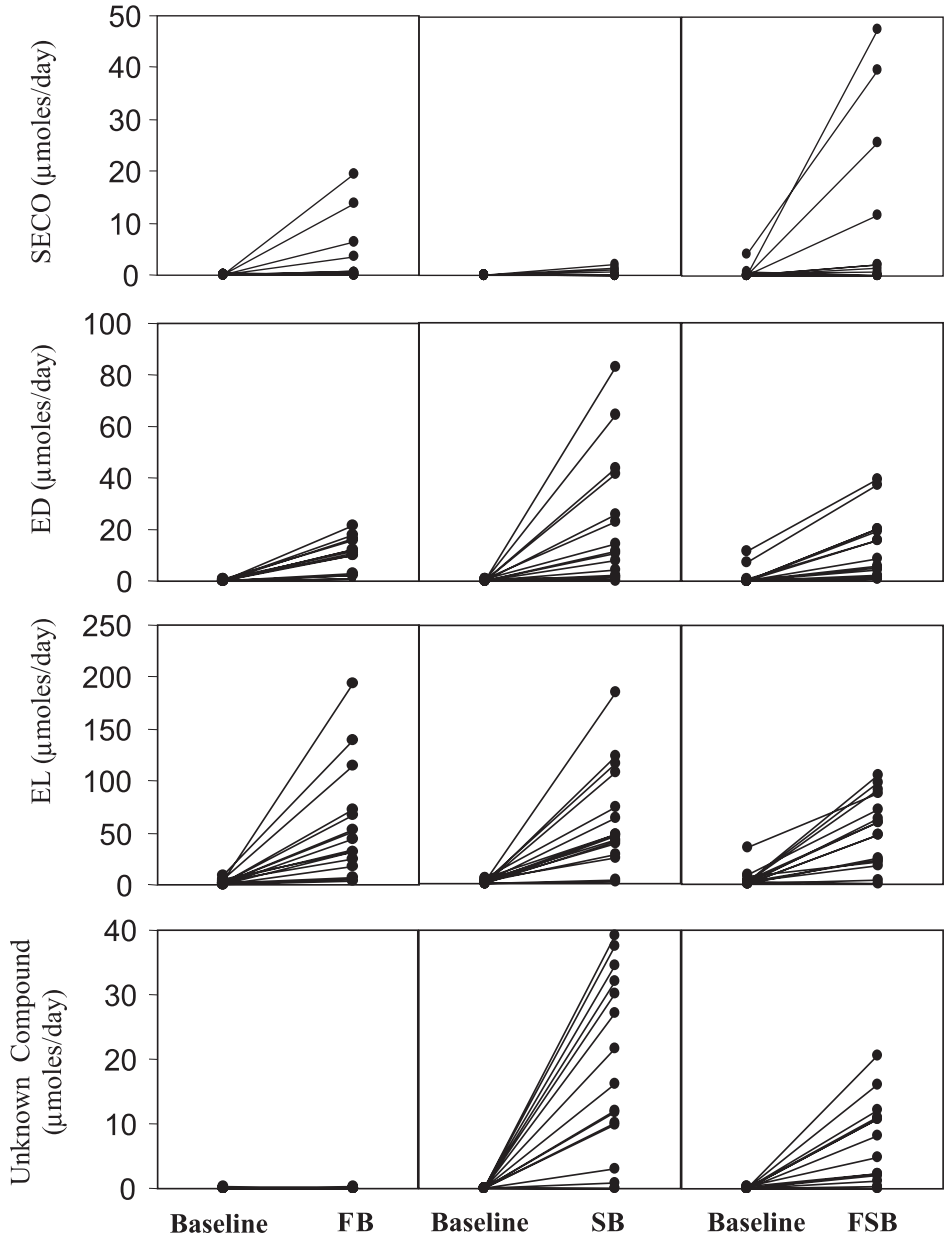

Figure 2. Urinary lignan excretion of individual subjects pre- and posttreatment bars.

Table 3. Urinary Lignan Excretion Pre- and Posttreatment Bars[a]

Treatment	SECO	ED	EL	ED+EL	Total (SECO+ED+EL)	Unknown Compound[b]
Baseline	0.0 ± 0.0	0.2 ± 0.1	2.5 ± 0.6	2.7 ± 0.6	2.7 ± 0.6	0.0 ± 0.0
FB	2.9 ± 1.4	9.2 ± 1.7	55.7 ± 13.2	65.0 ± 14.0	67.8 ± 13.8	0.0 ± 0.0
Change	2.9 ± 1.4[c]	9.0 ± 1.7[c]	53.2 ± 12.8[c]	62.2 ± 13.3[c]	65.1 ± 13.1[c]	0.0 ± 0.0*
Baseline	0.0 ± 0.0	0.2 ± 0.1	1.9 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	0.0 ± 0.0
SB	0.4 ± 0.2	21.0 ± 6.2	61.7 ± 12.2	82.7 ± 12.9	83.1 ± 12.9	17.9 ± 3.5
Change	0.3 ± 0.2[c]	20.8 ± 6.2[c]	59.8 ± 12.2[c]	80.6 ± 12.8[c]	81.0 ± 12.9[c]	17.9 ± 3.5[c,†]
Baseline	0.3 ± 0.2	0.8 ± 0.7	4.2 ± 2.1	5.0 ± 2.8	5.3 ± 2.8	0.0 ± 0.0
FSB	8.2 ± 0.9	12.3 ± 3.1	51.2 ± 8.5	63.5 ± 10.1	71.7 ± 9.6	6.4 ± 1.6
Change	7.9 ± 3.7[c]	11.5 ± 2.5[c]	47.0 ± 8.1[c]	58.5 ± 9.0[c]	66.4 ± 9.2[c]	6.4 ± 1.6[c,‡]

a: Values are mean ± SE; μmol/day. Abbreviations are as follows: SECO, secoisolariciresinol; ED, enterodiol; and EL, enterolactone; FB, flaxseed bar; SB, sesame seed bar; FSB, flaxseed + sesame seed bar.

b: Values are means ± SE; μmol equivalent internal standard/day; internal standard was 5α-androstane-3β,17β-diol. Changes in the same column with different superscripts (*, †, ‡) are significantly different at $P < 0.05$.

c: $P < 0.0001$ change from baseline.

nary lignans did not differ significantly among groups (Table 3). Except for the SECO aglycone of SDG, none of the sesame or flaxseed plant lignans were detected in the urine. However, an unknown compound (peak 4, compound A; Fig. 3) was consistently detected in the urine after the consumption of SB and FSB but not after FB (Fig. 2). The mass spectra (Fig. 4) suggest that this unknown compound is a lignan. The urinary level of this compound increased significantly ($P < 0.05$) with SB and FSB, with the increase with SB (17.9 μmol/day) significantly greater than that with FSB (6.4 μmol/day) (Table 3). The urinary SECO level was higher and the ED and EL levels were lower in the FSB group than can be predicted from those in the FB and SB groups. The total recoveries of ED, EL, and SECO in the urine were 31.8, 14.9, and 17.7% of the total plant lignans in FB, SB, and FSB, respectively. The recovery of combined ED, EL, and SECO in the urine was also lower in the FSB group than can be predicted from the recoveries in the FB and SB groups.

Discussion

This study demonstrated for the first time that, in postmenopausal women, 1) plant lignans in unground whole flaxseed, sesame seed, and their combination, in the bar formulation, are converted by the bacterial flora in the colon to mammalian lignans, as indicated by the significantly large increase in urinary excretion of ED and EL; 2) large amounts of urinary ED and EL are produced from sesame seed-containing bars, as much as that produced with flaxseed, indicating the presence of large amounts of mammalian lignan precursors; and 3) sesame plant lignans are converted as well to another compound, which is excreted in the urine.

This study did not directly compare the production of mammalian lignans from bars with unground seed with bars with ground seed. However, the urinary ED+EL excretion (62.2 μmol/day) after FB intake was higher than the 41.1 μmol/day observed in another study in which postmenopausal women also consumed 25 g/day ground flaxseed for 16 wk (8) and the 54.5 μmol/day excreted by premenopausal women who supplemented their diets with 25 g/day ground flaxseed for 7 days (11). In contrast, urinary ED+EL excretion of 59.4 μmol/day was reported in postmenopausal women after consuming 10 g/day ground flaxseed for 7 wk (9).

It is not possible to accurately compare the results of different studies as different subjects and flaxseed with perhaps different SDG concentrations were used (68). Differences in analytical methods to measure SDG and mammalian lignans can also produce different values (6). Subject differences in colonic microflora, intestinal transit time, or redox level of the colon (1,6) can also influence lignan metabolism, a fact that is also evident in this study. Subjects in the study (9), which produced higher urinary lignans per gram flaxseed intake, may have been greater mammalian lignan producers than the subjects in the present study. Nevertheless, despite individual variabilities, large urinary mammalian lignans observed in this study indicate that the bacterial conversion of the precursors in the whole seeds to mammalian lignans have taken place.

EL was produced in greater amounts than ED (53.2 vs. 9.0 μmol/day) in this study, which agrees with the results of Hutchins et al. (9) (56.2 vs. 3.2 μmol/day) and Brooks et al. (8) (31.1 vs. 10.0 μmol/day) in postmenopausal women but disagrees with the results of Nesbitt et al. (11) (10.9 vs. 43.6 μmol/day) and Cunnane et al. (69) (12.4 vs. 18.7 μmol/day) in premenopausal women. EL has been reported to possess greater beneficial effects than ED (38,70–72). Taken together, it would appear that flaxseed consumption leads to a greater excretion of EL in postmenopausal women and of ED in premenopausal women. However, more flaxseed studies are needed to confirm this as the differences could simply be due to differences in bacterial activities on the lignans independent of menopausal status.

Known precursors of ED and EL such as SDG, matairesinol, pinoresinol, and lariciresinol were found in SB but at much lower concentrations, particularly in SDG, when compared with those in FB. It was thus surprising that SB consumption resulted in a greater, albeit not significantly greater, increase in urinary lignans than FB. This could not

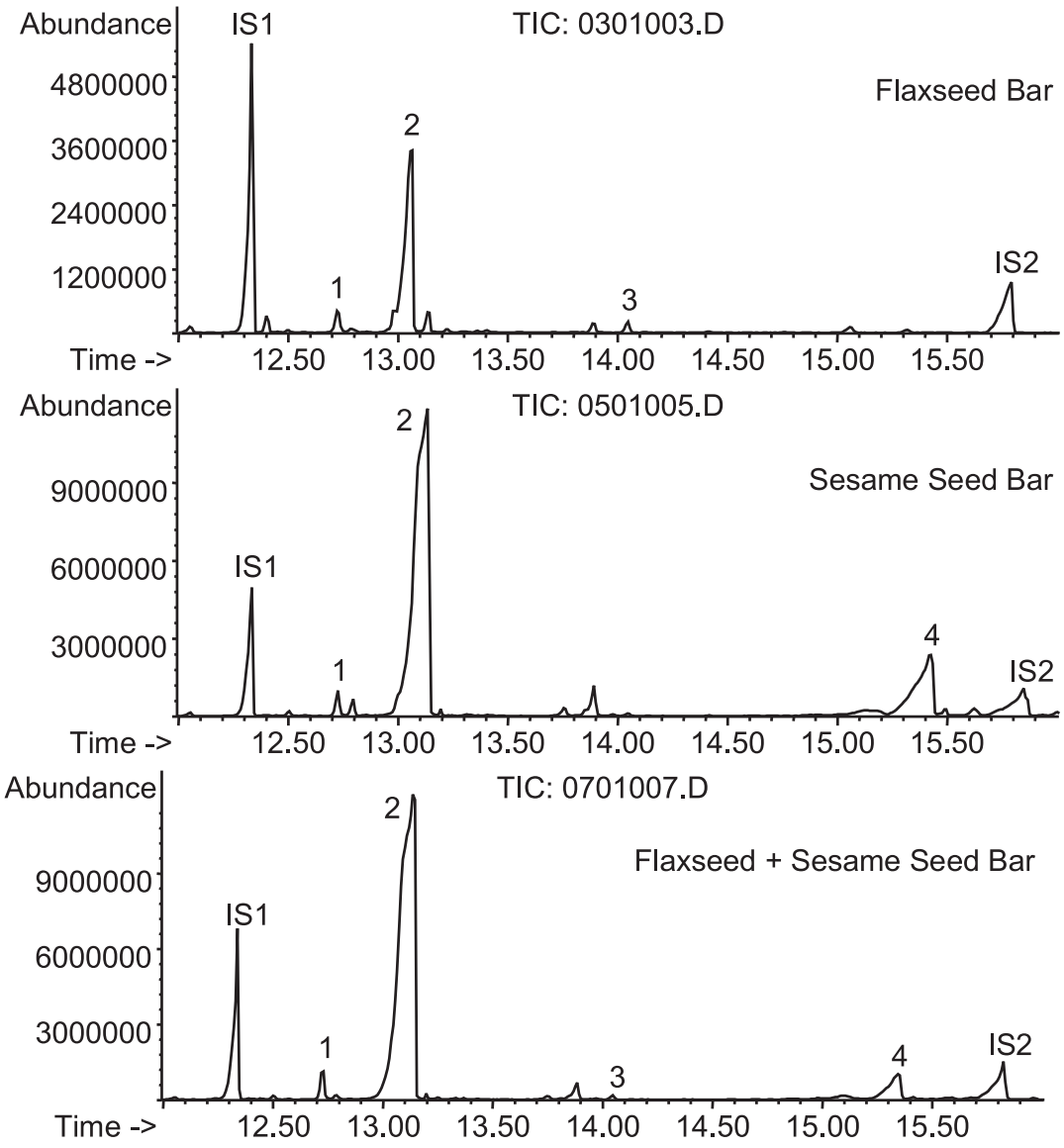

Figure 3. Gas chromatography-mass spectrometry chromatograms of urinary lignans after treatment bar consumption. IS1, 5α-androstane-3β,17β-diol (internal standard 1); 1, enterodiol; 2, enterolactone; 3, secoisolariciresinol; 4, compound A; and IS2, stigmasterol (internal standard 2).

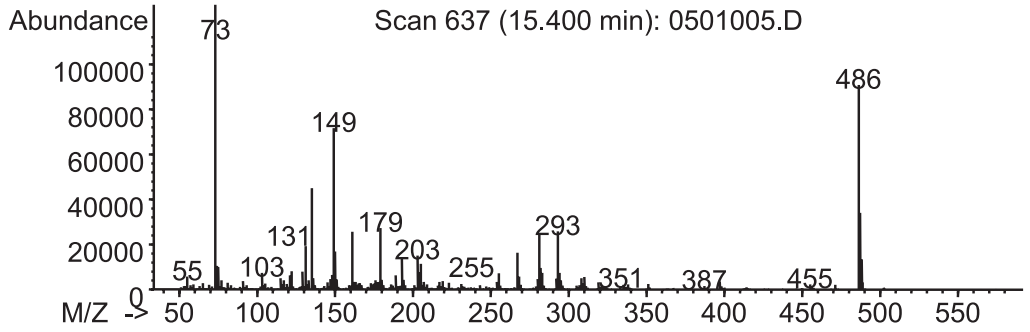

Figure 4. Typical mass spectrum of unknown urinary compound A detected after consumption of sesame seed bar or flaxseed + sesame seed bar.

have been due to foods rich in mammalian lignan precursors consumed in the regular diet of the SB and FSB groups because the subjects did not consume flaxseed, soybean, and herbal products other than those in the treatment bars. Although whole grains have higher amounts of mammalian lignan precursors compared with refined grains (39,40,73), they were not consumed in large amounts by the subjects as seen in the diet record. The recommended level of dietary fiber Canadians should aim for in the diet is 25–30 g/day (74); however, our subjects' baseline dietary fiber intake was only an average of 20.1 g/day. The small increase in dietary fiber intake during the study period can be attributed primarily to the food bar supplements. Furthermore, the twofold increase in urinary lignan excretion observed in healthy women who consumed 162 g whole meal rye bread compared with 153 g white wheat bread (73) is much smaller than the greater-than-eightfold increase in urinary lignan excretion after the flaxseed and/or sesame seed. Evidently, there are other mammalian lignan precursors in sesame seed, which may include its large amounts of SES, SMN, and sesaminol. These compounds have not previously been shown to be converted to the mammalian lignans. However, Nakai et al. (75) recently showed in rat studies that SES can be absorbed, metabolized in the liver to at least four compounds with catechol moieties, and excreted in bile and presumed also in the urine. The mass spectra of the unknown compound (compound A) detected in the urine of subjects after the intake of sesame seed-containing bars (SB or FSB) in this study suggest that it may be one of those four compounds. Whether SES and its metabolites that are excreted in bile undergo enterohepatic circulation and are partly metabolized by the bacterial flora to ED and EL (Fig. 1) remains to be established. The specific conversion of SMN and sesaminol to ED and EL is also yet to be determined.

When sesame seed was combined with flaxseed, the conversion of SECO and other precursors in flaxseed to ED and EL was lower than what was expected from the conversion seen with sesame seed or flaxseed alone. The urinary mammalian lignan recovery relative to the plant lignans was also lower in the FSB group than what was expected from the recoveries in the FB and SB groups. These suggest that the conversion of precursors such as SECO in flaxseed may have been suppressed in the presence of sesame lignans, but the reason for this is currently unknown. Nonetheless, the combined flaxseed and sesame seed appear to still produce mammalian lignans that did not differ significantly from that of flaxseed or sesame seed alone.

In conclusion, precursors in whole unground flaxseed and sesame seed in food bar formulation can be converted to mammalian lignans in postmenopausal women. These food bars may be used to study the anticancer effects and other health benefits of flaxseed and sesame seed, alone and in combination. Because the mammalian lignan production with sesame seed, alone or in combination with flaxseed, was equivalent to that from flaxseed, sesame seed may be used as an alternative to flaxseed as a very rich source of mammalian lignan precursors. The large amount of mammalian lignans produced with sesame seed, reported for the first time here, suggests that they should also be examined for their potential role on some of the reported beneficial effects of sesame seed and its major lignans.

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References

1. Axelson M, Sjövall J, Gustafsson BE, and Setchell KD: Origin of lignans in mammals and identification of a precursor from plants. *Nature* **298,** 659–660, 1982.
2. Borriello SP, Setchell KD, Axelson M, and Lawson AM: Production and metabolism of lignans by the human fecal flora. *J Appl Bacteriol* **58,** 37–43, 1985.
3. Heinonen S, Nurmi T, Liukkonen K, Poutanen K, Wahala K, et al.: In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J Agric Food Chem* **49,** 3178–3186, 2001.
4. Saarinen NM, Warri A, Makela SI, Eckerman C, Reunanen M, et al.: Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (Picea abies). *Nutr Cancer* **36,** 207–216, 2000.
5. Setchell KDR and Adlercreutz H: Mammalian lignans and phytoestrogen: recent studies on their formation, metabolism and biological role in health and disease. In *Role of the Gut Flora in Toxicity and Cancer*, Rowland IR (ed). London: Academic Press, 1988, pp 315–345.
6. Thompson LU: Analysis and bioavailability of lignans. In *Flaxseed in Human Nutrition*, Thompson LU and Cunnane SC (eds). Champaign, IL: AOCS Press, 2003, pp 92–116.
7. Rickard SE, Orcheson LJ, Seidl MM, Luyengi L, Fong HH, et al.: Dose-dependent production of mammalian lignans in rats and in vitro from the purified precursor secoisolariciresinol diglycoside in flaxseed. *J Nutr* **126,** 2012–2019, 1996.
8. Brooks JD, Ward WE, Lewis JE, Hilditch J, Nickell L, et al.: Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy. *Am J Clin Nutr* **79,** 318–325, 2004.
9. Hutchins AM, Martini MC, Olson BA, Thomas W, and Slavin JL: Flaxseed influences urinary lignan excretion in a dose-dependent manner in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **9,** 1113–1118, 2000.
10. Lampe JW, Martini MC, Kurzer MS, Adlercreutz H, and Slavin JL: Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Am J Clin Nutr* **60,** 122–128, 1994.
11. Nesbitt PD, Lam Y, and Thompson LU: Human metabolism of mammalian lignan precursors in raw and processed flaxseed. *Am J Clin Nutr* **69,** 549–555, 1999.
12. Lampe JW, Gustafson DR, Hutchins AM, Martini MC, Li S, et al.: Urinary isoflavonoid and lignan excretion on a western diet: relation to

soy, vegetable, and fruit intake. *Cancer Epidemiol Biomarkers Prev* **8,** 699–707, 1999.
13. Mueller SO, Simon S, Chae K, Metzler M, and Korach KS: Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor α (ER α) and ER β in human cells. *Toxicol Sci* **80,** 14–25, 2004.
14. Ingram D, Sanders K, Kolybaba M, and Lopez D: Case-control study of phyto-oestrogens and breast cancer. *Lancet* **350,** 990–994, 1997.
15. Dai Q, Franke AA, Jin F, Shu XO, Hebert JR, et al.: Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. *Cancer Epidemiol Biomarkers Prev* **11,** 815–821, 2002.
16. McCann SE, Muti P, Vitp D, Edge SB, Trevisan M, et al.: Dietary lignan intakes and risk of pre- and postmenopausal breast cancer. *Int J Cancer* **111,** 440–443, 2004.
17. Boccardo F, Lunardi G, Guglielmini P, Parodi M, Murialdo R, et al.: Serum enterolactone levels and risk of breast cancer in women with palpable cysts. *Eur J Cancer* **40,** 84–89, 2004.
18. Pietinen P, Stumpf K, Mannisto S, Kataja V, Uusitupa M, et al.: Serum enterolactone and the risk of breast cancer: a case control study in eastern Finland. *Cancer Epidemiol Biomarkers Prev* **10,** 339–344, 2001.
19. den Tonkelaar I, Keinan-Boker L, Veer PV, Arts CJ, Adlercreutz H, et al.: Urinary phytoestrogens and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **10,** 223–228, 2001.
20. Keinan-Boker L, van der Schouw YT, Grobbee DE, and Peeters PH: Dietary phytoestrogens and breast cancer risk. *Am J Clin Nutr* **79,** 282–288, 2004.
21. Kilkkinen A, Virtamo J, Vartiainen E, Sankila R, Virtanen MJ, et al.: Serum enterolactone concentration is not associated with breast cancer risk in a nested case-control study. *Int J Cancer* **108,** 277–280, 2004.
22. Thompson LU, Seidl MM, Rickard SE, Orcheson LJ, and Fong HH: Antitumorigenic effect of a mammalian lignan precursor from flaxseed. *Nutr Cancer* **26,** 159–165, 1996.
23. Thompson LU, Rickard SE, Orcheson LJ, and Seidl MM: Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. *Carcinogenesis* **17,** 1373–1376, 1996.
24. Rickard SE, Yuan YV, Chen J, and Thompson LU: Dose effects of flaxseed and its lignans on N-methyl-N-nitrosourea-induced mammary tumorigenesis in rats. *Nutr Cancer* **35,** 50–57, 1999.
25. Chen J, Tan KP, Ward WE, and Thompson LU: Exposure to flaxseed or its purified lignan during suckling inhibits chemically induced rat mammary tumorigenesis. *Exp Biol Med* **228,** 951–958, 2003.
26. Saarinen NM, Huovinen R, Warri A, Makela SI, Valentin-Blasini L, et al.: Enterolactone inhibits the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas in the rat. *Mol Cancer Ther* **10,** 869–876, 2002.
27. Wang L, Chen J, and Thompson LU: The inhibitory effect of flaxseed on the growth and metastasis of estrogen receptor negative human breast cancer xenografts is attributed to both its lignan and oil components. *Int J Cancer* **116,** 793–798, 2005.
28. Chen J, Wang L, and Thompson LU: Flaxseed and its components reduce metastasis after surgical excision of solid human breast tumor in nude mice. *Cancer Lett* 2005 May 20 [Epub ahead of print].
29. Li D, Yee JA, Thompson LU, and Yan L: Dietary supplementation with secoisolariciresinol diglycoside (SDG) reduces experimental metastasis of melanoma cells in mice. *Cancer Lett* **142,** 91–96, 1999.
30. Brooks JD and Thompson LU: Mammalian lignans and genistein decrease the activities of aromatase and 17β- hydroxysteroid dehydrogenase in MCF-7 cells. *J Steroid Biochem Mol Biol* **94,** 461–467, 2005.
31. Adlercreutz H, Bannwart C, Wahala K, Makela T, Brunow G, et al.: Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol* **44,** 147–153, 1993.
32. Wang C, Makela T, Hase T, Adlercreutz H, and Kurzer MS: Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. *J Steroid Biochem Mol Biol* **50,** 205–212, 1994.
33. Evans BA, Griffiths K, and Morton MS: Inhibition of 5α-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. *J Endocrinol* **147,** 295–302, 1995.
34. Mousavi Y and Adlercreutz H: Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *J Steroid Biochem Mol Biol* **41,** 615–619, 1992.
35. Prasad K: Hydroxyl radical-scavenging property of secoisolariciresinol diglucoside (SDG) isolated from flaxseed. *Mol Cell Biochem* **168,** 117–123, 1997.
36. Kitts DD, Yuan YV, Wijewickreme AN, and Thompson LU: Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Mol Cell Biochem* **202,** 91–100, 1999.
37. Fotsis T, Pepper M, Adlercreutz H, Hase T, Montesano R, et al.: Genistein, a dietary ingested isoflavonoid, inhibits cell proliferation and in vitro angiogenesis. *J Nutr* **125,** S790–S797, 1995.
38. Chen J and Thompson LU: Lignans and tamoxifen, alone and in combination, reduce human breast cancer cell adhesion, invasion and migration in vitro. *Breast Cancer Res Treat* **80,** 163–170, 2003.
39. Thompson LU, Robb P, Serraino M, and Cheung F: Mammalian lignan production from various foods. *Nutr Cancer* **16,** 43–52, 1991.
40. Mazur W: Phytoestrogen content of foods. *Baillieres Clin Endocrinol Metab* **12,** 729–742, 1998.
41. Thompson LU: Flaxseed, lignans and cancer. In *Flaxseed in Human Nutrition*, Thompson LU and Cunnane SC (eds). Champaign, IL: AOCS Press, 2003, pp 194–222.
42. Serraino M and Thompson LU: The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. *Nutr Cancer* **17,** 153–159, 1992.
43. Chen J, Stavro PM, and Thompson LU: Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor. *Nutr Cancer* **43,** 187–192, 2002.
44. Dabrosin C, Chen J, Wang L, and Thompson LU: Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts. *Cancer Lett* **185,** 31–37, 2002.
45. Chen J, Hui E, Ip T, and Thompson LU: 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.
46. Thompson LU, Chen J, Li T, Strasser-Weippl K, and Goss PE: Dietary flaxseed alters tumor biological markers in postmenopausal breast cancer. *Clin Cancer Res* **11,** 3828–3835, 2005.
47. Fukuda Y, Osawa T, Namiki M, and Saki T: Studies on antioxidative substances in sesame. *Agric Biol Chem* **49,** 301–306, 1985.
48. Ikeda S, Tohyama T, and Yamashita K: Dietary sesame seed and its lignans inhibit 2,7,8-trimethyl-2(2′-carboxylethyl-6hydroxychroman excretion into urine of rats fed gamma-tocopherol. *J Nutr* **132,** 961, 2002.
49. Kang MH, Naito M, Tsujihara N, and Osawa T: Sesamolin inhibits lipid peroxidation in rat liver and kidney. *J Nutr* **128,** 1018–1022, 1998.
50. Yamashita K, Ikeda S, and Obayashi M: Comparative effects of flaxseed and sesame seed on vitamin E and cholesterol levels in rats. *Lipids* **38,** 1249–1255, 2003.
51. Matsumura Y, Kita S, Morimoto S, Akimoto K, Furuya M, et al.: Antihypertensive effect of sesamin. I. Protection against deoxycorticosterone acetate-salt-induced hypertension and cardiovascular hypertrophy. *Biol Phar Bull* **18,** 1016–1019, 1995.
52. Kita S, Matsumura Y, Morimoto S, Akimoto K, Furuya M, et al.: Antihypertensive effect of sesamin. II. Protection against two-kidney, one-clip renal hypertension and cardiovascular hypertrophy. *Biol Pharm Bull* **18,** 1283–1285, 1995.
53. Matsumura Y, Kita S, Tanida Y, Taguchi Y, Morimoto S, et al.: Antihypertensive effect of sesamin. III. Protection against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats. *Biol Pharm Bull* **21,** 469–473, 1998.
54. Cooney RV, Custer LJ, Okinaka L, and Franke AA: Effects of dietary sesame seeds on plasma tocopherol levels. *Nutr Cancer* **39,** 66–71, 2001.

55. Hirose N, Inoue T, Nishihara K, Sugano M, Akimoto K, et al.: Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J Lipid Res* **32,** 629–638, 1991.
56. Ogawa H, Sasagawa S, Murakami T, and Yoshizumi H: Sesame lignans modulate cholesterol metabolism in the stroke-prone spontaneously hypertensive rat. *Clin Exp Pharm Phys* **22,** S310–S312, 1995.
57. Ide T, Hong DD, Ramasinghe P, Takahashi Y, Kushiro M, et al.: Interaction of dietary fat types and sesamin on hepatic fatty acid oxidation in rats. *Biochim Biophys Acta* **1682,** 80–91, 2004.
58. Sirato-Yasumoto S, Katsuta M, Okuyama Y, Takahashi Y, and Ide T: Effect of sesame seeds rich in sesamin and sesamolin on fatty acid oxidation in rat liver. *J Agric Food Chem* **49,** 2647–2651, 2001.
59. Hirose N, Doi F, Ueki T, Akazawa K, Chijiiwa K, et al.: Suppressive effect of sesamin against 7,12 dimethylbenz[a]-anthracene induced rat mammary carcinogenesis. *Anticancer Res* **12,** 1259–1265, 1992.
60. Miyahara Y, Komiya T, Katsuzaki H, Imai K, Nakagawa M, et al.: Sesamin and episesamin induce apoptosis in human lymphoid leukemia Molt 4B cells. *Int J Mol Med* **6,** 43–46, 2000.
61. Miyahara Y, Hibasami H, Katsuzaki H, Imai K, Osawa T, et al.: Sesaminol from sesame seed induces apoptosis in human lymphoid leukemia Molt 4B cells. *Int J Mol Med* **7,** 485–488, 2001.
62. Miyahara Y, Hibasami H, Katsuzaki H, Imai K, and Komiya T: Sesamolin from sesame seed inhibits proliferation by inducing apoptosis in human lymphoid leukemia Molt 4B cells. *Int J Mol Med* **7,** 369–371, 2001.
63. Jacklin A, Ratledge C, Welham K, Bilko D, and Newton CJ: The sesame seed oil constituent, sesamol, induces growth arrest and apoptosis of cancer and cardiovascular cells. *Ann NY Acad Sci* **1010,** 374–380, 2003.
64. Ahn YO: Diet and stomach cancer in Korea. *Int J Cancer* **Suppl 10,** 7–9, 1997.
65. Kurzer MS: Phytoestrogen supplement use by women. *J Nutr* **133,** S1983–S1986, 2003.
66. Association of Official Analytical Chemists: *Official Methods of Analysis.* Gaithersburg, MD: AOAC International, 1997.
67. Muir AD and Westcott ND: Quantitation of the lignan secoisolariciresinol diglucoside in baked goods containing flaxseed or flax meal. *J Agric Food Chem* **48,** 4048–4052, 2000.
68. Thompson LU, Rickard SE, Cheung F, Kenaschuk EO, and Obermeyer WR: Variability in anticancer lignan levels in flaxseed. *Nutr Cancer* **27,** 26–30, 1997.
69. Cunnane SC, Hamadeh MJ, Liede AC, Thompson LU, Wolever TM, et al.: Nutritional attributes of traditional flaxseed in healthy young adults. *Am J Clin Nutr* **61,** 62–68, 1995.
70. Garreau B, Vallette G, Adlercreutz H, Wahala K, Makela T, et al.: Phytoestrogens: new ligands for rat and human α-fetoprotein. *Biochim Biophys Acta* **1094,** 339–345, 1991.
71. Martin EM, Haorigui M, Pelissero C, and Nunez EA: Interactions between phytoestrogens and human sex hormone binding protein. *Life Sci* **58,** 429–436, 1996.
72. Sung M, Lautens M, and Thompson LU: Mammalian lignans inhibit the growth of estrogen-independent human colon tumor cells. *Anticancer Res* **18,** 1405–1408, 1998.
73. Juntunen KS, Mazur WM, Liukkonen KH, Uehara M, Poutanen KS, et al.: Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women. *Br J Nutr* **84,** 839–846, 2000.
74. Health Canada: *Report of the Expert Advisory Committee on Dietary Fibre*. Ottawa: Health Protection Branch, 1985.
75. Nakai M, Harada M, Nakahara K, Akimoto K, Shibata H, et al.: Novel antioxidative metabolites in rat liver with ingested sesamin. *J Agric Food Chem* **51,** 1666–1670, 2003.