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RESEARCH ARTICLE

Fatty acids and lignans in unground whole flaxseed and sesame seed are bioavailable but have minimal antioxidant and lipid-lowering effects in postmenopausal women

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Fatty acids and lignans in ground flaxseed and sesame seed are absorbed, metabolized, and exert some health benefits *in vivo*. However, it is unclear if they are absorbed, metabolized, and exert health benefits when consumed as unground whole seed; therefore, it was investigated in this study. In a randomized crossover study, 16 postmenopausal women supplemented their diets with food bars containing either 25 g unground flaxseed, sesame seed, or their combination (12.5 g each) (flaxseed+sesame seed bar, FSB) for 4 wk each, separated by 4 wk washout periods. Total serum n-3 fatty acids increased with flaxseed ($p<0.05$) and FSB ($p=0.064$) while serum n-6 fatty acids increased with sesame seed ($p<0.05$). Urinary lignans increased similarly with all treatments ($p<0.05$). Plasma lipids and several antioxidant markers were unaffected by all treatments, except serum γ-tocopherol (GT), which increased with both sesame seed ($p<0.0001$) and FSB ($p<0.01$). In conclusion, fatty acids and lignans from unground seed in food bars are absorbed and metabolized; however, except for serum GT, the 25 g unground seed is inadequate to induce changes in plasma lipids and several biomarkers of oxidative stress.

Keywords:
Antioxidants / Blood lipids / Flaxseed / Sesame seed / Tocopherols

1 Introduction

Flaxseed and sesame seed have gained interest for their health benefits due to their fatty acid and lignan contents [1–5]. Flaxseed is a rich source of the plant lignan, secoisolariciresinol diglucoside (SDG) that is metabolized *in vivo* to the enterolignans enterodiol (END) and enterolactone (ENL), which are then partly excreted in the urine [6, 7]. Intake of ground flaxseed (10–50 g/day) greatly increased the urinary END and ENL in women and men [2, 8–10]. Flaxseed is also a rich source of the n-3 polyunsaturated fatty acids (PUFA), α-linolenic acid (ALA; 18:3n-3), which is partly metabolized *in vivo* to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [11]. Serum concentrations of n-3 PUFA increased after consumption of ground flaxseed [1, 2, 12], indicating that ALA from ground flaxseed is bioavailable.

Sesame seed contains high concentrations of the plant lignans, sesamin (SES), sesamolin (SMN), and sesaminol, and a small amount of pinoresinol [13, 14]. SMN is converted to sesamol and sesamolinol *in vivo* [14], while SES is partly converted to ENL [15, 16]. Consumption of ground sesame seed increased the urinary levels of END and ENL [16]. Sesame seed is also a rich source of n-6 PUFA,

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Abbreviations: ALA, α-linolenic acid; **apo A1,** apolipoprotein A1; **AT,** α-tocopherol; **CD,** conjugated dienes; **DHA,** docosahexaenoic acid; **DT,** δ-tocopherol; **END,** enterodiol; **ENL,** enterolactone; **EPA,** eicosapentaenoic acid; **FB,** flaxseed bar; **FRAP,** ferric-reducing ability of plasma; **FSB,** flaxseed+sesame seed bar; **GLA,** γ-linolenic acid; **GT,** γ-tocopherol; **LA,** linoleic acid; **LDL-CD,** conjugated diene content of LDL; **MUFA,** monounsaturated fatty acids; **PUFA,** polyunsaturated fatty acids; **SB,** sesame seed bar; **SDG,** secoisolariciresinol diglucoside; **SECO,** secoisolariciresinol; **SES,** sesamin; **SFA,** saturated fatty acids; **SMN,** sesamolin; **TEAC,** trolox equivalent antioxidant capacity

primarily linoleic acid, (LA; C18:2n-6) and monounsaturated fatty acids (MUFA) [17] and thus can increase serum LA [18, 19]. Ground flaxseed (30–50 g/day) [1–3, 20, 21] and sesame seed (50 g/day) [18] have been shown to reduce blood lipids.

The high levels of PUFA in flaxseed and sesame seed are more susceptible to oxidation than the saturated fatty acids, and thus may lead *in vivo* to increased oxidative stress [22, 23], *i.e.* high levels of free radicals or reactive oxygen species that bind to proteins and DNA inside the cell and lead to disease, which are not counteracted by antioxidants, compounds capable of delaying or preventing the oxidation of oxidizable substrate. However, flaxseed lignans have antioxidant activity *in vitro* [24] and have modest antioxidant effects in animals [25]. Sesame seed and its lignans have tocopherol-enhancing effects in humans [18, 26] and rats [5, 27] and have been shown to reduce oxidative stress in animals [28, 29]. The antioxidant effects of lignans and possibly other seed components may be able to overcome any increase in oxidative stress induced by PUFA from increased consumption of flaxseed and/or sesame seed.

The above studies used ground seed or purified lignans or oil, and it is unclear whether unground whole seed in flaxseed- and sesame seed-containing foods would have the same nutrient availability and thus physiological effects as the ground seed. The lignans in whole flaxseed consumed with applesauce or custard sauce are less available than in ground or crushed flaxseed [30]. Low bioavailability of ALA was observed in whole flaxseed compared with ground/milled flaxseed in muffin formulations [31]. However, flaxseed is also commonly consumed in the food bar formulation. Because food bars need extensive chewing before swallowing, it is thought that such a formulation may be a good vehicle for the provision of unground whole flaxseed or sesame seed. It is unknown if sesame seed and flaxseed with different types of fatty acids (more monounsaturated and LA *versus* more n-3 fatty acids) and lignans (SES and SMN *versus* SDG) would have different physiological effects. Hence, the overall aim of this study was to examine, in postmenopausal women, the bioavailability of fatty acids and lignans and the antioxidant and lipid-lowering effects of food bars containing 25 g unground flaxseed, sesame seed, or their combination.

2 Materials and methods

2.1 Subjects

Sixteen healthy, omnivorous, non-smoking postmenopausal women (mean age 66.4 ± 1.7 years; 14.4 ± 2.0 years since menopause) volunteered for this study. There were no drop-outs. Mean weight and body mass index of the subjects were 73.1 ± 3.1 kg and 28.6 ± 1.5 kg/m^2, respectively, and remained constant throughout the study. Subjects were excluded if they were taking or had ever taken hormone replacement therapy or had taken antibiotics within the last 6 months, and/or lipid-lowering drugs within the last 4 wk prior to entering the study. Subjects were asked to discontinue consumption of foods containing flaxseed, sesame seed, or soy, and the use of herbal supplements 4 wk prior to the study. Regular use of basic vitamin and mineral supplements was allowed to continue for the duration of the study. All subjects provided written informed consent, and the study protocol (number 7242) was reviewed and approved by the University of Toronto Human Ethics Committee.

2.2 Study design

In a randomized crossover design, flaxseed bar (FB), sesame seed bar (SB), or flaxseed+sesame seed bar (FSB) was supplemented in subjects' regular diets. Subjects were randomized to a sequence as FB-SB-FSB, SB-FB-FSB, or FSB-SB-FB. Each treatment period lasted 4 wk with a 4 wk washout period between each treatment.

Similar to our previous study on flaxseed, subjects consumed a standard low-fiber, low-lignan dinner, 2 days prior to beginning the study [8]. A standard dinner was provided to reduce the measurement error for lignans as dinner containing different high levels of lignans on sampling days might increase the urinary lignan to levels unrelated to the food bar intake. On the day prior to the beginning of the study, subjects again consumed the standard low-fiber meals and began collecting a 24 h urine sample (1st urine of day discarded) through to the first urine of day 1 of the study period. Fasting blood samples were obtained on the morning of day 1. One treatment bar was then consumed at breakfast on study day 1 and daily until day 28. The evening prior to the last treatment bar consumption (day 27), subjects again consumed the standard dinner and began collecting a 24 h urine sample as previously described. Fasting blood samples were again collected on day 1 of the next 4 wk period. Subjects followed their normal diets throughout the rest of the study days (day 1–26). Food records (3 days) were completed during each treatment and washout period. Throughout the duration of the study, subjects were instructed to avoid other flaxseed-, sesame seed-, or soy-containing foods and herbal supplements.

2.3 Food bars

Each treatment bar had 25 g flaxseed (FB; Pizzey Milling, Angusville, MB, Canada), 25 g sesame seed (SB; Grain Process, Scarborough, ON, Canada), or 12.5 g flaxseed and 12.5 g sesame seed (FSB), prepared as previously described [32]. The bars were stored at -20°C until distribution to subjects, who also kept them in the freezer until needed for consumption. All food bars were prepared in one batch.

Standard methods were used for analysis of protein, carbohydrate, fat, and total, insoluble, and soluble dietary fiber contents of food bars [33]. GC-MS techniques were used for the analysis of the plant lignans (SDG,

matairesinol, lariciresinol, pinoresinol, sesaminol, SES, and SMN) [32] and fatty acid composition [34] of the treatment bars as previously described.

The α-tocopherol (AT), δ-tocopherol (DT), and γ-tocopherol (GT) were analyzed by HPLC using a modification of other methods [35–37]. Briefly, the tocopherols from ground samples were extracted twice with hexane containing 0.005% butylated hydroxytoluene and dried. Hexane, MeOH, and dl-AT acetate (Sigma Chemical, St. Louis, MO, USA) internal standard solution were then added. The samples were vortexed, centrifuged, filtered through a 0.45 μm pore size filter, and injected into the HPLC (Waters 2690 Separations Module; Waters, Milford, MA, USA). A R535 fluorescence detector (Shimadzu, Kyoto, Japan) set to an excitation wavelength of 285 nm and to an emission wavelength of 325 nm to detect tocopherols, and a Symmetry C18 reversed phase column (4.6 × 250 mm, 5 μm, 100 Å) (Waters) were used. As the different forms of tocopherols do not possess equal vitamin E activity, AT equivalents (μg) were calculated as well as the levels of the individual tocopherols ''as is''. GT and DT were multiplied by factors of 0.1 and 0.01, respectively, and the values summed to calculate total AT equivalents.

2.4 Trolox equivalent antioxidant capacity assay of test bars

The trolox equivalent antioxidant capacity (TEAC) of flaxseed, sesame seed, the three test bars, pure SDG, SES, AT, and L-(+)-Ascorbic acid were determined spectrophotometrically using a modification of other methods [38–40]. Briefly, a portion of ground food samples or pure compounds was combined with 100 μM 2,2-diphenyl-1-picrylhydrazyl (Aldrich Chemical, Milwaukee, WI, USA) solution and incubated for 4 h in a 37°C rotating water bath. The mixture was then filtered and absorbance of the filtrate read at 515 nm in an UV/Visible spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Uppsala, Sweden). Absorbance was measured against the standard curve of the reaction of trolox ((*S*)-(−)-6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (Aldrich Chemical) with 2,2-diphenyl-1-picrylhydrazyl.

2.5 Urinary lignans

Urinary concentrations of lignans (END, ENL, secoisolariciresinol (SECO; aglycone of SDG)) were measured by GC-MS as previously described [6, 8, 10, 32].

2.6 Blood analyses

Total fatty acids were extracted from the serum, converted to fatty acid methyl esters, and measured by GC [41]. Plasma lipids, *i.e.* total cholesterol, HDL, LDL, triacylglyceride, apolipoprotein A1 (apo A1), and apolipoprotein B were analyzed by the Core Lipid Laboratory at St. Michael's Hospital (Toronto, ON, Canada), according to the Lipid Research Clinics protocol.

Serum AT, DT, and GT were measured by HPLC (Waters 2690 Separations Module; Waters) [36]. Spectrophotometric methods were used to measure the ferric-reducing ability of plasma (FRAP) as a measure of total antioxidant power [42], the serum thiol groups as a measure of protein oxidation [43], and conjugated diene content of LDL (LDL-CD) to assess oxidation of LDL [44, 45].

2.7 Nutrient intakes

Nutrient intakes from the 3-day diet records, which include the food bars, were calculated and averaged by using the NUTRIWATCH nutrient analysis program (version 6.1.22E Delphi 1, based on the 1997 Canadian Nutrient File; Elizabeth Warwick, PEI, Cornwall, ON, Canada).

2.8 Statistical analyses

All statistical analyses used PROC MIXED in SAS version 8.1 (SAS Institute, Cary, NC, USA). All blood measurements and urinary lignans were corrected for order, treatment, order × treatment, all nutrient variables from the dietary analysis, age, years since menopause, weight, and BMI. Initially, all covariates were incorporated into each model. Those that were found to be not significant (with the exception of treatment) were excluded from the models systematically in a step-wise fashion, with the covariate with the largest, non-significant *p*-value being removed each time, and the model being refitted. Least squares means were estimated to detect if treatments resulted in any significant change between baseline and follow-up measurements. Tukey-adjusted pair-wise comparisons were performed to determine any differences between changes in treatments. The acceptable significance level was $p < 0.05$.

3 Results

3.1 Food bars

Due to the higher fat content of sesame seed, SB provided 193 kJ more than FB (Table 1). SB contained higher levels of MUFA and LA, while FB contained higher levels of ALA. FB also contained 4 g more dietary fiber than SB. No AT was detected in any of the test bars; however, all test bars contained GT and DT, with SB containing more of these tocopherols than FB. SB had a higher total vitamin E activity, as AT equivalents, than FB. FSB contained levels intermediate to FB and SB. Overall, SB contained a higher total lignan concentration than FB, with FSB being intermediate.

Table 1. Treatment bar composition

Macronutrient	FB	SB	FSB
Total energy (kJ)[a)]	685	878	776
Protein (g) (*% energy*)[a)]	5.2 (*12.7*)	6.0 (*11.4*)	5.6 (*12.1*)
Fat (g) (*% energy*)[a)]	11.1 (*60.7*)	15.9 (*68.2*)	13.4 (*64.7*)
Total SFA (g)	1.03	2.41	1.72
Total MUFA (g)	1.93	6.17	4.05
LA (18:2n-6) (g)	1.66	7.09	4.37
ALA (18:3n-3) (g)	6.20	0.05	3.01
Total PUFA (g)	7.87	7.14	7.38
Available CHO (g) (*% energy*)[a)]	10.9 (*26.6*)	10.7 (*20.4*)	10.8 (*23.2*)
Total dietary fiber (g)[a)]	7.1	3.1	4.7
Insoluble fiber (g)	4.4	3.0	4.2
Soluble fiber (g)	2.6	0.1	0.5
Total tocopherols (mg) (*mg AT equivalents*)	1.88 (*0.18*)	2.51 (*0.24*)	2.27 (*0.22*)
AT (mg)	0	0	0
GT (mg)	1.81	2.40	2.18
DT (mg)	0.07	0.11	0.09
SDG (μmol)[a)]	200.05	0.04	100.04
Matairesinol (μmol)[a)]	0.64	0.08	0.36
Pinoresinol (μmol)[a)]	2.47	5.58	4.02
Lariciresinol (μmol)[a)]	1.75	0.21	0.98
SES (μmol)[a)]	0	370.88	189.44
SMN (μmol)[a)]	0	90.44	45.22
Sesaminol (μmol)[a)]	0	69.37	34.69

CHO, carbohydrate

a) Previously reported in Coulman *et al.* [32]; reprinted with permission.

The TEAC of flaxseed was about 3.9 × greater than that of sesame seed (Fig. 1). As with the seed, the TEAC of FB was 3.9 × greater than SB, with FSB having intermediate values. The TEACs of FB and SB were 17 and 16% lower than their respective seeds. SDG accounted for 24% of flaxseed's antioxidant capacity. SES did not account for the sesame seed's antioxidant capacity as TEAC. On same weight basis, the TEAC of SDG was 1.4 × greater and 1.9 × less, compared with the known antioxidants, AT and ascorbic acid, respectively. On equimolar basis, however, SDG had 2.3 × and 2.1 × higher TEAC than AT and ascorbic acid, respectively. SES had no antioxidant capacity measured as TEAC, compared with SDG, AT, and ascorbic acid.

3.2 Food records

Significantly increased intakes from baseline were noted for protein, total, insoluble, and soluble dietary fiber, and PUFA with FB; energy, protein, total, insoluble, and soluble dietary fiber, cholesterol, total fat, SFA, MUFA, and PUFA with SB; total and insoluble dietary fiber and PUFA with FSB (Table 2). However, the change did not differ significantly between groups for any of the nutrients except for MUFA, with intake being significantly greater with SB group than FB group.

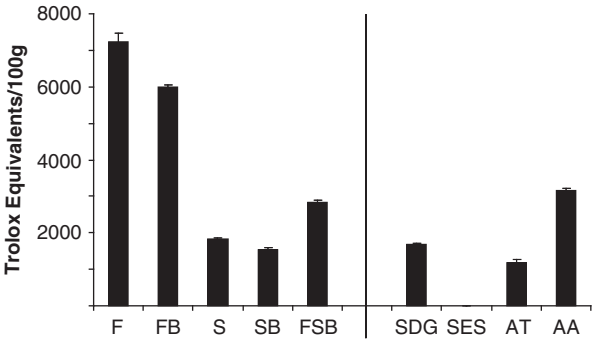

Figure 1. TEAC of flaxseed, sesame seed, treatment bars, and pure lignans and antioxidants (TEACs of SDG, AT, and AA are expressed as *per* equivalent amount of SDG in 100 g flaxseed (550 mg/100 g – Table 1)). Vertical bars are Mean ± SEM. F, flaxseed, S, sesame seed, AA, ascorbic acid.

3.3 Urinary lignans

Urinary excretion of SECO, END, ENL, END+ENL, and total lignans (SECO+END+ENL) has already been published [32]. It showed that urinary lignan excretion increased from baseline with all three treatments (4200, 7400, and 7000% for FB, SB, and FSB, respectively; $p<0.0001$). However, the increases did not differ significantly between groups.

3.4 Serum fatty acids

Total SFA, γ-linolenic acid (GLA), ARA, and DHA remained unchanged with all treatment groups. There was a trend for total MUFA to decrease with SB ($p=0.059$). Serum LA increased after consumption of SB ($p<0.05$), with the increases greater than after either FB or FSB ($p<0.05$) (Table 3). The increase in serum total n-6 PUFA ($p<0.05$) was also greater with SB than with FB ($p<0.05$), but not with FSB. Conversely, the increase in serum ALA with FB ($p<0.0001$) was greater than with SB and FSB ($p<0.05$). There was a trend for an increase in serum ALA with FSB ($p=0.054$). Serum EPA and docosapentanoic acid increased only with FSB ($p<0.05$); however, this did not differ from FB or SB. Total n-3 PUFA increased with FB ($p<0.05$) and there was a trend for an increase with FSB ($p=0.064$) but % change did not differ among groups.

3.5 Plasma lipids

Pretreatment levels of total cholesterol (5.59–5.85 umol/L), HDL (1.35–1.40 umol/L), LDL (3.43–3.56 umol/L), triacylglyceride (1.89–2.06 umol/L), and apolipoprotein B (1.17–1.23 g/L) were not significantly affected by any of the treatments. However, the 3.69% increase in apo A1 (pretreatment, 1.56–1.60 g/L) with SB almost reached significance ($p=0.052$).

Table 2. Three-day nutrient intakes of subjects

Treatment	Energy[a)] (kJ)	Protein[a)] (g)	CHO[a)] (g)	Total[a)] DF (g)	Insoluble DF (g)	Soluble DF (g)	Cholesterol (mg)
Baseline	7056±318	71.2±2.8	225.2±9.7	19.5±1.4	5.4±0.6	14.1±1.2	178±19
FB	7629±347	81.8±4.7	232.9±10.5	26.2±1.7	9.9±0.8	16.2±1.4	192±18
% Change	9.8±4.8	15.4±5.9*	5.1±4.7	39.2±8.4***	108.6±19.3***	18.2±7.1*	25.2±15.6
Baseline	6868±314	70.5±3.5	228.6±11.1	19.9±1.6	5.5±0.7	14.4±1.3	171±19
SB	7909±414	79.9±4.2	237.5±11.9	23.2±1.7	8.2±0.6	14.9±1.5	202±21
% Change	16.6±6.1**	16.1±6.9*	5.0±4.6	23.5±9.7**	81.1±22.4**	10.5±10.7	44.3±24.1*
Baseline	7378±372	74.3±3.3	229.0±7.7	21.0±1.4	6.2±0.8	14.8±1.3	201±21
FSB	7603±368	76.8±4.3	232.6±14.0	23.9±1.6	10.6±0.9	13.4±1.2	202±20
% Change	5.6±5.8	4.1±4.7	2.3±5.7	17.7±7.5*	117.3±36.8***	−4.1±8.0	15.7±14.4

Treatment	Total fat[a)] (g)	SFA (g)	MUFA (g)	PUFA (g)	Vitamin E (mg ATE)	Vitamin C (mg)
Baseline	56.0±5.3	17.8±1.8	21.5±2.1	11.3±2.1	103.3±33.0	367±91
FB	64.4±6.1	18.4±2.2	22.7±2.5	17.9±2.1	103.5±32.9	389±91
% Change	21.5±9.9	11.0±14.4	10.7±10.0[b)]	89.8±23.8***	23.4±14.3	18.3±14.1
Baseline	51.9±5.6	16.4±2.0	20.1±2.9	9.6±1.3	103.1±33.2	382±85
SB	73.5±5.8	20.0±2.1	30.8±2.9	17.7±1.3	103.0±32.8	363±86
% Change	57.0±16.1***	34.4±14.2*	81.6±23.5**[c)]	113.1±21.1***	28.9±18.7	−6.5±6.9
Baseline	62.6±8.3	19.2±2.2	25.0±4.6	12.8±2.0	104.0±33.4	368±85
FSB	65.6±5.0	19.1±1.9	24.9±2.4	17.2±1.6	103.1±32.9	352±84
% Change	21.4±13.2	15.5±15.2	25.8±17.0[b,c)]	68.3±23.1**	10.5±12.3	−1.9±7.2

CHO, carbohydrate, DF, dietary fiber. Values are expressed as Mean±SEM,
% change from baseline significant at *$p<0.05$, **$p<0.01$, and ***$p<0.001$.
(a) Previously reported in Coulman *et al.* [32]; reprinted with permission.
(b,c) % change values in the same column with different superscripts (a,b) are significantly different at $p<0.05$.

Table 3. Serum fatty acid levels pre- and post- treatment bars

Treatment	Total SFA	Total MUFA	18:2n-6 (LA)	18:3n-6 (GLA)	20:4n-6 (ARA)	Total n-6 PUFA
Baseline	27.1±0.9	27.9±0.9	30.9±1.2	0.5±0.1	6.3±0.5	40.1±1.5
FB	27.7±0.8	28.0±0.7	30.0±1.1	0.7±0.2	6.0±0.4	39.0±1.3
% Change	2.8±2.2	1.1±2.5	−2.5±1.8[a)]	26.9±22.9	−3.1±2.4	−2.5±1.6[a)]
Baseline	27.9±0.7	28.3±0.7	30.3±0.9	0.5±0.1	6.1±0.4	39.3±1.1
SB	27.3±0.6	27.1±0.7	31.6±0.9	0.5±0.1	6.4±0.6	40.9±1.2
% Change	−1.7±1.7	−4.1±1.6[##]	4.8±1.6*[b)]	3.1±6.8	4.7±4.7	4.4±1.8*[b)]
Baseline	27.4±0.7	27.3±0.9	31.2±1.2	0.5±0.1	6.3±0.5	40.3±1.4
FSB	27.7±0.8	27.4±0.8	30.4±1.0	0.6±0.1	6.3±0.5	39.6±1.3
% Change	1.1±1.3	1.0±2.1	−1.9±2.3[a)]	17.8±10.5	−0.6±2.6	−1.5±2.0[a,b)]

Treatment	18:3n-3 (ALA)	20:5n-3 (EPA)	22:5n-3 (DPA)	22:6n-3 (DHA)	Total n-3 PUFA
Baseline	1.0±0.1	0.8±0.1	0.5±0.0	2.2±0.1	4.9±0.3
FB	1.4±0.2	0.9±0.1	0.6±0.0	2.0±0.1	5.4±0.3
% Change	49.4±12.1**[a)]	22.0±9.0	7.9±3.1	−5.5±3.5	11.8±5.2*
Baseline	0.9±0.1	0.9±0.1	0.5±0.0	2.0±0.1	4.6±0.3
SB	0.8±0.1	0.8±0.1	0.5±0.0	2.1±0.1	4.7±0.2
% Change	−1.8±2.1[b)]	1.6±9.3	9.4±6.2	10.3±8.6	3.8±5.3
Baseline	0.9±0.1	0.9±0.1	0.5±0.0	2.2±0.1	5.0±0.3
FSB	1.0±0.1	1.0±0.1	0.6±0.0	2.2±0.1	5.3±0.2
% Change	17.0±8.4[###b)]	32.6±18.0*	12.1±5.5*	4.8±5.9	10.3±5.8[#]

ARA, arachidonic acid, DPA, docosapentanoic acid. Values are expressed as Mean±SEM % total fatty acids
% change from baseline *$p<0.05$, **$p<0.0001$, [#]$p=0.0641$, [##]$p=0.0589$, [###]$p=0.0536$.
(a,b) % change values in the same column with different lettered superscripts are significantly different at $p<0.05$.

3.6 Serum tocopherols

Only 8 of the 96 serum samples contained detectable levels of DT; thus, it was not presented. AT was not increased from baseline with any treatments, while GT was increased with both SB and FSB (Table 4). The % increase was greater with SB (64%) than FB (9%). There was a trend for AT+GT to be increased with SB ($p=0.057$).

Table 4. Serum tocopherol levels pre- and post-treatment bars

Treatment	AT (μmol/L)	GT (μmol/L)	AT+ GT (μmol/L)
Baseline	42.50±4.46	1.49±0.16	43.99±4.40
FB	44.54±4.80	1.61±0.21	46.15±4.74
% Change	6.3±4.5	9.0±9.8[a)]	6.2±4.2
Baseline	41.78±3.85	1.31±0.18	43.09±3.81
SB	44.95±4.43	2.00±0.22	46.96±4.39
% Change	7.4±3.1	64.0±14.6***[b)]	8.9±2.9[#]
Baseline	41.06±3.75	1.54±0.25	42.60±3.67
FSB	43.30±4.26	2.00±0.28	45.30±4.16
% Change	7.1±6.3	40.6±16.9**[a,b)]	8.0±6.0

Values are Mean±SEM, % change from baseline significant at $^{**}p<0.01$, and $^{***}p<0.0001$, % change from baseline $^{\#}p=0.057$. (a,b) Values with different lettered superscripts are significantly different at $p<0.05$.

3.7 Antioxidant measures

None of the treatments had any significant effect on FRAP, serum protein thiols, CD, or the ratio of CD to LDL (data not provided).

4 Discussion

This study demonstrated that: (i) fatty acids and lignans from unground seed in food bars are absorbed and metabolized by postmenopausal women, as seen by increased serum n-6 and n-3 fatty acids and urinary lignans and (ii) consumption of food bars containing 25 g whole unground flaxseed, sesame seed, and their combination by postmenopausal women do not significantly lower blood lipids or change several biomarkers of oxidative stress, despite increased serum concentrations of PUFA and increased serum GT in case of bars containing sesame seed.

We have already reported that the consumption of FB, SB, and FSB treatment bars led to significant increases in total urinary mammalian lignans, which did not differ significantly from each other, indicating that mammalian lignan precursors ingested from the bars were metabolized and absorbed [32]. The consumption of FB led to a 49% (1.96-fold) increase in serum ALA, similar to changes in other studies in healthy adults that demonstrated increases in serum ALA after consumption of ground flaxseed and/or flaxseed oil [1, 2, 12].

The consumption of SB led to 4.8 and 4.4% increases in serum LA and total n-6 PUFA, respectively, and a trend for a 4.1% decrease in serum total MUFA. Wu *et al.* also noted a 3.9% increase in serum LA after intake of 50 g/day sesame powders for 5 wk, although there was no change in serum oleic acid (MUFA) and there was a decrease in EPA [18]. Consumption of FSB tended to increase serum ALA; however, total MUFA and all of the n-6 PUFA were unchanged, suggesting that 12.5 g/day sesame seed may be too low a dose to induce changes in serum fatty acids. Interestingly, serum EPA increased after consumption of FSB only, but not FB, although FB contained twice the ALA and produced 32% more serum ALA than FSB. Studies in rats have shown that low intakes, near or below the requirement, of LA and ALA are synergistic in reducing the requirement for one another when growth [46] and tissue levels were measured [47]. These results suggest that the combination of sesame seed with flaxseed may enhance the conversion of ALA in flaxseed to its longer chain counterparts although this needs further investigation. Serum DHA was unchanged after consumption of all three bars, which is consistent with other studies where only flaxseed or its oil was fed [2, 48].

Although reported energy intake increased during SB, mean weight and BMI did not change over the course of the study, indicating that energy intake must not have really increased. This illustrates some of the problems associated with collecting dietary data, such as irregularity of food consumption, inaccurate estimation of portion sizes, or deliberate over-reporting of energy intake [49, 50]. The bars were not high in protein, and did not contain any cholesterol; hence it is surprising that protein intake increased with all treatments and that cholesterol intake increased with SB. This may be in part due to changes in background diet or subject errors in recording dietary data.

FB, SB, or FSB did not affect blood lipids. There was a trend ($p=0.052$) for apo A1 to increase with SB, although the increase in HDL with SB was not significant ($p=0.09$). These findings are in contrast to other human studies that found flaxseed to have lipid-lowering effects [1–3, 20, 21]. However, those studies used higher doses of flaxseed (30–50 g/day). The present study, however, supports the findings of Tarpila *et al.* [12] where no lipid-lowering effect was noted from a diet providing 20% energy from flaxseed- and flaxseed oil-supplemented foods in normo-lipidemic men and women, the results of Lemay *et al.* [51] who found no effect of 40 g/day crushed flaxseed in hyperlipidemic menopausal women, and the results of Stuglin and Prasad [52] who found no effect of muffins containing 33 g/day flaxseed in healthy men. Taken all together, the literature suggests that a dose higher than 25 g flaxseed may be needed to exert lipid-lowering effects. There is a paucity of human studies with which to compare our findings on sesame seed. Wu *et al.* found lipid-lowering effects in normolipidemic postmenopausal women who consumed 50 g sesame seed powder for 5 wk [18].

Since both lignans and fatty acids were absorbed from the bars as seen by increased urinary lignans and serum fatty acids, the lack of effect on serum lipids is likely due to 25 g being an insufficient dose of seed to induce changes. It remains to be seen whether treatment times longer than 4 wk and increased number of subjects with high baseline lipid levels will result in more significant changes than those observed in this study.

No effect of FB on serum tocopherol levels was observed, in agreement with Cunnane *et al.* who fed healthy adults 50 g/day flaxseed for 4 wk [2]. In rats, flaxseed decreased plasma AT and GT concentrations [28, 53]; however, flaxseed oil+SES increased plasma and liver GT compared with flaxseed oil alone [28]. In this study, SB and FSB increased

the serum GT, which is in agreement with other human studies that fed sesame seed [18, 26] and sesame seed oil [19]. Others found a 66% increase in serum GT after consumption of 50 g/day sesame seed powder for 5 wk [18], which is similar to the 64% increase seen in our study after SB. Vitamin E intake did not change over the course of our study; thus, the effects seen are likely due to a real tocopherol-sparing effect. Sesame seed lignans have been shown to spare tocopherols (in particular GT) by inhibiting cytochrome P450 3A, which is responsible for their catabolism into carboxychromans that are excreted in the urine [54, 55]. The major metabolite of GT, 2, 7, 8-trimethyl-2- (β-carboxyethyl)-6-hydroxychroman, possesses natriuretic activity [56], and inhibits cyclooxygenase activity [57], whereas the metabolite of AT does not. Although serum levels of GT are normally very low in comparison to AT [5, 27, 58], consumption of sesame seed may be a way to greatly enhance them.

Potential health benefits of tocopherols have been attributed to their ability to decrease oxidative stress and inflammation, which are associated with chronic diseases such as cardiovascular disease and cancer [59–61]. *In vitro* and animal studies suggest that GT may have higher antioxidant and anti-inflammatory properties than AT [60, 62]. However, although some studies have found serum GT, but not AT, to be inversely associated with cardiovascular disease occurrence [63, 64] recent reviews on the beneficial role of high intakes of AT and GT in human health showed conflicting results and thus suggested a need for further investigations [59–61].

The bars have antioxidant capacity *in vitro* as measured by TEAC but this did not translate into decreased oxidative stress, as assessed by the FRAP assay, serum protein thiols, and LDL-CD. The FB results are in contrast to other human studies that found increased lipid [2] and protein oxidation [3], after the consumption of 50 g ground flaxseed for 4 wk. Our results agree with the results of Wu *et al.* who found no change in LDL-CD, after 5 wk of ground sesame seed supplementation (50 g/day) [18]. However, they observed a reduction in LDL thiobarbituric acid reducing substances, which we did not measure. Rat studies have found sesame seed and its lignans to reduce [5, 27, 28] or not affect [54] plasma thiobarbituric acid reducing substances.

Antioxidant activity *in vitro* does not necessarily reflect the *in vivo* activity. For example, grape juice flavonoids did not have greater *in vivo* antioxidant effects compared with AT [65], although *in vitro*, flavonoids have been suggested to be more potent antioxidants, as measured by TEAC [66]. Suggested reasons for the differences between *in vivo* and *in vitro* results include lower absorption and retention of flavonoids than AT, and the study time being too short for circulating levels of flavonoids to stabilize [67, 68].

The lack of change in markers of oxidative stress may be due to the fact that dietary PUFA intake significantly increased during all the treatments and may have increased the oxidative stress [22, 23]. Components in flaxseed and sesame seed, such as the lignans, exerted antioxidant effects and overcame the pro-oxidant effects of PUFA.

There are many different antioxidant mechanisms, and different measurements may yield different results. For example, the FRAP assay measures non-enzymatic plasma antioxidants that are able to reduce iron *in vitro* under low pH. Components in flaxseed and sesame seed, such as the lignans, may have antioxidant activity other than the ability to reduce iron. Serum protein thiols and LDL-CD are measures of *in vivo* protein and lipid (LDL) oxidation. However, both protein and lipid oxidation involve many different mechanisms and oxidation products. There is no universal marker for either, and various assays may need to be performed to get a good sense of the oxidation occurring [69–71].

It is concluded that the lignans and fatty acids in 25 g of unground flaxseed and/or sesame seed in food bars are metabolized and absorbed at similar levels seen with ground flaxseed or sesame seed; however, this dose is inadequate to affect blood lipids over a 4 wk period. It remains to be seen if a longer treatment time and increased number of subjects will produce a greater effect. A 25 g sesame seed dose and even half the level (as in FSB) is adequate to raise serum GT. Although certain biomarkers of oxidative stress remained unchanged, it is possible that other components in the seeds, such as the lignans, overcame any pro-oxidant effects induced by the high level of PUFA.

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