Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and ex vivo androgen and progestin activity: a controlled crossover trial[1–3]

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ABSTRACT

Background: Currently there is considerable interest in the potential health benefits of oil seeds, such as soy and flaxseed, especially in relation to cardiovascular disease and cancer.

Objective: We therefore evaluated health aspects of partially defatted flaxseed in relation to serum lipids, indicators of oxidative stress, and ex vivo sex hormone activities.

Design: Twenty-nine hyperlipidemic subjects (22 men and 7 postmenopausal women) completed two 3-wk treatment periods in a randomized, crossover trial. Subjects were given muffins that contributed ≈20 g fiber/d from either flaxseed (≈50 g partially defatted flaxseed/d) or wheat bran (control) while they consumed self-selected National Cholesterol Education Program Step II diets. Both muffins had similar macronutrient profiles. Treatment phases were separated by ≥2 wk.

Results: Partially defatted flaxseed reduced total cholesterol (4.6 ± 1.2%; $P = 0.001$), LDL cholesterol (7.6 ± 1.8%; $P < 0.001$), apolipoprotein B (5.4 ± 1.4%; $P = 0.001$), and apolipoprotein A-I (5.8 ± 1.9%; $P = 0.005$), but had no effect on serum lipoprotein ratios at week 3 compared with the control. There were no significant effects on serum HDL cholesterol, serum protein carbonyl content, or ex vivo androgen or progestin activity after either treatment. Unexpectedly, serum protein thiol groups were significantly lower (10.8 ± 3.6%; $P = 0.007$) at week 3 after the flaxseed treatment than after the control, suggesting increased oxidation.

Conclusions: These data indicate that partially defatted flaxseed is effective in lowering LDL cholesterol. No effects on lipoprotein ratios, ex vivo serum androgen or progestin activity, or protein carbonyl content were observed. The significance of increased oxidation of protein thiol groups with flaxseed consumption requires further investigation. *Am J Clin Nutr* 1999;69:395–402.

KEY WORDS Flaxseed, soluble fiber, lignans, vegetable protein, α-linolenic acid, serum cholesterol, hyperlipidemia, androgen, progestin, sex hormone activity, protein carbonyl content, protein thiol groups, protein thiol oxidation, cardiovascular disease, cancer, oxidative stress, antioxidants, humans, functional foods

INTRODUCTION

There is considerable interest in the potential health benefits of oil seeds, such as soy and flaxseed, especially regarding cardiovascular disease and cancer. This interest in oil seeds relates to their high content of polyunsaturated fatty acids [particularly α-linolenic acid (1–3)], vegetable protein (4–6), soluble fiber (7), and flavonoids and related compounds (8–10), which may possess cholesterol-lowering (11), antioxidant (12), and sex hormone agonistic (13, 14) and antagonistic (15, 16) activities.

There is evidence that whole flaxseed may lower serum cholesterol in both normal (17, 18) and hyperlipidemic (19) subjects. Whole flaxseed contains 41% oil by weight, of which 70% is polyunsaturated; more than half of the total fatty acid is α-linolenic acid (20). However, no studies have been carried out with partially defatted flaxseed (<10% fat by wt) to determine whether the nonlipid components, especially the viscous fiber seed coat, are responsible for the cholesterol-lowering effects. We therefore selected partially defatted flaxseed as a more concentrated source of the viscous seed coat gum to study the effects of flaxseed on serum cholesterol in the absence of high n−3 fatty acid intake. Flaxseed is also a rich source of lignans, with potential weak estrogenic and antiestrogenic activity similar to that of the isoflavones found in soy (9, 21). These plant-derived sex hormone analogues have attracted attention as possible anticancer agents, especially for breast and prostate cancers (22, 23). In addition to their estrogenic activity, if lignans block androgen or progesterone receptors, they may alter the cardiovascular disease risk profile by changing HDL-cholesterol metabolism (24). Lignans, like flavonoids (12), have antioxidant activity (25) and therefore may also be of benefit in the prevention of cardiovascular disease (12, 26, 27) and cancer (28, 29). We therefore assessed the potential health benefits of partially defatted flaxseed in hyperlipidemic

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TABLE 1
Muffin composition and contribution to daily diet[1]

	Control muffin	Flaxseed muffin
Daily supplement (g)	186	229
Energy		
(MJ)	1.74	1.75
(kcal)	415	418
Protein		
(g)	21.4	24.2
(% of energy)	20.6	23.2
Total fat		
(g)	11.0	11.3
(% of energy)	23.8	24.3
SFA		
(g)	1.1	1.1
(% of energy)	2.5	2.4
MUFA		
(g)	5.3	3.9
(% of energy)	11.6	8.4
PUFA		
(g)	4.1	5.9
(% of energy)	8.8	12.8
Available carbohydrate		
(g)	57.8	55.0
(% of energy)	55.7	52.6
Total dietary fiber		
(g)	17.2	19.4
(g/MJ)	9.9	11.1
(g/1000 kcal)	41.4	46.4

[1] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

subjects with regard to serum lipids, serum protein thiol and carbonyl groups as markers of oxidative stress (30–32), and ex vivo serum androgen and progestin activities (33, 34).

SUBJECTS AND METHODS

Subjects

Subjects were men and postmenopausal women with hyperlipidemia [LDL cholesterol > 4.1 mmol/L (160 mg/dL) or triacylglycerol > 2.3 mmol/L (200 mg/dL)] (35) who had no clinical or biochemical evidence of diabetes, liver disease, or renal disease. After recruitment, subjects were instructed to follow a National Cholesterol Education Program (NCEP) Step II diet (35). They received biweekly dietary counseling on the NCEP Step II diet for a run-in period of ≥2 mo to stabilize their baseline serum lipid concentrations.

Thirty-seven subjects participated during the run-in period, and 36 remained for random assignment to the test (flaxseed) and control (wheat bran) phases. Four subjects completed only 1 of the 2 phases and failed to enter the final phase because of changes in personal circumstances and general availability. Three subjects withdrew during the course of 1 of the 2 phases because of either recurrent, unrelated health problems (2 subjects in control phase) or dislike of the muffins (1 subject in flaxseed phase). Twenty-nine subjects (22 men, 7 postmenopausal women) completed both phases of the study. Most subjects had normal weights [body mass index (in kg/m^2): 24.9 ± 0.5 ($\bar{x}$ ± SEM); range: 19.6–29.8]. Mean subject age was 57 ± 2 y (range: 41–73 y). After following an NCEP Step II diet for the minimum 2-mo run-in period, all but 9 subjects still had baseline serum lipid concentrations above the desirable range (35). Thirteen subjects had LDL-cholesterol concentrations > 4.1 mmol/L, 3 had triacylglycerol concentrations > 2.3 mmol/L, and 4 had both LDL-cholesterol concentrations > 4.1 mmol/L and triacylglycerol concentrations > 2.3 mmol/L. Two men were taking hypolipidemic agents (hydroxymethylglutaryl-CoA reductase inhibitors) and 3 were being treated with β-adrenergic blocking agents. One man and one woman were taking L-thyroxin. Two women were receiving hormone replacement therapy. Medications and dosages were held constant during the course of the study, and subjects were also asked to maintain a consistent level of physical activity. The study was approved by the Ethics Committee of the University of Toronto. Informed consent was obtained from all subjects.

Study design

In this randomized, crossover study the test and control phases were separated by a washout period of ≥2 wk. Subjects were blinded to the muffin type, but the flaxseed muffins were darker and had a heavier consistency. Subjects were instructed to consume an NCEP Step II diet throughout the study, including the washout period. Fasting blood samples were obtained and blood pressure and body weight were measured on day 0 and at the end of week 3 in both phases. Seven-day diet records were obtained during the last week of each phase and were analyzed to assess compliance.

Supplements

Muffins were baked in 2 batches and were kept frozen at −20°C; both test and control muffins were baked in each batch. They were provided to the subjects frozen and were kept frozen until required for consumption. Subjects thawed the muffins overnight in the refrigerator or heated them in a microwave oven for immediate consumption. The test and control muffins had similar macronutrient profiles (**Table 1**). The daily supplement consisted of 4 test or control muffins. In the test muffins, this dose provided ≈50 g partially defatted flaxseed meal. In the control muffins, wheat bran and whole-meal flour replaced flaxseed and white flour. Canola oil was added to the control muffin mix to balance the residual oil in the partially defatted flaxseed so that the total fat content of the 2 types of muffins would be equivalent. Fatty acid analysis of the muffins indicated that α-linolenic acid accounted for 31% and 8% of total fat in the test and control muffins, respectively (17, 18). Subjects were instructed by a dietitian to reduce their consumption of cereals and breads to minimize any potential effect of the muffins on the dietary macronutrient profile.

Serum and dietary analyses

Serum was stored at −70°C and all serum samples from one subject were analyzed in a single run. Serum was analyzed for total cholesterol, triacylglycerol, and HDL cholesterol after magnesium chloride precipitation with an automated clinical chemistry analyzer (CH1000; Technicon Inc, Tarrytown, NY) by using the chemical methods of the Lipid Research Clinics Program (36). LDL-cholesterol concentrations were calculated. The precision and accuracy of the total cholesterol, triacylglycerol, and HDL-cholesterol measurements were certified by the Centers for Disease Control and Prevention–National Heart, Lung, and Blood Institute Lipid Standardization Program. Internal and external quality-control procedures were followed (36). Previous studies showed that the average between-run CVs for such analyses were as follows: total cholesterol, 1.5% (range: 0.8–3.2%); HDL cholesterol, 3.2% (range: 1.6–5.3%); and triacylglycerol, 3.0% (range: 1.9–5.0%) (37).

Serum concentrations of apolipoproteins A-I and B were measured with a Behring BN100 nephelometer (Behring Werke AG, Marburg, Germany) in samples that had been stored at −70 °C (38). Jenkins et al (37) reported previously that the average within-run CVs were 3.4% for apolipoprotein A-I (range: 3.0–3.5%) and 2.7% for apolipoprotein B (range: 1.8–2.9%). Serum lipoprotein(a) concentrations were measured with a commercial enzyme-linked immunosorbent assay (Macra Lp(a) Kit; Strategic Diagnostics, Newark, DE).

Serum protein thiol groups were measured spectrophotometrically by using 5,5′dithio-bis(nitrosobenzoic acid) (DTNB) (39). Serum samples were diluted with 0.25 mol tris-EDTA buffer/L, pH 8.2, and were incubated with 100 μmol DTNB/L (final concentration) and methanol for 15 min at room temperature. Samples were centrifuged (3000 × *g* for 5 min at room temperature) and the absorbance of the supernate was measured at 412 nm. Thiols were calculated by using the molar extinction coefficient of 13.6. Serum protein carbonyl groups were measured spectrophotometrically with the 2,4-dinitrophenylhydrazine (DNPH) binding assay (40). Serum samples were incubated with 5 mmol DNPH/L (final concentration) for 1 h at room temperature, were precipitated with 10% trichloroacetic acid, and were then centrifuged (3000 × *g* for 10 min at 4 °C). The pellet was washed 3 times with ethanol:ethyl acetate (1:1, by vol) and was redissolved in 6 mol guanidine/L, pH 2.3. Absorbance of the solution was measured at 366 nm against a blank, and protein carbonyl groups were calculated by using the molar extinction coefficient of 22.0. CVs of replicates were 1.6 ± 1.0% and 9.8 ± 11.4% for protein thiols and carbonyls, respectively.

We recently developed a tissue culture system suitable for assessing agonistic and antagonistic activity of steroid hormones ex vivo (33, 34). In this system, breast cancer cell lines (BT-474 or T-47D) that are positive for steroid hormone receptors are stimulated with the agonist of interest, and prostate-specific antigen protein is measured after 8 d in the tissue culture supernate with a highly sensitive immunofluorometric assay (41). Androgens and progestins, but not estrogens, up-regulate this gene. To study antagonistic activity, the cell line is first treated with the antagonist and then stimulated with a progestin (norgestrel) or an androgen (dihydrotestosterone). By comparing experiments with and without the antagonist, the androgen- and progestin-blocking activity can be calculated as a percentage. The experimental procedures are described in detail elsewhere (33, 34). We showed that agonistic and antagonistic activity can be assessed in serum samples after a 3-fold dilution in culture media (42).

Seven-day diet records, which were compiled by the subjects during week 3 of each phase, were analyzed for macronutrients and total dietary fiber by using a database in which most foods were derived from US Department of Agriculture data (43). These data were supplemented with our own analyses of foods such as flaxmeal and other muffin components; we used methods of the Association of Official Analytical Chemists for macronutrients (44) and total dietary fiber (45). Fatty acids were measured in Folch extracts of foods by gas chromatography (17, 18).

Statistical analyses

Results are expressed as means ± SEMs. The significance of percentage differences between and within treatments was assessed with Student's *t* test for paired data (two-tailed). The treatment effect was assessed with the PROC GLM procedure in SAS (version 6.12; SAS Institute, Inc, Cary, NC), with treatment, sex, and their interaction as categorical (class) variables; subject as a random variable nested within sex; and the baseline value as a covariate (46).

TABLE 2
Calculated dietary intakes for week 3 of control and flaxseed treatment periods[1]

	Control (n = 29)	Flaxseed (n = 29)
Energy		
(MJ/d)	8.08 ± 0.39	8.15 ± 0.45
(kcal/d)	1933 ± 94	1950 ± 107
Total protein		
(g/d)	86 ± 4	90 ± 4
(% of energy)	18 ± 1	19 ± 0
Available carbohydrate		
(g/d)	266 ± 11	265 ± 13
(% of energy)	56 ± 1	55 ± 1
Total dietary fiber		
(g/d)	42 ± 2	43 ± 2
(g/MJ)	5.4 ± 0.3	5.5 ± 0.2
(g/1000 kcal)	22 ± 1	23 ± 1
Total fat		
(g/d)	52 ± 5	54 ± 6
(% of energy)	23 ± 1	24 ± 1
SFA		
(g/d)	14 ± 2	13 ± 2
(% of energy)	6 ± 0	6 ± 0
MUFA		
(g/d)	21 ± 2	20 ± 2
(% of energy)	9 ± 0	9 ± 0
PUFA		
(g/d)	14 ± 1	16 ± 1[2]
(% of energy)	6 ± 0	7 ± 0[2]
Dietary cholesterol		
(mg/d)	129 ± 19	133 ± 17
(mg/MJ)	15.5 ± 1.7	16.2 ± 1.5
(mg/1000 kcal)	65 ± 7	68 ± 6
Alcohol		
(g/d)	7 ± 2	7 ± 2
(% of energy)	2 ± 1	2 ± 1

[1] $\bar{x}$ ± SEM. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] Significantly different from control period, $P < 0.02$.

A total of 29 subjects were studied in both the test and control phases. Of the 29 subjects, 4 (2 men, 2 women) were studied twice, once when receiving the first batch of test and control muffins and again when receiving the second batch of muffins. Mean values from their 2 studies were used in all calculations. Twenty subjects (13 men, 7 women) and 15 subjects (12 men, 3 women) provided adequate amounts of serum for measurements of protein thiol and carbonyl groups, respectively. Twelve subjects (10 men, 2 women) and 10 subjects (8 men, 2 women) provided sufficient serum for determination of ex vivo androgen and progestin activity, respectively.

RESULTS

The subjects reported consuming 92.5 ± 1.8% of the flaxseed muffins and 93.5 ± 1.5% of the control muffins. The mean intakes of macronutrients during the flaxseed muffin phase (≈ 19% of energy from protein, 55% from available carbohydrate, 24%

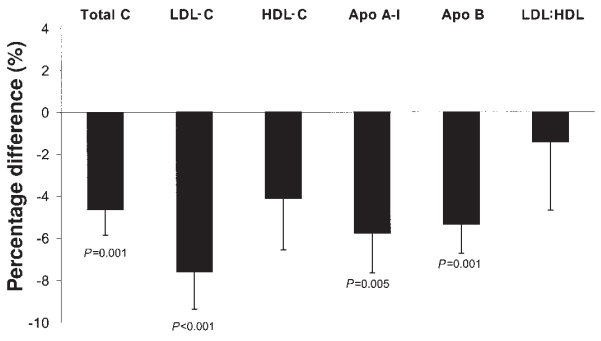

FIGURE 1. Mean (±SEM) percentage differences between flaxseed and control in serum lipoprotein and apolipoprotein (apo) concentrations of hyperlipidemic subjects after 3 wk of supplementation. Compared with the control, flaxseed significantly reduced total cholesterol, LDL cholesterol (LDL-C), apo A-I, and apo B concentrations but did not significantly lower HDL cholesterol (HDL-C) or LDL:HDL (Student's *t* test for paired data, two-tailed). There were no significant differences between baseline values obtained before the 2 treatments. $n = 29$ for total C, HDL-C, apo A-I, and apo B. LDL-C could not be calculated in 3 subjects because triacylglycerol concentrations were elevated (>4.0 mmol/L) and thus $n = 26$ for LDL-C and LDL:HDL.

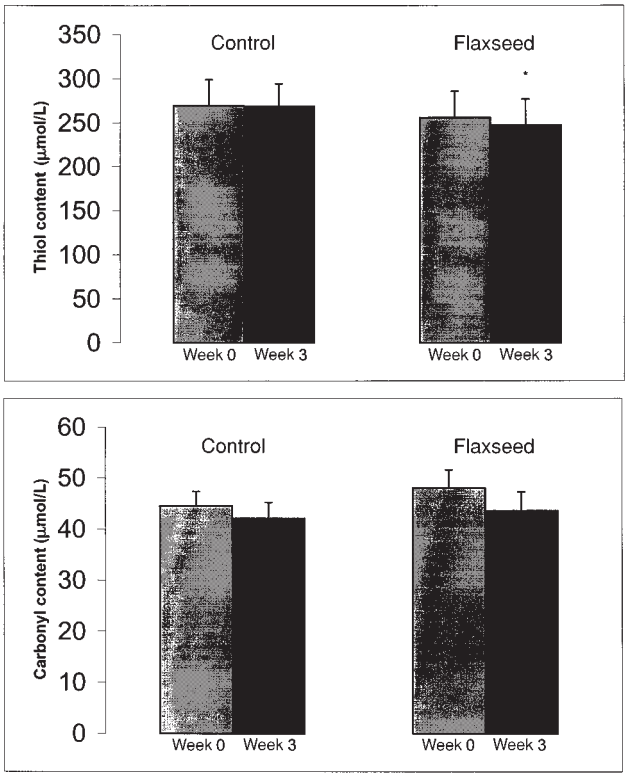

FIGURE 2. Mean (±SEM) serum protein thiol content ($n = 20$) and serum protein carbonyl content ($n = 15$) at baseline (week 0) and after 3 wk of treatment with either partially defatted flaxseed or wheat bran (control). *Significantly lower than control (10.8 ± 3.6%; $P = 0.007$, Student's *t* test for paired data, two-tailed), indicating increased thiol oxidation with flaxseed supplementation.

from fat, and 2% from alcohol) were not significantly different from intakes during the control muffin phase (**Table 2**). During the flaxseed phase, mean intakes of energy (8.15 MJ/d), total dietary fiber (43 g/d), and dietary cholesterol (133 mg/d) were also not significantly different from intakes during the control phase. There was no significant change in body weight during either phase and no significant difference in body weight between phases at weeks 0 or 3 (**Table 3**).

Baseline (week 0) serum lipid and lipoprotein concentrations did not differ significantly between the flaxseed and control phases (Table 3). From baseline to week 3 of the flaxseed muffin phase, there were significant reductions in total cholesterol (5.5 ± 1.2%), LDL cholesterol (9.7 ± 1.8%), and apolipoprotein B (5.9 ± 1.5%), but a significant increase in triacylglycerol (10.2 ± 4.8%). No significant changes in blood lipids occurred between baseline and week 3 of the control phase. When serum lipoprotein and apolipoprotein concentrations at the end of the flaxseed phase were compared with those at the end of the control phase, the following were significantly lower with the flaxseed treatment: total cholesterol (4.6 ± 1.2%), LDL cholesterol (7.6 ± 1.8%), apolipoprotein B (5.4 ± 1.4%), and apolipoprotein A-I (5.8 ± 1.9%) (**Figure 1**). There were no significant differences in blood pressure between the control and flaxseed treatments at week 3. There were also no significant differences in treatment effects between men and women.

In the 20 subjects for whom data were available, serum protein thiol content did not change significantly during either treatment (**Figure 2**). However, thiol concentrations were significantly lower at the end of the flaxseed phase than at the end of the control phase (10.8 ± 3.6%). Protein carbonyl content ($n = 15$) did not differ significantly either across or between treatments (Figure 2).

We observed no significant differences in ex vivo androgen and progestin agonistic and antagonistic activities of serum between the control and flaxseed phases at either week 0 or week 3 (data not shown). Androgen antagonistic activity decreased during the control phase by 7.5 ± 3.2% ($P = 0.038$).

DISCUSSION

Dietary supplementation with partially defatted flaxseed reduced serum concentrations of total cholesterol, LDL cholesterol, and apolipoprotein B compared with the control treatment. Although the decrease in HDL cholesterol was not significant, apolipoprotein A-I concentrations were reduced significantly during flaxseed supplementation. Ratios of LDL to HDL cholesterol and of apolipoprotein B to apolipoprotein A-I were not affected by flaxseed supplementation. Despite the lack of reduction in lipoprotein ratios, the changes we observed with partially defatted flaxseed supplementation have been described as beneficial for cardiovascular health (7). Partially defatted flaxseed had no effect on ex vivo androgenic or progesterogenic activity. However, flaxseed supplementation reduced protein thiol groups compared with the control, possibly indicating increased oxidative activity. The decrease in protein thiol groups, which indicates increased oxidative stress, would currently be seen as an undesirable effect. Increased oxidative stress may damage proteins, cellular membranes, and genetic material (28). On the other hand, generation of oxygen radicals appears to be involved in the initiation of apoptosis (47) and the natural defense against transformed or foreign cells (48, 49).

TABLE 3
Body weight, blood lipid concentrations, and blood pressure at weeks 0 and 3 of control and flaxseed treatment periods[1]

	Control				Flaxseed			
	Week 0	Week 3	Percentage change[2]	*P*	Week 0	Week 3	Percentage change[2]	*P*
Body weight (kg)	72.0 ± 2.1	72.0 ± 2.0	0.0 ± 0.2	0.926	71.7 ± 2.1	71.6 ± 2.1	−0.0 ± 0.2	0.935
Total C (mmol/L)	6.55 ± 0.18	6.38 ± 0.17	−2.0 ± 1.9	0.287	6.42 ± 0.16	6.06 ± 0.15	−5.5 ± 1.2	<0.001
HDL-C (mmol/L)	1.21 ± 0.06	1.22 ± 0.06	1.6 ± 1.8	0.404	1.20 ± 0.07	1.17 ± 0.06	−1.4 ± 2.8	0.611
LDL-C (mmol/L)	4.39 ± 0.15	4.26 ± 0.12	−1.8 ± 2.6	0.495	4.36 ± 0.13	3.92 ± 0.11	−9.7 ± 1.8	<0.001
Triacylglycerol (mmol/L)	2.14 ± 0.16	2.06 ± 0.16	0.6 ± 5.6	0.914	2.09 ± 0.22	2.18 ± 0.18	10.2 ± 4.8	0.044
Apo A-I (g/L)	1.61 ± 0.05	1.62 ± 0.05	0.4 ± 1.2	0.766	1.59 ± 0.05	1.52 ± 0.05	−3.7 ± 2.0	0.070
Apo B (g/L)	1.66 ± 0.05	1.63 ± 0.05	−1.5 ± 2.0	0.447	1.63 ± 0.04	1.53 ± 0.04	−5.9 ± 1.5	0.001
Total C:HDL-C	5.65 ± 0.21	5.44 ± 0.21	−3.0 ± 2.1	0.169	5.71 ± 0.27	5.49 ± 0.24	−2.7 ± 2.2	0.222
LDL-C:HDL-C	3.68 ± 0.16	3.54 ± 0.16	−3.0 ± 2.9	0.303	3.71 ± 0.19	3.46 ± 0.18	−5.2 ± 3.1	0.097
Apo B:apo A-I	1.05 ± 0.04	1.02 ± 0.03	−1.7 ± 1.9	0.371	1.06 ± 0.04	1.03 ± 0.04	−1.5 ± 2.0	0.476
Lipoprotein(a) (mg/L)	20.9 ± 4.1	23.4 ± 4.3	11.4 ± 7.6	0.142	22.1 ± 4.3	20.7 ± 4.1	6.2 ± 13.5	0.651
Blood pressure (mm Hg)								
Systolic	129 ± 3	125 ± 3	−3.0 ± 1.1	0.014	128 ± 4	124 ± 2	−2.0 ± 1.8	0.284
Diastolic	81 ± 2	80 ± 1	−1.1 ± 1.2	0.373	81 ± 1	79 ± 1	−1.6 ± 1.4	0.261

[1] $\bar{x}$ ± SEM; $n = 29$ except for LDL-C and LDL-C:HDL-C ($n = 26$), lipoprotein(a) ($n = 27$), and blood pressure ($n = 28$); C, cholesterol; Apo, apolipoprotein.
[2] Percentage change = (week 3 − week 0) × 100/week 0.

This situation may be analogous to the double-edged sword effect seen with long-chain n−3 fatty acids in marine oil. These fatty acids are more susceptible to oxidation, may increase hepatic glucose output, and may raise LDL-cholesterol concentrations (50, 51); on the other hand, they reduce VLDL-triacylglycerol concentrations and decrease platelet aggregation (52, 53). In previous studies in which full-fat flaxseed was consumed, no significant reduction was seen in thiobarbituric acid–reactive substances (TBARS), as indicators of increased lipid oxidative stress, despite high concentrations of lignans as potential antioxidant phenolics. This lack of effect might have been related to the relatively high content of α-linolenic acid in the full-fat flaxseed (17, 18).

The components of flaxseed to which health benefits have been ascribed include its high contents of lignans, vegetable protein, and α-linolenic acid and its seed coat gum (54). This gum is a highly viscous mixture of acidic and neutral polysaccharides that have been characterized as primarily glucuronic acids, rhamnose, arabinose, xylose, and galactose (55). This polysaccharide gum makes up ≈8% of full-fat flaxseed (55–57). The 50 g partially defatted flaxseed consumed daily in the present study provided ≈5–6 g flaxseed gum. The reduction in LDL cholesterol in our study was between 7% and 8%, similar to reductions measured in other studies in which 5–10 g viscous soluble fibers, including guar, pectin, psyllium, and β-glucan, were consumed in foods or given as supplements (37, 58–62). As with lipid changes observed with other viscous fiber sources, the reduction in serum cholesterol that we observed was probably related to greater fecal losses of bile acid (62, 63) and increased primary bile acid synthesis (64, 65). Partially defatted flaxseed had no effect on the ratios of LDL to HDL cholesterol or of apolipoprotein B to apolipoprotein A-I. A similar lack of change in lipoprotein ratios was reported for other diets high in soluble fiber, despite significant reductions in LDL cholesterol (58). Unchanged lipoprotein ratios have also been reported after other dietary manipulations recommended for reducing LDL cholesterol (35), including reductions in saturated fat and dietary cholesterol and increases in polyunsaturated fatty acid intake (66).

If the decreases in serum LDL cholesterol were due to flaxseed gum, it appears that this component is more hypocholesterolemic per gram than most other viscous fibers. However, the supplement also provided 24.2 g vegetable protein/d from flaxseed. Soy proteins in amounts of 30–50 g/d have been shown to lower serum cholesterol (4–6, 67) and it has been suggested that the amino acid composition of the protein may be responsible for this effect (5, 6). Another explanation may be the content of phenolic substances, such as isoflavonoids (4, 11). Because neither the flaxseed protein nor the phenolic flaxseed lignans have been tested individually for their hypolipidemic effect, one or both components may have contributed to the cholesterol reduction observed.

Attention has focused on the ability of soy isoflavonoids and flaxseed lignans to block sex hormone receptors in the prevention of hormone-dependent cancers. It has been proposed that the low incidences of breast cancer in Japan and China relate to the large amount of soy consumed (8, 9). Studies have shown that soy isoflavonoids block estrogen activity in vitro (8, 9, 15, 16). Inhibition of tumor growth in the breast, prostate, skin, and liver has been observed in animal models (68–70), suggesting that soy may have other endocrine and nonendocrine effects. Similar data are now emerging for flaxseed (71, 72). Until now, studies have focused on estrogen activity. This study is the first attempt of which we are aware to assess androgen and progestin activities ex vivo by analyzing serum from subjects who consumed flaxseed. No agonistic or antagonistic effects that might have suggested a beneficial effect of flaxseed in prostate cancer prevention were observed. Furthermore, reduced androgen activity might have increased HDL-cholesterol concentrations (24), but this was not observed. It is possible that the lack of effect was because the lignans were primarily in the conjugated glucuronide form in urine, and this form has little such activity (10).

The phenolic lignans may also have antioxidant activity. Certain dietary antioxidants appear to offer protection from cardiovascular disease (12, 26, 27), possibly by reducing LDL-cholesterol oxidation and therefore atherogenicity. Antioxidants may also reduce cancer risk (29) by reducing oxidative damage to DNA and thereby preserving the genome (28, 73). Because we previously found no major effect of whole flaxseed on markers of lipid peroxidation, including serum TBARS and urinary malondialdehyde excretion (17, 18), we measured oxidation of plasma proteins as an indicator of longer-term oxidant activity. Serum protein thiol groups were selected because they have been

shown to be particularly sensitive indexes of oxidation (31, 32, 74, 75). The flaxseed supplement reduced serum protein thiol groups, indicating increased oxidation. If confirmed, this would be interpreted as an undesirable effect. However, some prooxidant activity may be beneficial in cancer prevention and tumor cell destruction (76). T lymphocytes destroy foreign cells and pathogens by mechanisms including free radical generation (77). The generation of intracellular oxygen radicals also appears to play a role in the induction of apoptosis (47). Malignant tissue has diminished peroxidizability (78–81), and antioxidants appear to accumulate in malignant cells. Whether the effect of flaxseed on serum protein thiol groups has any bearing on these activities remains to be determined.

In conclusion, these data confirm that supplementing the diet with partially defatted flaxseed results in reductions in serum LDL-cholesterol concentrations similar to those observed with full-fat flaxseed (17–19). The flaxseed gum is likely the major active ingredient in flaxseed responsible for the lipid-lowering action, but the isolated gum requires testing, as do the vegetable protein and phenolic lignan components. Androgen antagonistic activity in serum did not increase, and therefore the data do not support a potential protective role of lignans in prostate cancer related to altered sex hormone activity. No evidence was found for an antioxidant role of flaxseed components. However, the reduction in protein thiol groups raises the question of whether increased prooxidant activity resulting from flaxseed consumption has any physiologic significance.

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