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Dietary Flaxseed Independently Lowers Circulating Cholesterol and Lowers It beyond the Effects of Cholesterol-Lowering Medications Alone in Patients with Peripheral Artery Disease[1–4]

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Abstract

Background: Dietary flaxseed lowers cholesterol in healthy subjects with mild biomarkers of cardiovascular disease (CVD).

Objective: The aim was to investigate the effects of dietary flaxseed on plasma cholesterol in a patient population with clinically significant CVD and in those administered cholesterol-lowering medications (CLMs), primarily statins.

Methods: This double-blind, randomized, placebo-controlled trial examined the effects of a diet supplemented for 12 mo with foods that contained either 30 g of milled flaxseed [milled flaxseed treatment (FX) group; n = 58] or 30 g of whole wheat [placebo (PL) group; n = 52] in a patient population with peripheral artery disease (PAD). Plasma lipids were measured at 0, 1, 6, and 12 mo.

Results: Dietary flaxseed in PAD patients resulted in a 15% reduction in circulating LDL cholesterol as early as 1 mo into the trial (P = 0.05). The concentration in the FX group (2.1 ± 0.10 mmol/L) tended to be less than in the PL group (2.5 ± 0.2 mmol/L) at 6 mo (P = 0.12), but not at 12 mo (P = 0.33). Total cholesterol also tended to be lower in the FX group than in the PL group at 1 mo (11%, P = 0.05) and 6 mo (11%, P = 0.07), but not at 12 mo (P = 0.24). In a subgroup of patients taking flaxseed and CLM (n = 36), LDL-cholesterol concentrations were lowered by 8.5% ± 3.0% compared with baseline after 12 mo. This differed from the PL + CLM subgroup (n = 26), which increased by 3.0% ± 4.4% (P = 0.030) to a final concentration of 2.2 ± 0.1 mmol/L.

Conclusions: Milled flaxseed lowers total and LDL cholesterol in patients with PAD and has additional LDL-cholesterol–lowering capabilities when used in conjunction with CLMs. This trial was registered at clinicaltrials.gov as NCT00781950. *J Nutr* 2015;145:749–57.

Keywords: flaxseed, cholesterol lowering, peripheral artery disease, platelet aggregation, statins

Introduction

Dietary flaxseed has provided beneficial cardiovascular effects in a number of animal studies. These include improving vascular reactivity (1), inhibiting the progression of atherosclerosis (2), promoting the regression of existing atherosclerotic plaques (3), inhibiting the incidence of arrhythmias during ischemia/reperfusion challenge (4), and lowering circulating concentrations of cholesterol (5). Several clinical trials have used flaxseed as a dietary supplement to investigate its efficacy on bone density (6), menopausal symptoms (6), blood glucose (7), lipid profile (8), and blood pressure (9, 10). The beneficial actions of dietary flaxseed have been attributed to its rich content of fiber, lignans, and the omega-3 (ω-3) FA, α-linolenic acid (18:3n–3; ALA)[11] (1–5).

Although much is known of the effects of dietary flaxseed in both animal models and human clinical trials, several important variables remain to be studied. For example, the effects of dietary flaxseed on circulating concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and TGs in patients with documented cardiovascular disease (CVD) may be quite different from the response exhibited by subjects without clinical symptoms of CVD or in those only presenting with risk factors (8, 11, 12). Furthermore, the effects of flaxseed in patients

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already administered cholesterol-lowering medication (CLM) are unknown. In view of a number of studies that have shown significant drug-food interactions (13, 14), this is an important point to consider. Finally, it is unclear if flaxseed may also inhibit platelet aggregation in a clinical population, which would be beneficial when blood clots may represent a serious health concern. The marine ω-3 FAs EPA (20:5n–3) and DHA (22:6n–3) can inhibit platelet aggregation (15). Dietary flaxseed with its rich content of ALA may have a similar effect in cardiovascular patients at risk of clinical events. Therefore, understanding the effects of dietary flaxseed on a variety of clinical variables in the circulation of a human population, particularly in those with pre-existing CVD, becomes an important clinical topic to investigate.

Patients with peripheral artery disease (PAD) exhibit many characteristics of CVD and because of this they are at high risk of myocardial infarctions and stroke (16, 17). They frequently have diabetes, hyperlipidemia, hypertension, and atherosclerotic coronary artery disease and altered blood coagulation properties (9, 16–18). As a result, they are commonly on a combination of lipid-lowering, antithrombotic, antihypertensive, and blood glucose–lowering therapies (9, 17). In view of the effects of flaxseed on CVD in animal models, it is possible that flaxseed may be beneficial in a patient population with PAD. The FLAX-PAD (FLAXseed and Peripheral Artery Disease) Trial (NCT00781950) was initiated to study the effects of consuming dietary flaxseed in a patient population with PAD (9). The significant antihypertensive action of flaxseed in this patient population has been recently reported (10, 19).

The aim of this part of the FLAX-PAD Trial, therefore, was 2-fold: first, to determine if consumption of dietary flaxseed, in PAD patients and in a subgroup being administered CLMs, would alter the cholesterol and TG profile, and, second, to determine if platelet aggregation would be altered. It was hypothesized that dietary flaxseed would lower circulating cholesterol concentrations in PAD patients. These effects on the PAD patients would be complicated by the ongoing administration of cholesterol-lowering drugs (i.e., statins) and antithrombotic medication (i.e., clopidogrel). It is also possible that the dosage of medications required to achieve the desired effects on circulating cholesterol and platelet aggregation would be changed because of consumption of flaxseed. For this reason, a double-blinded, placebo-controlled, randomized trial (9) was initiated in a patient population with documented PAD to study the effects of milled flaxseed on a variety of blood characteristics over a 1-y ingestion period.

Methods

The FLAX-PAD Trial design. This was a single-site, double-blind, placebo-controlled study performed in Winnipeg, Canada. Twelve months has been recommended as a duration that is ideal to study dietary effects (9, 20). The study was conducted after approval from the Natural Health Products Directorate of Health Canada, the University of Manitoba Research Ethics Board, and the St. Boniface Hospital Research Review Committee. Each patient who participated provided written consent. A Safety Monitoring Committee was created and insured patient safety throughout the course of the study. Details concerning the FLAX-PAD experimental design are found elsewhere (9). Briefly, 347 patients with documented PAD were initially screened for entry into the study. Of these, 237 were excluded because they did not meet the study inclusion criteria, there was an unwillingness to participate, or for other reasons (**Figure 1**) (10). The 110 patients that remained were randomly selected by a computer program into the milled flaxseed treatment (FX; $n = 58$) and placebo (PL; $n = 52$) groups. Of the patients that were administered CLM at study onset, 40 patients (88.9%) in the FX + CLM subgroup ($n = 45$) were on statins [simvastatin (10–80 mg/d), rosuvastatin (10–40 mg/d), atorvastatin (10–80 mg/d), and pravastatin (20 mg/d)], of which 4 of these were on a combination therapy merging a statin with either ezetimibe or a fibrate. The remaining 5 patients (11.1%) were on other CLMs [ezetimibe (10 mg/d), bezafibrate (400 mg/d 3 times per week), and fenofibrate (145 and 160 mg/d)]. In the PL + CLM subgroup ($n = 36$), 35 patients (97.2%) were taking statins [simvastatin (10–20 mg/d), rosuvastatin (10–40 mg/d), and atorvastatin (5–80 mg/d)] of which 4 patients were taking a statin in combination with either a fibrate [gemfibrozil or Lipidil (Laboratoires Fournier S.A.)], ezetimibe, or with fenofibrate and ezetimibe. Only 1 patient (2.8%) was taking ezetimibe at 10 mg/d. The number of patients in the FX-only and PL-only subgroups was 11 and 15, respectively. All patients were monitored by the attending physician and CLMs were adjusted as necessary to keep blood concentrations of lipids in normal ranges. All patients had an ankle/brachial index of <0.9, which was the clinical criteria for the presence of PAD in this study.

The investigational products provided to the patients included buns, snack bars, muffins, bagels, pasta, and tea biscuits, each containing 30 g of milled flaxseed or a placebo that contained milled wheat. Small bags of the milled product were also available. This allowed the subjects to mix the product into other foods such as yogurt, cereals, or drinks to give them variety in their diet over the course of 12 mo. In this case, the wheat was mixed with a very small amount of wheat germ, coconut oil, and wheat bran to ensure that the color and texture of the food product resembled the same food that contained flaxseed. The muffins, bagels, and snack bars were also formulated with different flavorings to give the patients sufficient variety to ensure compliancy over the time of the study. The placebo product contained the same flavorings but did not

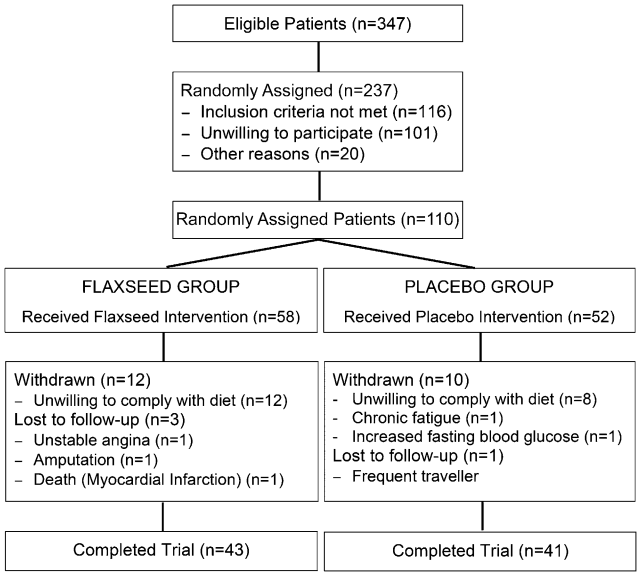

FIGURE 1 Participant eligibility, screening, randomization, and follow-up of PAD patients for the 12-mo FLAX-PAD Trial. FLAX-PAD, FLAXseed and Peripheral Artery Disease; PAD, peripheral artery disease.

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[3] Supplemental Tables 1–3 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[4] These authors contributed equally to the completion of this paper.

[11] Abbreviations used: ALA, α-linolenic acid; CLM, cholesterol-lowering medication; CVD, cardiovascular disease; FLAX-PAD, FLAXseed and Peripheral Artery Disease; FX, milled flaxseed treatment; PAD, peripheral artery disease; PL, placebo.

contain flaxseed. Food composition and sensory results are described for the muffins, bagels, and snack bars in detail elsewhere (21, 22). The energy and nutrient composition of the available food products are presented in **Supplemental Table 1**. One food item was ingested per day over the course of the study with subjects gradually introduced to the food products beginning with 10 g in week 1, 20 g in week 2, and then continuing with 30 g for the duration of the study. Each test subject received a 1-mo supply of food products. They decided what type of foods and which flavors they desired. The individualized monthly ration of food products was then delivered to each subject and was stored by the patient in their own freezer to ensure freshness over the month. Although the food products ingested varied from person to person, the flaxseed content of the diet was distinct in comparison with the placebo group. The individualized choice of flavors and food items was critical to maintain compliance throughout the 12 mo. All personnel that collected or analyzed data were blinded to the intervention. Only after all data were calculated was the unblinding completed.

Body measurements and blood collection. Body weight, waist circumference, and BMI were recorded at 0-, 6-, and 12-mo time points. Patients were requested to fast for 12 h before the morning of their blood draw (35 mL). Ten milliliters was collected in citrate-containing Vacutainer tubes (Becton Dickinson) for immediate platelet analysis and 5 mL was collected into EDTA-containing tubes for FA methyl ester and enterolignan analyses. Plasma was obtained by centrifugation as described previously (1, 2). Plasma was separated into small aliquots and stored immediately at −80°C to be measured at a later date.

Plasma analyses. Plasma total cholesterol, LDL cholesterol, HDL cholesterol, and TGs were measured by validated techniques in the St. Boniface Hospital Biochemistry Laboratory. Plasma FAs were measured by GC, as described in detail (1, 2). Plasma enterolignans were quantified by gas chromatography–mass spectrometry as previously described (10, 23). Platelet aggregation studies were measured using freshly collected blood on a Chrono-Log 490–2D Platelet aggregometer (Chrono-Log Corp.) as previously reported (24). Collagen (5 μg/mL, number 385) and thrombin (0.3 units/mL, number 386; Chrono-Log Corp.) were the agonists used to perform platelet aggregation experiments.

Statistical analyses. Continuous variables were expressed as mean ± SD unless otherwise stated. Categorical variables were expressed as proportions. Analyses were performed to compare the 2 groups. A frequency test was used to determine if data were normally distributed. Categorical variables were compared with χ^2-test. Continuous variables were compared using an independent samples *t* test. A repeated-measures ANOVA was used to compare balanced data sets. The Greenhouse-Geisser model was used to measure main effects for time and group and group × time interactions. Pairwise comparisons over time were adjusted for multiple comparisons using least-significant differences. Between-group comparisons in the present manuscript will be discussed as "differences" and time effects will be referred to as "change." Spearman correlations were used to evaluate main associations between flaxseed components and blood lipids. Differences were considered significant for values of $P < 0.05$. Trends were noted if $P \leq 0.1$. These analyses were performed using SPSS 22.0 (International Business Machines).

For unbalanced data analysis, an advanced mixed 2-factor repeated-measures procedure was used to determine differences between groups and among time points. Group and time were between and within factors, respectively. Three models including compound symmetry, Huynh-Feldt, and unstructured were run. The best model was chosen based on the information of Akaike information criteria and Schwarz's Bayesian information criteria. If the mixed procedure provided a significant difference ($P < 0.05$) for group, time, or group × time interaction, the least-squared means were calculated and compared. To control for type 1 errors for multiple comparisons, a Bonferroni correction was applied to the α-level so that $P = 0.008$ was the critical value of statistical significance for continuous variables and $P = 0.017$ for values represented as absolute or percent change. All statistical tests were determined using SAS statistical software, version 9.2 (Statistical Analysis System; SAS Institute, Inc.).

Results

Body morphometrics. Body weight, waist circumference, and BMI measurements were measured at baseline and at 6 and 12 mo. There were no within-group changes over time for either of the study groups or between-group differences at any time point for any of the 3 measured variables ($P \geq 0.05$; data not shown).

Lipid measurements. Baseline concentrations of all lipids were similar between PL and FX intervention groups. When all patients, including those with missing time points, were integrated into the analysis, flaxseed ingestion resulted in an 11–15% reduction of circulating concentrations of total and LDL cholesterol as early as 1 mo into the trial ($P = 0.05$), which was maintained for 6 mo compared with placebo (**Figure 2**A, B). At 12 mo, these differences were no longer observed. Plasma total cholesterol and LDL cholesterol were significantly attenuated by flaxseed ingestion at 6 mo compared with baseline values ($P = 0.005$ and $P \leq 0.001$, respectively). Conversely, there were no changes in the PL group over time. Circulating HDL-cholesterol concentrations did not change within or between groups at any time point during the trial (Figure 2C). Circulating TGs were also not statistically different between groups throughout the study; however, the concentration in the FX group increased significantly at 12 mo compared with the mean baseline value ($P = 0.002$; Figure 2D). When plasma lipids were compared using only patients that completed the 1-y trial (**Table 1**), LDL

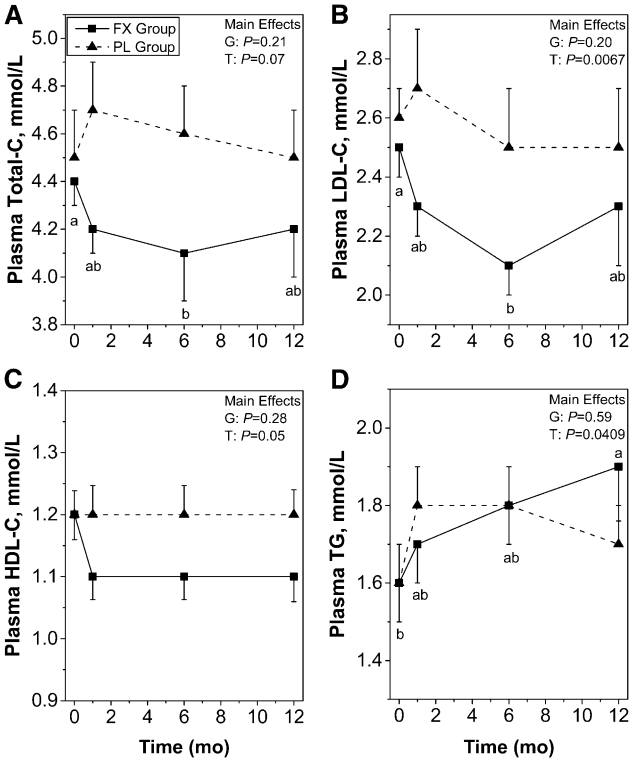

FIGURE 2 Plasma lipid concentrations in patients with PAD in the FX and PL groups at baseline and at 1-, 6-, and 12-mo time points. Values are represented as means (SEMs). Plasma lipids include Total-C (A), LDL-C (B), HDL-C (C), and TG (D). Within a group, labeled means without a common letter differ, $P \leq 0.008$. Flaxseed: baseline, $n = 58$; 1 mo, $n = 52$; 6 mo, $n = 45$; 12 mo, $n = 43$. Placebo: baseline, $n = 52$; 1 mo, $n = 47$; 6 mo, $n = 41$; 12 mo, $n = 41$. FX, milled flaxseed treatment; G, group; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PAD, peripheral artery disease; PL, placebo; T, time; Total-C, total cholesterol.

cholesterol in those consuming flaxseed decreased at 6 mo compared with baseline values ($P = 0.007$), as did HDL cholesterol ($P = 0.042$). A similar trend was observed for total cholesterol at these 2 time points ($P = 0.07$). No other differences between groups or changes over time in plasma lipids including TGs, total cholesterol:HDL cholesterol, and LDL cholesterol: HDL cholesterol were observed.

A risk factor for developing PAD is an increase in circulating plasma cholesterol (25). Eighty percent of the patients enrolled in the trial had hyperlipidemia (9). Therefore, it was not surprising that a majority of the patients (74%) were administered CLM before starting the trial. As expected, patients administered CLM (+CLM) exhibited lower basal concentrations of total cholesterol, LDL cholesterol, total cholesterol: HDL cholesterol, and LDL cholesterol:HDL cholesterol than those not taking these medications ($P < 0.01$; **Table 2**). Baseline concentrations of all plasma lipids were similar when comparing FX + CLM and PL + CLM subgroups ($P \geq 0.05$). TGs were significantly lower in the FX-only subgroup than in the PL-only subgroup ($P = 0.030$), with all other lipids remaining similar between the 2 groups.

It was important to determine if the circulating concentrations of cholesterol that were controlled by the administration of CLM exhibited any additional cholesterol-lowering effects provided by the flaxseed (or, conversely, if the flaxseed would inhibit the cholesterol-lowering effects of the CLM). The data over time as percent change was examined in individuals that completed the 1-y trial to determine if total cholesterol and LDL-cholesterol concentrations could be further reduced by dietary flaxseed in patients using CLMs. A trend noting a group difference was observed for total cholesterol ($P = 0.06$; **Table 3**), but the most noticeable attenuation in blood lipids was observed with LDL cholesterol in the FX + CLM subgroup (**Figure 3**A). After 12 mo of consuming 30 g/d of dietary flaxseed, LDL cholesterol was attenuated by 8.5% in the FX + CLM subgroup compared with a 3.0% increase in the PL + CLM subgroup ($P = 0.030$). This group difference for LDL cholesterol was also evident by the significant main effect for group ($P = 0.020$) when compared as absolute change (**Supplemental Table 2**). To determine the effects of flaxseed alone in this patient population, we examined LDL cholesterol in a subgroup of individuals not taking administered CLMs (Figure 3B). LDL cholesterol was significantly attenuated after 6 mo compared with initial values ($P = 0.009$, Supplemental Table 2, and $P = 0.014$, Figure 3B). No significant between-group differences were present at any time point (Supplemental Table 2; $P = 0.38$). No other differences in plasma lipids including TGs, total cholesterol, HDL cholesterol, total cholesterol:HDL cholesterol ratio, and LDL cholesterol: HDL cholesterol ratio were observed between any of the treatment groups after 1, 6, or 12 mo (Table 3). Despite a significant main effect for time in total cholesterol ($P = 0.020$; Table 3) for the FX- and PL-only subgroups, the limited sample size prohibited detection of any changes over time in either of the treatment groups. Patient subgroups (FX + CLM and FX only; PL + CLM and PL only) were not compared because of the large differences in sample size.

The next step was to assess if basal concentrations of circulating cholesterol influenced the subsequent effects of flaxseed and CLM. Patients were organized by dietary treatment into subgroups where baseline concentrations of total cholesterol were borderline to high (≥5.3 mmol/L). Twelve patients in the FX group fit these criteria. Two of these patients discontinued the study before the first month so only 10 patients were included in the analysis. Of these individuals, 6 were already taking CLM at the onset. In the PL group, there were 11 hypercholesterolemic patients at the onset with only 2 patients taking CLM. Two patients were unavailable for their 6-mo follow-up but were present for their 12-mo appointment.

TABLE 1 Plasma lipid concentrations measured at baseline and 1-, 6-, and 12-mo time points for patients with PAD in both FX and PL treatment groups[1]

					Effects (P)		
Lipids	Baseline	1 mo	6 mo	12 mo	G	T	G × T
TG, mmol/L					0.57	0.10	0.07
FX	1.6 ± 0.7	1.8 ± 0.8	1.8 ± 0.8	1.9 ± 0.9			
PL	1.7 ± 0.9	1.7 ± 0.7	1.8 ± 0.9	1.7 ± 0.7			
Total-C, mmol/L					0.12	0.033	0.53
FX	4.4 ± 1.2	4.2 ± 1.0	4.1 ± 1.1	4.2 ± 1.3			
PL	4.7 ± 1.3	4.7 ± 1.4	4.6 ± 1.4	4.5 ± 1.3			
LDL-C, mmol/L					0.35	0.0022	0.19
FX	2.5 ± 1.0[a]	2.3 ± 1.0[a,b]	2.2 ± 1.0[b]	2.3 ± 1.1[a,b]			
PL	2.6 ± 1.0	2.6 ± 1.0	2.5 ± 1.0	2.4 ± 0.9			
HDL-C, mmol/L					0.22	0.051	0.13
FX	1.20 ± 0.33[a]	1.12 ± 0.27[a,b]	1.11 ± 0.25[b]	1.12 ± 0.25[a,b]			
PL	1.21 ± 0.29	1.22 ± 0.33	1.19 ± 0.30	1.22 ± 0.25			
Total-C:HDL-C ratio					0.77	0.58	0.62
FX	3.8 ± 1.2	3.9 ± 1.1	3.8 ± 1.3	3.8 ± 1.3			
PL	4.0 ± 1.3	4.0 ± 1.3	3.9 ± 1.1	3.8 ± 1.1			
LDL-C:HDL-C ratio					0.97	0.30	0.57
FX	2.2 ± 1.0	2.1 ± 1.0	2.1 ± 1.1	2.1 ± 1.1			
PL	2.2 ± 0.9	2.2 ± 0.9	2.1 ± 0.8	2.0 ± 0.8			

[1] Values are means ± SDs. $n = 43$ (FX) or 41 (PL). Labeled means in a row without a common letter differ, $P < 0.05$. Differences between groups were not statistically significant, $P \geq 0.05$. FX, flaxseed group; G, group; G × T, group × time; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PAD, peripheral artery disease; PL, placebo group; T, time; Total-C, total cholesterol.

TABLE 2 Subgroup analysis of plasma lipid concentrations at baseline represented by the FX or PL group in PAD patients that were or were not taking CLMs[1]

	Diet + CLM			Diet only		
Variable	FX + CLM	PL + CLM	*P*	FX only	PL only	*P*
Sample size, *n*	37	26		7	15	
TG, mmol/L	1.7 ± 0.7	1.5 ± 0.7	0.42	1.2 ± 0.4	2.0 ± 1.1	0.03
Total-C, mmol/L	4.3 ± 1.1	4.1 ± 0.8†	0.54	5.1 ± 0.9	5.7 ± 1.5	0.33
LDL-C, mmol/L	2.3 ± 1.0†	2.1 ± 0.5‡	0.52	3.4 ± 0.8	3.6 ± 1.1	0.69
HDL-C, mmol/L	1.2 ± 0.3	1.3 ± 0.3	0.75	1.1 ± 0.2	1.1 ± 0.2	0.49
Total-C:HDL-C ratio	3.6 ± 1.1†	3.4 ± 0.7‡	0.30	4.9 ± 1.0	5.1 ± 1.4	0.75
LDL-C:HDL-C ratio	1.9 ± 1.0†	1.8 ± 0.5‡	0.31	3.3 ± 0.7	3.2 ± 1.0	0.86

[1]Values are means ± SDs. Symbols indicate different from FX-only or PL-only subgroups within the same dietary group, †$P < 0.01$, ‡$P < 0.001$. CLM, cholesterol-lowering medication; FX, flaxseed group; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PAD, peripheral artery disease; PL, placebo group; Total-C, total cholesterol.

Despite a main effect over time for LDL cholesterol ($P = 0.010$), no significant changes over time were detected in either group when post hoc tests were applied and there were no between-group differences ($P = 0.67$; data not shown). A similar trend was observed for total cholesterol with $P = 0.05$ as a main effect over time and $P = 0.10$ for the group effect. These trends can be attributed to the drop in total cholesterol of 0.9 mmol/L in the flaxseed group compared with only 0.2 mmol/L in the placebo group after 6 mo of intervention.

It is possible that the cholesterol-lowering action of dietary flaxseed may have induced a reduction in the prescribed dosage of CLMs used over the course of the trial. Recall that patients were monitored individually by the attending physician. Because the health of our patients was our priority CLM doses could be adjusted at any time as warranted by the physician. These results are therefore suggested based on the placebo-controlled nature of our trial. No changes in administered medications were reported in the FX + CLM subgroup until the 1-y visit. At this visit, 1 person (2.7%) had stopped taking statin medication (rosuvastatin at 40 mg/d), 33 patients (89.2%) had their CLM dose unchanged, and 3 patients (8.1%) increased their statin dose (simvastatin at 40 mg/d to atorvastatin at 80 mg/d and 2 patients increased their atorvastatin from 20 mg/d to 40 and 80 mg/d). Each of these changes occurred at or following 7 mo of flaxseed intervention. In the PL + CLM subgroup, 4 patients (15.4%) had their CLM dose increased, 1 at 1 mo (atorvastatin at 40–60 mg/d), 2 at 6 mo (atorvastatin at 5–10 mg/d and ezetimibe at 10 mg/d to cholestyramine at 2 packs/d), and 1 at 12 mo (rosuvastatin at 10–20 mg/d). One patient stopped their statin use at 6 mo, only to be restarted on the same dose 2 mo later. Only 1 patient had their statin dose reduced at 12 mo (atorvastatin at 80–40 mg/d). In the PL-only group (i.e., none of the patients were taking CLM at the start of the trial), 33.3% were being administered CLM by the end of the study-monitoring period. This is in contrast to the FX-only group in which only 1 patient (14.3%) began a low-dose statin therapy at 6 mo (rosuvastatin at 10 mg/d), which was quickly discontinued shortly thereafter.

Consumption of milled flaxseed induced a 1-fold increase in circulating ALA concentrations and a 10- to 50-fold increase in plasma total enterolignans or the enterolignans species enterodiol and enterolactone (10). Absolute changes in these components after 1, 6, and 12 mo are listed in **Supplemental Table 3**. The association of plasma ALA or enterolignans with changes in plasma total cholesterol was examined in patients who ingested flaxseed. No inverse correlations were observed between plasma ALA, total enterolignans, enterodiol, or enterolactone with either total cholesterol and LDL cholesterol or TGs as a result of consuming flaxseed ($P \geq 0.05$; data not shown).

Platelet aggregation. Platelet aggregation was monitored using both collagen and thrombin as agonists. Using all patients enrolled in the trial, a significant main effect for time ($P < 0.01$) was observed with each agonist for both percent aggregation and rate of aggregation (**Table 4**). When thrombin was used as the agonist, percent aggregation was attenuated in the FX group at 6 mo compared with baseline ($P = 0.005$) and rate of aggregation changed comparably for both FX and PL groups. Collagen did not influence any changes in the FX group in either percent or rate of aggregation. There were no differences between the FX and PL groups at any of the measured time points ($P \geq 0.05$) despite these changes over time (Table 4).

TABLE 3 Percent change from baseline in plasma lipid concentrations in PAD patients by dietary FX and PL as a function of administered CLMs or the stand-alone diet[1]

	Change over time, %			Effects (*P*)[2]		
Lipids	After 1 mo	After 6 mo	After 12 mo	G	T	G × T
TG, %				0.43	0.63	0.45
FX + CLM	11.6 ± 5.4	16.4 ± 6.5	22.1 ± 6.3			
PL + CLM	11.0 ± 5.6	10.7 ± 6.2	7.9 ± 6.3			
TG, %				0.44	0.84	0.51
FX only	11.3 ± 8.8	20.9 ± 14.7	10.4 ± 7.6			
PL only	8.6 ± 6.6	−1.2 ± 9.4	4.3 ± 9.5			
Total-C, %				0.06	0.59	0.88
FX + CLM	−2.3 ± 1.6	−3.4 ± 2.2	−3.0 ± 2.5			
PL + CLM	2.8 ± 2.3	−0.3 ± 2.3	1.8 ± 2.8			
Total-C, %				0.42	0.02	0.44
FX only	−0.7 ± 3.4	−17.0 ± 5.5	−9.1 ± 8.2			
PL only	1.9 ± 3.9	−6.6 ± 5.1	−9.5 ± 4.6			
HDL-C, %				0.36	0.45	0.66
FX + CLM	−5.1 ± 1.8	−4.1 ± 2.9	−3.0 ± 3.4			
PL + CLM	−1.1 ± 2.2	−2.6 ± 2.3	1.4 ± 2.8			
HDL-C, %				0.13	0.93	0.74
FX only	−5.8 ± 3.2	−7.4 ± 8.4	−11.4 ± 3.8			
PL only	1.7 ± 3.5	−0.4 ± 3.9	2.4 ± 3.5			
Total-C/HDL-C, %				0.68	0.79	1.00
FX + CLM	4.0 ± 2.2	3.3 ± 3.9	2.1 ± 3.7			
PL + CLM	5.5 ± 3.6	2.7 ± 2.0	1.8 ± 2.9			
Total-C/HDL-C, %				0.42	0.32	0.36
FX only	6.2 ± 3.8	−4.3 ± 12.6	4.3 ± 11.5			
PL only	1.3 ± 4.3	−5.4 ± 5.4	−10.4 ± 4.9			
LDL-C/HDL-C, %				0.52	0.52	0.85
FX + CLM	4.6 ± 5.0	2.5 ± 6.8	−2.4 ± 4.9			
PL + CLM	6.6 ± 5.1	2.4 ± 2.9	1.9 ± 4.1			
LDL-C/HDL-C, %				0.53	0.09	0.39
FX only	7.2 ± 5.2	−13.3 ± 14.9	0.3 ± 16.3			
PL only	0.5 ± 5.3	−8.7 ± 6.8	−17.0 ± 6.5			

[1] All values are means ± SEMs. The absence of letters within a row indicates no significant changes over time, $P > 0.017$. Flaxseed groups: FX + CLM after 1 mo, $n = 41$; after 6 mo, $n = 37$; after 12 mo, $n = 36$; and FX only after 1 mo, $n = 11$; after 6 mo, $n = 7$; after 12 mo, $n = 6$. Placebo groups: PL + CLM after 1 mo, $n = 32$; after 6 mo, $n = 26$; after 12 mo, $n = 26$; and PL only after 1 mo, $n = 15$; after 6 mo, $n = 13$; after 12 mo, $n = 15$. CLM, cholesterol-lowering medication; FX, flaxseed; G, group; G × T, group × time; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PAD, peripheral artery disease; PL, placebo; T, time; Total-C, total cholesterol.
[2] Significant if $P < 0.05$.

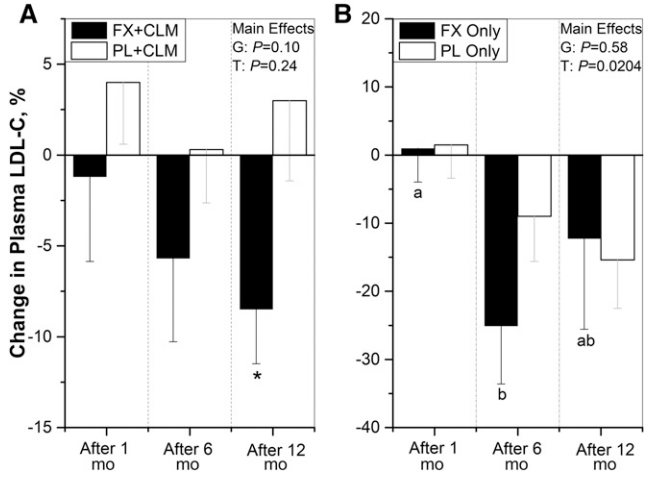

FIGURE 3 Mean percent change from baseline in plasma LDL-C in patients with PAD in FX or PL subgroups consuming the diet plus CLMs or just the diet after 1, 6, and 12 mo. Values are represented as means (SEMs). This subgroup of patients is consuming either a flaxseed or placebo diet in conjunction with administered CLMs (FX + CLM or PL + CLM) (A). This subgroup of patients is only consuming the study diet (FX only or PL only) (B). Different from placebo, $*P < 0.05$. Within a group, labeled means without a common letter differ, $P \leq 0.017$. FX + CLM: after 1 mo, $n = 41$; after 6 mo, $n = 37$; after 12 mo, $n = 36$. PL + CLM: after 1 mo, $n = 32$; after 6 mo, $n = 26$; after 12 mo, $n = 26$. FX only: after 1 mo, $n = 11$; after 6 mo, $n = 7$; after 12 mo, $n = 6$. PL only: after 1 mo, $n = 15$; after 6 mo, $n = 13$ (2 patients could not make their 6-mo appointment); after 12 mo, $n = 15$. CLM, cholesterol-lowering medication; FX, milled flaxseed treatment; G, group; LDL-C, LDL cholesterol; PAD, peripheral artery disease; PL, placebo; T, time.

Anticoagulant medications were monitored over time between treatment groups to see if dietary flaxseed affected any differences in administration. Of the 110 patients enrolled in the trial, 54 of the 58 patients in the FX group (93%) were being administered anticoagulant medications before commencing the study. Only 3 patients in the FX group had their medications adjusted over the study. In the PL group, 47 patients (90%) were taking antiplatelet medications at the study onset. Only 4 patients in the PL group had their platelet medications adjusted. No differences in antiplatelet medication resulted from dietary supplementation with flaxseed compared with placebo.

Discussion

A secondary endpoint of the FLAX-PAD Trial was to examine the effects of flaxseed on blood lipids in a patient population with pre-existing CVD (9). Unlike other studies to date, ours is inclusive of patients being administered CLM, which is an exclusion criterion in many other studies (12, 26–28). Of the 110 patients enrolled in the study, 74% were on CLM. LDL cholesterol dropped by 15% and total cholesterol by 11% in the FX group compared with the PL group after just 1 mo of consuming dietary flaxseed and was maintained at these concentrations for up to 6 mo (Figure 2). However, only within the flaxseed group and not between-treatment groups were significant decreases detected. This is consistent with other reports of flaxseed reducing circulating cholesterol in hemodialysis patients (12), in patient populations with hyperlipidemia (8, 26, 29, 30), and in postmenopausal women (27, 28) in short-term studies. In healthy control populations, the effects of flaxseed are less consistent with a modest cholesterol-lowering effect at higher flaxseed concentrations (40–50 g/d) (5, 20, 31, 32) and no effect at doses used in the present study (30 g/d) (24, 33). Health Canada has recently approved a cholesterol-lowering health claim for 40 g of flaxseed (34). This was based on several studies with this dosage (20, 26, 27, 35). Our data are consistent with these data and confirm that a 30-g dosage of flaxseed is at the lower dosage range for inducing significant total cholesterol– and LDL-cholesterol–lowering effects. After 12 mo of flaxseed consumption in our FLAX-PAD Trial, lipid concentrations did not differ. One explanation for this may be that some of the patients were not adhering to the dietary protocol toward the end of the 12 mo. Dietary compliance was monitored through plasma markers of flaxseed consumption. Secoisolariciresinol diglucoside metabolites, known as enterolignans, and ALA are 2 very sensitive indicators of flaxseed ingestion (23, 24, 36). As shown previously (10), patients in the FLAX-PAD Trial who

TABLE 4 Platelet aggregation in platelet-rich plasma of PAD patients at 0-, 1-, 6-, and 12-mo time points for FX and PL groups using all study participants[1]

					Effects (P)[2]		
Platelet measurements	Baseline	1 mo	6 mo	12 mo	G	T	G × T
Percent aggregation, %					0.21	0.0019	0.60
Collagen in FX, 5 μg/mL	75.7 ± 18.1	72.5 ± 21.7	81.3 ± 15.4	77.8 ± 19.5			
Collagen in PL, 5 μg/mL	72.3 ± 18.6^{a,b}	67.3 ± 25.1^{b}	78.5 ± 19.5^{a}	79.3 ± 19.9^{a}			
Percent aggregation, %					0.21	0.0019	0.60
Thrombin in FX, 0.3 U/mL	99.1 ± 8.8^{a}	95.4 ± 11.0^{a,b}	93.6 ± 11.7^{b}	92.1 ± 13.1^{a,b}			
Thrombin in PL, 0.3 U/mL	96.4 ± 6.8	94.6 ± 13.8	95.0 ± 12.1	90.5 ± 20.3			
Maximum rate of aggregation, % change/min					0.80	0.0064	0.30
Collagen in FX, 5 μg/mL	103 ± 34.6	97.9 ± 44.9	111 ± 34.1	106 ± 40.3			
Collagen in PL, 5 μg/mL	103 ± 39.2^{a,b}	90.7 ± 45.3^{b}	111 ± 38.6^{a}	116 ± 40.1^{a}			
Maximum rate of aggregation, % change/min					0.80	0.0064	0.30
Thrombin in FX, 0.3 U/mL	293 ± 58.0^{a}	299 ± 58.0^{a}	261 ± 55.6^{b}	251 ± 54.1^{b}			
Thrombin in PL, 0.3 U/mL	280 ± 42.1^{a}	280 ± 64.5^{a}	261 ± 65.2^{a,b}	247 ± 61.9^{b}			

[1] All values are means ± SDs. Labeled means in a row without a common letter differ, $P \leq 0.008$. Flaxseed: baseline, $n = 58$; 1 mo, $n = 51$; 6 mo, $n = 45$; 12 mo, $n = 43$. Placebo: baseline, $n = 52$; 1 mo, $n = 45$; 6 mo, $n = 41$; 12 mo, $n = 41$. FX, flaxseed group; G, group; G × T, group × time; PAD, peripheral artery disease; PL, placebo group; T, time.
[2] Significant if $P < 0.05$.

ingested flaxseed had a 1-fold increase in circulating ALA concentrations and a 10- to 50-fold increase in plasma enterolignans compared with placebo ($P < 0.05$). Despite the large fold increases in plasma enterolignans and ALA after 12 mo in the FX group compared with the PL group, changes from baseline were lower at this time point than values measured after 6 mo (Supplemental Table 3). In fact, plasma ALA was significantly reduced in the FX group after 12 mo compared with 6-mo values ($P = 0.002$). Another possible explanation may be caused by desensitization to flaxseed. HDL-cholesterol concentrations did not differ between groups when all subjects were included in the analysis (Figure 2), which is in agreement with other studies (8, 27, 28), but did have a modest significant decrease at 6 mo compared with baseline values in the flaxseed group when only patients that completed the trial were used (Table 1). Two reports involving hypercholesterolemic patients note lower HDL-cholesterol concentrations with a higher prevalence of adult men (26) and in a pediatric population (37). In the present study, male patients were most predominant, comprising 73% (PL group) and 74% (FX group) of the patients within this particular cohort. It is difficult to measure this outcome as a result of gender influence in our study because of the skewed ratio of male-to-female patients; however, the prevalence of male gender may be a contributing factor toward the observed lower HDL-cholesterol concentrations at 6 mo in these patients. The effect of flaxseed on TGs is less understood. Associated increases in TGs with flaxseed consumption have been noted in several clinical trials (37, 38), with no changes (8, 27, 28) and decreases (12, 35) in others.

A limitation of this study was the small sample size that may have concealed significant between-group differences for total and LDL cholesterol. Original calculations for achieving a minimum of 80% power at an α-level of 0.05 were based on incidence of myocardial infarctions and strokes (9). Recalculating the power of the study for LDL cholesterol, a power of 80% was achieved at baseline with a sample size of 110 participants but was only 74% at 1 mo ($n = 99$), 63% at 6 mo ($n = 84$), and 62% at 12 mo ($n = 83$) when using a repeated-measures ANOVA design. It is estimated that a minimum sample size of 132 participants would be needed to achieve a minimum of 80% power accounting for 20% sample loss and should be used in future studies to detect significant differences between treatment groups. An SD of 0.96 mmol/L was used for LDL cholesterol as determined in the present study, which is similar to published results in PAD patients (0.91 mmol/L) (39). Similar sample sizes and power would be expected for total cholesterol.

The vast majority (74%) of PAD patients enrolled in this study was administered cholesterol-lowering drugs to treat their existing hyperlipidemic condition (9) and of these patients, 90% were administered statins. This allowed us to examine drug-food interactions in the present study. Several basic questions, therefore, arose because of the potential interactions of flaxseed with CLMs. First, did flaxseed interfere (positively or negatively) with the cholesterol-lowering action of the drugs in the present study? As shown in Figure 3A, in the FX + CLM group flaxseed and CLM resulted in a significantly greater decrease in LDL cholesterol beyond that achieved in the PL + CLM group. Thus, it clearly does not interfere with the cholesterol-lowering action of CLM. In a small subgroup of patients who were not administered CLMs at the onset (Figure 3B), flaxseed independently lowered LDL cholesterol, which is in agreement with previous data obtained in the absence of medication (26–28), thus suggesting that flaxseed works independently of the CLM to lower LDL cholesterol.

The lack of effect of dietary flaxseed on circulating total cholesterol and LDL cholesterol in hypercholesterolemic patients was surprising because this is in contrast to previous reports (12, 27, 28, 30). However, a significant main effect of time on LDL cholesterol, with a similar trend for total cholesterol, suggests that the small sample size within this subgroup may have influenced the analysis. The ineffectiveness of dietary flaxseed on circulating TG and HDL-cholesterol concentrations is consistent with previous reports (28, 40) and a recent meta-analysis that reported that 70–90% of trials demonstrated neutral effects of flaxseed on these lipid variables (8).

Dietary flaxseed may suppress the immediate need for administered statins or other CLMs in newly diagnosed patients. In the subgroups in which patients were not taking any CLMs at the onset, those that were consuming milled flaxseed did not require any CLMs to control their cholesterol concentrations over the course of the 12 mo. However, 33% of the individuals consuming the placebo diet were prescribed CLMs by the end of the study period. Flaxseed may provide a possible strategy for individuals looking for natural ways to control their plasma cholesterol concentrations. This may reduce the immediate need for prescription medications and their potential negative side effects. Additional studies with larger sample sizes are needed to accurately answer this question.

The component within flaxseed that was responsible for the cholesterol-lowering effect of flaxseed has not been identified in the present study. However, we can rule out 2 candidate bioactives. Because of the lack of an inverse correlation of ALA and enterolignans with measured cholesterol, it would strongly suggest that the cholesterol-lowering effect of flaxseed does not involve either of these 2 compounds. Most of the published evidence in humans does not support flax oil (and therefore ALA) as having a primary role in cholesterol lowering (8, 41). Alternatively, higher doses of flax lignans in healthy (42) or unhealthy (43) individuals or defatted flaxseed in hyperlipidemic patients (30) have been suggested to play a role in lowering plasma cholesterol. These authors did not correlate the lignan metabolites, enterodiol or enterolactone, with LDL cholesterol; therefore, although "high lignan" (42) or "high secoisolariciresinol diglucoside" (43) doses yielded decreases in LDL cholesterol, these 2 key lignan metabolites were likely not involved as outlined in our study. It is more likely that soluble mucilage fiber, resulting from flaxseed lignan extracts (43), may have yielded the attenuated cholesterol values observed in these studies. The dietary fiber content of flaxseed is 28% by weight, of which 33% is soluble fiber (26). It is more probable that the high fiber content of flaxseed is responsible for the LDL-cholesterol–lowering actions of flaxseed. This is consistent with other conclusions in the literature (27, 41, 44).

PAD patients are at great risk of thrombosis and the subsequent complications of myocardial infarctions and stroke (45). Because the fish-derived ω-3 FAs EPA and DHA have antithrombotic effects on platelets (46–49) and flaxseed is rich in another ω-3 FA, ALA, it is possible that flaxseed consumption in the PAD patients may be of antithrombotic benefit. ALA has inhibited both collagen- and thrombin-induced platelet aggregation in animal studies (48). Clinical studies in healthy subjects have shown that dietary supplementation with flaxseed or ALA had no effect on platelet aggregation (11, 24, 32, 33). The FLAX-PAD Trial is the first to assess the aggregatory actions of dietary flaxseed in a population with clinical evidence of hypercoagulation. The significant inhibition in percent aggregation induced by flaxseed in the present trial was restricted to the agonist thrombin. This would suggest that selected changes in

G-protein coupled receptors or in the leucine-rich repeat family of receptors may have occurred in the FX group in comparison with the PL group (50). However, no significant differences were calculated between treatment groups at any time with either collagen or thrombin.

In summary, dietary flaxseed has the capacity to lower total and LDL cholesterol even in the presence of cholesterol-lowering drugs such as statins. It does not appear to interfere with the cholesterol-lowering capacity of statins. In view of these actions and its effect on platelet aggregation, dietary flaxseed can be recommended for patients at risk of CVD. A 10% decrease in total or LDL cholesterol would be predicted to induce a clinically significant reduction in the incidence of myocardial infarctions and stroke over time (51). However, our conclusions are limited by the sample size, particularly when the analysis involved smaller subgroups. Larger trials may be recommended. Despite this, the potential for flaxseed to lower both plasma cholesterol concentrations and decrease blood pressure (10) simultaneously is noteworthy. More than 2 of 3 patients in the United States do not have control of both their blood pressure and their cholesterol levels (52). Controlling both cholesterol and blood pressure can substantially reduce the risk of heart disease by one-half or more (52). In view of this need, the potential for a dietary supplement such as flaxseed to achieve this dual action of lowering blood pressure (as previously shown) (10) and cholesterol (as shown here) is very appealing in a patient population with significant documented CVD.

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