The Lipid-Lowering Effects of Phytosterols and (n-3) Polyunsaturated Fatty Acids Are Synergistic and Complementary in Hyperlipidemic Men and Women1,2

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Abstract

Fish oils rich in (n-3) long-chain PUFA (LCPUFA) can reduce circulating triglycerides and raise HDL-cholesterol. Phytosterols have been shown to reduce total cholesterol and LDL-cholesterol in normocholesterolemic and hyperlipidemic populations. We investigated the combined effects of phytosterols and (n-3) LCPUFA on plasma lipid profile in hyperlipidemic individuals. This study was a 3-wk randomized, double-blind, placebo-controlled, 2×2 factorial trial in 4 parallel groups of 60 hyperlipidemic individuals. Subjects were randomized to receive either sunola oil or 1.4 g/d (n-3) LCPUFA capsules with or without 2 g phytosterols per day while maintaining their habitual diet. The combination of phytosterols and (n-3) LCPUFA reduced plasma total cholesterol by 13.3% (P = 0.001), which differed from (n-3) LCPUFA alone (P < 0.001). LDL-cholesterol concentrations followed the same pattern as that of plasma cholesterol with a 12.5% decrease (P = 0.002) in the combination group. The HDL-cholesterol concentration was increased by (n-3) LCPUFA (7.1%; P = 0.01) alone and in combination with phytosterols (8.6%; P = 0.04), whereas phytosterol treatment alone had no effect. Plasma triglyceride concentration was lowered by (n-3) LCPUFA (22.3%; P = 0.004) alone and in combination with phytosterols (25.9%; P = 0.005), whereas phytosterol treatment alone had no effect. In conclusion, the combined supplementation with phytosterols and (n-3) LCPUFA has both synergistic and complementary lipid-lowering effects in hyperlipidemic men and women. J. Nutr. 138: 1086–1090, 2008.

Introduction

Hyperlipidemia is associated with an increased risk in the development of cardiovascular disease (1). Dietary and/or pharmacological management of hyperlipidemia remains an effective strategy to reduce overall cardiovascular risk (2).

Phytosterols are structurally analogous to cholesterol and have been shown to substantially reduce intestinal cholesterol absorption by 30–40% (3,4). Phytosterol-enriched foods, such as margarine spreads, reduce plasma total cholesterol by 4–9% and LDL-cholesterol by 10–15% with an average dose of 2 g/d (5,6). Phytosterols, however, do not affect plasma concentrations of other lipids, i.e. triglycerides or HDL-cholesterol.

The principle (n-3) long-chain PUFA (LCPUFA) in marine oils, eicosapentaenoic acid [EPA; 22:5(n-3)] and docosahexaenoic acid [DHA; 22:6(n-3)], have been shown to possess a wide range of physiological effects, from alterations in circulating plasma lipids to eicosanoid and cytokine production (7). There is considerable evidence to support reductions in triglycerides and improvements in circulating HDL-cholesterol in response to high dosage (1–5 g/d) (n-3) LCPUFA supplementation (8,9).

Lipid aberrations rarely occur in isolation and are highly interactive; therefore, to effectively treat combined hyperlipidemia, the simultaneous management of plasma lipids as well as lipoproteins is required. We designed this study to investigate the lipid-lowering effects of dietary supplementation with phytosterols and (n-3) LCPUFA in a hyperlipidemic population sample.

Methods

Subjects. Participants with established hyperlipidemia were recruited from the general community of Newcastle, Australia. The primary selection criteria included: plasma total cholesterol concentration ≥6.0 mmol/L (2.31 g/L); triglyceride concentration ≥1.5 mmol/L (1.32 g/L); aged between 35 and 70 y; no cardiovascular disease, diabetes mellitus, chronic inflammatory diseases, untreated hypertension (≥140/95 mm Hg), or liver/renal disease; not taking antiinflammatory or lipid-lowering medication; not consuming a phytosterol-enriched spread and/or fish oil supplements; and no strong aversion or any known allergies/intolerances to the foods involved in the study. On the basis of these criteria, 60 subjects

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3 Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, (n-3) long-chain PUFA group; FOP, (n-3) long-chain PUFA + phytosterol group; LCPUFA, long-chain PUFA; SO, sunola oil group; SOP, sunola oil + phytosterol group.
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(male n = 27 and female n = 33) were enrolled and completed the trial. All participants had a usual weekly consumption of no more than 2 fish meals per week and maintained their habitual diet throughout the study, based on analysis of 24-h food recalls.

A power calculation based on an anticipated 10% (0.60 mmol/L) reduction in the primary outcome, plasma total cholesterol concentration at the 0.05 level of significance, 80% power, and a 10% dropout rate suggested that 15 subjects would be required for each group.

The Human Research Ethics Committee, University of Newcastle approved this study protocol. All participants gave written informed consent prior to commencement. This study was registered on the Australian Clinical Trials Registry (trial no. 00081597).

**Experimental design.** The present study was a randomized, double-blind, placebo-controlled, 2 × 2 factorial intervention in 4 parallel groups. The study period was 3 consecutive wk. Participants were randomized using permuted stratified block randomization controlled for gender. Supplement containers were labeled before the trial with a code so that neither principle researchers nor volunteers knew what capsules were consumed.

Individuals were randomized to receive either sunola oil (SO) capsules alone or in combination with 25 g/d of a spread containing 2 g/d phytosterols (SP) or 1.4 g/d (n-3) LCPUFA (EPA and DHA) (FO) capsules alone or in combination with 25 g/d of a phytosterol-enriched spread (FOP). There were 15 participants in each group. The (n-3) LCPUFA capsules were provided as 4 × 1 g tuna oil capsules (NuMega Ingredients) providing 1.4 g/d of (n-3) LCPUFA (80 mg EPA + 280 mg DHA per capsule) in a triacylglycerol form. Participants were instructed to take 4 capsules with their main meals each day. The phytosterol-enriched spread used in this study was a commercially available canola oil-based spread (Logical original), which was provided as individually measured tubs (25 g each) to replace all habitual margarine/butter consumption (Table 1). The plant sterols were predominantly β-sitosterol, campesterol, and stigmasterol. Participants were instructed to use the spread on bread/crackers, melted over vegetables, mixed into mashed potatoes/pumpkin, rice, etc. and not to use it for high-temperature cooking or frying. Unintentional intake of phytosterols from other food sources was not possible to monitor or restrict. Compliance was monitored by regular telephone contact with participants, weighing of tubs and capsule count-back before and after the trial period, interviewing volunteers about their use of the spread at the end of the trial, evaluating their dietary records, and analysis of plasma fatty acid composition. Volunteers also recorded whether they had consumed their supplements and/or spread on a diary card each day.

**Measurements.** All data were collected at baseline and postintervention. A general medical questionnaire, including medical history, current medical condition(s) and prescribed medications, weight (kg), and height (m) were calculated to the nearest 0.1 using a calibrated balance beam scale (PCS Measurement); waist and hip girth (cm) were measured while subjects were dressed in light clothing and without shoes; BMI was calculated as weight/height² (kg/m²); and 24-h food recalls were collected and entered into a food database system (FoodWorks, Xyris). Participants did not receive additional dietary counseling during the study.

**Plasma lipid analysis.** Blood samples from fasting subjects (>10 h) were collected at baseline and postintervention. Plasma was prepared by centrifuging (Heraeus Biofuge Sartorius) 3000 × g; 10 min at 4°C and the resulting plasma, buffy coat, and RBC subfractions were collected and stored at −80°C until analyzed.

Plasma total cholesterol, HDL-cholesterol, and triglyceride concentrations were measured by automated methods on a VP auto analyzer, using standardized reagents (Hunter Area Pathology Service). LDL-cholesterol concentration was calculated using the Friedewald equation (10).

**Fatty acid composition of plasma lipids.** Plasma fatty acid concentration was determined by GC using an established method by Lepage and Roy (11). Methylated samples containing C19:0 (0.00002 mg/sample) as an internal standard were analyzed using a 30-m × 0.25-mm (DB-225) fused carbon-silica column, coated with cyanopropylphenyl (J & W Scientific). Plasma fatty acids were analyzed at baseline and postintervention.

**Statistical analysis.** All data are presented as means ± SEM. The 95% CI for the differences in the changes between the groups are also given. Significance was set at P-value < 0.05. Changes from baseline were determined using nonparametric analyses (Wilcoxon's Signed Rank test). The effect of treatment on the percentage change of lipid variables between groups was determined using 2-way between-group ANOVA with post hoc comparisons (Tukey’s honestly significant difference). A 2-way ANOVA was used to determine whether there was a significant main effect for each independent variable by testing for between-subject effects; also, an interaction effect [phytosterol × (n-3) LCPUFA] was tested between the 2 independent variables in their effect on the dependent variable. This method was also used to test for synergy and/or complementarity of the 2 independent variables [phytosterols and (n-3) LCPUFA]. Statistical analyses were performed according to the intention to treat. All results were analyzed using SPSS version 15.0 for Windows.

**Results**

This study included 60 participants (27 male and 33 female) with a mean age of 55.4 ± 1.0 y and BMI of 26.9 ± 0.5 kg/m². Groups did not differ in general anthropometric measures at baseline or postintervention (Table 2).

The capsules were well tolerated, with an overall compliance of 98% ± 0.5%. Mean intake of phytosterols (as determined by capsule count-back) was 23.5 ± 0.8 g/d, mostly accompanied by bread/crackers. Analysis of 24-h food recalls indicated no changes in macro- and/or micro-

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**TABLE 1** Energy and nutrient composition of the phytosterol spread¹,²

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit/25 g</th>
<th>Unit/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>625.0</td>
<td>2500.0</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>16.7</td>
<td>67.0</td>
</tr>
<tr>
<td>SFA, g</td>
<td>4.2</td>
<td>16.5</td>
</tr>
<tr>
<td>MUFA, g</td>
<td>8.5</td>
<td>33.8</td>
</tr>
<tr>
<td>PUFA, g</td>
<td>4.0</td>
<td>16.2</td>
</tr>
<tr>
<td>(n-3) Fatty acids, g</td>
<td>1.2</td>
<td>5.2</td>
</tr>
<tr>
<td>ALA, g</td>
<td>1.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Phytosterols, g</td>
<td>2.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

¹ Data provided by manufacturer (Meadow Lea Foods).
² MUFA, monounsaturated fatty acid; ALA, alpha linolenic acid.

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**TABLE 2** Baseline characteristics of hyperlipidemic subjects who consumed SO, FO, SOP, or FOP for 3 wk³

<table>
<thead>
<tr>
<th>Variable</th>
<th>SO</th>
<th>FO</th>
<th>SOP</th>
<th>FOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.9 ± 2.6</td>
<td>56.6 ± 2.0</td>
<td>57.8 ± 1.5</td>
<td>52.6 ± 2.2</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>78.3 ± 4.5</td>
<td>75.5 ± 4.0</td>
<td>79.1 ± 3.7</td>
<td>79.9 ± 3.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.5 ± 1.0</td>
<td>26.4 ± 1.3</td>
<td>27.4 ± 1.4</td>
<td>27.2 ± 0.9</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.9 ± 0.02</td>
<td>0.8 ± 0.02</td>
<td>0.9 ± 0.01</td>
<td>0.9 ± 0.01</td>
</tr>
<tr>
<td>Gender, n/M/F</td>
<td>7/8</td>
<td>6/9</td>
<td>7/8</td>
<td>7/8</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>6.4 ± 0.2</td>
<td>6.6 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>Plasma LDL-cholesterol, mmol/L</td>
<td>4.2 ± 0.4</td>
<td>4.4 ± 0.1</td>
<td>4.3 ± 0.3</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Plasma HDL-cholesterol, mmol/L</td>
<td>1.3 ± 0.07</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.07</td>
<td>1.4 ± 0.08</td>
</tr>
<tr>
<td>Plasma triglyceride, mmol/L</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

³ Values are means ± SEM, n = 15.
nutrient intakes between baseline and postintervention for any of the 4 groups (Table 3).

**Plasma lipids.** Plasma lipid concentrations did not differ among the groups at baseline (Table 2). Plasma lipid variables did not change in the SO group. Plasma HDL-cholesterol increased ($P = 0.01$) and triglycerides decreased from baseline in the FO group ($P = 0.004$). Total cholesterol ($P = 0.12$), LDL-cholesterol ($P = 0.12$), and triglycerides ($P = 0.07$) tended to decrease, whereas the total:HDL-cholesterol ratio decreased ($P = 0.02$) from baseline in the SOP group. In the FOP group, plasma total cholesterol ($P = 0.001$), LDL-cholesterol ($P = 0.002$), triglyceride ($P = 0.005$), and total:HDL-cholesterol ratio ($P = 0.003$) decreased and HDL-cholesterol ($P = 0.04$) increased from baseline.

We explored the treatment effects on the percentage change from baseline in each of the groups. The percentage change in plasma total cholesterol from baseline in the FOP group differed from that in the SO group ($P = 0.002; 95\% \text{ CI}, -20\% \text{ to } -3.5\%$) and FO group ($P < 0.001; 95\% \text{ CI}, -24\% \text{ to } -8.5\%$) (Fig. 1A). Similarly, the percentage change in LDL-cholesterol in the FOP group differed from that of the FO group ($P < 0.001; 95\% \text{ CI}, -29\% \text{ to } -7\%$) (Fig. 1B). Also, the reduction in LDL-cholesterol in the SOP group differed from the FO group ($P = 0.04; 95\% \text{ CI}, -22\% \text{ to } -0.3\%$). The decrease in plasma triglyceride concentration in the FO group ($P = 0.005$) was greater than that in the SO group and the reduction in triglyceride concentration in the FOP group differed from the SO group ($P = 0.003$) (Fig. 1C). Changes in HDL-cholesterol in the FOP group differed from the SO group ($P = 0.05; 95\% \text{ CI}, -0.2\% \text{ to } 21\%$) (Fig. 1D). The change in the total:HDL-cholesterol ratio from baseline differed between the FOP and SO groups ($P = 0.006; 95\% \text{ CI}, -24\% \text{ to } -3\%$) (Fig. 1E).

We tested whether phytosterols and (n-3) LCPUFA alone and their interaction affected plasma lipid concentrations. Plasma

**FIGURE 1** Percentage change in plasma total cholesterol (A), LDL-cholesterol (B), HDL-cholesterol (C) and triglyceride (D) concentrations and total:HDL-cholesterol ratio (E) from baseline in hyperlipidemic subjects who consumed SO, FO, SOP, or FOP for 3 wk. Values are means ± SEM, $n = 15$. Symbols designate changes from baseline: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Means for a variable without a common letter differ, $P < 0.05$.
total and LDL cholesterol were affected by phytosterols ($P \leq 0.001$) and by the interaction ($P \leq 0.01$). Dietary (n-3) LCPUFA affected plasma HDL cholesterol and triglyceride concentrations and the total:HDL cholesterol ratio ($P \leq 0.023$). The ratio also was affected by phytosterols ($P = 0.015$).

**Plasma fatty acids.** Plasma fatty acid concentrations did not differ among groups at baseline. In both the FO and FOP groups, both EPA (44.5%, $P = 0.002$; 44.7%, $P = 0.002$) and DHA (41.1%, $P = 0.02$; 54.5%, $P = 0.001$) increased from baseline, demonstrating compliance with the (n-3) LCPUFA supplementation. The percentage change in plasma EPA concentration in the FO group differed from that of the SO and SOP groups ($P < 0.001, 95\%\ CI, 26–127$; $P = 0.007, 95\%\ CI, 13–115$). This was also the case with the FOP group ($P < 0.001, 95\%\ CI, 26–127$; $P = 0.007, 95\%\ CI, 13–115$). Similarly, the percentage change in plasma DHA concentration in the FO and FOP groups differed from that of the SO ($P < 0.001, 95\%\ CI, 26–122$; $P < 0.001, 95\%\ CI, 64–160$) and SOP groups ($P = 0.008, 95\%\ CI, 12–107$; $P < 0.001, 95\%\ CI, 49–145$). The interaction of phytosterols and (n-3) LCPUFA affected the plasma EPA and DHA concentrations ($P < 0.001$).

**Discussion**

In this study, we demonstrate the efficacy of concomitant supplementation with a phytosterol-enriched spread and (n-3) LCPUFA supplementation in individuals with hyperlipidemia. Our findings provide evidence of a synergistic reduction in total and LDL-cholesterol and complementary effects on triglycerides and HDL-cholesterol concentration, using concomitant dietary supplementation with phytosterols and (n-3) LCPUFA. The combined therapy may therefore be ideal for optimization of plasma lipid fractions for maximum cardioprotection.

Reductions in total cholesterol (6.2%) and LDL-cholesterol (5.7%) in the SOP group are consistent with other studies of similar duration and intervention (12–16), although we did not observe a significant change from baseline. This study also showed that combining a phytosterol-enriched spread with (n-3) LCPUFA supplements significantly reduced total cholesterol and LDL-cholesterol concentration by 13.3 and 12.5%, respectively. Moreover, the combined treatment provided a synergistic reduction in plasma total cholesterol ($P = 0.009$), which was greater than (n-3) LCPUFA ($P < 0.001; 95\%\ CI, 24–8$) or SO alone ($P = 0.002; 95\%\ CI, 20–3$) as well as a synergistic reduction in LDL-cholesterol concentration ($P = 0.011$), which was greater than (n-3) LCPUFA alone ($P < 0.001; 95\%\ CI, 29–7$).

A number of studies have investigated the effect of phytosterols on lipids and lipoproteins in conjunction with a low-fat diet (6,13,17–20). In the study by Cleghorn et al. (13), moderately hypercholesterolemic participants following a reduced-fat diet (<30% energy from fat) and replaced their butter consumption with 25 g/d phytosterol-enriched spread (2 g/d sterol esters) for 4 wk; their total cholesterol and LDL-cholesterol were reduced by 8.9 and 12.3%, respectively. Three studies (17,18,20) that used the National Cholesterol Education Program Step 1 diet in combination with a phytosterol spread (1.6–2.3 g/d) had a mean total and LDL-cholesterol reduction of 5.4 and 7.6%, respectively. It is conceivable that the impact of a reduced-fat diet is in itself enough to attain appreciable reductions in plasma lipid profile. Conversely, our FOP group had a considerably greater reduction in total and LDL-cholesterol while maintaining a habitual diet (33.3% energy from fat). Our combination treatment also differs from such studies in the practicality of its application and minimal dietary constraint.

The mechanism by which phytosterols reduce total cholesterol and LDL-cholesterol is not entirely understood; however, their competition with and displacement of cholesterol from bile salts and micelles is well recognized (3,21,22). Given the discrepancies within (n-3) LCPUFA studies, which generally show a small increase or no effect on plasma total and LDL-cholesterol (23–25), the mechanism by which the FOP of phytosterols and (n-3) LCPUFA elicit reductions in plasma total cholesterol remains unclear. (n-3) fatty acids can alter hepatic regulation of LDL-cholesterol via alterations in the rate of LDL formation and receptor-dependent LDL uptake (26–28). Nonetheless, the authors speculate that these findings may be associated with the combined effects of both phytosterols and (n-3) LCPUFA in the higher affinity for micellar absorption and, hence, increases in cholesterol displacement and clearance. This mechanism is subject to further investigation. It is likely that the coexistence of (n-3) LCPUFA and phytosterols in the gastrointestinal tract may cause greater micellar displacement of cholesterol, resulting in greater inhibition of cholesterol absorption. A small amount of dietary phytosterols do get absorbed into the circulation; therefore, it is also likely that lipoprotein displacement (analogous to micellar competition) occurs, resulting in a greater reduction of total and LDL-cholesterol. (n-3) LCPUFA supplementation is associated with increased LDL particle size, whereas phytosterols are known to reduce LDL concentration. The net effect of the FOP treatment may be a reduction in both circulating LDL concentration and increased LDL particle size, contributing to a reduction in atherogenic risk.

Moreover, the hypotriglyceridemic and HDL-raising effects of (n-3) LCPUFA are sustained even in the presence of phytosterol. In this study, we noted optimization of the cholesterol-lowering effect of phytosterols by combining (n-3) LCPUFA for triglyceride-lowering and HDL-cholesterol improvements. The FO group had a sizeable reduction in triglycerides (22.3 and 25.9%), whereas the FOP group had increased HDL-cholesterol (7.1 and 8.6%).

We acknowledge the relatively short duration of this intervention trial; however, changes in lipids and lipoproteins in response to phytosterols and (n-3) LCPUFA have been highly responsive. Considering the antiinflammatory, antiaggregatory, and antithrombogenic effects of (n-3) LCPUFA, the synergistic and complementary cholesterol and triglyceride-lowering effects along with the increases in HDL-cholesterol demonstrated in this study makes the combined phytosterol and (n-3) LCPUFA therapy an ideal alternative or adjunct treatment for maximum reduction in cardiovascular risk. Perhaps functional foods containing the 2 active components, i.e. phytosterols and (n-3) LCPUFA, in appropriate dose levels can be developed for the ease of consumption and improved compliance. Indeed, long-term safety and efficacy as well as compliance need to be demonstrated in a larger multicentered trial to confirm our findings from this study.

Optimization of the plasma lipid profile is imperative to providing maximum cardioprotection. In this study, we have demonstrated that the use of a phytosterol-enriched spread, combined with (n-3) LCPUFA supplementation, appreciably improves overall plasma lipid profile in individuals with combined hyperlipidemia. The additional lipid-lowering effects provided by the FOP may eliminate the need or moderate reliance on long-term lipid-lowering drug therapy. This combined therapy may be a useful addition to existing regimens for the effective management of hyperlipidemia.
Acknowledgments
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Literature Cited


