# Phytosterols partially explain differences in cholesterol metabolism caused by corn or olive oil feeding

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**Abstract** To examine whether phytosterols in polyunsaturated oils account for their differential action on lipid metabolism compared with monounsaturated oils, 16 normolipidemic individuals consumed three 10-day experimental diets containing corn oil (high in polyunsaturated fatty acids and phytosterols), olive oil (high in monounsaturated fatty acids and low in phytosterols), or olive oil supplemented with phytosterols given at twice the level naturally found in corn oil (high in monounsaturated fatty acids and phytosterols). Plasma total cholesterol concentrations after both the olive oil and the olive oil–phytosterol treatments were higher ($P < 0.001$) than those after the corn oil treatment. Olive oil treatment resulted in greater ($P < 0.05$) plasma LDL-cholesterol and triglyceride concentrations compared to corn oil treatment. Addition of the phytosterol mixture to the olive oil diet resulted in suppression of the significant differences in LDL-cholesterol and triglyceride concentrations between corn and olive oil. Free cholesterol fractional synthetic rates determined by deuterium incorporation were lower ($P < 0.05$) with olive oil treatment compared to corn oil treatment; the significance of this difference was abolished with the addition of phytosterols to the olive oil diet. These results suggest that phytosterols are partly responsible for the differences in plasma cholesterol levels and synthesis observed between polyunsaturated and monounsaturated oils.**—Howell, T. J., D. E. MacDougall, and P. J. H. Jones.** Phytosterols partially explain differences in cholesterol metabolism caused by corn or olive oil feeding. *J. Lipid Res.* 1998. **39:** 892–900.

**Supplementary key words** polyunsaturated fatty acids • monounsaturated fatty acids • low density lipoprotein cholesterol • triglycerides • cholesterol synthesis • deuterium incorporation

Although causes of coronary heart disease are multifactorial, high serum cholesterol concentrations are established risk markers. Dietary fat selection is known to exert a major influence on circulating cholesterol levels; they are raised with consumption of fats containing saturated fatty acids (SFA), and reduced with fats rich in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (1–4). It has been postulated that consumption of MUFA results in a protective action on high density lipoprotein (HDL) cholesterol concentration whereas PUFA consumption results in a lowering of both low density lipoprotein (LDL) and HDL concentrations (4–8); however, there is still need for consensus on this effect. In volunteers fed diets rich in PUFA versus other fatty acids, researchers have demonstrated enhanced lipoprotein remnant removal rates (9), enhanced fecal elimination (10–12), and increased synthesis (13–15) of cholesterol. Despite this knowledge, there is still dispute over mechanisms by which unsaturated fats reduce circulating cholesterol concentrations in humans, and whether such mechanisms are consistent among different unsaturated fats.

Most oils high in PUFA are naturally abundant in phytosterols, whereas MUFA-rich oils such as olive oil generally possess lower plant sterol concentrations. Research has demonstrated that cholesterol absorption efficiency varies inversely with the quantity of total phytosterols contained within an oil (14, 16, 17). Thus, the enhanced turnover and synthesis of cholesterol observed after PUFA feeding could be the result of diminished absorption subsequent to micellar exclusion of cholesterol by phytosterols (18). Consequently, the differential effect on circulation lipoprotein concentrations observed between oils high in PUFA versus MUFA may be partly explained by the action of phytosterols contained within the PUFA-rich oil. To date this question has not been systematically addressed.

The present study was designed to investigate the influence of addition of a phytosterol mixture similar to that found in corn oil to an olive oil diet on cholesterol metabolism in normolipidemic humans. It was hypoth-

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BMI, body mass index; $D_2O$, deuterium oxide; D, deuterium; TLC, thin-layer chromatography; FSR, fractional synthetic rate; FC, free cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; CI, confidence interval.

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esized that the observed effects on circulating plasma lipid concentrations and de novo cholesterol synthesis rates after supplementation of an olive oil diet with phytosterols would not differ significantly from those resulting from a corn oil diet that naturally contains phytosterols. The study also compared the effects of corn and olive oils on plasma lipoprotein concentrations and de novo synthesis rates of cholesterol, hypothesizing that there would be no difference among any of the diet treatments.

## SUBJECTS AND METHODS

### Subjects

Sixteen healthy volunteers with plasma total cholesterol levels of less than 4.9 mmol/L, LDL-cholesterol below 3.0 mmol/L, plasma triglyceride below 2.4 mmol/L, and BMI of less than 27 were recruited. Subjects were non-smokers and were not taking any medication known to affect lipid metabolism. All subjects gave informed written consent. The study protocol was approved by the Human Investigation Ethical Review Committee of McGill University.

### Experimental design

Three 10-day diet treatments were administered in a randomized, Latin-square design. Each treatment period was separated by a minimum 2-week washout period where the subjects consumed their habitual ad libitum diets. During each treatment subjects consumed a solid food diet designed to contain 50% of energy as carbohydrate, 35% fat, and 15% protein. Non-oil constituents were identical across diets. Diets contained 2/3 fat as either *i)* corn oil, *ii)* olive oil, or *iii)* olive oil plus a phytosterol mixture fed at a level of 0.4 g per 1000 kcal consumed. Phytosterols added to the olive oil diet were chosen from products currently available in health food stores. These phytosterols (Nu-Life Nutritional Products, Vancouver, Canada) were almost identical in composition to those found in the corn oil used in the study (see Table 1). These free phytosterols possess over twice the solubility in oils than those naturally found in corn oil (19), thus should be readily miscible with the olive oil used presently. However, criticism has been leveled previously at how representative the use of crystalline free cholesterol is as a means of increasing dietary levels. We held the same concern for adding free phytosterol to the diet. In order to avoid the potential pitfall of inadequate representation of our added powdered phytosterol to that found naturally in corn oil, the following steps were taken. First, to maximize dispersion across diet, the phytosterol mixture was suspended in a portion of the olive oil supplemented to each meal, then thoroughly mixed into a cholesterol-containing component of the meal before cooking. Second, the level of phytosterols added to olive oil was doubled over that contained in the corn oil. It was anticipated that these approaches would address the limitations of directly adding powdered phytosterol to diet. The supplementary phytosterols were administered in equal amounts to each of the three daily meals in the olive oil suspension. These three isocaloric meals were prepared and consumed daily on site under supervision at the Metabolic Research Unit, Macdonald Campus, McGill University. All ingredients were weighed to the nearest 0.5 g.

For each diet cycle, subjects received identical caloric loads, individually determined by the Mifflin predictive equation (20) and multiplied by an additional activity factor of 1.7 to yield total daily energy requirements for younger, active individuals (21). Body weights were monitored daily during each diet cycle to ensure compliance and correct caloric intake. Any adjustments needed to caloric intake were made only within the first 3 days of the first cycle. This number of calories remained consistent over all three diet phases.

On days 9 and 10 of each dietary phase, fasting blood samples were collected just before and 24 h after dosing with deuterium oxide ($D_2O$) for the determination of plasma lipid concentrations and deuterium (D) incorporation into free and esterified cholesterol. An oral bolus dose of 0.7 g $D_2O$/kg estimated body water (99.8 atom percent excess, CDN Isotopes Inc., Montreal, Quebec) was administered at 8:00 am to each subject after the initial blood sample was taken on day 9. To maintain body water deuterium enrichment levels, drinking water consumed during the subsequent 24 h included trace amounts of $D_2O$ (1.4 g/kg water consumed).

### Macronutrient analysis of diets

Complete homogenized mixtures of the 2-day meal cycles for each diet phase were frozen then chemically analyzed for macronutrient content. Moisture content was determined in duplicate samples of meal mixtures by lyophilizing (Flexi-Dry MP System, FTS Systems Inc, Stone Ridge, NY) at −80°C for 48 h. Triplicate freeze-dried samples were then ashed in an isothermic muffle furnace at 550°C for 24 h. For protein, nitrogen content was determined on freeze-dried samples using an automatic nitrogen analyzer (Leco Corp., St. Joseph, MI). Crude fat content was determined by first pre-extracting samples with water, then extracting with petroleum ether, followed by acid hydrolysis and a second petroleum ether extraction. Proximate compositions were reported as grams of macronutrient per 100 g wet

weight. Carbohydrate concentrations of food samples were calculated by subtracting the sum of moisture, protein, crude fat, and ash values from 100 g. Final values are presented as percent of total energy (see Table 2).

### Fatty acid and phytosterol analyses

After lipid extraction (22) and boron trifluoride methylation (23) of homogenized meal samples, fatty acid methyl esters were analyzed on a Hewlett-Packard 5890 Series II gas–liquid chromatograph equipped with flame ionization detectors and a 30 m × 0.25 mm ID fused-silica SP-2330 capillary column (Supelco Inc., Bellefonte, PA). The carrier gas was helium at 1.0 mL/min with the inlet splitter set at 50:1. Temperature programmed runs were made as follows: initial temperature of 80°C for 1 min, ramp 10°C/min to 200°C, hold for 10 min, ramp 4°C/min to 250°C, hold 15 min, injector and detector set at 250°C. Fatty acid methylesters were identified by matching retention times with 99% pure commercial standards (Nu-Chek Prep).

Phytosterol analyses were carried out after lipid extraction and saponification of the dietary oils, homogenized meals, and plasma samples. The nonsaponifiable lipid contents were analyzed using a 30 m × 0.25 mm ID SAC™-5 capillary column (Supelco Inc., Bellefonte, PA). The helium carrier gas flow rate was 1.0 mL/min with the inlet splitter set at 50:1. Column temperature was 275°C with the injector and detector temperatures set at 300°C. Sterol peaks were identified by comparison of retention times with those of authentic standards and quantitated with the use of a 5-α-cholestane internal standard (Sigma Chemical Co., St. Louis, MO). To eliminate the effect of varying cholesterol content, the non-cholesterol sterol values were expressed in terms of μmol/mmol of cholesterol (24).

### Plasma lipid analysis

Fasting blood samples obtained on days 9 and 10 of each diet treatment were collected in Vacutainer tubes containing EDTA (0.1%). Plasma and erythrocytes were separated within 2 h and stored at −80°C. Plasma total cholesterol (25) and plasma triglyceride concentrations (26) were assayed enzymatically (Sigma Co., St. Louis, MO). HDL cholesterol levels were measured similarly after dextran sulfate–magnesium precipitation of apoB-containing lipoproteins (27). LDL cholesterol concentrations were calculated using the Friedewald formula (28). To control for analytical variation, individual subject's samples from each diet phase were analyzed at the same time in duplicate.

### Cholesterol synthesis determinations

Methods for determination of de novo cholesterol synthesis have been described previously (29, 30). Briefly, to determine the synthesis of free cholesterol in the rapidly turning over pool, erythrocyte total lipids were extracted in duplicate then separated with thin-layer chromatography (TLC) using hexane–diethyl ether–acetic acid 105:45:1.5 (v/v/v). For determination of deuterium enrichment of esterified cholesterol, plasma total lipids were similarly extracted and separated. Free cholesterol and cholesteryl ester bands were identified using standards before being scraped off the TLC plates and eluted from silica. Cholesteryl ester fractions were saponified from the silica gel with methanolic potassium hydroxide. Free cholesterol samples were eluted from the silica gel with the use of hexane–chloroform–diethyl ether 5:2:1 (v/v/v). After drying under nitrogen, cholesterol samples were transferred into pre-annealed Pyrex combustion tubes (18 cm × 6 mm) containing 0.5 g cupric oxide (BDH Chemicals, Toronto, ON) and 1 mm diameter silver wire (2–2.5 cm). Combustion tubes were then sealed under vacuum and heated at 520°C for 4 h. The resultant water was then vacuum distilled into pre-annealed Pyrex reduction tubes (10 cm × 6 mm) containing 0.06 g zinc reagent (Biogeochemical Laboratories, Bloomington, IN).

To measure plasma water deuterium enrichments, baseline plasma samples were diluted 2-fold while 24-h plasma samples were diluted 7-fold. These dilutions reduced the deuterium enrichment to within the range of the mass spectrometric working standards. Samples were sealed under vacuum into pre-annealed Pyrex reduction tubes containing 0.06 g of zinc. Tubes containing water from cholesterol and plasma samples were reduced at 520°C for 30 min before analysis of deuterium enrichment. Deuterium enrichment was determined by isotope ratio mass spectrometry (VG Isomass 903D, Cheshire, England). The mass spectrometer was calibrated daily against water standards of known isotropic composition. Samples for each subject were analyzed in duplicate, concurrently with a single set of standards.

Calculation of fractional synthetic rates (FSR) of free cholesterol in erythrocyte and esterified cholesterol in plasma was based on the methods using tritiated water by Dietschy and Spady (31) as adapted by Jones et al. (29, 30). In brief, synthesis was calculated using the difference between deuterium abundance of erythrocyte or plasma cholesterol at $t = 0$ and $t = 24$ h relative to the enrichment of the body water pool.

### Statistical analyses

Descriptive data are expressed as mean ± SD, whereas inferential data pertaining to the hypotheses are expressed as mean ± SEM. The inferential data determined for each treatment group were analyzed for within and between differences using a repeated measures analysis of variance (ANOVA) with gender used as a co-vari-

ate. Where the repeated measures ANOVA attained a significance of $P < 0.05$, specific group differences were evaluated using Tukey's pairwise comparisons post-hoc analysis. The significant between-group differences were expressed as mean differences along with the 95% confidence interval (i.e., mean difference (95% CI)).

## RESULTS

Characteristics of study participants prior to the commencement of the study are shown in **Table 1**. Subjects' ages (23 ± 2 years), BMI 23.3 ± 2.4 kg/m$^2$), and initial blood screening plasma lipid concentrations were within defined ranges. Average body weight did not vary significantly among the three diet periods (68.3 ± 0.6 kg).

Macronutrient composition, fatty acid and sterol profiles, and cholesterol contents of the diets are shown in **Table 2**. The diet was designed to contain 50% energy as carbohydrate, 35% fat, and 15% protein. Overall, there was good agreement between analytical and calculated data. Carbohydrate, fat, protein, and cholesterol contents did not differ between diet treatments. Fatty acid composition and phytosterol content variation between diets directly reflected the fatty acid and sterol composition of the oils (**Table 3**). The supplemental phytosterol mixture administered as a non-esterified powder was similar in composition to the phytosterols naturally found in corn oil (**Table 4**).

**Table 5** gives the ratio of plasma campesterol/cholesterol (mmol/mol) in 13 subjects after consumption of the three 10-day dietary phases. The mean campesterol/cholesterol ratio observed after consumption of the olive oil diet (21.5 ± 2.5 mmol/mol) tended (NS) towards being lower than that seen after consumption of olive oil–phytosterol (29.1 ± 4.0 mmol/mol) and corn oil (28.2 ± 5.6 mmol/mol) dietary phases. No difference in campesterol/cholesterol ratio was observed between the supplemented olive oil and corn oil dietary phases.

TABLE 1. Characteristics of the study subjects

| Characteristic | Women (n = 8) | Men (n = 8) | Mean (n = 16) |
|---|---|---|---|
| Age, y | 22 ± 1 | 24 ± 2 | 23 ± 2 |
| Body weight, kg | 59 ± 5 | 77 ± 9 | 68 ± 11 |
| Height, m | 1.63 ± 0.09 | 1.78 ± 0.05 | 1.71 ± 0.10 |
| Body mass index, kg/m$^2$ | 22.3 ± 2.4 | 24.3 ± 2.1 | 23.3 ± 2.4 |
| Total cholesterol, mmol/L | 4.06 ± 0.39 | 3.69 ± 0.47 | 3.87 ± 0.47 |
| LDL-C, mmol/L | 2.03 ± 0.45 | 2.00 ± 0.57 | 2.00 ± 0.52 |
| HLD-C, mmol/L | 1.61 ± 0.26 | 1.30 ± 0.23 | 1.46 ± 0.29 |
| Triglycerides, mmol/L | 0.92 ± 0.18 | 0.86 ± 0.32 | 0.89 ± 0.25 |

Values expressed as mean ± SD. Abbreviations: LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

TABLE 2. Composition of experimental diets

| | Corn Oil Diet | Olive Oil Diet |
|---|---|---|
| | *% of total energy* | |
| Carbohydrate[a] | 52.7 ± 3.7 | 52.4 ± 3.9 |
| Protein | 15.0 ± 1.5 | 15.0 ± 1.4 |
| Fat | 32.3 ± 2.8 | 32.7 ± 2.8 |
| Fatty acids | | |
| Saturated | 6.7 | 7.4 |
| C8:0 | 0.1 | 0.1 |
| C10:0 | 0.4 | 0.2 |
| C12:0 | 0.7 | 0.5 |
| C14:0 | 0.6 | 0.6 |
| C16:0 | 3.8 | 4.6 |
| C18:0 | 1.2 | 1.5 |
| MUFA | 8.6 | 20.9 |
| C16:1n7 | 0.3 | 0.5 |
| C18:1n9 | 8.3 | 20.4 |
| PUFA | 17.0 | 4.3 |
| C18:2n6 | 16.7 | 3.9 |
| C18:3n3 | 0.3 | 0.4 |
| | *mg/1000 kcal* | |
| Cholesterol | 128.9 | 128.9 |
| Phytosterols | | |
| Total | 218.9 | 66.6 |
| Campesterol | 48.6 | 0.0 |
| Stigmasterol | 7.2 | 0.0 |
| β-Sitosterol | 163.1 | 66.6 |

Values expressed as mean ± SD. Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[a]Calculated by subtracting wet weights of moisture, protein crude fat, and ash from 100 g, therefore, overestimated by 1–5% due to crude fibre content.

Plasma lipid level responses to diet treatment are shown in **Fig. 1**. Plasma total cholesterol concentrations on the olive oil (3.71 ± 0.15 mmol/L) and olive oil–phytosterol (3.65 ± 0.13 mmol/L) diets were higher ($P = 0.0001$) than those observed on the corn oil diet (3.32 ± 0.11 mmol/L). There was no significant difference between the olive oil and olive oil–phytosterol diets (mean difference: 0.06 mmol/L (95% CI: −0.13 to 0.25 mmol/L)).

The olive oil treatment (2.17 ± 0.12 mmol/L) resulted in elevated ($P < 0.05$) plasma LDL-cholesterol concentration compared with the corn oil treatment (1.99 ± 0.12 mmol/L). With the addition of phytosterols to the olive oil diet (2.11 ± 0.12 mmol/L), plasma LDL-C concentration decreased so as to be no longer significantly different from that of the corn oil diet (0.12 mmol/L (−0.05 to 0.29 mmol/L)); however, this decrease in LDL-C was not statistically different from the olive oil diet (−0.07 mmol/L (−0.24 to 0.10 mmol/L)).

TABLE 3. Composition of dietary oils

| | Dietary Oil | |
|---|---|---|
| | Corn Oil | Olive Oil |
| Phytosterol content | *mg/100 g* | |
| Campesterol | 210 | 0 |
| Stigmasterol | 65 | 0 |
| β-Sitosterol | 555 | 225 |
| Sitostanol | nd | trace |
| Total | 830 | 225 |
| Fatty acid composition | *% of total fat* | |
| Saturated | 13.9 | 17.8 |
| C14:0 | 0.0 | 0.0 |
| C16:0 | 12.3 | 16.5 |
| C18:0 | 1.6 | 1.3 |
| MUFA | 27.8 | 72.9 |
| C16:1n7 | 0.0 | 0.9 |
| C18:1n9 | 27.8 | 72.0 |
| PUFA | 56.8 | 9.3 |
| C18:2n6 | 55.5 | 8.2 |
| C18:3n3 | 1.3 | 1.1 |

Abbreviations: nd, none deteccted; trace, <10 mg detected; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Plasma triglyceride concentrations responded in a similar manner with an increase ($P < 0.05$) of 0.15 mmol/L (95% CI: 0.01 to 0.28 mmol/L) after the olive oil treatment (0.85 ± 0.07 mmol/L) when compared to the corn oil treatment (0.70 ± 0.04 mmol/L). With the addition of the phytosterol mixture to the olive oil diet, plasma triglyceride concentrations followed the same trend as was seen with LDL-C, where there was a non-significant decrease of −0.05 mmol/L (−0.18 to 0.09 mmol/L) relative to the olive oil diet alone. However, the mean of 0.80 ± 0.06 mmol/L was no longer significantly different from the corn oil group (0.10 mmol/L (−0.03 to 0.23 mmol/L)).

There were no significant differences between HDL-C concentrations after corn oil (1.05 ± 0.05 mmol/L), olive oil (1.14 ± 0.06 mmol/L), or olive oil plus phytosterol (1.17 ± 0.05 mmol/L) treatments. There were gender differences detected where females had consistently higher ($P = 0.03$) plasma HDL-C concentrations than did males over each diet treatment. Nevertheless, both genders responded similarly to diet treatment with no within-group differences or diet combined with gender group differences detected. For this reason, groups were not stratified.

Free cholesterol (FC) fractional synthetic rates (FSR) for the three diet treatments are shown in **Fig. 2**. FC-FSR after the corn oil treatment (0.061 ± 0.009 pools/day) were higher ($P < 0.05$) (0.034 pools/day (95% CI: 0.008 to 0.059 pools/day)) than those after the olive oil treatment (0.028 ± 0.004 pools/day). With the addition of phytosterols to olive oil, the FC-FSR increased by 0.019 pools/day, although this increase was not statistically significant (95% CI: −0.007 to 0.044 pools/day). Nevertheless, this increase did render the FC-FSR of the phytosterol treatment group no longer significantly different from the corn oil treatment (−0.0152 pools/day (95% CI: −0.0410 to 0.0106 pools/day).

Deuterium incorporation rates into esterified cholesterol did not differ among the corn oil (0.0197 ± 0.0078 pools/day), olive oil (0.0225 ± 0.0057 pools/day), and olive oil–phytosterol (0.0198 ± 0.0064 pools/day) treatments.

TABLE 4. Characteristics of phytosterol content of each diet treatment

| Phytosterol | Supplement[a] | Corn Oil Diet | Olive Oil Diet |
|---|---|---|---|
| | Composition (*% of total*) | | |
| Campesterol | 23.7 | 25.3 | 0.0 |
| Stigmasterol | 14.4 | 7.8 | 0.0 |
| β-Sitosterol | 61.9 | 66.9 | 100.0 |
| | Mean Daily Intake (range[b]) (*g*) | | |
| Total | 1.45 (1.17–1.69) | 0.63 (0.49–0.75) | 0.22 (0.17–0.26) |
| β-Sitosterol | 0.98 (0.81–1.13) | 0.47 (0.36–0.56) | 0.22 (0.17–0.26) |

[a]Supplement administered as an olive oil suspension at a constant amount per 1000 kcal.
[b]Range of total daily intakes for individual subjects.

TABLE 5. Plasma campesterol/cholesterol ratios of subjects after receiving diets containing different oils

| Subject | Olive Oil + Phytosterol Diet | Corn Oil Diet | Olive Oil Diet |
|---|---|---|---|
| | *mmol campesterol/mol cholesterol* | | |
| 1 | 24.6 | 17.9 | 7.3 |
| 2 | 29.9 | 9.7 | 13.0 |
| 3 | 18.6 | 18.5 | 6.3 |
| 4 | 11.7 | 21.0 | 20.9 |
| 5 | 37.8 | 31.1 | 17.3 |
| 6 | 61.5 | 24.5 | 28.1 |
| 7 | 33.2 | 28.1 | 30.3 |
| 8 | 50.9 | 13.0 | 33.4 |
| 9 | 15.4 | 83.5 | 19.6 |
| 10 | 17.9 | 55.1 | 30.0 |
| 11 | 18.2 | 19.8 | 24.7 |
| 12 | 27.6 | 29.1 | 30.1 |
| 13 | 31.3 | 15.3 | 18.8 |
| Mean ± SEM | 29.1 ± 4.0 | 28.2 ± 5.6 | 21.5 ± 2.5 |

## DISCUSSION

Although some research suggests that dietary intake of oils rich in MUFA and PUFA has similar effects on plasma lipid concentrations (3, 32, 33), a sizable body of data concludes that, of these fats, PUFA possess greater efficacy in lowering total and LDL-C concentra-

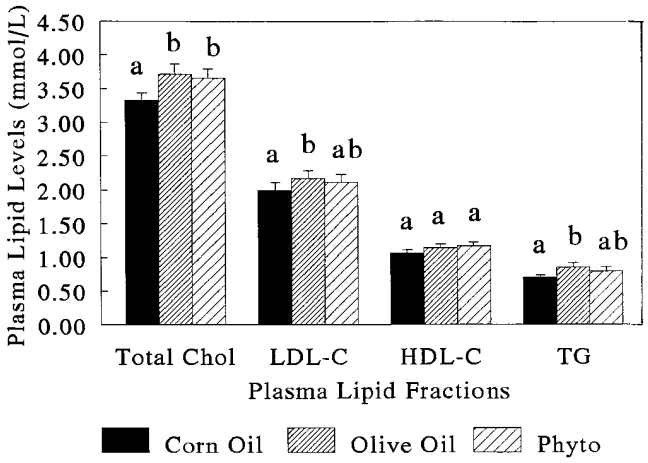


**Fig. 1.** Plasma lipid concentrations of total cholesterol (Total Chol), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) in healthy male and female subjects (n = 16) consuming either a corn oil-based diet (Corn Oil), an olive oil-based diet (Olive Oil), or an olive oil plus phytosterol diet (Phyto) for 10 days. Results are expressed as mean ± SEM. Diet treatment group means within each parameter having different subscripts differ significantly ($P < 0.05$ using Tukey's pairwise comparisons).

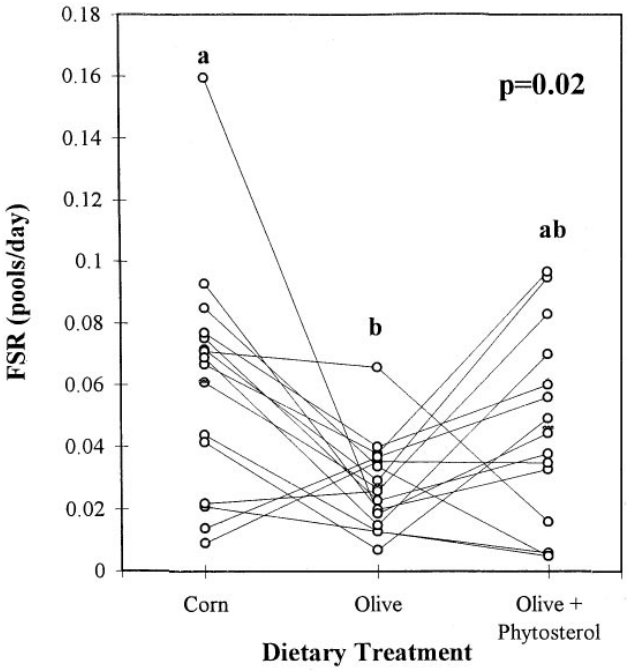


**Fig. 2.** Free cholesterol fractional synthetic rates (FSR) in healthy male and female subjects (n = 16) consuming either a corn oil-based diet (Corn), an olive oil-based diet (Olive), or an olive oil plus phytosterol diet (Olive + Phytosterol) for 10 days. Dietary treatment group means having different subscripts differ significantly ($P < 0.05$ using Tukey's pairwise comparisons).

tions (4, 5, 8, 14, 15, 34, 35). Results from the present study agree with these previous data demonstrating that MUFA-containing olive oil consumption was associated with higher plasma total cholesterol, LDL-C, and triglyceride concentrations when compared to PUFA-containing corn oil consumption. These differences between corn and olive oil have been attributed to either fatty acid composition (5, 8) or possibly to variations in phytosterol content (14, 35, 36). To our knowledge, the present study design is the first to systematically determine whether phytosterols naturally present in corn oil explain the differential cholesterol metabolism observed between these two oils, and thus offer a reason for the disparate results across studies comparing MUFA- versus PUFA-containing oils. Addition of phytosterols to the olive oil diet did not significantly influence plasma lipid concentrations compared to the non-supplemented olive oil diet. Nevertheless, phytosterol administration did suppress the significant difference observed between the corn and olive oil treatments, indicating the presence of a cholesterol-modulating action. Previous metabolic ward studies have also shown that PUFA, but not MUFA, rich oils decrease HDL cholesterol concentrations (4–8). The results of the present study could not confirm these findings, indicating that both types of fats studied exerted similar influences on HDL cholesterol concentrations.

The second objective of the present study was to determine whether phytosterols play a role in controlling cholesterogenesis during corn oil feeding. Contrary to what might be expected, enhanced rates of cholesterol biosynthesis with consumption of PUFA versus other fats has been observed previously (10, 12–15, 37, 38), as well as in the present study. These results suggest a fundamental difference in how various plant oils elicit their cholesterol-lowering effects. It can be speculated that corn oil, through its phytosterol content, reduces cholesterol absorption from gut which in turn lowers body pools and enhances synthesis rate through desuppression of cellular hydroxy-methylglutaryl-CoA (HMG-CoA) reductase activity. Presently, when phytosterols were added to the olive oil diet, cholesterol synthesis rates were enhanced so that there was no longer a significant difference from rates seen after the corn oil diet. These results suggest that phytosterols, perhaps working through inhibition of cholesterol absorption, are at least in part responsible for the differential effects on cholesterol synthesis observed between MUFA- and PUFA-containing oils. However, despite the changes observed in cholesterol metabolism with the addition of phytosterols to the olive oil diet, plasma total cholesterol concentrations were not altered. Fur-

thermore, although LDL-C and triglyceride concentrations and cholesterol synthesis rates were influenced by phytosterol supplementation to olive oil, the resultant values were not significantly different from those obtained after the non-supplemented olive oil diet. Clearly, other factors must also be at work to explain these discrepant findings.

The current study demonstrated higher plasma triglyceride concentrations after olive oil treatment compared with corn oil treatment. In most clinical trials comparing effects of MUFA- and PUFA-containing oils, circulating triglyceride concentrations were not altered (5, 8, 14, 15, 35); however, epidemiological studies have demonstrated that plasma triglyceride concentrations correlate positively with MUFA and negatively with PUFA intakes (39, 40). It has been well established that very low density lipoprotein (VLDL) particles are the main transporters of triglycerides in plasma, therefore, plasma triglyceride concentrations have been categorized as surrogate indicators of plasma VLDL concentrations. In addition, dietary cholesterol has been shown to stimulate output of all VLDL lipids (41). Therefore, it is possible that if corn oil phytosterols blocked dietary cholesterol from entering the circulation, plasma VLDL concentrations would be suppressed during corn versus olive oil feeding. Furthermore, this study determined that addition of phytosterols to the olive oil diet suppressed plasma triglyceride concentrations to levels that were no longer significantly different from those after the corn oil treatment, consistent with the theory that phytosterols in corn oil may indirectly affect triglyceride metabolism through decreasing absorption of dietary cholesterol.

Other dietary factors could also potentially explain the differential cholesterol metabolism observed during corn versus olive oil consumption. Squalene, found in high concentrations in olive oil (35, 36, 42), may down-regulate HMG-CoA reductase activity through promotion of cholesterol synthesis further along its biosynthetic pathway. However, in general, ingestion of squalene would be expected to enhance cholesterol synthesis, which, in this study was lower in the olive oil-treated subjects. Fatty acid compositional differences between the two oils also likely account for dietary fat-related effects on cholesterol metabolism. Dietary PUFA may lower LDL-cholesterol levels through an alteration in membrane lipid composition or fluidity which enhances hepatic LDL receptor function and clearance (43). This augmented LDL clearance with PUFA-rich oil consumption can be attributed to increased membrane fluidity seen with PUFA- over MUFA-containing oils. Linoleic acid, for instance, exhibits 50% greater molar potency in augmenting LDL metabolism than does oleate (44). Furthermore, in a study where PUFA-rich oils were substituted for those rich in SFA, LDL receptor activity increased from 25% to 80% of control and reduced LDL-cholesterol production rate from nearly 200% to 155% (45). In the same study, SFA caused a dose-dependent increase in LDL-cholesterol production rates and markedly increased the plasma LDL-cholesterol levels while the PUFA-containing oils did not affect either of these (45). These effects have yet to be demonstrated for MUFA lipids.

Although results from this study failed to demonstrate a strong relationship between phytosterol administration and modifications in cholesterol metabolism, effects observed suggest that phytosterols naturally present in corn oil cannot be excluded as factors influencing cholesterol metabolism. One explanation for the lack of a more substantial effect could be the form of phytosterols administered in this study. Phytosterols are normally present in esterified and non-esterified forms, matrixed within the lipid soluble component of the plant. In the present study, free phytosterols were administered as a crystalline powder; a form perhaps not as likely to disperse and distribute in a manner resembling native plant sterol. Similar concerns have been expressed over the lack of efficacy in raising circulating cholesterol levels when free powdered cholesterol was added to diets (46), in that such forms of cholesterol do not mimic the naturally occurring sterol. With cholesterol, administration of either higher levels or a more natural form such as egg white is required to observe a cholesterol-raising effect (47). In order to bypass this same limitation in the present work, phytosterol powder was administered as an olive oil suspension and provided at twice the level found naturally in corn oil. The similar plasma campesterol/cholesterol ratios observed between the corn oil and olive oil–phytosterol-supplemented groups in the present work support the rationale for increasing the supplemental dose administered to the olive oil diet by a factor of two. A higher circulating ratio in the olive oil–phytosterol group would have indicated an enhanced absorption of plant sterol, indicating its higher solubility.

A second reason for lack of substantial treatment effect may related to the brief duration of feeding period. Although diets were strictly controlled, a feeding period of 10 days may not have been long enough to realize the full effects of the diet treatments on cholesterol metabolism. It has been documented that the cholesterol-modifying effects of MUFA- and PUFA-containing fats require a period of at least 14 days to attain equilibrium (48).

In summary, our findings offer a possible explanation for the disparity observed across studies compar-

ing cholesterol-modulating effects of PUFA- versus MUFA-containing oils. It is speculated that dietary phytosterol levels exist as an important determinant of overall cholesterol modulating efficacy of a dietary fat. Results of the present study allow us to conclude that dietary oil phytosterol content is at least partially responsible for the differential effects between corn and olive oil on plasma lipid concentrations and cholesterol synthesis rates.

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