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Phytosterols mixed with medium-chain triglycerides and high-oleic canola oil decrease plasma lipids in overweight men

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Abstract

Phytosterols (PSs) have been recently added to various mediums. Nevertheless, matrices with functional properties, such as medium-chain triglycerides (MCTs), should be precisely examined for supplementary advantages. The objective of this study was to identify the existence of combined biological actions of a functional oil enriched in PSs within MCTs and high-oleic canola (HOC), relative to a control (olive oil), in overweight, hyperlipidemic men using a rigorously controlled dietary intervention. Twenty-three overweight, hyperlipidemic men consumed both types of oil in a randomized, crossover trial for 6 weeks each. Fasted plasma samples were collected on the first and last 2 days of each study period. Body weight decreased -1.22 ± 0.35 kg ($P = .0019$) and -1.68 ± 0.47 kg ($P = .0016$) after the 6-week study period in the olive oil and functional oil groups, respectively. The end points for total cholesterol and low-density lipoprotein cholesterol (LDL-C) in the functional oil group ($P = .0006$) were lower than in the olive oil group ($P = .0002$). Total cholesterol values decreased from comparable baseline to end point of 4.71 ± 0.16 mmol/L ($P < .0001$) in the functional oil phase and 5.14 ± 0.19 mmol/L ($P = .0001$) in the olive oil phase ($P = .0592$). In addition, LDL-C demonstrated a similar drop, to an end point of 3.12 ± 0.16 mmol/L ($P < .0001$) and 3.54 ± 0.18 mmol/L ($P = .0002$), for the functional oil and olive oil groups, respectively, with significant changes ($P = .0221$). High-density lipoprotein cholesterol levels did not change in either treatment. Triacylglycerol end points decreased in functional oil and olive oil groups ($P = .0195$ and .0105, respectively) to the same extent from baseline. Results indicate that PSs mixed within an MCT- and HOC-rich matrix lower plasma LDL-C, without significantly changing the high-density lipoprotein cholesterol concentrations, in hyperlipidemic, overweight men, and may therefore decrease the risk of cardiovascular events.

1. Introduction

Numerous trials have established the ability of plant sterols (PSs) to reduce total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) [1]. In addition, high-density lipoprotein cholesterol (HDL-C) and triacylglycerol (TG) concentrations remained unaltered [1]. Plant sterols should be consumed with a meal to stimulate biliary flow to obtain an optimal LDL-C–lowering effect, and, indeed, various types of food vehicles can be used [2]. Plant sterols are generally given in a fat medium because this increases PS solubility and improves its consumption [1-4]. Most studies have used margarines and mayonnaise sources of PS [1-4]; however, other fat sources, especially those with functional properties, should be considered.

A potential oil to blend PSs with would be medium-chain triglyceride (MCT) oil. It has been shown to induce beneficial increases in energy expenditure and decreases in body fat [5-9] and thus potentially help reduce obesity. In addition, an experiment demonstrated that PSs in an MCT matrix could reduce TC more than PSs in a conventional oil containing long-chain triglycerides [10]. Although there are concerns regarding hypertriglyceridemic effects of MCT [11,12], 2 studies [13,14] used MCT in obesity prevention, blended with PS for their hypocholesterolemic properties, and demonstrated favorable effects on blood lipids concentrations.

Another possibility of a novel matrix could be an oleic acid–rich oil, given that it may be beneficial for the prevention of hyperlipidemia through lowering both TC and TG concentrations. Therefore, we hypothesized that the consumption of PSs in a mixture of MCT oil and high-oleic canola (HOC) oil would prevent undesirable increases in blood lipid concentrations. The objective of this study

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Table 1
Macronutrient composition of the study diet (12 552 kJ/d)

Composition	Protein (% energy)	Carbohydrates (% energy)	Fat (% energy)	Fiber (g)
Day 1				
Breakfast	4.6	16.5	13.7	9
Lunch	3.9	14.4	11.8	6
Supper	4.8	16.2	14.1	8
Total % energy	13.3	47.1	39.6	23
Day 2				
Breakfast	3.8	18.3	13.2	4
Lunch	2.7	14.9	12.0	7
Supper	4.4	16.6	14.1	11
Total % energy	10.9	49.8	39.3	22
Day 3				
Breakfast	4.9	16.6	13.3	4
Lunch	4.5	14.0	12.7	10
Supper	4.8	16.2	13.0	10
Total % energy	14.2	46.8	39.0	24

was therefore to identify the existence of combined biological actions of a functional oil enriched in PSs within MCTs and HOC, relative to a control oil in overweight, hypercholesterolemic men using a rigorously controlled dietary intervention.

2. Subjects and methods

2.1. Subjects

Thirty-two hyperlipidemic, overweight men were recruited from the surrounding community of Montreal through newspaper advertising. Subjects were 18 to 45 years of age, with a body mass index between 25 and 33 kg/m^2 and plasma LDL-C of more than 3 mmol/L. Before enrollment, subjects were required to provide a medical history and to undergo a complete physical examination. Subjects were excluded if they had used oral hypolipidemic therapy or had diabetes, hypertension, hypothyroidism, or other known metabolic disorders. Fasting blood samples were collected for serum biochemistry and hematology to test LDL-C levels and normality of other parameters. Before study entry, subjects received a complete description of the protocol, and informed consent was obtained from participants. The study protocol was reviewed and accepted by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences of McGill University.

2.2. Experimental design and diets

A randomized, single-blind, crossover study consisting of 2 independent phases of 6 weeks each and an intermediary washout period of 4 to 8 weeks was conducted. The experimental diets consisted of prepared meals, which were precisely weighed by the kitchen staff at the Mary Emily Nutrition Clinical Research Unit of McGill University. Diets were based on a 3-day rotating menu. Subjects were required to consume at least 1 of the 3 meals at the clinic under supervision of the clinical staff. Diets were served as 3 isoenergetic meals per day (Table 1) and provided ~45% of energy as carbohydrates, ~15% as protein, and ~40% as fat, of which 75% was delivered as treatment fat. The remaining 25% of total fat was found in the standard food items, identical in both diets. Treatment fat, either as functional or control oil, was directly incorporated. The control oil, extra-virgin olive oil, was included into the meals to improve participants' blinding. The functional oil (Delta SL, Bunge North America) consisted of 3 major components: 45% to 47% HOC interesterified with 45% to 47% MCTs, and 6% to 10% of sterol esters were physically blended in after the interesterification. Each meal contained comparable amounts of fat derived from the test oil. Nonfat and nonsterol constituents were identical across diets. The nutrient intake of the diet was adjusted to tailor each individual's specific energy requirements using the equation of Mifflin et al [15] to control energy balance, to which an activity factor of 1.7 was added to compensate for energy expended in physical activity (PA). Patients were asked to maintain a constant level of PA throughout the entire study; however, the direct energy cost of PA was not measured. The different energy densities of MCT and long-chain triglyceride, 34 and 38 kJ/g, respectively, were accounted for in the calculation of energy intake to ensure that functional and extra-virgin olive oil diets were isoenergetic. During the first week of phase 1, energy intake was readjusted to correct energy balance. Energy intake was fixed thereafter and was identical during both dietary phases. Body weight was monitored daily upon arrival at the clinic. No extra food was allowed between meals except for decaffeinated, energy-free carbonated beverages, and herbal teas, which were provided by the clinic. The nutrient content of the diets was determined using Food Processor (ESHA Research, Salem, OR).

2.3. Plasma analyses

Blood samples were collected after an overnight fast on days 1, 2, 41, and 42 of each experimental phase. Samples were collected in duplicate to decrease the day-to-day variability. Blood samples were then centrifuged at 1500 rpm for 20 to 25 minutes, and plasma, serum, and red blood cells were immediately separated into 0.5- to 1-mL aliquots and stored at −20°C for future analysis. Plasma lipid aliquots were sent to and analyzed at the Lachine Clinic (Montreal, Canada). An enzymatic colorimetric test (enzymatic kit, Roche Diagnostics, Indianapolis, IN) was used for TC and TG [16,17]. The TC determination was based on Δ^4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed [18]. This determination is based on the work by Roeschlau et al [16], using a lipoprotein lipase derived from microorganisms.

Table 2
Changes in blood lipids in hypercholesterolemic men after 6 weeks of consuming diets rich in either olive or functional oil

	Olive oil (n = 23)				Functional oil (n = 23)				Between-group *P*	
	Baseline	±SEM	End point	±SEM	Baseline	±SEM	End point	±SEM	Baseline *P*	End point *P*
Weight										
Average	86.34	2.38	85.12	2.36	86.33	2.21	84.65	2.20	.9875	.3477
Difference			1.22	0.35			1.68	0.47		.2123
TC										
Average	5.73	0.18	5.14	0.19	5.68	0.21	4.71	0.16	.7075	.0006[a]
Difference			0.60	0.13			0.97	0.17		.0592
LDL-C										
Average	4.00	0.18	3.54	0.18	3.95	0.19	3.12	0.16	.6917	.0002[a]
Difference			0.46	0.1			0.83	0.15		.0221[a]
HDL-C										
Average	0.97	0.07	0.93	0.04	0.91	0.04	0.89	0.03	.1063	.1533
Difference			0.04	0.04			0.02	0.02		.3607
TG										
Average	1.69	0.15	1.48	0.13	1.81	0.14	1.53	0.11	.2170	.4309
Difference			0.22	0.09			0.27	0.10		.6254

[a] Significant differences observed.

Determination of HDL-C in plasma was done using polyethylene glycol–modified enzymes and the dextran sulfate technique [19]. The low-density lipoprotein subfraction was indirectly quantified using the equation by Friedewald et al [20].

2.4. Statistics

Descriptive analysis of the data was expressed as mean ± SEM. Data for blood lipid concentrations for each phase were analyzed using a paired *t* test to determine significant changes between baseline and end point. An unpaired *t* test was used to establish differences between the 2 diets at baseline and at end point. The data were merged for analysis on SAS statistical software (SAS Institute, Cary, NC). $P < .05$ was used to determine significance.

3. Results

Thirty-three subjects were recruited; however, only 23 subjects completed the study. Five subjects dropped out during the first phase because of medical problems not related to the study (n = 1), because they disliked the meals (n = 2), or the time commitment required for the study (n = 2). In the second phase, additional subjects dropped out because of transportation problems (n = 4) and personal problems (n = 1). The 28% dropout rate was seen because of the lengthy and intensive study protocol. Subjects were young (mean age, 37 years), overweight (mean body mass index, 28 kg/m^2), hyperlipidemic men. All individuals tolerated the diet without any reported adverse events. Subjects could not differentiate between the oils.

The weights of the subjects at baseline, the average of the first 2 days, were similar for both oils (Table 2). Both the functional and control oil groups lost weight (−1.68 ± 0.47 kg, $P = .0016$; −1.22 ± 0.35 kg, $P = .0019$, respectively) after 6 weeks.

The end point for TC after functional oil feeding was lower ($P = .0006$) than that after the control oil phase, whereas the baseline TC data of 2 groups were not different ($P = .7075$) (Table 2). The TC values decreased ($P < .0001$) from 5.68 ± 0.21 to 4.71 ± 0.16 mmol/L, baseline to end point; therefore, a −17.0% change in the functional oil phase. A similar development was seen ($P = .0001$) with the control oil from 5.73 ± 0.18 mmol/L at baseline to 5.14 ± 0.19 mmol/L at end point, but this was to a lesser extent (−10.4%). Functional oil consumption resulted in 9.5% lower ($P = .0002$) LDL-C concentrations compared with control oil, although the baseline values for control oil and functional oil were similar ($P = .6917$). Low-density lipoprotein cholesterol concentrations decreased ($P < .0001$) from baseline 3.95 ± 0.19 mmol/L to end point 3.12 ± 0.16 mmol/L by −21.0% with the functional oil. The control oil also showed a decrease ($P = .0002$) from 4.00 ± 0.18 to 3.54 ± 0.18 mmol/L, a change of −11.5% in LDL-C. The magnitude of changes of TC did not differ between oils ($P = 0.0592$); however, LDL-C changes were statistically different ($P = .0221$). Values for high-density lipoprotein did not exhibit statistically significant difference in baseline ($P = .1063$) and end points ($P = .1533$) in the functional oil group compared with the control oil group. Triacylglycerol values decreased significantly during consumption of functional oil ($P = .0195$) and olive oil ($P = .0105$) from baseline. However, there were no differences seen between the baseline ($P = .2170$) and end point ($P = .4309$) data for TG values for functional oil vs control oil.

4. Discussion

Results from this study suggest that a functional oil containing PSs within an MCT and HOC mixture provides an effective means of favorably modulating blood lipid profiles in overweight, hypercholesterolemic men.

Functional oil contained 1.3 g/4184 kJ/d of diet of PSs mixed with MCT and HOC oil, in a high-fat diet over 6 weeks. Published results [1-3] indicate that addition of 1.5 to 3 g/d of PSs to the diet causes a 7% to 16% decrease in TC and an 8% to 15% reduction in LDL-C concentrations. Studies have shown that consuming higher doses of PS does not necessarily produce larger LDL-C–lowering effects [21]. An above-average decrease in TC and LDL-C concentrations in functional oil was probably due to the fact that diets were strictly controlled in conjunction with a high rate of compliance of participants in a suitable matrix. Oil-based products enriched with PS have shown to lower TC and LDL-C; however, oils with functional properties such as MCT have often been overlooked.

The MCTs that were included in the mixture may provide a further benefit of increased energy expenditure, thus potentially assisting in weight loss. This property of MCTs is thought to be mainly due to the fact that they are metabolized differently in comparison to long-chain triglycerides. Medium-chain triglycerides undergo direct transport to the liver via the portal vein, then are oxidized for energy, whereas long-chain triglycerides are absorbed by the intestinal lymphatic ducts and transported, as chylomicrons, through the thoracic duct, to reach the systemic circulation. Several studies report that MCTs are cholesterol neutral [22,23]; however, others reported hypercholesterolemic effects of MCT [12,24] because of high saturated fat content. Nevertheless, the functional oil in the present study did not increase TC and LDL-C, but on the contrary decreased concentrations. A recent study concluded that mixed micelles containing MCT lipolysis products have a reduced solubilizing capacity for cholesterol, therefore amplifying the effectiveness of PSs in displacing cholesterol [10]. This property enhances the benefits of MCT in cholesterol-lowering PS products. In addition, previous studies on similar functional oil formulations [13,14] have shown comparable results. First, Bourque et al [13] examined the effect of a diet supplemented with a functional oil composed of MCTs (50% of fat), PSs (22 mg/kg body weight), and n-3 fatty acids (5% of fat) in overweight women. The results demonstrated a significant reduction in mean plasma TC concentration by 9.1%. Low-density lipoprotein cholesterol was also significantly lower by 16% on functional oil. Subsequently, St-Onge et al [14] evaluated the effects of a similar functional oil as Bourque et al in normolipidemic, overweight men for 4 weeks compared with olive oil. The TC and LDL-C concentrations decreased significantly by 12.5% and 13.9% when subjects consumed functional oil, respectively, compared with 4.7% and no change for olive oil, respectively [14]. The difference in comparison to our study is the magnitude of changes for TC and LDL-C in functional oil and olive oil, which were higher in the present study. This might be due to the different type of population used, men vs women, normolipidemic vs hyperlipidemic, and the different doses of MCTs and PSs used in formulations. In addition, the current study was 6 vs 4 weeks [13,14]; the longer time might have further promoted the efficacy of PS in reducing blood lipid concentrations of subjects. Consequently, it can be inferred that the dose of PS of 1.3 g/4184 kJ/d in an MCT and HOC mixture was successful in optimizing TC reduction, including LDL-C lowering in hyperlipidemic, overweight men.

Triacylglycerol concentrations decreased similarly after feeding both the PS-containing functional oil and olive oil, indicative perhaps of the general characteristics of the diet provided by the clinic, as observed in past studies of a similar design [4]. The low simple sugar, alcohol-free, regular 3 meals per day cycle with reduced caffeine intake likely played an additional role in lowering circulating TG concentrations. In addition, previous research has shown that monounsaturated fatty acid–rich diets are associated with improvements in various endothelial functions and may have favorably affected TG concentrations in both control oil and functional oil groups. Furthermore, PSs have been shown not to affect TG concentrations [2]. Reduction of TG in the functional oil group addresses the question concerning MCT tendency to raise blood TG in humans [11] and shows that hypertriglyceridemia did not occur with the functional oil formulation. However, in a previous study on humans, the amount of MCTs consumed in test diets was higher than that in the present study. On the contrary, some reports have reported unchanged TG concentrations after MCT feeding [22,23,25]. In the current study, TG concentrations decreased in the functional oil group to an equal extent as observed in the control group, which might be due to the TG-suppressing effect modulated by other components such as the HOC or the lack of MCT-raising effect of TG.

Other factors may have contributed to the observed blood lipid changes, including the fact that the test diet fed was generally higher in fiber content (23 g/d); subjects subsequently lost weight during the trial. The absolute changes in weight after 6 weeks were not significantly different between the 2 oils; therefore, weight loss was not likely a factor on lipid concentration differences. Finally, the PA of subjects was not measured in the study; however, it can be assumed that there were no differences because HDL-C did not vary for either control or functional oil.

In summary, a functional oil mixture of PS in MCT and HOC demonstrated beneficial effects on plasma lipids by substantially lowering TC and LDL-C concentrations in comparison to a more conventional olive oil. Functional foods, which have added benefits over and beyond their basic nutritional value, such as the present oil, could contribute to a management strategy for hyperlipidemia.

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References

[1] Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Stresa Workshop Participants. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 2003;78:965-78.

[2] Berger A, Jones PJ, Abumweis SS. Plant sterols: factors affecting their efficacy and safety as functional food ingredients. Lipids Health Dis 2004;7:3-5.

[3] Law M. Plant sterol and stanol margarines and health. BMJ 2000; 320:861-4.

[4] Vanstone CA, Raeini-Sarjaz M, Parsons WE, Jones PJ. Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. Am J Clin Nutr 2002; 76:1272-8.

[5] Bray GA, Lee M, Bray TL. Weight gain of rats fed medium-chain triglycerides is less than rats fed long-chain triglycerides. Int J Obes 1980;4:27-32.

[6] Baba N, Bracco EF, Hashim SA. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. Am J Clin Nutr 1982;35:678-82.

[7] Nosaka N, Maki H, Suzuki Y, Haruna H, Ohara A, Kasai M, et al. Effects of margarine containing medium-chain triacylglycerols on body fat reduction in humans. J Atheroscler Thromb 2003;10:290-8.

[8] St-Onge MP, Jones PJH, Ross R, Parsons WE. Medium chain triglycerides increase energy expenditure and decrease adiposity in overweight men. Obes Res 2003;11:395-402.

[9] St-Onge MP, Jones PJ. Greater rise in fat oxidation with medium-chain triglyceride consumption relative to long-chain triglyceride is associated with lower initial body weight and greater loss of subcutaneous adipose tissue. Int J Obes Relat Metab Disord 2003; 27:1565-71.

[10] von Bonsdorff-Nikander A, Christiansen L, Huikko L, Lampi AM, Piironen V, Yliruusi J, et al. A comparison of the effect of medium- vs. long-chain triglycerides on the in vitro solubilization of cholesterol and/or phytosterol into mixed micelles. Lipids 2005;40:181-90.

[11] Hill JO, Peters JC, Swift LL, Yang D, Sharp T, Abumrad N, et al. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. J Lipid Res 1990;31:407-16.

[12] Tholstrup T, Ehnholm C, Jauhiainen M, Petersen M, Hoy CE, Lund P, et al. Effects of medium-chain fatty acids and oleic acid on blood lipids, lipoproteins, glucose, insulin, and lipid transfer protein activities. Am J Clin Nutr 2004;79:564-9.

[13] Bourque C, St-Onge MP, Papamandjaris AA, Cohn JS, Jones PJ. Consumption of an oil composed of medium chain triacylglycerols, phytosterols, and N-3 fatty acids improves cardiovascular risk profile in overweight women. Metabolism 2003;52:771-7.

[14] St-Onge MP, Lamarche B, Mauger JF, Jones PJ. Consumption of a functional oil rich in phytosterols and medium-chain triglyceride oil improves plasma lipid profiles in men. J Nutr 2003;133:1815-20.

[15] Mifflin MD, St-Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241-7.

[16] Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. Z Klin Chem Klin Biochem 1974;12:226.

[17] Nagele U, Hagele EO, Sauer G, Wiedemann E, Lehmann P, Wahlefeld AW, et al. Reagent for the enzymatic determination of serum total triglycerides with improved lipolytic efficiency. J Clin Chem Clin Biochem 1984;22:165-74.

[18] Esterbauer H, Gebicki J, Puhlm H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med 1992;13:341-90.

[19] Harris N, Galpchian V, Rifai N. Three routine methods for measuring high-density lipoprotein cholesterol compared with the reference method. Clin Chem 1996;42:738-43.

[20] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18: 499-502.

[21] Clifton PM, Noakes M, Ross D, Fassoulakis A, Cehun M, Nestel P. High dietary intake of phytosterol esters decreases carotenoids and increases plasma plant sterol levels with no additional cholesterol lowering. J Lipid Res 2004;45:1493-9.

[22] Temme EH, Mensink RP, Hornstra G. Effects of medium chain fatty acids (MCFA), myristic acid, and oleic acid on serum lipoproteins in healthy subjects. J Lipid Res 1997;38:1746-54.

[23] Nosaka N, Kasai M, Nakamura M, Takahashi I, Itakura M, Takeuchi H, et al. Effects of dietary medium-chain triacylglycerols on serum lipoproteins and biochemical parameters in healthy men. Biosci Biotechnol Biochem 2002;66:1713-8.

[24] Cater NB, Heller HJ, Denke MA. Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentrations in humans. Am J Clin Nutr 1997;65:41-5.

[25] Asakura L, Lottenberg AM, Neves MQ, Nunes VS, Rocha JC, Passarelli M, et al. Dietary medium-chain triacylglycerol prevents the postprandial rise of plasma triacylglycerols but induces hypercholesterolemia in primary hypertriglyceridemic subjects. Am J Clin Nutr 2000;71:701-5.