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Olive oil containing olive oil fatty acid esters of plant sterols and dietary diacylglycerol reduces low-density lipoprotein cholesterol and decreases the tendency for peroxidation in hypercholesterolaemic subjects

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Plant sterols (PS) and MUFA are well-documented cholesterol lowering agents. We aimed to determine the effect of PS esterified to olive oil fatty acids (PS-OO) on blood lipid profile and lipid peroxidation in hypercholesterolaemic subjects. Twenty-one moderately overweight, hypercholesterolaemic subjects consumed three consecutive treatment diets, each lasting 28 d and separated by 4-week washout periods, using a randomized crossover design. Diets contained 30 % energy as fat, 70 % of which was provided by olive oil (OO), and differed only in the treatment oils: OO, PS esterified to sunflower oil fatty acids (PS-SO), and PS-OO. Both PS-SO and PS-OO treatments provided 1·7 g PS /d. PS-OO and PS-SO consumption resulted in a decrease ($P=0{\cdot}0483$) in LDL-cholesterol (LDL-C) concentrations compared with the OO diet. Although total cholesterol and apo B-100 levels were not significantly affected, PS-SO and, to some extent, PS-OO reduced the total:HDL-cholesterol (HDL-C) ratio ($P=0{\cdot}0142$) and the apo B-100:apo A-I ratio ($P=0{\cdot}0168$) compared with the OO diet. There were no differences across diets in lipoprotein(a) (Lp(a)) and lipid peroxidation levels. However, following consumption of OO and PS-SO, Lp(a) concentrations increased ($P=0{\cdot}0050$ and 0·0421, respectively), while PS-OO treatment did not affect Lp(a) levels. Furthermore, there was a decrease ($P=0{\cdot}0097$) in lipid peroxidation levels with PS-OO treatment during the supplementation phase. Our results suggest that supplementing an OO-rich diet with PS-OO favourably alters the plasma lipid profile and may decrease the susceptibility of LDL-C to lipid peroxidation in hypercholesterolaemic subjects.

Plant sterols: Olive oil fatty acids: Blood lipids: Oxidative stress: Hypercholesterolaemic subjects: Lipid peroxidation: Lipoprotein (a): LDL cholesterol: Apo B: Diacylglycerol

CHD is the leading cause of morbidity and mortality in Western countries. An elevated concentration of LDL-cholesterol (LDL-C) is a well-established independent risk factor for atherosclerosis[1]. In addition, LDL oxidation has been implicated by several studies as one of the initial steps of atherogenesis, and therefore associated with higher risk of CHD[2,3]. Elevated concentrations of oxidised LDL have recently been identified as a strong predictor for subsequent acute CHD events in healthy men[4].

Plant sterols (PS) have long been reported to have significant cholesterol-lowering properties[5]. Studies conducted throughout the past decades suggested that an intake of 1·5–2 g PS/d is needed to achieve an optimal LDL-C lowering effect[6]. It is generally accepted that PS decrease circulating cholesterol concentrations by suppressing intestinal absorption of cholesterol due to the higher affinity of PS to micelles compared to cholesterol, resulting in less cholesterol being incorporated in chylomicrons[7,8]. Several reports have demonstrated that the solubility of PS may play an important role in the process of PS incorporation into micelles[9]. Indeed, low intestinal bioavailability of purified phytosterols was shown to be elevated by esterification to fatty acids, dissolving in dietary diacylglycerol (DAG) oil, or by emulsifying with lecithin micelles[10–13]. To date, the most common process used to enhance the solubility of PS is by esterifying PS with *n*-6 PUFA, such as soyabean oil and sunflower oil (SO) fatty acids. PS can therefore be incorporated into fatty foods, such as margarines and spreads[14]. Emerging new approaches consist of esterifying PS to fatty acids associated with additional health benefits, such as fish oil fatty acids[15].

Early epidemiological evidence showed a lower incidence of CHD in Mediterranean countries[16] where olive oil (OO) is the primary source of fat[17]. The consumption of OO, which contains high levels of oleic acid (a MUFA), was inversely associated with IHD, presumably due to hypolipidaemic effects[18]. This notion has been further confirmed in several clinical intervention trials[19–21]. MUFA supplementation was also shown to have protective effects against lipid peroxidation[19,22]. Likewise, PS were also reported to have antioxidant properties[23].

Abbreviations: DAG, diacylglycerol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; Lp(a), lipoprotein (a); OO, Olive oil; SO, sunflower oil; PS, plant sterols; TBARS, thiobarbituric acid-reactive substance; TC, total cholesterol.

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The objective of this study was to assess whether a novel formulation of PS that had been esterified with OO fatty acids (PS-OO) would exert the effects of its components on the blood lipid profile and lipid peroxidation. We tested this hypothesis in mildly overweight, hypercholesterolaemic subjects who consumed an OO-rich diet that was further supplemented either with PS-OO or with PS esterified to SO fatty acids (PS-SO).

Methods

Study population

Twenty-four volunteers (eleven males, thirteen postmenopausal females) were recruited from the Montreal area by an advertisement posted in local newspapers. The inclusion criteria were as follows: baseline LDL-C $>2{\cdot}6$ mmol/l (100 mg/dl), BMI ranging from 24 to 30 kg/m^2 and aged 30–65 years. Subjects were excluded if they had taken medications known to affect lipid metabolism, such as cholestyramine, colestipol, gemfibrozil, probucol, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, fish oil capsules and supplements containing PS, during the previous 3 months. Subjects who had been diagnosed with diabetes mellitus, kidney disease or liver disease, and those who smoked, consumed more than two glasses per day of alcoholic beverages and/or took two or more doses per week of laxatives or concentrated sources of fibre were also excluded. Subjects with thyroid disease (*n* 2) and with high blood pressure (*n* 4) were included in the study since they had been stable in response to thyroid and blood pressure treatments, and their medications were maintained throughout the trial. One subject on hormone replacement therapy was also included in the study and her regimen was maintained at the same dose throughout the study period.

Fasting blood samples were obtained for screening purposes. Before enrolment in the study, the candidates provided a medical history and underwent a complete physical examination conducted by the study physician. The study protocol was reviewed and approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University (protocol number REB# 808-0403). All subjects received explanations about the protocol and written consent forms were obtained from each participant.

Experimental design, protocol and diets

The study was a semi-randomized, crossover, double blind, clinical intervention trial using a Latin square sequence. It consisted of three 28 d phases separated by a 4-week washout interval. During each dietary treatment phase, the subjects were provided with an OO-based, weight-maintaining, North-American diet. During the washout period, the subjects consumed their own habitual diets. All subjects received the control OO diet during the first phase, after which they were randomly assigned to the two other dietary treatments.

The basic diet contained approximately 15 % energy as protein, 55 % energy as carbohydrates, 30 % energy as fat, of which approximately 70 % was provided by OO, 80 mg cholesterol per 4186·8 kJ (1000 kcal); and 12 g fibre per 4186·8 kJ (1000 kcal). The OO of the basic diet provided 0·02 g/d naturally occurring free PS. The basic diet composition is shown in Table 1. Table 2 lists the composition of the treatment oils. During the two randomized phases, an isoenergetic amount of OO was replaced by either (i) 21·4 g low-fat PS-SO margarine providing the equivalent of 1·7 g soyabean sterols (Take control®, Unilever Bestfoods NA, Baltimore, MD) or (ii) 9·1 g PS-OO containing the equivalent of 1·7 g soyabean sterols enzymatically esterified to OO fatty acids, as well as 1·4 g DAG (Enzymotec Ltd, Migdal HaEmeq, Israel). No antioxidants were supplemented to the PS-OO matrix or other tested matrices.

The diets were provided to the subjects by the Mary Emily Clinical Nutrition Research Unit at McGill University. These diets consisted of three isoenergetic meals, prepared according to a 3-d cycle menu. All meals (breakfast, lunch and supper) were prepared in the metabolic kitchen of the clinic where the foods were weighed precisely to 0·5 g during meal preparation. Treatment oils were ingested at breakfast and under supervision to monitor compliance. The subjects were instructed to consume only foods and beverages provided by the clinic.

All three experimental diets were isoenergetic. Individual basal energy requirements were calculated by the Mifflin equation[24], and were then multiplied by a physical activity factor of 1·7 to meet the total energy requirements of mildly to moderately active healthy adults. Energy intake was adjusted during the first 2 weeks of the control phase to maintain a constant body weight. A comparable energy level was maintained during the two subsequent phases. Subjects were encouraged to keep a constant exercise level throughout the study to ensure that body weights remained unchanged.

Fasting blood samples were collected from the subjects on days 1, 2, 28 and 29 of each phase. On day 28 of each phase, postprandial plasma TAG concentrations were measured 4 h after breakfast. A complete blood count was done on day 29 for monitoring purposes.

Plasma lipids

Blood samples were collected in vacutainer tubes and centrifuged for 15 min at 1000 ***g*** at 4°C, within 30 min, to separate plasma from erythrocytes. Plasma and erythrocytes were immediately stored at −80°C until lipid analysis. Total cholesterol (TC), HDL-cholesterol (HDL-C), and TAG concentrations were measured by automated methods on the multianalyzer Dimension RxL Max utilizing enzymatic reagents Flex (Dade Behring Diagnostic, Marburg, Germany). LDL-C

Table 1. Average composition of the control olive oil-based diet over a period of 3 d

(Values are means with their standard errors)

Diet component	Mean	SE
Proteins (% energy)	14·4	0·2
Carbohydrates (% energy)	54·0	1·0
Fat (% energy)	31·2	0·9
SFA (% energy)	6·8	0·6
MUFA (% energy)	18·6	0·6
PUFA (% energy)	3·5	0·1
Cholesterol (mg/1000 kcal)	80·3	1·0
Fibre (g/1000 kcal)	12·8	0·3

Table 2. Fatty acid and plant sterol composition of study formulations*

	OO	PS-SO	PS-OO
Plant sterol esters (g/100 g oil or margarine)	ND	12·1	28·6
Free plant sterol equivalents (g/100 g oil or margarine)			
β-sitosterol	0·2	3·7	8·4
Campesterol	>0·1	1·9	4·6
Stigmasterol	>0·1	1·4	3·9
Brassicasterol	ND	0·2	0·5
Others	0·1	0·5	1·0
Total	0·2	7·8	18·3
Fatty acids (% by weight total fatty acids)			
12 : 0	ND	0·2	ND
14 : 0	>0·1	0·2	ND
16 : 0	12·9	8·3	12·8
16 : 1	1·0	0·1	0·9
17 : 0	ND	0·1	ND
18 : 0	2·9	6·2	3·0
18 : 1	69·8	41·8	70·6
18 : 2	11·5	36·4	10·7
18 : 3 *n*-3	0·8	5·5	0·8
20 : 0	0·5	0·5	0·5
20 : 1	0·3	0·3	0·2
22 : 0	0·2	0·4	0·2
24 : 0	0·1	0·2	0·1
Other fatty acids	0·2	ND	0·1

OO, control olive oil; PS-SO, plant sterols esterified with sunflower oil fatty acids; PS-OO, plant sterols esterified with olive oil fatty acids; ND, not detected.
* Typical values.

was calculated by the Friedewald equation[25] for individuals with TAG levels <4·5 mmol/l, while LDL-C was measured directly by the abovementioned enzymatic methods when TAG levels were >4·5 mmol/l. Apo A-I, apo B-100 and lipoprotein (a) (Lp(a)) levels were measured on the BN ProSpec Nephelometer (Dade, Behring Diagnostics, Marburg, Germany) utilizing the Dade Behring N Antisera assays to determine apo A-I, apo B-100, and Lp(a), respectively (Behring Diagnostics).

Plasma cholesterol precursor and plant sterols

Plasma PS concentrations were determined from the non-saponifiable material of plasma lipids as reported previously[26]. Briefly, an internal standard, 5α-cholestane, was added to 0·5 ml plasma sample. Plasma samples were saponified with 0·5 M methanolic KOH for 1 h at 100°C and the non-saponifiable materials were extracted with petroleum diethyl ether. After extraction, the samples were derivatized with 1·3 ml TMS reagent (pyridine–hexamethyldisilazan–trimethylchlorosilane; 9:3:1, by volume); Sigma-Aldrich Canada Ltd, Oakville, ON, Canada). The samples were then injected into GLC (HP 5890 Series II, Hewlett Packard, Palo Alto, CA, USA), equipped with a flame ionization detection and autoinjector system and with a 30 m capillary column. The column temperature was 285°C and the isothermal running conditions were maintained for 30 minutes. Detector and injector temperatures were set to 310°C and 300°C, respectively. Lathosterol, campesterol, stigmasterol and β-sitosterol peaks were identified by comparison with authenticated standards (Sigma-Aldrich Canada Ltd).

Plasma lipid peroxidation

Apo B-containing lipoproteins were precipitated with manganese chloride–heparin by ultracentrifugation[27]. The LDL fraction was re-suspended in normal saline after centrifugation. The thiobarbituric acidreactive substance (TBARS) assay[28] was used to measure lipid peroxidation in the plasma LDL subfraction (OXItek, ZeptoMetrix Corporation, Buffalo, NY, USA).

Statistical analyses

Based on previous publications with a comparable design[29], twenty subjects would be required in order to detect a clinically significant difference (−0·48 mmol/l) in LDL-C levels, the primary outcome, using 0·84 mmol/l as a standard deviation, at the 0·05 level of significance and 80 % power. A total enrolment of twenty-four patients was originally estimated as being required to enable a study dropout rate of 15 %.

All data were expressed as means and their standard errors. Statistical significance was set at $P<0·05$ for all analyses. Variables that were not normally distributed were log transformed before analysis. Differences in plasma variables were tested by repeated-measures ANOVA with the type of dietary matrix in each intervention arm as the within-subject factor and with endpoint values as the dependent variable. Baseline values were inserted into the model as covariates if their interaction with dietary matrices was found to be statistically significant. Subsequently, contrast analyses were used to identify differences between pairs of diets. When variables failed to demonstrate any treatment effect, the two-tailed paired Student's *t* test was used to compare baseline and endpoint values within each diet phase. Furthermore, a modified Cohen's effect size was calculated for endpoint values to evaluate changes from the baseline OO diet. Data were analyzed with the use of SAS software (version 8.0; SAS Institute Inc, Cary, NC, USA).

Results

Study subjects

Twenty-four subjects (eleven males, thirteen females) were recruited and twenty-one subjects (eleven males, ten females) completed the entire trial. The three females who dropped out during the first phase reported difficulties with the transportation to the clinic (*n* 1) or with daily clinic visits (*n* 1) and personal affairs (*n* 1). The baseline characteristics of study subjects who completed the trial are displayed in Table 3. No side effects were reported after consuming the treatment oils. Results from the complete blood count at the end of each phase were within the normal range for all subjects (data not shown). The mean baseline bodyweight for OO, PS-SO, and PS-OO (73·9 (SE 2·7) kg, 74·7 (SE 2·8) kg, and 74·4 (SE 2·8) kg, respectively) and the percentage changes in bodyweight values (−0·7 (SE 0·3) %, −0·4 (SE 0·2) %, and −0·7 (SE 0·2) %, respectively) did not differ between treatments. Baseline values (following washout periods) of all the characteristics presented in Table 3 were not statistically different between dietary phases (data not shown).

Table 3. Baseline characteristics of the study subjects (*n* 11 males, 10 females)

(Values are means with their standard errors)

	Mean	SE
Age (years)	54·19	1·62
Weight (kg)	73·69	2·72
BMI (kg/m^2)	25·93	0·62
Total cholesterol (mmol/l)	6·09	0·18
LDL-cholesterol (mmol/l)	3·91	0·12
HDL-cholesterol (mmol/l)	1·28	0·07
TAG (mmol/l)	1·77	0·25

Plasma lipid concentrations

Table 4 lists plasma lipid concentrations at the end of each treatment phase. Supplementation of an OO-based diet with either PS-SO or PS-OO resulted in reduced ($P=0·0218$ and 0·0185, respectively) LDL-C levels compared with control OO, but there was only a mild tendency towards a reduction ($P=0·0839$) in TC levels. TC:HDL-C ratios following PS-SO treatment were lower ($P=0·0018$) compared with control OO, but they were not significantly different from those observed with PS-OO. Consumption of the PS-matrices did not influence plasma HDL-C or TAG (fasting and postprandial) concentrations (Table 4).

The effects of the dietary fats and PS matrices on apo concentrations are also presented in Table 4. Apo A-I concentrations were higher ($P=0·0052$ and $P<0·0001$) following PS-OO administration relative to control OO-diet and PS-SO, respectively, presumably due to differences in baseline values ($P<0·0001$). PS-containing diets had a strong tendency to induce ($P = 0·0577$) lower (10–11%, change from baseline) apo B-100 concentrations. This was associated with a mild tendency towards decreased ($P=0·1030$) LDL-C:apo B ratios, especially following the PS-OO treatment (an effect size of 0·47). Apo B-100:apo A-I ratios were lower ($P = 0·0052$) with PS-SO (6%, relative to baseline) and, to a lesser degree ($P = 0·2057$), with PS-OO (4%, relative to baseline) administrations compared with the control OO-diet. The effect of PS-SO consumption (an effect size of 0·35) on apo B-100:apo A-I ratios did not differ ($P=0·8698$) from the effect of PS-OO supplementation (an effect size of 0·31).

Plasma Lp(a) concentrations were not altered ($P=0·1182$) by the dietary treatments at the end of the feeding phases. When endpoint values were compared to baseline concentrations, however, consumption of PS-OO did not influence Lp(a) levels, while the control OO-based diet and PS-SO treatments resulted in an increase ($P=0·0050$ and $P=0·0421$, respectively) of Lp(a) concentrations (Fig. 1).

Plasma lipid peroxide concentrations

The dietary treatments did not impact ($P=0·1295$) endpoint plasma TBARS concentrations. However, when baselines and endpoints were compared (Fig. 2), the consumption of PS-OO was shown to have resulted in reduced (−13 %; $P=0·0097$) TBARS levels, whereas the OO-based diet (−10 %; $P=0·0993$) and the PS-SO (−3 %; $P=0·1640$) treatments failed to produce a comparable effect.

Plasma neutral sterol concentrations

Consumption of PS-SO and PS-OO resulted in a statistically significant increase in plasma campesterol, stigmasterol, and β-sitosterol concentrations compared with the control OO-based diet (Table 5). Similar observations were obtained when plasma PS concentrations were normalized to cholesterol levels, although to a somewhat larger extent. The concentration of cholesterol precursor, lathosterol and its ratio to cholesterol were elevated ($P=0·0031$ and 0·0268, $P=0·0564$ and 0·0047) by PS-SO and PS-OO treatments, respectively, compared with the control OO-diet.

Table 4. Fasting plasma lipid and apo concentrations in overweight, hyperlipidaemic subjects (*n* 21) consuming different oil supplements varying in fatty acid and plant sterol content in separate diets each lasting 4 weeks

(Values are means with their standard errors)

	OO		PS-SO		PS-OO		
Plasma lipid	Mean	SE	Mean	SE	Mean	SE	*P* value*
Cholesterol							
Total (mmol/l)	5·90	0·22	5·61	0·21	5·49	0·25	0·0839
LDL (mmol/l)	3·83^a	0·17	3·59^b	0·16	3·48^b	0·19	0·0483
HDL (mmol/l)	1·29	0·07	1·29	0·07	1·23	0·07	0·1904
Total:HDL	4·75^a	0·18	4·53^b	0·20	4·70^{ab}	0·22	0·0142
TAG							
Fasting (mmol/l)	1·86	0·27	1·62	0·19	1·75	0·25	0·1402
Postprandial (mmol/l)	2·63	0·21	2·56	0·23	2·70	0·28	0·9942
Apo							
ApoA-I (g/l)	1·30^a	0·06	1·30^a	0·06	1·31^b	0·05	0·0001†
ApoB-100 (g/l)	1·12	0·05	1·06	0·06	1·09	0·06	0·0577
ApoB-100:Apo A-I	0·89^a	0·04	0·84^b	0·05	0·84^{ab}	0·05	0·0168
LDL cholesterol:apoB-100	3·49	0·17	3·47	0·14	3·28	0·12	0·1030

OO, control olive oil; PS-SO, plant sterols esterified with sunflower oil fatty acids; PS-OO, plant sterols esterified with olive oil fatty acids.

* *P* values obtained by repeated-measures ANOVA, with †baseline concentrations included in the model as covariates. Values of plasma lipid concentrations were normalized using a log transformation.

[a,b] Values not sharing a common superscript letter are significantly different at $P<0·05$.

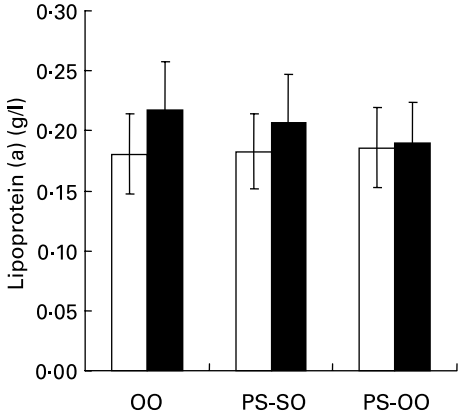

Fig. 1. Lipoprotein(a) (Lp(a)) levels in blood were measured at baseline (□) and after treatment (■). Values are means, with their standard errors shown by vertical bars, of Lp(a) concentrations of twenty-one patients. Statistical significance between the baseline and endpoint values was determined by the two-tailed paired-Student's *t* test for the OO ($P=0{\cdot}005$) and the PS-SO ($P=0{\cdot}0421$) treatments but not for PS-OO ($P=0{\cdot}8123$). OO, control olive oil; PS-SO, plant sterols esterified with sunflower oil fatty acids; PS-OO, plant sterols esterified with olive oil fatty acids.

Discussion

Our results suggest that supplementation of an OO-based diet with OO fatty acids esterified to PS and mixed with dietary DAG reduces LDL-C levels and could lower LDL susceptibility to oxidation compared with an OO-based diet in hypercholesterolaemic mildly-overweight subjects. Consumption of both PS-OO and PS-SO diets favoured comparable beneficial reductions of CHD-related risk factors. However, in the context of an OO background diet, PS-OO feeding improved plasma antioxidant properties and protected against the increase in Lp(a) levels over the study period, while PS-SO supplementation did not show such an action.

In the current study, PS-OO and PS-SO supplementation to the base OO-diet tended to reduce TC concentrations by -8 and $-6\,\%$, relative to baseline respectively. Importantly, we have shown that ingestion of 1·7 g/d PS esterified to OO or SO fatty acids significantly decreased LDL-C concentrations by 6–9 % compared to the base OO-diet. These decreases are consistent with results from a recent meta-analysis[6], in which an intake of 1·5–1·9 g PS /d was associated with a 7·0–10·1 % reduction in LDL-C levels. Moreover, plasma levels of apo B-100 tended to lower with consumption of PS diets, but to a somewhat lesser extent than LDL-C concentrations. These observations suggest that the PS treatments may have affected the cholesterol content of the LDL particles more than their number. On the other hand, there was a significant reducing effect of PS-containing treatments on apo B-100:apo A-I ratio, suggesting that, in fact, these diets favour a beneficial suppression of apo B-100 levels compared with the control OO-diet. Taken together, our findings suggest that supplementation with PS provides superior protection against coronary artery disease risk factors than a healthy OO-based diet.

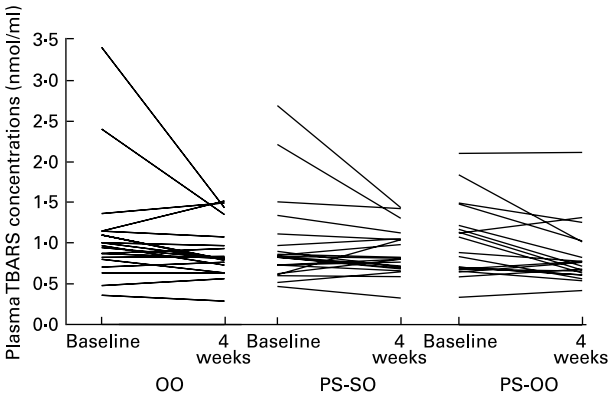

Fig. 2. Plasma thiobarbituric acid reactive substance (TBARS) levels in the LDL fraction were measured at baseline and endpoint (4 weeks) of each of three feeding phases (*n* 21 subjects). Statistical significance between the baseline and endpoint values was determined using the two-tailed paired-Student's *t* test only for the PS-OO treatment. ($P=0{\cdot}0097$), but not for OO ($P=0{\cdot}0993$) and the PS-SO ($P=0{\cdot}1640$) treatments. OO: control olive oil; PS-SO: plant sterols esterified with sunflower oil fatty acids; PS-OO: plant sterols esterified with olive oil fatty acids.

In this study, the PS concentrations in plasma increased following consumption of both PS-SO and PS-OO as compared with the base OO-diet. Plasma PS concentrations are naturally low since they are poorly absorbed. Nevertheless, high PS intake has been consistently shown to substantially increase circulating PS concentrations[30,31]. In the current study, however, the degree of elevation in plasma PS concentrations and their ratios to cholesterol due to PS consumptions were 2–3-fold lower than what we had previously observed, when PS were administered equally across two to three daily meals[31]. Taken together with the somewhat limited LDL-C lowering effect, these findings suggest that a single morning dose of PS may result in a lower PS bioavailability and therefore lesser efficacy. The link between PS bioavailability and treatment efficacy warrants further investigation.

Compared with the baseline values, the serum lipid peroxides associated with LDL particles were significantly reduced following PS-OO treatment. Early publications suggested that OO consumption resulted in LDL enrichment with oleic acid and, consequently, in a greater resistance to oxidation[32]. Furthermore, a diet enriched in MUFA, rather than PUFA, was shown to inhibit LDL oxidation[19,21,33]. The presence of high levels of PS in diet[34] or *in vitro*[35] has been associated with decreased lipid peroxidation. Interestingly, similar observations were recently noted following administration of MUFA-enriched PS mixed with DAG to atherosclerotic apo E deficient mice[36]. Therefore, the antioxidant properties of MUFA-diets and PS could have contributed, at least in part, to the reduction in TBARS concentrations observed in our study on PS-OO.

In the current study, OO-diet and PS-SO supplementation resulted in increased Lp(a) levels over the study period, whereas no such deleterious effect was observed following PS-OO consumption. Elevated concentrations of Lp(a) have been suggested to be a risk factor for a variety of atherosclerotic and thrombotic disorders[37,38]. The atherogenicity of Lp(a) may be mediated, at least in part, by associated proinflammatory oxidized phospholipids[38,39], but the link between Lp(a) and LDL oxidation in response to dietary modifications remains unclear. Although OO was shown in some studies to protect LDL from oxidation[40,41], there are several reports in which its consumption resulted in elevated Lp(a) concentrations[42,43]. Likewise, PUFA-enriched diets have been shown to increase Lp(a) levels[44], presumably via interacting

Table 5. Plasma plant sterol and cholesterol precursor concentrations following the consumption of each dietary treatment by mildly-overweight hypercholesterolaemic subjects (*n* 21)

(Values are means with their standard errors)

	OO		PS-SO		PS-OO		
Plasma sterol	Mean	SE	Mean	SE	Mean	SE	*P* value*
Lathosterol (μmol/l)	6·03[a]	0·75	6·42[b]	0·53	7·21[ab]	0·90	0·0085†
Campesterol (μmol/l)	9·26[a]	0·83	18·11[b]	1·75	18·68[b]	2·07	<0·0001
Stigmasterol (μmol/l)	4·56[a]	0·67	4·65[b]	0·73	5·20[b]	0·79	0·0282†
β-sitosterol (μmol/l)	5·48[a]	0·61	7·62[b]	0·94	7·73[b]	0·96	0·0007
Lathosterol:cholesterol	1·06[a]	0·14	1·22[b]	0·14	1·43[b]	0·19	0·0205†
Campesterol:cholesterol	1·61[a]	0·15	3·36[b]	0·36	3·71[b]	0·47	<0·0001
Stigmasterol:cholesterol	0·84[a]	0·14	0·93[ab]	0·18	1·16[b]	0·25	0·0121
β-sitosterol:cholesterol	0·93[a]	0·08	1·38[b]	0·15	1·50[b]	0·18	<0·0001

OO, control olive oil; PS-SO, plant sterols esterified with sunflower oil fatty acids; PS-OO, plant sterols esterified with olive oil fatty acids.

* *P* values obtained by repeated-measures ANOVA, with †baseline concentrations included in the model as covariates. Values of plasma lipids concentrations were normalized using a log transformation.

[a,b] Values not sharing a common superscript letter are significantly different at $P<0·05$.

with several transcription factors, such as NF-κB and PPAR α[45,46]. In contrast, dietary DAG ingestion[47] was recently associated with a slight but significant reduction in Lp(a) concentrations, possibly as a result of decreased hepatic fat contents. In our current study, the PS-OO treatment, which contained equivalent amounts of OO fatty acids as the control OO-diet, in addition with DAG maintained low Lp(a) levels. Although the levels of DAG administered in the PS-OO dietary matrix were considerably lower than the dose of DAG that had been shown to be effective in human clinical studies[42,48], its presence could have contributed to maintaining Lp(a) levels. Taken together with the reduction in plasma TBARS concentrations, these results suggest that PS-OO may exert protective actions against oxidative stress.

In conclusion, our findings demonstrate that providing PS-containing matrices to hypercholesterolaemic, mildly overweight subjects fed with an OO-based diet results in optimized plasma lipid concentrations. Furthermore, consumption of PS-OO, but not PS-SO, may reduce the susceptibility of LDL to oxidative stress, which, in turn, could protect against increases in Lp(a) concentrations. Therefore, in the context of an OO-based diet, supplementation with PS-OO matrixed with DAG in OO may offer a greater level of protection against CHD than the traditional PS-SO formula to hypercholesterolaemic individuals.

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