

# An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets

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**Abstract** We investigated the effect of olive oil, rapeseed oil, and sunflower oil on blood lipids and lipoproteins including number and lipid composition of lipoprotein subclasses. Eighteen young, healthy men participated in a double-blinded randomized cross-over study (3-week intervention period) with 50 g of oil per 10 MJ incorporated into a constant diet. Plasma cholesterol, triacylglycerol, apolipoprotein B, and very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) cholesterol concentrations were 10–20% higher after consumption of the olive oil diet compared with the rapeseed oil and sunflower oil diets [analysis of variance (ANOVA),  $P < 0.05$ ]. The size of IDL, VLDL, and LDL subfractions did not differ between the diets, whereas a significantly higher number (apolipoprotein B concentration) and lipid content of the larger and medium-sized LDL subfractions were observed after the olive oil diet compared with the rapeseed oil and sunflower oil diets (ANOVA,  $P < 0.05$ ). Total HDL cholesterol concentration did not differ significantly, but HDL<sub>2a</sub> cholesterol was higher after olive oil and rapeseed oil compared with sunflower oil (ANOVA,  $P < 0.05$ ). In conclusion, rapeseed oil and sunflower oil had more favorable effects on blood lipids and plasma apolipoproteins as well as on the number and lipid content of LDL subfractions compared with olive oil. Some of the differences may be attributed to differences in the squalene and phytosterol contents of the oils. —Pedersen, A., M. W. Baumstark, P. Marckmann, H. Gylling, and B. Sandström. An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets. *J. Lipid Res.* 2000. 41: 1901–1911.

**Supplementary key words** blood lipids • cholesterol • dietary fatty acids • dietary oils • HDL cholesterol • insulin • ischemic heart disease • nonesterified fatty acids • phytosterols • squalene • triacylglycerols

Dietary fatty acid composition influences plasma lipids and lipoproteins associated with the development of atherosclerosis and ischemic heart disease (IHD). The effect of dietary fatty acids on plasma total cholesterol, and on low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, has been subject to many studies and prediction algorithms have been developed (1–6). However, the conventional risk markers of IHD are not sufficient for identifying individuals at high risk of IHD (7) and additional blood parameters such as elevated fasting plasma insulin, apolipoprotein B (apoB), triacylglycerols (TAG), and nonesterified fatty acids (NEFA) have been studied in relation to an increased risk of IHD (8–11). Very low density lipoprotein (VLDL) or/and intermediate density lipoprotein (IDL) mass concentrations have been found to be related to disease progression (12–15). Results from clinical studies have suggested a strong relationship between a predominance of small, dense LDL particles in plasma and an increased risk of myocardial infarction or coronary atherosclerosis as assessed from angiography (9, 16–19), and prospective studies have confirmed this association (20, 21). The increased atherogenicity of small, dense LDL may be explained by a lower binding affinity to the LDL receptor (22), increased susceptibility to oxidation (23), or an increased capacity for

Abbreviations: ALP, atherogenic lipoprotein phenotype; ANOVA, analysis of variance; apo, apolipoprotein; CRP, C-reactive protein; FC, free cholesterol; HDL, high density lipoprotein; HL, hepatic lipase; IDL, intermediate density lipoprotein; IHD, ischemic heart disease; LDL, low density lipoprotein; MUFA, monounsaturated fatty acids; OO, olive oil; NEFA, nonesterified fatty acids; PL, phospholipid; PUFA, polyunsaturated fatty acids; RO, rapeseed oil; SFA, saturated fatty acids; SO, sunflower oil; TAG, triacylglycerol; VLDL, very low density lipoprotein.

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binding to the intimal proteoglycans (24). A predominance of small, dense LDL particles is also a main characteristic of the atherogenic lipoprotein phenotype (ALP), which is considered highly associated with IHD (25, 26).

Only limited information exists concerning the effect of diet on VLDL, IDL, and LDL and HDL subclasses. In a study of healthy men, the subjects with predominantly smaller LDL particles (pattern B) exhibited a significantly greater reduction in the mass of medium- and small-sized LDL subfractions compared with those with predominantly larger LDL particles (pattern A) when changing from a high fat to a low fat diet (27). This indicates that individuals with an ALP (pattern B) may benefit more from dietary modifications than individuals without an ALP (pattern A) (27). In the same group of subjects (28), a high saturated fat intake was associated with an increased mass of large cholesterol-enriched LDL particles. *n*-3 fatty acid supplementation to hypertriglyceridemic subjects has resulted in an increased number of large, buoyant LDL (29) and an increased LDL particle size (30) compared with baseline. Thus, the limited available evidence suggests that the amount and type of dietary fat influence the LDL subfraction profile, but it is not clear whether common edible vegetable oils leading to varying blood lipid levels also differ with respect to lipoprotein subclass profiles.

In the present study, we therefore compared the effects of three experimental diets rich in different vegetable oils (olive oil [OO], rapeseed oil [RO], and sunflower oil [SO]) on blood lipids and on the number, size, and composition of LDL and HDL subfractions. It was hypothesized that RO, because of its lower content of saturated fatty acids (SFA) compared with OO and its higher content of an *n*-3 fatty acid, would be associated with the most favorable lipoprotein subclass profile. Furthermore, we expected that SO, because of its higher content of polyunsaturated fatty acids (PUFA), would lead to lower blood lipid concentrations, but higher, less favorable total and LDL:HDL cholesterol ratios compared with the OO and RO diets. The effects on blood coagulation factor VII (31) and the oxidation resistance of VLDL and LDL particles (N. S. Nielsen, P. Marckmann, and C-E. Høy, unpublished observations) were also studied, but are presented separately.

## SUBJECTS AND METHODS

### Subjects

Eighteen male students were recruited for the study by local advertisement. The subjects were aged 20–28 years (mean, 24 years), weighed from 62 to 99 kg (mean, 79 kg), and had body mass indexes from 18 to 27 kg/m<sup>2</sup> (mean, 23 kg/m<sup>2</sup>). All subjects were nonsmokers and did not use any medication. They were apparently healthy, did not exercise excessively, and had no family history of atherosclerotic disease or hypertension. Mean fasting lipid concentrations at inclusion were as follows: plasma total cholesterol, 4.74 mM (range, 3.09 to 6.25 mM); HDL-cholesterol, 1.10 mM (range, 0.84 to 1.50 mM); and plasma total TAG, 1.2 mM (range, 0.41 to 3.29 mM). The aim of the study was explained orally to each subject and written information was

given, before the subjects gave their written consent. The research protocol was approved by the Scientific Ethics Committee of the municipalities of Copenhagen and Frederiksberg (01-272/95).

### Experimental design

The study was performed as a double-blinded, randomized, cross-over experiment with three periods of 3 weeks separated by wash-out periods of 5–12 weeks. Six groups of three subjects consumed the diets in the following order: OO, RO, SO/OO, SO, RO/RO, OO, SO/RO, SO, OO/SO, OO, RO/SO, RO, OO, respectively. Subjects were carefully instructed not to change habitual diets during the wash-out periods and to keep the same physical activity level during the study. Subjects received all foods from our department and were not allowed to consume other foods in the study periods, except for water, plain coffee, and tea (coffee and tea in small amounts). Subjects were allocated to fixed energy levels according to body weight, age, and physical activity indexes. Body weight was measured every second day in each period, and a weight variation of more than 1 kg resulted in either the allocation to a different energy level or to consumption of extra rolls (same nutrient composition as the total diet). The mean energy intake during the study was 15.4 MJ/day (range, 13–18 MJ). Consumption of extra rolls was noted in a diary, as were daily records of physical activity, any sign of illness, medication, coffee and tea intake, and any deviation from the diet. On weekdays lunch was consumed in the department under supervision, whereas beverages, dinner, snack, and breakfast for the next morning were provided daily as a package with guidelines for its preparation. Food and beverages for the weekend were provided on Fridays. Leftovers were brought back to the department to be registered. The amounts of returned foods were small and did not affect the average fat intake and fat composition for any of the subjects. Most food for each intervention period was prepared in one batch in the metabolic kitchen, weighed, coded in color according to the oil used, and frozen until use. Duplicate portions of each diet were collected during 2 weeks in each period of the study. The portions were pooled per diet and analyzed for energy content and nutritional composition.

### Diet

The three diets were identical except for the quality of fat. Nineteen percent of the total dietary energy intake, that is, 50 g/10 MJ, derived from either extra virgin OO (Navarino, Danton Trading, Århus, Denmark), physically refined RO (in a pilot scale) (Department of Biotechnology, Technical University of Denmark, Lyngby, Denmark), or chemically refined SO (Solex W, Århus Olie, Århus, Denmark). All other food items were held constant and identical in the three experimental diet periods. Menus were repeated every week and consisted of common prepared and cooked foods. The test oils were included in bread, rolls, cakes, dressing, and dinner dishes consisting of vegetables, meat, pasta, rice, or potatoes.

### Food analysis

Duplicate portions of each of the three 3-week diets were collected, homogenized, lyophilized, and chemically analyzed. Nitrogen was determined according to the principle of Dumas (32) on an automatic nitrogen analyzer (NA 1500; Carlo Erba Strumentazione, Milan, Italy). Fat content was measured after extraction according to the procedure by Folch, Lees, and Stanley (33), and the content of dietary fiber was determined enzymatically (34). The diet macronutrient composition is shown in **Table 1**.

The fatty acid composition of the 3-week diets was analyzed by gas chromatography (8420; Perkin-Elmer, Birkerød, Denmark) after extraction with diethyl ether-petroleum ether and subse-

TABLE 1. Analyzed macronutrient composition (% of total energy) and fiber and cholesterol content (g/10 MJ) of olive oil, rapeseed oil, and sunflower oil diets

	OO Diet	RO Diet	SO Diet
Fat	35	35	35
Saturated fatty acids	11	9	10
Monounsaturated fatty acids	21	18	9
Polyunsaturated fatty acids	3	7	15
Carbohydrates	53	52	53
Protein	12	13	12
Fiber (g/10 MJ)	25	22	24
Cholesterol (mg/10 MJ) <sup>a</sup>	257	259	257

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil.

<sup>a</sup>The cholesterol content of the experimental diet without added oil was calculated by the use of a national database (Dankost 2.0, Danish Catering Center A/S, Herlev, Denmark) and the analyzed cholesterol content of the oils was added.

quent methylation with methanolic BF<sub>3</sub> (35) by the National Food Administration in Denmark (Table 2). The squalene and sterol contents of the oils (Table 3) were determined from non-saponifiable material by gas-liquid chromatography on a 50-m-long capillary column (Ultra 1<sup>R</sup>; Hewlett-Packard, Palo Alto, CA) as trimethylsilyl derivatives, using 5 $\alpha$ -cholestane as internal standard (36), at the University of Helsinki (Helsinki, Finland).

### Blood sampling

Fasting blood samples were taken after 10 min of supine rest before the study and on days 21 and 22 in each diet period. Blood samples were collected in tubes without additives for the analysis of serum C-reactive protein (CRP), and in ethylenediaminetetraacetic acid tubes for the analysis of plasma insulin, NEFA, cholesterol, TAG, apolipoproteins, squalene, sterols, fatty acid composition of plasma cholesteryl esters, and lipoprotein fractionation. All plasma samples were immediately placed on ice, and centrifuged at 3,000 *g* for 15 min at 4°C. Samples for serum CRP were kept at -20°C, samples for plasma insulin, NEFA, squalene, sterols, and fatty acid composition of plasma cholesterol esters at -80°C, and samples for lipids, apolipoproteins, and lipoprotein fractionation were shipped the same day (to Freiburg University, Freiburg, Germany) and kept cooled (4°C) until analysis. Elevated CRP concentration in one subject on one occasion and technical problems with samples from one subject on one occasion resulted in the exclusion of these observations.

### Blood analysis

Serum CRP was assessed by a commercial immuno-turbidimetric method (Roche, Basel, Switzerland), plasma insulin by an enzyme-

TABLE 2. Analyzed fatty acid composition (mol%) of the three experimental diets containing 50 g/10 MJ of olive oil, rapeseed oil, and sunflower oil

Fatty Acids	OO Diet	RO Diet	SO Diet
Saturated fatty acids, total	31	27	29
Lauric acid (C <sub>12:0</sub> )	1	1	1
Myristic acid (C <sub>14:0</sub> )	3	4	3
Palmitic acid (C <sub>16:0</sub> )	19	15	16
Stearic acid (C <sub>18:0</sub> )	5	4	6
Monounsaturated fatty acids, total	60	50	27
Oleic acid (C <sub>18:1, n-9</sub> )	55	45	24
Polyunsaturated fatty acids, total	8	21	42
Linoleic acid (C <sub>18:2, n-6</sub> )	7	15	41
$\alpha$ -Linolenic acid (C <sub>18:3, n-3</sub> )	0.8	6	0.8

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil.

TABLE 3. Sterol and squalene concentrations of the test oils (mg/kg)

	Olive Oil	Rapeseed Oil	Sunflower Oil
Cholesterol	10	27	7
Campesterol	55	2,753	543
Sitosterol	958	3,892	2,417
Squalene	3,651	10	134

linked immunosorbent assay method (Dako, Glostrup, Denmark), and plasma NEFA by a commercial enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). The fatty acid composition of plasma cholesteryl esters was determined after separation by thin-layer chromatography. Plasma cholesteryl esters were methylated with BF<sub>3</sub> (35) and the methyl esters were analyzed by gas chromatography (HP 6890; Hewlett-Packard) at the Technical University of Denmark. Plasma squalene and noncholesterol sterols were determined as described in the food analysis section.

VLDL ( $d < 1.0063$  g/ml), IDL ( $1.0063$  g/ml  $< d < 1.019$  g/ml), LDL ( $1.019$  g/ml  $< d < 1.063$  g/ml), and HDL ( $1.063$  g/ml  $< d < 1.21$  g/ml) were prepared by sequential flotation according to Lindgren et al. (37, 38). HDL subfractions (HDL<sub>2b</sub>,  $1.063$  g/ml  $< d < 1.100$  g/ml; HDL<sub>2a</sub>,  $1.100$  g/ml  $< d < 1.125$  g/ml; HDL<sub>3</sub>,  $1.125$  g/ml  $< d < 1.21$  g/ml) were prepared by equilibrium density gradient centrifugation according to Anderson et al. (39, 40) with minor modifications. Total LDL was fractionated into six density classes by equilibrium density gradient centrifugation as described previously (41). The density ranges of the subfractions as determined by precision refractometry (38) of blank gradients were as follows: LDL-1,  $d < 1.031$ ; LDL-2,  $1.031 < d < 1.034$ ; LDL-3,  $1.034 < d < 1.037$ ; LDL-4,  $1.037 < d < 1.040$ ; LDL-5,  $1.040 < d < 1.044$ ; LDL-6,  $d > 1.044$  g/ml. All centrifugation steps were performed at a temperature of 18°C using partially filled (6 ml) polycarbonate bottles in a 50 Ti rotor. Phospholipid (PL), free cholesterol (FC), total cholesterol (TC), and TAG were measured by automated (EPOS; Eppendorf, Hamburg, Germany) enzymatic methods. Reagent kits for TC and TAG were obtained from Boehringer Mannheim (Mannheim, Germany), kits for FC were purchased from Wako Chemicals, and kits for PL were from bioMérieux (Nürtingen, Germany). Apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II) were measured by end-point nephelometry (Behring, Marburg, Germany). ApoB was standardized according to the Centers for Disease Control (CDC, Atlanta, GA) standard. This standard was confirmed by amino acid analysis in an LDL-3/LDL-4 pool (41). Particle composition of apoB-containing particles was assessed by calculating the molar ratio of lipid per apoB, using previously published molecular weights (41). The average particle volume of each subfraction was calculated from the above-described number of lipid molecules per particle and the volume of the lipid molecules and the estimated volume of one apoB molecule (41). A radius corresponding to this volume was calculated, assuming a spherical lipoprotein particle. Radii calculated in this way are in good agreement with radii measured by X-ray small-angle scattering (41).

The described method for the analysis of cholesterol and TAG in lipoproteins resulted in a mean recovery for cholesterol of 0.85 (range, 0.79–0.92) and for TAG of 0.91 (range, 0.84–1.03). The discrepancy may be due to the fact that concentrations in plasma were measured in samples other than those used to determine concentrations in lipoproteins. HDL cholesterol was also measured by a classic polyethylene glycol precipitation method (Quantolip; Immuno AG, Vienna, Austria) and resulted in slightly higher HDL cholesterol levels than those presented in

this article. However, neither of the methods revealed significant differences in HDL cholesterol between the diets. In addition, total, chylomicron, VLDL, and LDL plus HDL cholesterol concentrations were measured in a different laboratory (Research Department of Human Nutrition, Frederiksberg, Denmark) (after separation by sequential ultracentrifugation) with kits from Boehringer Mannheim and the results showed lower cholesterol concentrations in total plasma and higher concentrations in the individual lipoproteins, resulting in better recovery for these data. However, the statistical analyses of the two sets of data resulted in similar results. Therefore, it was decided that the data including measurements of subfractions should be presented despite a risk of minor compositional bias due to the incomplete recovery.

The intra-assay coefficients of variation for the determinations were as follows: insulin, 5.0%; NEFA, 1.8%; sterols, 2.5%; LDL subfraction concentrations of FC, PL, and TC, 2–4.5%; of TAG, 4–8%; and of apoB, 3.0–5.8%, depending on the subfraction. The interassay coefficients of variation were as follows: insulin, 5.9%; NEFA, 1.7%; sterols, 4.8%.

### Statistical analysis

Results from each diet intervention period were compared by analysis of variance (ANOVA). If the ANOVA indicated significant differences ( $P \leq 0.05$ ), it was followed by post-hoc statistical analysis using modified *t*-tests according to the Bonferroni method ( $P$  values above 0.1 were not Bonferroni corrected). Plasma TAG, HDL<sub>2b</sub> cholesterol, and cholesterol ester linoleic acid concentrations were not distributed normally and were log transformed. No period or carryover effects were observed. Power calculations (power 0.85, significance level  $P < 0.05$ ) showed that with a sample size of 18 subjects it should be possible to detect a 0.2-mmol/l difference in total plasma cholesterol and a 0.1-mmol/l difference in plasma TAG (42). The SAS statistical package (SAS Institute, Cary, NC) was used for the statistical analyses.

## RESULTS

### Plasma concentrations of blood lipids, apolipoproteins, insulin, and NEFA

Fasting plasma total cholesterol concentrations were approximately 12% higher after consumption of the OO

TABLE 4. Blood lipids, lipoproteins, and lipid ratios in 18 healthy men after 3 weeks of consuming a diet rich in olive oil, rapeseed oil, or sunflower oil<sup>a</sup>

	OO Diet <sup>b</sup>	RO Diet <sup>b</sup>	SO Diet <sup>b</sup>
	<i>mM</i>		
Plasma total triacylglycerol	0.86 (0.07)	0.73 (0.04)	0.72 (0.05)
Plasma total cholesterol	4.15 (0.18)	3.67 (0.19)	3.74 (0.16)
VLDL cholesterol	0.33 (0.03)	0.25 (0.02) <sup>d</sup>	0.27 (0.02) <sup>c</sup>
LDL cholesterol	2.16 (0.14)	1.73 (0.14) <sup>e</sup>	1.89 (0.11) <sup>e</sup>
HDL cholesterol	0.97 (0.05)	0.98 (0.06)	0.90 (0.05)
LDL:HDL cholesterol	2.35 (0.22)	1.87 (0.19) <sup>c,f</sup>	2.23 (0.20)
Total:HDL cholesterol	4.44 (0.32)	3.90 (0.28) <sup>c</sup>	4.31 (0.28)

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

<sup>a</sup> Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction.

<sup>b</sup> Values are means (SEM).

<sup>c</sup>  $P < 0.05$ , <sup>d</sup>  $P < 0.01$ , <sup>e</sup>  $P < 0.001$  compared with olive oil.

<sup>f</sup>  $P < 0.05$  compared with sunflower oil.

diet compared with the RO and SO diets (ANOVA,  $P < 0.0001$ ), and fasting plasma TAG and apoB concentrations were approximately 20% higher after OO compared with RO and SO (ANOVA,  $P \leq 0.003$ ); see **Table 4** and **Fig. 1**. Plasma total cholesterol and TAG and apoB concentrations did not differ between the RO and SO diets. Plasma apoA-I concentrations were higher after OO and RO than after SO (ANOVA,  $P = 0.002$ ) (Fig. 1). Plasma apoA-II concentrations did not differ significantly between the three diets [mean (SEM), mg/dl; OO, 38.6 (1.1); RO, 36.5 (1.2); SO, 36.2 (1.0)], nor did fasting plasma insulin [mean (SEM), pM; OO, 30.6 (1.9); RO, 29.3 (1.8); SO, 28.4 (1.2)]. Plasma NEFA concentrations tended to be higher after OO than after SO, while no significant difference was observed between RO and the other oils [mean (SEM), mM; OO, 0.40 (0.04); RO, 0.38 (0.04); SO, 0.33 (0.03); ANOVA,  $P = 0.05$ ; OO vs. SO,  $P = 0.07$ ].

### VLDL, IDL, total LDL

The concentrations of cholesterol, TAG, PL, and apoB in VLDL, IDL, and LDL are shown in **Table 5**. The VLDL, IDL, and LDL cholesterol and PL concentration were significantly higher after the OO diet than after the RO and SO diets. The VLDL TAG concentration was significantly higher after OO than after SO, and the LDL TAG concentration was significantly higher after OO than after RO and SO (Table 5).

The concentration of apoB in VLDL, IDL, and total LDL particles, corresponding to the number of lipoprotein particles, was significantly higher after the OO diet than after the RO and SO diets, while no difference in apoB concentration was observed between the RO and SO diets (Table 5).

The number of lipid molecules in each lipoprotein particle is given in **Table 6**. No significant difference in the number of cholesterol molecules in each VLDL or LDL particle was observed. The number of cholesterol molecules (and cholesteryl ester, data not shown) in each IDL particle was higher after OO than after RO (ANOVA,  $P = 0.04$ ; OO vs. RO,  $P = 0.09$ ), while the number of TAG molecules in each IDL particle was significantly lower after OO than after SO, with results after RO intermediate. As a consequence of the virtually unchanged lipid composition in the lipoprotein particles, the calculated average size of VLDL, IDL, and LDL did not differ between the three diets [mean radius (SEM), nm: VLDL, 16.1 (0.2); IDL, 12.0 (0.1); LDL, 9.7 (0.1)].

### LDL subfractions

The cholesterol, TAG, and PL concentrations in the LDL subclasses exhibited the same pattern through all subclasses: concentrations were higher after OO than after RO and SO, with results after SO intermediate. With a few exceptions the differences were significant for the larger (LDL-1, LDL-2) and medium-sized (LDL-3, LDL-4) LDL subfractions and not significant for the small, dense LDL particles (LDL-5, LDL-6). Cholesterol and PL concentrations were significantly higher after OO than after RO in LDL-1 to LDL-4, and higher after OO than after SO in

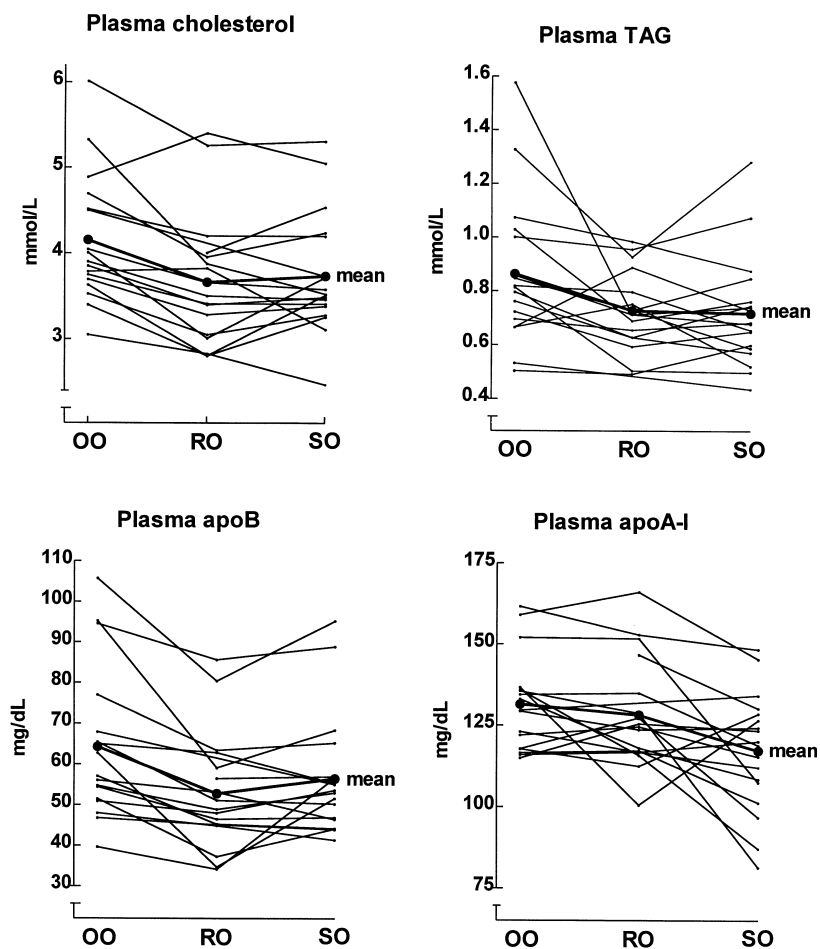


Fig. 1. Individual and mean concentrations of plasma cholesterol, TAG, apoB, and apoA-I in 18 healthy males after 3 weeks of consuming OO, RO, or SO.

LDL-1 to LDL-3. LDL-1, LDL-2, and LDL-3 TAG concentrations were higher after OO than after RO and SO. No significant differences in cholesterol, TAG, and PL between the RO and SO diets were observed.

The concentration of apoB (corresponding to number of LDL subfractions) was significantly higher after OO than after RO in LDL-1 to LDL-5, and significantly higher after OO than after SO in LDL-1 to LDL-3 (Fig. 2). The apoB concentration and the lipid content of

LDL subclasses did not differ between the RO and SO diets.

The number of cholesterol molecules per LDL particle did not differ significantly between the diets for any LDL subfraction (Table 6). However, in all LDL subclasses, the number of TAG molecules per particle was higher after RO than after OO. This was statistically significant for total LDL, LDL-1, LDL-3, and LDL-6. There were no differences in the calculated average size of the

TABLE 5. Lipid and apolipoprotein B concentration in apoB-containing lipoproteins in 18 healthy males after 3 weeks of consuming a diet rich in olive oil, rapeseed oil, or sunflower oil<sup>a</sup>

	Cholesterol <sup>b</sup>			Triacylglycerols <sup>b</sup>			Phospholipids <sup>b</sup>			Apolipoprotein B <sup>b</sup>		
	OO	RO	SO	OO	RO	SO	OO	RO	SO	OO	RO	SO
	<i>mM</i>			<i>mM</i>			<i>mg/dl</i>			<i>mg/dl</i>		
VLDL	0.33 (0.03)	0.25 <sup>d</sup> (0.02)	0.27 <sup>c</sup> (0.02)	0.56 (0.05)	0.46 <sup>c</sup> (0.03)	0.47 (0.04)	15.8 (1.4)	12.7 <sup>d</sup> (0.9)	13.0 <sup>c</sup> (1.1)	4.4 (0.4)	3.5 <sup>d</sup> (0.2)	3.5 <sup>d</sup> (0.3)
IDL	0.12 (0.02)	0.08 <sup>d</sup> (0.01)	0.08 <sup>d</sup> (0.01)	0.04 (0.003)	0.04 (0.002)	0.04 (0.003)	3.3 (0.4)	2.5 <sup>d</sup> (0.2)	2.6 <sup>c</sup> (0.2)	2.0 (0.3)	1.5 <sup>c</sup> (0.1)	1.4 <sup>d</sup> (0.1)
LDL	2.16 (0.14)	1.73 <sup>e</sup> (0.14)	1.89 <sup>e</sup> (0.11)	0.10 (0.007)	0.09 <sup>c</sup> (0.005)	0.09 <sup>d</sup> (0.004)	49.9 (3.0)	40.6 <sup>e</sup> (2.9)	43.2 <sup>e</sup> (2.4)	50.3 (3.3)	39.5 <sup>e</sup> (2.7)	42.7 <sup>d</sup> (2.8)

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein.

<sup>a</sup> Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction.

<sup>b</sup> Values are means (SEM).

<sup>c</sup>  $P < 0.05$ , <sup>d</sup>  $P < 0.01$ , <sup>e</sup>  $P < 0.001$  compared with olive oil.

TABLE 6. Number of lipid molecules per particle (molar ratio of lipid to apoB) in 18 healthy males after 3 weeks of consuming a diet rich in olive oil, rapeseed oil, or sunflower oil<sup>a</sup>

	Cholesterol <sup>b</sup>			Triacylglycerols <sup>b</sup>		
	OO	RO	SO	OO	RO	SO
VLDL	3,840 (109)	3,687 (84)	3,924 (142)	6,739 (269)	6,766 (170)	6,754 (259)
IDL	2,947 (103)	2,707 (96) <sup>c</sup>	2,889 (93)	1,315 (83)	1,511 (75)	1,615 (97) <sup>d</sup>
LDL	2,215 (32)	2,250 (45)	2,301 (57)	109 (5)	127 (6) <sup>d</sup>	114 (4)
LDL-1	2,643 (50)	2,684 (73)	2,815 (60)	156 (10)	194 (12) <sup>c</sup>	183 (12)
LDL-2	2,469 (43)	2,587 (57)	2,565 (54)	104 (8)	120 (9)	107 (7)
LDL-3	2,296 (49)	2,422 (48)	2,458 (60)	84 (6)	101 (7) <sup>c</sup>	89 (4)
LDL-4	2,241 (34)	2,359 (48)	2,330 (49)	85 (4)	97 (7)	87 (4)
LDL-5	2,103 (32)	2,187 (40)	2,193 (46)	88 (4)	98 (6)	92 (4)
LDL-6	1,821 (34)	1,855 (43)	1,813 (38)	126 (6)	144 (8) <sup>c</sup>	131 (5)

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein.

<sup>a</sup> Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction.

<sup>b</sup> Values are means (SEM).

<sup>c</sup>  $P < 0.05$ , <sup>d</sup>  $P < 0.01$  compared with olive oil.

LDL subfractions between the three diets [mean diameter (SEM), nm: LDL-1, 10.3 (0.1); LDL-2, 10.0 (0.1); LDL-3, 9.8 (0.1); LDL-4, 9.7 (0.1); LDL-5, 9.6 (0.1); LDL-6, 9.3 (0.1)].

### HDL

Total HDL cholesterol tended to be higher after OO and RO than after SO, although this difference was not statistically significant (ANOVA,  $P = 0.06$ ). The lipid (cholesterol, TAG, and PL) and apolipoprotein (apoA-I and apoA-II) contents in HDL<sub>2a</sub> were significantly higher after OO and RO than after SO, whereas higher TAG and PL (OO) concentrations were observed after OO and SO compared with RO in HDL<sub>3</sub> (Table 7). No significant differences in cholesterol, TAG, and PL concentration in HDL, HDL<sub>2a</sub>, or HDL<sub>2b</sub> between the OO and RO diet were observed.

The higher apoA-I concentration in plasma after OO and RO compared with SO was accompanied by increased concentrations of apoA-I and apoA-II in HDL<sub>2a</sub> after OO and RO (Table 8). No other differences in apoA-I or apoA-II in the HDL subfractions between the diets were observed.

### Ratios

The LDL cholesterol:HDL cholesterol ratio was significantly higher after OO and SO compared with RO (Table 4). Similarly, the total cholesterol:HDL cholesterol ratio, referred to as an atherogenic index (43), was significantly higher after OO than after RO. The total:HDL cholesterol ratio was higher after SO compared with RO, but only with borderline significance (ANOVA,  $P = 0.01$ ; RO vs. SO,  $P = 0.06$ ); see Table 4.

### Plasma squalene and sterol concentrations

The plasma squalene and noncholesterol sterol concentrations are presented as ratios to plasma cholesterol ( $100 \times$  mmol/mol of cholesterol) in order to eliminate the variation in cholesterol because the sterols are transported mainly in LDL. The plasma ratios of the cholesterol precursors squalene and desmosterol were significantly higher after the OO diet compared with the RO and SO diets (Table 9). The plasma  $\Delta^8$ -lathosterol:cholesterol ratio was higher after OO and RO compared with SO, whereas the plasma  $\Delta^7$ -lathosterol:cholesterol ratio was higher after RO compared with SO. The plasma sitosterol:cholesterol ratio was significantly lower after consumption of the OO diet compared with the RO and SO diets, whereas the plasma campesterol:cholesterol ratio was significantly different between all three diets: highest after the RO diet and lowest after the OO diet, with intermediate concentrations after the SO diet (Table 9).

### Fatty acid composition of plasma cholesterol esters

The fatty acid composition of cholesteryl esters in plasma was analyzed to assess dietary compliance. The results confirm a high compliance. The oleic, linoleic, and  $\alpha$ -linolenic acid concentrations in plasma cholesteryl esters closely re-

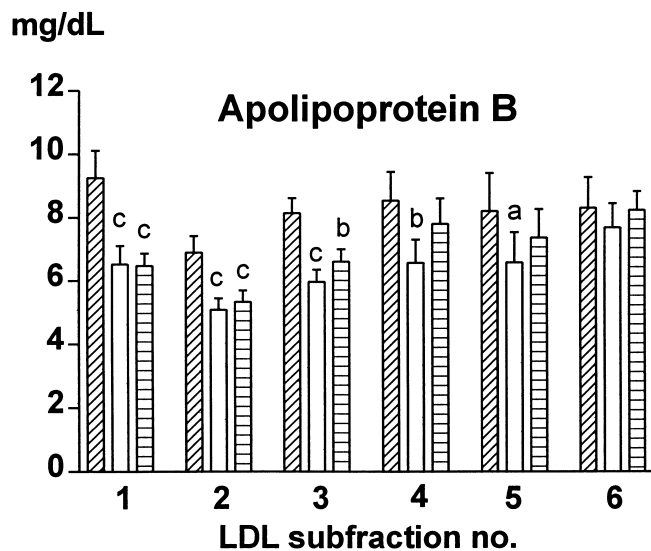


Fig. 2. Concentration of apoB in LDL subfractions in 18 healthy males after 3 weeks of consuming OO (hatched), RO (clear), or SO (horizontal lines). Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction. <sup>a</sup>  $P < 0.05$ , <sup>b</sup>  $P < 0.01$ , <sup>c</sup>  $P < 0.001$  compared with OO.

TABLE 7. Lipid concentration in HDL fractions in 18 healthy males after 3 weeks of consuming a diet rich in olive oil, rapeseed oil, or sunflower oil<sup>a</sup>

	Cholesterol <sup>b</sup>			Triacylglycerols <sup>b</sup>			Phospholipids <sup>b</sup>		
	OO	RO	SO	OO	RO	SO	OO	RO	SO
	<i>mM</i>			<i>mM</i>			<i>mg/dl</i>		
HDL	0.97 (0.05)	0.98 (0.06)	0.90 (0.05)	0.068 (0.003)	0.063 (0.003)	0.062 (0.002)	55.8 (2.8)	54.6 (3.0)	48.4 (2.9)
HDL <sub>2b</sub>	0.24 (0.03)	0.25 (0.03)	0.22 (0.03)	0.016 (0.001)	0.016 (0.001)	0.016 (0.001)	12.1 (1.8)	12.7 (1.7)	10.6 (1.4)
HDL <sub>2a</sub>	0.33 (0.02) <sup>c</sup>	0.33 (0.02) <sup>c</sup>	0.29 (0.02)	0.025 (0.001) <sup>c</sup>	0.023 (0.001) <sup>c</sup>	0.021 (0.001)	20.2 (1.2) <sup>d</sup>	20.1 (1.4) <sup>d</sup>	17.0 (1.3)
HDL <sub>3</sub>	0.40 (0.01)	0.39 (0.01)	0.39 (0.01)	0.028 (0.002)	0.023 (0.001)	0.025 (0.001) <sup>e</sup>	23.1 (0.6) <sup>c</sup>	21.5 (0.6)	20.9 (0.8)

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil; HDL, high density lipoprotein.

<sup>a</sup> Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction.

<sup>b</sup> Values are means (SEM).

<sup>c</sup> *P* < 0.05, <sup>d</sup> *P* < 0.01 compared with sunflower oil.

<sup>e</sup> *P* < 0.05 compared with rapeseed oil.

flected the presence of the particular fatty acid in the test diets (31). In accordance, the oleic:linoleic acid ratio in plasma cholesteryl esters was highest after consumption of OO and lowest after consumption of SO, with intermediate values after consumption of RO [mean (SEM): OO, 0.62 (0.03); RO, 0.34 (0.002); SO, 0.20 (0.01) ANOVA, *P* < 0.0001; all comparisons, *P* < 0.0001].

## DISCUSSION

In this strictly controlled dietary experiment in healthy men, we studied the effects of consumption of diets rich in monounsaturated fatty acids (MUFA) (OO and RO) or in PUFA (SO) on number, size, and composition of LDL and HDL subclasses. In addition, the effects on total cholesterol, VLDL, IDL, LDL, and HDL cholesterol, and plasma insulin, TAG, and NEFA were studied. For many of the measured variables the effect of OO differed distinctly from the effect of the other two oils. The subjects' baseline plasma cholesterol concentrations were relatively low (mean, 4.74 mM), but close to those observed for this age group in large population studies in Denmark (44). In addition, previous studies have shown that changes in dietary fatty acid composition affect blood lipid concentrations even in those subjects with low initial concentrations (45, 46). In this study, nine (50%) of the subjects had plasma cholesterol <5 mM. However, their responses to

the three experimental diets were similar to those with plasma cholesterol >5 mM.

The OO diet resulted in higher concentrations of VLDL, IDL, and LDL particles (number and lipid content) than the RO and SO diets. The observed differences were statistically significant for VLDL, IDL, total LDL, and the large and medium-sized LDL subfractions (LDL-1 to LDL-4). No significant differences between the diets were observed in number or composition of the smallest, dense LDL-6 particles, but OO compared with RO consumption resulted in a higher number of the small LDL-5 (Fig. 2). In contrast to the observed strong association between a predominance of small, dense LDL and an increased risk of IHD, it is currently unknown to what extent an increased number of large LDL particles, as observed after the OO diet, is unfavorable in regard to IHD. A study of normolipidemics reported that LDL from subjects with a predominance of large or small LDL particles exhibited weaker LDL receptor-binding affinity than LDL from subjects with a predominance of medium-sized LDL particles (47). In addition, increased concentrations of large, buoyant LDL particles have been found in subgroups of patients with IHD (48, 49).

One study demonstrated an increased LDL particle size after n-3 fatty acid supplementation (30). In this study, an RO rich diet containing a relatively high amount of  $\alpha$ -linolenic acid did not affect the size of the LDL subfractions (or any other lipoproteins) differently than the OO

TABLE 8. Apolipoprotein concentration in HDL fractions in 18 healthy males after 3 weeks of consuming a diet rich in olive oil, rapeseed oil, or sunflower oil<sup>a</sup>

	Apolipoprotein A-I <sup>b</sup>			Apolipoprotein A-II <sup>b</sup>		
	OO	RO	SO	OO	RO	SO
	<i>mg/dl</i>			<i>mg/dl</i>		
HDL	91.4 (3.3)	90.4 (4.0)	84.3 (4.3)	30.7 (1.0)	28.6 (1.2)	28.5 (1.1)
HDL <sub>2b</sub>	11.4 (2.0)	12.5 (2.3)	9.8 (1.5)	2.8 (0.3)	2.8 (0.3)	2.8 (0.2)
HDL <sub>2a</sub>	26.0 (1.5) <sup>c</sup>	26.0 (1.8) <sup>c</sup>	23.1 (1.8)	9.4 (0.5) <sup>c</sup>	9.6 (0.5) <sup>c</sup>	8.5 (0.6)
HDL <sub>3</sub>	54.1 (1.7)	49.8 (1.6)	50.3 (2.2)	20.5 (0.9)	19.2 (0.6)	20.2 (0.9)

Abbreviations: OO, olive oil; RO, rapeseed oil; SO, sunflower oil; HDL, high density lipoprotein.

<sup>a</sup> Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction.

<sup>b</sup> Values are mean (SEM).

<sup>c</sup> *P* < 0.05 compared with sunflower oil.

TABLE 9. Plasma squalene and noncholesterol sterols in 18 healthy males after 3 weeks of consuming a diet rich in olive oil, rapeseed oil, or sunflower oil<sup>a</sup>

	Olive Oil <sup>b</sup>	Rapeseed Oil <sup>b</sup>	Sunflower Oil <sup>b</sup>
	<i>mmol/mol of cholesterol × 10<sup>-2</sup></i>		
Squalene	58 (3)	37 (2) <sup>c</sup>	31 (2) <sup>c</sup>
Δ <sup>8</sup> -Lathosterol	20 (2)	20 (1)	16 (1) <sup>d</sup>
Desmosterol	98 (4)	70 (3) <sup>c</sup>	73 (4) <sup>c</sup>
Δ <sup>7</sup> -Lathosterol	169 (11)	181 (8)	156 (8) <sup>e</sup>
Cholestanol	136 (6)	144 (9)	137 (6)
Campesterol	191 (10)	433 (22) <sup>c</sup>	271 (20) <sup>c,f</sup>
Sitosterol	165 (8)	205 (12) <sup>c</sup>	204 (10) <sup>c</sup>

<sup>a</sup> Statistical analysis: ANOVA followed by pairwise comparison by Student's *t*-test with Bonferroni correction.

<sup>b</sup> Values are mean (SEM).

<sup>c</sup> *P* < 0.001 compared with olive oil.

<sup>d</sup> *P* < 0.05 compared with olive oil and rapeseed oil.

<sup>e</sup> *P* < 0.01 compared with rapeseed oil.

<sup>f</sup> *P* < 0.0001 compared with rapeseed oil.

and SO diets. In one report, a high saturated fat intake (46 E%) (especially myristic and palmitic acid) was associated with an increased mass of large, cholesterol-enriched LDL particles (28). Although the differences in the amount of myristic and palmitic acid in the OO diet (3 and 19 mol%, respectively) compared with the RO (4 and 15 mol%, respectively) and SO diets (4 and 16 mol%, respectively) were small it may have contributed to the higher number of the large and medium-sized LDL subfractions after the OO diet observed in this study. An alternative explanation could be a different impact of the fatty acid components on hepatic lipase (HL) activity. An inverse relationship between the concentration and size of large, buoyant LDL particles and HL activity has been reported (28, 50, 51).

It has been suggested that n-3 fatty acids from fish oil affect the composition of lipoprotein subfractions favorably by lowering the TAG content (29), but the interpretation of the composition data is not clear. A reduced TAG content might be favorable because TAG-poor LDL particles are catabolized faster than TAG-rich particles (29). However, a reduction of the TAG content may also result in a reduction of the size of the particle, which is not considered favorable. Our data showed that RO consumption resulted in a higher number of TAG particles in most LDL subfractions compared with OO and SO without affecting the size of the lipoproteins.

In this study, HDL cholesterol concentrations tended to be higher after OO and RO compared with SO (*P* = 0.06). In accordance, Mata et al. (43) found lower HDL cholesterol and apoA-I levels after a PUFA-rich diet compared with a MUFA-rich diet, while Carmena et al. (52) found lower HDL cholesterol after SO compared with OO. Information about the effect of diet on HDL subclasses is limited. We observed a lower concentration of cholesterol, TAG, and PL in HDL<sub>2a</sub> after SO compared with OO and RO. The relatively high intake of n-6 PUFA during the SO diet (15 E%) may be the reason for the lower apoA-I concentration in plasma and HDL<sub>2a</sub> observed after SO (53). In contrast, Valsta et al. (54) observed no differ-

ences in HDL<sub>2</sub> or HDL<sub>3</sub> in healthy women and men between RO and SO diets, while Dreon et al. (55) reported a higher HDL<sub>2</sub> cholesterol after PUFA compared with MUFA. An increment in HDL<sub>3</sub> after fish oil supplements has been reported in hypertriglyceridemic patients (56), while in type IIb hyperlipoproteinemia, n-3 fatty acids significantly increased HDL<sub>2b</sub> without alterations in HDL<sub>3</sub> (29). In this study the MUFA-rich diets containing OO and RO had more favorable effects on HDL subclasses than the PUFA-rich diet containing SO.

The conventional risk markers of IHD, plasma total cholesterol and LDL cholesterol, were significantly higher after 3 weeks of consuming a diet rich in OO compared with RO and SO. The observed difference between OO and RO/SO is not surprising, because similar results were obtained in other human experiments comparing OO and RO (57) and OO and SO (52, 58). In addition, it has been shown that an OO diet resulted in similar plasma cholesterol concentrations as a palmolein diet rich in SFA (59, 60), while RO resulted in a lower plasma cholesterol than palmolein (61). However, other comparisons of OO with RO or SO resulted in virtually similar blood lipid and LDL cholesterol concentrations (62, 63). In accordance with our results, similar blood lipid concentrations after RO- and SO-containing diets have been observed in a number of other studies (53, 64–66).

In this study the plasma cholesterol difference between OO and RO could be explained only partly by differences in fatty acid composition. According to algorithms of Hegsted et al. (4) and Keys, Anderson, and Grande (1) this study should result in a difference in plasma total cholesterol of 0.2–0.3 mM between OO and RO. The observed difference was larger than predicted, namely 0.5 mM, while the plasma cholesterol difference between OO and SO was virtually identical to the predicted difference.

OO is rich in squalene (3,651 mg/kg oil) compared with RO (10 mg/kg oil) and SO (134 mg/kg oil) (Table 3). The higher intake of squalene during the OO diet resulted in significantly higher plasma squalene:cholesterol ratios (Table 9). In addition, the plasma desmosterol:cholesterol ratio was also higher after the OO diet compared with RO and SO diets, indicating that a higher level of cholesterol synthesis took place during the OO diet. This is likely to have contributed to the higher cholesterol concentration after OO compared with RO and SO. In agreement, Miettinen and Vanhanen (67) found that squalene had cholesterol-raising properties when 1 g of squalene was added to RO. Plant sterols are absorbed to some extent (campesterol, 10–14%; sitosterol, 1–5%) (68) and the lower contents of campesterol and sitosterol in OO compared with RO and SO (Table 3) were reflected directly in the plasma ratios of these sterols (Table 9). Plant sterols interfere with intestinal cholesterol absorption (69) and have the ability to lower total and LDL cholesterol (70). The plasma levels of campesterol and sitosterol in healthy subjects have been found to be inversely related to the overall cholesterol synthesis (71). Because plasma ratios of campesterol and sitosterol to cholesterol were lower after the OO diet



compared with the RO and SO diets, this may offer an additional explanation for the higher plasma and LDL cholesterol concentrations observed after consumption of the OO diet.

The higher plasma TAG concentration after OO than after RO and SO observed in this study has been reported in some studies (43, 64), while a number of studies have not found OO to be hypertriglyceridemic compared with other oils (55, 63, 72). A meta-analysis designed to compare effects of MUFA and PUFA diets on blood lipids and lipoproteins reported consistently lower TAG concentrations after high PUFA diets (73). Thus, the lower content of PUFA in OO, compared with RO and SO, may contribute to the higher TAG concentrations after OO. In addition, if squalene enhances the hepatic cholesterol content because of an increased cholesterol synthesis, then the LDL receptor activity would be downregulated affecting the removal of both LDL, VLDL and IDL particles. This might explain the observed increase in VLDL TAGs (and total TAGs) observed in this study and by others after consumption of OO/squalene (67).

As hypothesized, this study resulted in higher LDL: HDL and total:HDL cholesterol ratios after the OO and SO diets compared with the RO diet. This is in accordance with Valsta et al. (53) who found a more favorable HDL<sub>2</sub>:LDL ratio after RO compared with SO, and with Carmena et al. (52) who reported no difference in total: HDL cholesterol ratio between OO and SO. In contrast, other studies have reported no differences in the LDL: HDL cholesterol ratio between RO and SO (65, 74) and conflicting results regarding the total:HDL cholesterol ratio when comparing OO and SO (43, 58). The total:HDL cholesterol ratio has been reported to be a better predictor of IHD than other conventional risk markers (total cholesterol, LDL cholesterol or TAG) in a number of studies (75). Thus, in this study the RO diet resulted in the most favorable lipid ratios compared with both OO and SO. In addition, all other measured conventional risk markers of IHD (HDL cholesterol, apoA, LDL cholesterol, apoB) and the LDL and HDL subfractions were more favorably affected by RO and SO compared with OO. Thus, taken together, the RO diet seemed to have more favorable effects with regard to blood lipid and lipoprotein subfractions compared with SO and RO. However, results from analyses of coagulation factors suggest that OO has an antithrombotic effect compared with SO (and RO) that might counteract its less favorable plasma lipid effects (31, 76).

In conclusion, the results from this study suggest that RO and SO rich diets compared with OO have more favorable effects on plasma lipid and lipoprotein concentrations in young healthy subjects. The differences may in part be attributed to differences in the amount of nonfatty acid components of the oils. In addition, when vegetable oils accounted for a substantial part of the total fat intake, the oil quality was shown to affect the LDL and HDL subclass profiles differently. Whether these differences may be due to differences in fatty acid composition is still to be resolved. ■

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