Different effects of diets rich in olive oil, rapeseed oil and sunflower-seed oil on postprandial lipid and lipoprotein concentrations and on lipoprotein oxidation susceptibility

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Elevated concentrations of fasting and non-fasting triacylglycerol-rich lipoproteins (TRL) as well as oxidative changes of lipoproteins may increase the risk of ischaemic heart disease. To compare the effects of different diets rich in unsaturated fatty acids on the concentrations and *in vitro* oxidation of fasting and postprandial lipoproteins eighteen males consumed diets enriched with rapeseed oil (RO), olive oil (OO), or sunflower-seed oil (SO) in randomised order for periods of 3 weeks followed by a RO test meal. In the postprandial state the concentrations of cholesterol and triacylglycerol (TAG) in TRL were higher after consumption of OO compared with RO and SO ($P<0.04$), possibly related to differences in the fasting state. The propagation rates for VLDL and LDL oxidation were higher in the postprandial compared with the fasting state irrespective of diet. In the fasting state, the propagation rates were highest after SO ($P<0.001$), and in the postprandial state, SO gave rise to a shorter VLDL lag time ($P=0.03$) and a higher propagation rate than OO consumption ($P=0.04$). Overall, the SO diet resulted in a higher postprandial propagation rate of LDL ($P<0.001$) compared with RO and OO, while there was no effect of diet on LDL oxidation lag time. Our results suggest that RO and SO diets lower the postprandial cholesterol and TAG concentrations compared with OO, while RO and OO diets result in similar and lower *in vitro* susceptibility to oxidation of lipoproteins than SO.

Dietary oils: Lipoproteins: Oxidation susceptibility: Vitamins

Subjects with IHD (Simpson *et al.* 1990; Grooth *et al.* 1991; Patsch *et al.* 1992) and offspring of IHD patients (Uiterwaal *et al.* 1994) have exaggerated triacylglycerol (TAG) responses to fat-containing meals, suggesting that a delayed catabolism of chylomicron (CM) remnants is involved in the pathogenesis of atherosclerosis (Karpe *et al.* 1994). The atherogenic remnant hypothesis, originally proposed by Zilversmit (1979), suggests that CM remnants carrying dietary cholesterol enter the artery wall and cause cholesterol deposition leading to the development of atherosclerosis and IHD. In accordance, non-fasting TAG concentrations after a standard fat load can predict progression of atherosclerosis (Nordestgaard & Tybjaerg Hansen, 1992). An involvement of TAG-rich lipoproteins in atherosclerosis is supported by results from a histological study showing liposomal structures

similar to surface remnants of TAG-rich lipoproteins in human atherosclerostic plaques (Chung *et al.* 1994). In addition, TAG enriched apolipoprotein (apo) B-containing lipoproteins have been isolated from human atherosclerostic plaques (Rapp *et al.* 1994). Furthermore, subjects with the atherogenic lipoprotein phenotype (Austin *et al.* 1990) exhibit increased postprandial TAG concentrations after fatty meals (Karpe, 1997).

Recently, a study reported very different TAG and apo B-48 responses to identical test meals in Northern and Southern Europeans, suggesting that the habitual diet composition and/or genetic factors may influence the acute postprandial response (Zampelas *et al.* 1998). Another study reported that intake of a Mediterranean-type background diet rich in olive oil (OO) resulted in earlier and higher peak plasma TAG concentrations compared with a

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diet rich in saturated fatty acids (SFA) in response to identical test meals (Roche *et al.* 1998). The postprandial TAG response observed after OO consumption (Roche *et al.* 1998) resembled that of the Southern Europeans (Zampelas *et al.* 1998) indicating that a background diet rich in OO or monounsaturated fatty acids (MUFA) may result in different postprandial lipid responses compared with other fats.

Oxidative damage of lipoproteins is suggested to participate in the initial events leading to CHD. Previous studies on lipoprotein oxidation have mainly focused on the LDL particle. However, it has been suggested that oxidation of VLDL may also be involved in the development of atherosclerosis (Keidar *et al.* 1992; Rapp *et al.* 1994; Whitmann *et al.* 1998). A postprandial increase in the plasma concentration of CM tends to result in an increase in VLDL concentration due to competition for lipoprotein lipase (Tinker *et al.* 1999). A prolonged residence time of VLDL may result in its increased interaction with the arterial wall and thereby possibly increase the risk of lipid peroxidation. A number of factors general for lipid oxidation influence the oxidation susceptibility of LDL particles (Esterbauer *et al.* 1992) and very likely also of VLDL particles. First, the fatty acid composition of the diet influences the sensitivity of lipoproteins to oxidation. Dietary linoleic acid increases the content of polyunsaturated fatty acids (PUFA) in lipoproteins thereby increasing their sensitivity to oxidation, while dietary oleic acid results in LDL particles less prone to oxidation (Berry *et al.* 1991; Esterbauer *et al.* 1992; Kleinveld *et al.* 1993; Reaven *et al.* 1993). Second, the content of endogenous antioxidants, in particular α-tocopherol, may affect the oxidation susceptibility of LDL particles (Esterbauer *et al.* 1992; Winklerhofer-Roob *et al.* 1995). Third, smaller and more dense particles are more susceptible to oxidation than larger buoyant particles (Bonanome *et al.* 1992; Reaven *et al.* 1993; Tribble *et al.* 1995). In addition, the number of LDL particles may be important for oxidation. Saturation of LDL receptors leading to a longer half-life of LDL results in a greater susceptibility to oxidation.

LDL isolated in the postprandial state may be more atherogenic than LDL isolated in the fasting state, possibly due to a higher content of oxidation products (thiobarbituric acid-reacting substances) postprandially (Lechleitner *et al.* 1994). In addition, an increased content of PUFA in VLDL increases their susceptibility to oxidation (Hau *et al.* 1996). Therefore, investigation of postprandial response is important, since the serum TAG (and thereby VLDL) concentration is elevated during most of the day compared with the fasting level.

The objective of the present study was to compare the effects of the intake of diets rich in OO or rapeseed oil (RO), having with comparable fatty acid profiles, with and the more unsaturated sunflower-seed oil (SO). In this paper we focus on the effects on blood lipid parameters and on the susceptibility to oxidation of lipoproteins isolated in the fasting and the postprandial state. The effects on blood coagulation markers as well as on fasting lipids and lipoprotein subfractions have been published (Larsen *et al.* 1999; Pedersen *et al.* 2000).

Subjects and methods

Subjects

Eighteen healthy males were recruited for the study by local advertisement. Their mean age was 23·9 (range 20–28) years and their BMI was 22·9 (range 18·4–27·0) kg/m^2. All subjects were non-smokers and were not using any medication including dietary supplements. They were apparently healthy, did not exercise excessively (<2 h heavy physical exercise/week) and had no family history of atherosclerotic disease or hypertension. Mean fasting lipid concentrations were: serum total cholesterol 4·71 (range 2·46–6·01) mmol/l; HDL-cholesterol 1·09 (range 0·57–1·39) mmol/l; plasma total TAG 1·2 (range 0·43–1·58) mmol/l. Before the study informed consent was obtained from the volunteers according to the Second Helsinki Declaration and the protocol was approved by the Ethical Committee of Frederiksberg and Copenhagen (file no. 01-272/95). All subjects completed all phases of the study.

Study design and diets

The study design and experimental diets were previously described (Pedersen *et al.* 1999). In short, the experiment was a double-blinded randomised cross over study with

Table 1. Composition of the three experimental diets enriched in rapeseed oil, olive oil or sunflower-seed oil

(% of energy)

	Rapeseed oil	Olive oil	Sunflower-seed oil
Protein*	12·4	12·4	12·2
Fat*	32·8	31·9	32·1
Carbohydrate*	54·9	55·7	55·6
Vitamin E†			
Vitamin E (α-TE/MJ)	1·21	0·90	1·52
α-Tocopherol (mg/MJ)	1·11	0·88	1·49
Fibre* (g/MJ)	2·31	2·58	2·56

TE, tocopherol equivalents.

* Protein, fat, and fibre content were analysed; carbohydrate content was calculated.

† Values of vitamin E were determined from Dankost 2·0, and contributions from oils added.

three periods of 3 weeks with strict dietary control and 4–12 weeks wash-out periods in between. At the end of each 3-week period (on day 22) the volunteers were given two meals (1·6 and 5·8 MJ) separated by 1 h 45 min. Both test meals consisted of a low-fat basic rice dish (9 % of energy from fat) enriched with RO to a total of 42 % energy from fat. The first meal provided 17·4 g fat, 9·1 g protein and 42·8 g carbohydrate and the second meal provided 63·6 g fat, 32·3 g protein and 152·8 g carbohydrate. The subjects were asked not to take any medication or vitamin supplements 3 months before and during the study.

The diets were planned to have a total fat content of about 30 % energy. Fifty g per 10 MJ of either RO, OO or SO-seed was incorporated into the diets corresponding to 19 % energy (Table 1). Lunch was consumed at the Institute on weekdays while other meals as well as weekend meals were pre-packaged for consumption at home.

The oils used in the study were an extra virgin olive oil (Navarino; Danton Trading, Aarhus, Denmark), a chemically refined sunflower-seed oil (Solex W; Aarhus olie A/S, Aarhus, Denmark), and a physically refined rapeseed oil (Department of Biotechnology, Technical University of Denmark, Lyngby, Denmark). The fatty acid composition of the diets with the oil incorporated is given in Table 2.

Duplicate portions of experimental diets and of test oils were chemically analysed, as previously described (Larsen *et al.* 1999). Fibre was analysed according to Asp *et al.* (1983). For composition see Table 1.

Blood sampling and sample preparation

Blood samples were obtained from fasting subjects before the start of the study. On the test day (day 22) a fasting blood sample was taken (after 12 h fasting) and non-fasting blood samples were taken at 13.15 hours (4 h 15 min after the first meal), at 14.45 hours (5 h 45 min after the first meal) and at 17.45 hours (8 h 45 min after the first meal). A total of 190 ml of blood was collected on a test day. Blood samples were taken after 10 min of supine rest and

collected in tubes without additives for the analysis of C-reactive protein, and in EDTA-containing evacuated tubes (1 g/l) for other analyses. All samples were immediately placed on ice (except serum C-reactive protein) and centrifuged at 3000 *g* for 15 min at 4°C. Samples for serum C-reactive protein, plasma cholesterol and TAG were kept at −20°C: samples for lipoprotein fractionation were kept at 4°C until the next day. Samples for isolation of lipoproteins for determination of oxidation susceptibility had sucrose added (final concentration 10 % w/v) before freezing at −80°C (Rumsey *et al.* 1992).

All samples from each subject were analysed within a single run, except for the determination of cholesterol and TAG in lipoproteins, which were done in fresh plasma after every test day. Serum C-reactive protein was assessed by a commercial immuno-turbidumetric method (Roche, Basel, Switzerland).

On three occasions samples were excluded from analysis; once due to elevated C-reactive protein concentration in one subject (22 mg/l), once due to technical problems with samples from one subject and once due to one subject's inability to consume the test meals.

Analyses

Fat from the food and from LDL was extracted using the Folch method (Folch *et al.* 1957). The extracted fats from the diets and the LDL were methylated with KOH as catalyst (2 M in methanol, MeOH) (Christopherson & Glass, 1969). The fatty acid composition of the methyl esters were determined using a gas–liquid chromatograph as previously described (Sorensen *et al.* 1998). Fatty acids were identified by comparison with commercial standards (Nu-Check-Prep.; Elysian, MN, USA), quantified by the peak areas which were adjusted using response factors and the composition calculated as mol %.

From plasma, the CM (CM + large VLDL), VLDL (VLDL+CM), and LDL+HDL fractions were separated by sequential ultracentrifugation. CM were isolated by overlaying 3 ml of plasma with 2·5 ml of saline of density 1·006 kg/l according to the nomogram of Dole & Hamlin (1962). The UC tubes (Quick-seal polyallomer, 13 × 64 mm, Beckman Instruments, Palo Alto, CA, USA) were centrifuged for 23 min at 20°C at 100 000 *g* (L7-55: Beckmann Instruments) using a fixed angle rotor (50·4 Ti; Beckmann Instruments). The tubes were sliced 45 mm from the bottom, and the top fraction ($S_f > 400$) was transferred and adjusted to a total volume of 5 ml with saline. The bottom fraction was transferred to another UC tube, adjusted to 5·5 ml with saline of density 1·006 kg/l and centrifuged for 16 h at 4°C at 170 000 *g*. After tube slicing 35 mm from the bottom, the top fraction containing CM remnants and VLDL, and the bottom fraction containing LDL+HDL were transferred to separate tubes and both adjusted to a final volume of 5 ml. Cholesterol and TAG concentrations in total plasma and CM, VLDL and LDL+HDL fractions were assessed by commercial enzymic methods (Boehringer-Mannheim B-M CHOD-PAP 236-691, B-M 543-004, and GPO-PAP 701 912; Boehringer Mannheim GmbH, Mannheim, Germany) on a Cobas Mira analyser (Roche, Basel, Switzerland). HDL

Table 2. Fatty acid composition of the experimental diets enriched in rapeseed oil, olive oil or sunflower-seed oil*

(mol/100 mol total fatty acids; mean values of two determinations)

Fatty acid	Rapeseed oil	Olive oil	Sunflower-seed oil
<C16	10·1	10·1	11·4
C16:0	13·9	18·0	15·6
C16:1	1·3	1·7	1·2
C18:0	4·5	5·2	6·0
C18:1	46·7	56·0	24·5
C18:2	14·7	6·4	38·8
C18:3	6·8	1·3	1·1
>C18	1·8	1·0	1·3
SFA	28·7	33·5	33·4
MUFA	49·5	58·3	26·2
PUFA	21·8	8·2	40·4

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
* For details of diets and procedures, see Table 1 and p. 491.

cholesterol and HDL TAG concentrations were determined after precipitation with polyethylene glycol (Quantolip, Immuno AG, Vienna, Austria). LDL-cholesterol concentration was calculated as the difference between HDL-cholesterol concentration and the cholesterol concentration in the LDL+HDL fraction, similarly for LDL-TAG concentration. Control sera HK96 (DEKS, Clinical Biochemistry, Cph. County Herlev Hospital/University of Copenhagen, Denmark) and Precinorm L (Boehringer Mannheim GmbH, Mannheim, Germany) were used for quality control of the analyses. The intra-assay CV for the determinations were: cholesterol 1·0 %, HDL cholesterol 1·8 %, TAG 1·0 %. The inter-assay CV for the determinations were: cholesterol 1·5 %, HDL-cholesterol 3·6 %, TAG 0·7 %.

The procedures for preparation of lipoproteins for oxidation analysis were previously described (Sorensen *et al.* 1998) with modifications in the method of isolation as follows. Briefly, 3 ml of plasma from each subject that had been frozen and stored at $-80°C$ was rapidly thawed and used for isolation of VLDL and LDL by gradient ultracentrifugation at $285\,000\,g$ and 4°C. After 30 min the CM was isolated and the rest of the sample was ultracentrifuged for an additional 18 h to separate VLDL and LDL. Protein content was determined by the method of Lowry *et al.* (1951). Lipid peroxidation was performed *in vitro* as previously described (Sorensen *et al.* 1998). In short, the kinetics of the VLDL or LDL oxidation were determined by continuously monitoring the changes of absorbance at 234 nm (Princen *et al.* 1992, 1995) in a thermostat-controlled spectrophotometer (set at 37°C) with an automatic sample changer (UV-2101PC spectrophotometer, CPS controller and CPS-260 cell positioner; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). From the reaction curve, the lag time and propagation rate were determined. Absorbance curves of VLDL or LDL preparations obtained from each volunteer on the three diets were determined in parallel. Each VLDL or LDL preparation was subjected to three consecutive oxidation runs of 300 min within 24 h; the values shown for lag time and propagation rate are means of the three values. In every oxidation run one reference VLDL or LDL sample, prepared from a reference plasma stored at $-80°C$, was used as a control. Oxidation runs with >10 % deviation from the mean lag time or propagation rate of former measurements of the control were omitted.

The content of α-tocopherol, δ-tocopherol, γ-tocopherol, α-carotene and β-carotene in plasma and LDL was determined according to the method by Kaplan *et al.* (1990). One hundred μl α-tocopheryl acetate (9·89 μg/ml) was added as internal standard to the plasma−LDL fraction. The entire extraction procedure was performed in darkness. The vitamins were extracted twice with 3 ml hexane by mixing and centrifugation (5 min, $2000\,g$, room temperature) and the solvent evaporated in a vacuum centrifuge (Christ Alpha RVC; Christ, Oosterode am Harz, Germany). The sample was reconstituted in 150 μl acetonitril−chloroform (80:20, v/v) (HPLC-grade; Rathburn Chemicals Ltd, Walkerburn, Scotland) and filtered (Chromafil, AO-45/3; Macherey-Nagel, Düren, Germany). The samples were analysed by HPLC using an on-line degasser (ERC-3415; ERC Inc, Tokyo, Japan), a pump (Waters 510), an autosampler (Waters 717), a multiwavelength detector (490 Waters; Waters Corporation, Milford, MA, USA) and an LC-18 column 250 mm × 4·6 mm with 5 μm spherical particles (Supelco, Bellafonte, PA, USA). The mobile phase consisted of acetonitril−chloroform−2-propanol−water (78:16:3·5:2·5, by vol.) (HPLC-grade; Rathburn Chemicals Ltd, Walkerburn, Scotland) and the flow rate was 2 ml/min. The content of vitamins was determined using u.v. detection at 292 nm (for tocopherol) and 450 nm (for carotene) by comparison with a commercial standard (Sigma, St. Louis, MO, USA) using peak area for quantification.

Table 3. Fasting, postprandial peak and area under the curves (AUC) of plasma-, chylomicron (CM)-, VLDL-, LDL- and HDL-triacylglycerol (TAG) concentrations (mmol/l) after consumption of two consecutive test meals. The preceding diet was rich in olive oil (OO), rapeseed oil (RO) or sunflower-seed oil (SO)*

(Mean values and standard errors of the mean)

		RO		OO		SO		ANOVA†	*P* values‡			ANOVA§
		Mean	SEM	Mean	SEM	Mean	SEM	(*P* value)	RO-OO	OO-SO	RO-SO	(*P* value)
Plasma TAG:	Fasting	0·68	0·03	0·79	0·05	0·67	0·04	0·001	0·001	0·001	0·9	–
	Peak	1·91	0·17	2·06	0·16	1·75	0·14	0·05	0·1	0·01	0·4	0·6
	AUC (mmol h/l)	11·46	0·93	12·61	0·96	10·51	0·84	0·01	0·05	0·001	0·3	0·4
CM-TAG:	Fasting	0·12	0·01	0·16	0·02	0·12	0·01	0·001	0·001	0·01	0·3	–
	Peak	1·25	0·16	1·36	0·14	1·10	0·12	0·07	0·2	0·05	0·4	0·3
	AUC (mmol h/l)	6·82	0·79	6·21	0·78	5·33	0·66	0·04	0·1	0·01	0·3	0·2
VLDL-TAG:	Fasting	0·32	0·02	0·37	0·03	0·32	0·02	0·001	0·001	0·001	0·9	–
	Peak	0·41	0·03	0·45	0·03	0·40	0·03	0·01	0·01	0·01	0·9	0·7
	AUC (mmol h/l)	3·33	0·27	3·00	0·19	2·97	0·22	0·0002	0·0002	0·0005	0·7	0·2
LDL-TAG:	Fasting	0·12	0·007	0·13	0·01	0·12	0·007	0·06	0·06	0·05	0·8	–
	Peak	0·15	0·01	0·16	0·01	0·14	0·01	0·01	0·2	0·01	0·007	0·04
HDL-TAG:	Fasting	0·11	0·006	0·13	0·006	0·12	0·006	0·01	0·07	0·01	0·1	–
	Peak	0·14	0·005	0·16	0·008	0·14	0·005	0·05	0·05	0·01	0·7	0·4
	AUC (mmol h/l)	1·24	0·05	1·12	0·03	1·13	0·04	0·03	0·03	0·01	0·7	0·3

* For details of diets and procedures, see Tables 1 and 2, and p. 491.
† ANOVA without the fasting value in the model.
‡ *Post-hoc* pair wise comparison by Student's *t* test.
§ ANOVA with the fasting value as a covariate in the model.

Table 4. Fasting, postprandial peak, postprandial minimum and area under the curves (AUC) of plasma-, chylomicron (CM)-, VLDL-, LDL- and HDL-cholesterol concentrations (mmol/l) after consumption of two consecutive test meals. The preceding diet was rich in olive oil (OO), rapeseed oil (RO) or sunflower-seed oil (SO)*

(Mean values and standard errors of the mean)

		RO Mean	RO SEM	OO Mean	OO SEM	SO Mean	SO SEM	ANOVA† (P value)	P values‡ RO-OO	OO-SO	RO-SO	ANOVA§ (P value)
Plasma-cholesterol:	Fasting	3.50	0.17	4.07	0.19	3.62	0.14	0.001	0.001	0.001	0.2	–
	Minimum	3.39	0.16	3.91	0.17	3.50	0.14	0.001	0.001	0.001	0.2	0.9
CM-cholesterol:	Fasting	0.04	0.004	0.06	0.008	0.05	0.01	0.2	–	–	–	–
	Peak	0.14	0.02	0.16	0.02	0.12	0.01	0.05	0.07	0.01	0.3	0.01
	AUC (mmol h/l)	0.96	0.11	0.81	0.10	0.71	0.09	0.004	0.02	0.001	0.4	0.009
VLDL-cholesterol:	Fasting	0.17	0.01	0.20	0.02	0.16	0.02	0.001	0.01	0.001	0.7	–
	Peak	0.22	0.02	0.24	0.02	0.22	0.02	0.01	0.01	0.01	0.9	0.5
LDL-cholesterol:	Fasting	2.20	0.16	2.73	0.18	2.33	0.14	0.001	0.001	0.001	0.07	–
	Minimum	2.06	0.15	2.54	0.16	2.18	0.13	0.001	0.001	0.001	0.08	0.9
HDL-cholesterol:	Fasting	1.12	0.06	1.13	0.05	1.09	0.05	0.4	–	–	–	–
	Minimum	1.05	0.06	1.07	0.06	1.03	0.06	0.5	–	–	–	0.5

* For details of diets and procedures, see Tables 1 and 2, and p. 491.
† ANOVA without the fasting value in the model.
‡ *Post-hoc* pair-wise comparison by Student's *t*test.
§ ANOVA with the fasting value as a covariate in the model.

Statistics

Results are presented as mean values with their standard errors of the mean. All variables were normally distributed. To overcome the problem of interdependence of repeated measurements the data analyses are based on summary statistics (Matthews *et al.* 1990). Maximal postprandial concentration (peak) and area under the postprandial response curve were selected as physiologically relevant summary measurements for TAG and cholesterol levels, and mean values for oxidation data, fatty acid composition and vitamin content. An ANOVA with subject and preceding diet as independent variables was applied to the summary measures relevant for the particular variable. ANOVA was repeated with the fasting value as covariate in the model for TAG and cholesterol. No period or carry-over effects were observed. For each fat, paired *t* tests were used to analyse changes between fasting and postprandial values. Correlations were tested by calculating the Pearson's correlation coefficient (r) with r^2 giving the percentage variation. Power calculations (power 0·85, significance level $P < 0.05$) showed that with a sample size of eighteen subjects it should be possible to detect differences in postprandial plasma TAG peak concentrations of 0·25 mmol/l (Altman, 1995).

Results

Triacylglycerol and cholesterol concentrations

Food records and plasma analysis of TAG- and cholesterol-fatty acids confirmed good subject compliance (Larsen *et al.* 1999). Fasting and postprandial total plasma- and lipoprotein-TAG concentrations differed significantly between diets (Table 3). Intake of OO for 3 weeks was associated with significantly higher postprandial plasma-, CM- and VLDL-TAG concentrations than RO and SO while the RO- and SO-rich diets resulted in similar responses except for HDL-TAG. Differences in postprandial plasma-, CM- and VLDL-TAG concentrations between diets did not maintain statistical significance when the fasting value was included as a covariate in the statistical analysis (Table 3). Similarly, differences in postprandial HDL-TAG concentrations between the diets were not statistically different from the fasting value in the statistical model. However, the HDL-TAG peak appeared later after OO and RO than after SO with the fasting value included in the statistical analysis (time after the second meal: OO 3 h 35 min (SEM 0.16 h), RO 3 h 5 min (SEM 0.18 h), SO 3 h 34 min (SEM 0.17 h), ANOVA, $P = 0.001$).

Fasting and postprandial total and lipoprotein cholesterol concentrations differed significantly between the diets in the same way as TAG concentrations, except for HDL-cholesterol, where no differences in fasting or postprandial concentrations were observed (Table 4). Postprandial total plasma-, CM-, VLDL-, and LDL-cholesterol were significantly higher following 3 weeks intake of an OO-rich diet compared with RO- or SO-rich diets. The postprandial differences in total plasma- and LDL-cholesterol did not maintain statistical significance when the fasting values were included in the model (Table 4). Fasting CM cholesterol concentrations did not differ between the

diets. However, the postprandial CM peak and area under the curve cholesterol concentrations were higher after OO compared with RO and SO. These differences maintained statistical significance after inclusion of the fasting value in the model. VLDL mean cholesterol concentrations maintained borderline statistical significance ($P=0.05$) after inclusion of the fasting value in the statistical model.

In vitro *very-low-density lipoprotein oxidation*

We determined the lag time and propagation rate during oxidation of VLDL particles isolated in the fasting state and postprandially after the intervention periods (Table 5). In the fasting state the lag time showed no differences between the diets, while 3 weeks consumption of SO resulted in a higher fasting propagation rate than OO or RO ($P<0.0001$). Postprandially, a SO-rich diet gave rise to a significantly shorter lag time compared with the RO and OO diets ($P=0.03$) while the SO diet resulted in a higher VLDL propagation rate, postprandially, than the OO diet ($P=0.04$). Irrespective of the diets, the propagation rate was higher in the postprandial state than in the fasting state.

Low-density lipoprotein in vitro oxidation, fatty-acid composition and content of tocopherols

Oxidation susceptibility. No differences in lag time of LDL oxidation between any of the diets were found in the fasting or postprandial state (Table 6). Three weeks consumption of the SO diet resulted in a higher fasting and postprandial propagation rate compared with the RO and OO diets ($P<0.001$).

Fatty-acid composition. The fatty acid composition of the LDL particle was determined and the content of SFA, MUFA, and PUFA as well as the unsaturation index was calculated for samples taken in the fasting and postprandial state after 3 weeks consumption of the diets (Table 7). LDL isolated in the fasting state had a similar content of SFA after the three diets, while the content of MUFA in LDL was highest after the OO diet and lowest after the SO diet ($P<0.001$). The content of PUFA in LDL was highest after SO and lowest after the OO diet ($P<0.001$), while the relative content of α-linolenic acid

was higher after the RO diet compared with the SO and OO diets ($P<0.001$). As a result of the differences in the content of unsaturated fatty acids in LDL the unsaturation index of these particles in the fasting state was lower after the OO diet compared with the other diets ($P<0.001$). The fatty-acid composition of LDL isolated in the postprandial state showed no difference in the relative amount of SFA, but differences were found in the content of MUFA in postprandial LDL, which was lower after the SO diet than after the RO and OO diets ($P<0.001$). The SO diet resulted in the highest and the OO diet in the lowest content of PUFA in LDL ($P<0.001$). The SO diet with the highest content of linoleic acid resulted in LDL with the highest content of this fatty acid ($P=0.002$), as was the case for the linolenic acid after the RO diet ($P=0.0012$). As observed in the fasting state, the unsaturation index was lower in LDL isolated postprandially after the OO diet compared with the other diets ($P<0.001$).

Vitamin content. The content of vitamin E (α-, δ-, and γ-tocopherol) and of α- and β-carotene in the LDL particles was determined (Table 8). The content of γ-tocopherol in LDL was significantly higher after the RO diet compared with the OO and SO diets ($P<0.001$). No differences were found between the diets with respect to the content of δ-tocopherol, α-tocopherol, or α-carotene in LDL in the fasting state. Postprandially, the content of α-tocopherol was higher in LDL after 3 weeks' consumption of the SO diet compared with RO ($P=0.045$) while there were no differences between the diets in the content of δ-tocopherol, γ-tocopherol, α-carotene, or β-carotene in LDL. The postprandial α-tocopherol level was increased compared with the fasting level after the OO diet ($P=0.02$).

Statistical analyses were conducted to determine the relationship between oxidation parameters and vitamin E or unsaturation index. There was a correlation between unsaturation index and propagation rate of SO in the fasting state (r 0.83, $P<0.001$) and RO in the postprandial state (r 0.83, $P<0.001$).

Discussion

A number of studies have compared the effect of oleic acid-rich and linoleic acid-rich diets (Bonanome *et al.*

Table 5. Fasting and postprandial VLDL oxidation characteristics after 3 weeks intake of rapeseed oil, olive oil or sunflower-seed oil diet. Postprandial measurements were obtained after consumption of two consecutive rapeseed oil test meals*

(Mean values and standard errors of the mean)

		Rapeseed oil		Olive oil		Sunflower-seed oil	
		Mean	SEM	Mean	SEM	Mean	SEM
Lag time (/min):	Fasting	187.3	24.4	192.4	8.3	140.9	9.4
	Postprandial	173.5[a]	13.2	190.0[a]	20.3	147.3[b]	13.6
Propagation rate (nmol/mg per min):	Fasting	6.31[ab]	0.40	4.78[b]	0.09	10.06[c]	0.53
	Postprandial	10.43†	1.00	7.99†[a]	0.49	11.56†[b]	1.05†

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).
* For details of diet and procedures, see Tables 1 and 2, and p. 491.
† Postprandial value (samples taken 5 h 45 min after the first meal) was significantly different from fasting value ($P<0.05$).

Table 6. Fasting and postprandial LDL oxidation characteristics after 3 weeks intake of rapeseed-oil, olive-oil or sunflower-seed oil diet. Postprandial measurements were obtained after consumption of two consecutive rapeseed-oil test meals*

(Mean values and standard errors of the mean)

		Rapeseed oil		Olive oil		Sunflower oil	
		Mean	SEM	Mean	SEM	Mean	SEM
Lag time (/min):	Fasting	106·1	7·9	114·1	10·9	102·5	8·9
	Postprandial	112·0	10·5	117·6	7·0	99·8	7·3
Propagation rate (nmol/mg per min):	Fasting	8·80[a]	0·47	6·98[a]	0·59	11·20[b]	0·56
	Postprandial	8·81[a]	0·45	8·01†[a]	0·35	11·02[b]	0·65

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.01$).

* For details of diets and procedures, see Tables 1 and 2, and p. 491.

† Postprandial value was significantly different from fasting value ($P < 0.001$).

1992; Nydahl *et al.* 1993; Reaven *et al.* 1994; Nydahl *et al.* 1995; Carmena *et al.* 1996) since an OO-rich diet has been considered beneficial in preventing IHD (International Consensus Statement on Olive Oil and the Mediterranean Diet: Implications for Health in Europe, 1997). In the present study we aimed to compare two oleic acid-rich diets differing in the α-linolenic acid content and tocopherol composition with a linoleic acid-rich diet. At the end of each 3 weeks dietary intervention period the subjects were given two consecutive meals. The two meal design was chosen to achieve reasonably marked postprandial TAG responses. RO was chosen as the common fat in all the test meals in order to study the impact of the background diets on the acute metabolic responses to a standard fat load.

Three weeks consumption of an OO diet resulted in significantly higher postprandial plasma and lipoprotein TAG and cholesterol concentrations compared with RO and SO diets (Table 3). In another study OO resulted in higher postprandial TAG and apo B-48 concentrations than SFA (Roche *et al.* 1998). Thus, the suggested cardioprotective role of OO (Trichopoulou *et al.* 1993) appears not to be related to postprandial lipid and lipoprotein concentrations.

The OO-rich diet also resulted in higher fasting plasma TAG and cholesterol levels compared with consumption of RO- and SO-enriched diets. In other studies similar changes of plasma lipids after diets rich in either RO, OO or SO have been observed (McDonald *et al.* 1989; Lichtenstein *et al.* 1993; Nydahl *et al.* 1993; Gustafsson *et al.* 1994; Carmena *et al.* 1996). The effect may be ascribed to the different contents of oleic, linoleic and α-linolenic acid in the oils as reflected in the plasma TAG and cholesteryl esters (Larsen *et al.* 1999).

The higher non-fasting concentrations of lipids and lipoproteins observed after consumption of OO in the present study is presumably related to the higher fasting TAG and cholesterol concentrations. We observed a significant positive correlation between fasting TAG and postprandial TAG area under the curve concentrations (r 0·66, $P = 0.003$). A similar relationship between fasting and postprandial TAG concentrations has been observed in a number of studies (Meydani *et al.* 1989; O'Meara *et al.* 1992; Potts *et al.* 1994). Despite rather large differences in the MUFA and PUFA composition of the RO and SO diets, fasting lipid and lipoprotein concentrations did not differ and neither were any differences observed

Table 7. Fatty-acid composition (mol %) and unsaturation index of LDL after 3 weeks intake of rapeseed oil (RO), olive oil (OO) or sunflower-seed oil (SO). Postprandial samples were obtained after consumption of two consecutive RO test meals*

(Mean values and standard errors of the mean)

	Fasting						Postprandial					
	RO		OO		SO		RO		OO		SO	
Fatty acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16:0	23·3[a]	0·6	25·2[b]	0·5	23·4[a]	0·7	21·4	1·2	23·8	0·8	23·3	0·3
18:0	7·8	0·6	7·5	0·4	8·9	0·6	7·4[a]	1·2	7·0[a]	0·6	9·0[b]	0·3
18:1	18·9[a]	0·3	23·9[b]	0·5	11·4[c]	0·3	21·7[a]	2·5	23·6[a]	0·5	13·8[b]	0·4
18:2	28·1[a]	1·6	23·3[b]	0·7	37·6[c]	1·7	29·1[a]	2·2	26·2[a]	1·3	34·5[b]	0·6
18:3	1·2[a]	0·1	0·3[b]	0·0	0·2[b]	0·0	1·9[a]	0·5	0·7[b]	0·0	0·8[b]	0·1
SFA	34·4	1·0	35·7	0·7	35·7	1·1	31·4	1·9	33·5	1·0	35·4	0·5
MUFA	24·3[a]	0·4	28·7[b]	0·5	15·2[c]	0·4	26·6[a]	2·6	28·3[a]	0·5	18·0[b]	0·4
PUFA	41·3[a]	1·2	35·7[b]	0·5	49·1[c]	1·2	42·0[a]	1·4	38·1[b]	1·0	46·7[c]	0·6
Unsaturation index	132·7[a]	1·8	124·7[b]	1·6	137·2[a]	1·9	134·8[a]	1·8	128·0[b]	2·4	135·9[a]	1·9

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[a,b,c] Mean values within a row, within fasting and postprandial samples respectively, with unlike superscript letters were significantly different ($P < 0.01$). For exact P values, see p. 494.

* For details of diets and procedures, see Tables 1 and 2 and p. 491.

† Unsaturation index is calculated as mol of a given fatty acid/100 mol total fatty acids multiplied by the number of double bonds in that fatty acid summarised over all the fatty acids.

Table 8. Fasting and postprandial LDL content of vitamin E and A (μg/mg LDL protein) after 3 weeks intake of rapeseed oil (RO), olive oil (OO) or sunflower-seed oil (SO) diet. Postprandial measurements were obtained after consumption of two consecutive rapeseed oil test meals*

(Mean values and standard errors of the mean)

	Fasting						Postprandial					
	RO		OO		SO		RO		OO		SO	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
α-Tocopherol	16·13	2·15	12·71	1·63	13·80	1·46	11·86[a]	1·25	17·42†[c]	1·95	21·14[cb]	2·93
δ-Tocopherol	0·26	0·08	0·19	0·08	0·25	0·06	0·14	0·06	0·3	0·09	0·23	0·08
γ-Tocopherol	2·70[a]	0·67	0·62[b]	0·12	0·73[b]	0·11	1·44[a]	0·20	1·71[a]	0·43	1·06[a]	0·21
α-Carotene	0·05	0·01	0·03	0·01	0·05	0·01	0·05	0·01	0·05	0·01	0·08	0·01
β-Carotene	2·68	0·37	1·90	0·30	2·43	0·36	2·34	0·37	2·79	0·65	3·62	0·55

[a,b,c] Mean values within a row, within fasting and postpradial samples respectively, with unlike superscript letters were significantly different ($P < 0.05$) For exact P values, see p. 494.

* For details of diets and procedures, see Tables 1 and 2, and p. 491.

† Postprandial value was significantly different from fasting value ($P < 0.02$).

in postprandial lipid and lipoprotein responses to test meals after consumption of these diets.

Studies comparing effects of intake of diets rich in unsaturated fatty acids other than fish oil on postprandial responses are scarce. In a study of healthy subjects comparing OO and SFA in the preceding diet, plasma TAG concentrations in the early postprandial period were higher after the OO-rich diet (Roche *et al.* 1998). In accordance, Southern Europeans exhibited much higher postprandial TAG concentrations in the early postprandial phase than those of Northern Europeans, which may be explained by the observation that MUFA contributed significantly more (18 *v.* 11 % energy, respectively) to total energy intake by Southern compared with Northern Europeans (Zampelas *et al.* 1998).

In the present study the difference in MUFA content between the RO and SO diets (18 *v.* 9 % energy, respectively) resulted in similar postprandial lipid or lipoprotein concentrations after test meals and a similar MUFA content in the OO and RO diets (21 and 18 % energy, respectively) resulted in different postprandial responses. Thus, the observed difference in postprandial lipid and lipoprotein concentrations appears not to be due to the preceding consumption of MUFA *per se*. Our results suggest that other components other than dietary MUFA influence postprandial lipid and lipoprotein concentrations and that higher fasting lipid and lipoprotein concentrations reflect higher non-fasting lipid and lipoprotein concentrations. The higher fasting total- and LDL-cholesterol concentrations after OO are partly attributable to differences in the fatty-acid composition of the diets but may also be due to effects of the non-fatty acid constituents of the oils, squalene and sterols (for data and discussion, see Pedersen *et al.* 2000).

Unfortunately, the present study did not include measures of remnant particles, which could have helped to differentiate between intestinally and hepatically derived lipoproteins. A background diet containing *n*-3 fatty acids resulted in lower postprandial concentrations of CM remnants as measured by TAG-rich lipoprotein retinyl palmitate concentration compared with a SFA-rich diet (Weintraub *et al.* 1988). However, 8 weeks consumption

of an OO- or SFA-rich diet resulted in similar apo B-48 (a marker of CM and CM remnants) responses, although the postprandial increase in apo B-48 was greater after the OO-rich diet (Roche *et al.* 1998). Thus, in the present study in which test meals were preceded by consumption of diets rich in different unsaturated fatty acids for 3 weeks, we can not exclude the possibility that differences exist in the postprandial CM and CM remnant clearance rates between the OO, RO and SO diets.

Ingestion of a diet rich in SO resulted in VLDL particles more prone to oxidation, as assessed from propagation rate and lag time, than a diet rich in RO or OO. This was probably due to a higher content of PUFA in the SO diet and perhaps also influenced by the content of antioxidants. This is supported by the finding by Hau *et al.* (1996) showing a correlation between propagation rate and the number of double bonds in PUFA in VLDL. Our results show a higher propagation rate of VLDL in the postprandial state compared with the fasting state, irrespective of oil fatty-acid composition. In accordance, Lechleitner *et al.* (1994) observed that postprandial LDL induced a more pronounced cholesterol accumulation in macrophages and more thiobarbituric acid-reacting substances compared with fasting LDL and suggested that this could be due to a greater susceptibility to oxidation.

In the present study the LDL particle showed an increased susceptibility to oxidation in the fasting as well as in the postprandial state after intake of SO compared with OO (and RO), as demonstrated by a higher propagation rate. The increased propagation rate after SO is probably due to a larger amount of PUFA in the LDL particle. Other studies have shown that a diet rich in PUFA compared with a diet rich in MUFA results in a higher rate of LDL oxidation (Bonanome *et al.* 1992; Turpeinen *et al.* 1995) and that LDL rich in oleic acid and poor in linoleic acid has a reduced oxidation rate (Kleinveld *et al.* 1993). Although LDL from RO and SO both had higher unsaturation indexes than OO-LDL, only the SO-LDL was more prone to oxidation than the OO-LDL. This was observed both in the fasting and the postprandial state. We observed no effect on the lag time of the higher content of α-tocopherol after the SO diet in the postprandial state.

The increased content of tocopherol may not have been high enough to affect the oxidation susceptibility in terms of lag time. The amount of γ-tocopherol was much higher in RO than in the other oils and resulted in a higher LDL γ-tocopherol content. However, LDL γ-tocopherol was only about one-tenth of the amount of α-tocopherol in LDL. This may be a result of a higher consumption of γ-tocopherol compared with α-tocopherol or a different distribution of α- and γ-tocopherol in the different lipoproteins as demonstrated by Meydani *et al.* (1989). It is probably not a result of selectivity in the liver to channel γ-tocopherol from CM into the bile and α-tocopherol into VLDL as suggested by Traber & Kayden (1989) since we have found large amounts of γ-tocopherol in VLDL in other studies (NS Nielsen, unpublished results). The relationship between content of antioxidants and lag time has been examined with conflicting results. In theory the antioxidants should reduce the susceptibility to oxidation. The susceptibility of LDL to oxidation did decrease linearly after supplementation with α-tocopherol in some studies (Dieber-Rotheneder *et al.* 1991; Princen *et al.* 1992; Reaven *et al.* 1993; Reaven *et al.* 1994; Princen *et al.* 1995) while other studies found no association (Kleinveld *et al.* 1993; Turpeinen *et al.* 1995). Furthermore, it has been suggested that antioxidants may act as pro-oxidants (Bowry *et al.* 1992). It has also been shown that supplementation with β-carotene has no effect on *in vitro* LDL oxidation susceptibility (Princen *et al.* 1992).

The propagation rate of LDL obtained after the SO and RO diets correlated with the unsaturation index in the fasting and postprandial states, respectively. The lower propagation rate after the OO diet compared with SO can be explained by the lower content of PUFA in VLDL and LDL after OO. In addition, the significantly lower concentration of TAG in VLDL and of cholesterol in LDL after SO and RO compared with OO may suggest marginally smaller lipoprotein particles that may explain the increased susceptibility to oxidation after SO compared with lipoprotein particles after the OO diet (Bonanome *et al.* 1992; Reaven *et al.* 1993; Tribble *et al.* 1995). The lower propagation rate of LDL after RO compared with SO, may result from the exponential relationship between number of double bonds and oxidation lability (Gunstone, 1984).

In conclusion, we found that in terms of plasma lipid levels the RO and SO diets may be preferred to the OO diet, while in terms of susceptibility of lipoproteins to *in vitro* oxidation an OO-rich diet may be preferred to a RO and especially a SO diet. Thus, the claimed cardioprotective role of OO seems not to be mediated through more favourable effects on postprandial lipid and lipoprotein responses compared with other oils, but may be related to lower oxidation susceptibility.

Acknowledgements

References

Altman DG (1995) Clinical trials. In *Practical Statistics for Medical Research*, pp. 440–476. London: Chapman & Hall.

Asp NG, Johanson CG, Hallmer H & Siljeström M (1983) Rapid enzymatic assay of insoluble and soluble dietary fiber. *Journal Agriculture and Food Chemistry* **31**, 476–482.

Austin MA, King M-C, Vranizan KM & Krauss RM (1990) Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* **82**, 495–506.

Berry EM, Eisenberg S, Haratz D, Friedlander Y, Norman Y, Kaufmann NA & Stein Y (1991) Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins – the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. *American Journal of Clinical Nutrition* **53**, 899–907.

Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M, Maiorino M & Ursini F (1992) Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arteriosclerosis and Thrombosis* **12**, 529–533.

Bowry VW, Ingold KU & Stocker R (1992) Vitamin E in human low-density lipoprotein. When and how this antioxidant becomes a pro-oxidant. *Biochemical Journal* **288**, 341–344.

Carmena R, Ascaso JF, Camejo G, Varela G, Hurt-Camejo E, Ordovas JM, Maritnez-Valls J, Bergström M & Wallin B (1996) Effect of olive and sunflower oils on low density lipoprotein level, composition, size, oxidation and interaction with arterial proteoglycans. *Atherosclerosis* **125**, 243–255.

Christopherson SW & Glass RL (1969) Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. *Journal of Dairy Science* **52**, 1289–1290.

Chung BH, Tallis G, Yalamoori V, Anantharamaiah GM & Segrest JP (1994) Liposome-like particles isolated from human atherosclerotic plaques are structurally and compositionally similar to surface remnants of triglyceride-rich lipoproteins. *Arteriosclerosis and Thrombosis* **14**, 622–635.

Dieber-Rotheneder M, Puhl H, Waeg G, Striegl G & Esterbauer H (1991) Effect of oral supplementation with D-α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *Journal of Lipid Research* **32**, 1325–1332.

Dole VP & Hamlin JT (1962) Particulate fat in lymph and blood. *Physiological Reviews* **42**, 674–701.

Esterbauer H, Gebicki J, Puhl H & Jürgens G (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine* **13**, 341–390.

Folch J, Lees M & Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Grooth PHE, van Stiphout WAHJ, Krauss XH, Jansen H, van Tol A, van Ramhorst E, Chin-On S, Hofman A, Cresswell SR & Havekes L (1991) Postprandial lipoprotein metabolism in normolipidemic men with and without coronary heart disease. *Arteriosclerosis and Thrombosis* **11**, 653–662.

Gunstone FD (1984) Reaction of oxygen and unsaturated fatty acids. *Journal of the American Oil Chemist Society* **61**, 441–447.

Gustafsson I-B, Vessby B, Öhrvall M & Nydahl M (1994) A diet rich in monounsaturated rapeseed oil reduces the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects. *American Journal of Clinical Nutrition* **59**, 667–674.

Hau M-F, Smelt AHM, Bindels AJGH, Sijbrands EJG, Van der Laarse A, Onkenhout W, van Duyvenvoorde W & Princen HMG (1996) Effects of fish oil on oxidation resistance of

VLDL in hypertriglyceridemic patients. *Arteriosclerosis, Thrombosis, and Vascular Biology* **16**, 1197–1202.

International Consensus Statement on Olive Oil and the Mediterranean Diet: Implications for Health in Europe (1997) *European Journal of Cancer Prevention* **6**, 438–421.

Kaplan LA, Miller JA, Stein EA & Stampfer MJ (1990) Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma. *Methods in Enzymology* **189**, 155–167.

Karpe F (1997) Postprandial lipid metabolism in relation to coronary heart disease. *Proceedings of the Nutrition Society* **56**, 671–678.

Karpe F, Steiner G, Uffelman K, Olivecrona T & Hamsten A (1994) Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* **106**, 83–97.

Keidar S, Kaplan M, Rosenblat M, Brook GJ & Aviram M (1992) Apolipoprotein E and lipoprotein lipase reduce macrophage degradation of oxidized very-low-density lipoprotein (VLDL), but increases cellular degradation of native VLDL. *Metabolism* **41**, 1185–1192.

Kleinveld HA, Naber AHJ, Stalenhoef AFH & Demacker PNM (1993) Oxidation resistance, oxidation rate, and extent of oxidation of human low density lipoprotein depend on the ratio of oleic acid content to linoleic acid content: studies in vitamine E deficient subjects. *Free Radical Biology and Medicine* **15**, 273–280.

Larsen LF, Jespersen J & Marckmann P (1999) Are olive oil diets antithrombotic? Diets enriched with olive, rapeseed, or sunflower oil affect postprandial factor VII differently. *American Journal of Clinical Nutrition* **70**, 976–982.

Lechleitner M, Hoppichler F, Föger B & Patch JR (1994) Low-density lipoproteins of the postprandial state induce cellular cholesteryl ester accumulation in macrophages. *Arteriosclerosis and Thrombosis* **14**, 1799–1807.

Lichtenstein AH, Ausman LM, Carrasco W, Jenner JL, Gualtieri LJ, Goldin BR & Schaefer EJ (1993) Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a national cholesterol education program step 2 diet. *Arteriosclerosis and Thrombosis* **13**, 1533–1542.

Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

McDonald BE, Gerrard JM, Bruce VM & Corner EJ (1989) Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on in vivo thromboxane A$_2$ and prostacyclin production in healthy young men. *American Journal of Clinical Nutrition* **50**, 1382–1388.

Matthews JN, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. *British Medical Journal* **300**, 230–235.

Meydani M, Cohn JS, Macauley JB, McNamara JR, Blumberg JB & Schaefer EJ (1989) Postprandial changes in the plasma concentration of α- and gamma-tocopherol in human subjects fed a fat-rich meal supplemented with fat-soluble vitamins. *Journal of Nutrition* **119**, 1252–1258.

Nordestgaard BG & Tybjaerg Hansen A (1992) IDL, VLDL, chylomicrons and atherosclerosis. *European Journal of Epidemiology* **8**, 92–98.

Nydahl M, Gustafsson I-B, Öhrvall M & Vessby B (1993) Similar serum lipoprotein cholesterol concentrations in healthy subjects on diets enriched with rapeseed and with sunflower oil. *European Journal of Clinical Nutrition* **48**, 128–137.

Nydahl M, Gustafsson I-B, Öhrvall M & Vessby B (1995) Similar effects of rapeseed oil (canola oil) and olive oil in a lipid-lowering diet for patients with hyperlipoproteinemia. *Journal of the American College of Nutrition* **14**, 643–651.

O'Meara NM, Lewis GF, Cabana VG, Iverius PH, Getz GS & Polonsky KS (1992) Role of basal triglyceride and high density lipoprotein in determination of postprandial lipid and lipoprotein responses. *Journal of Clinical Endocrinological Metabolism* **75**, 465–471.

Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM Jr & Patsch W (1992) Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arteriosclerosis and Thrombosis* **12**, 1336–1345.

Pedersen A, Baumstark MW, Marckmann P, Gylling H & Sandström B-M (2000) 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. *Journal of Lipid Research* **41**, 1901–1911.

Pedersen A, Marckmann P & Sandström B-M (1999) Postprandial lipoprotein, glucose and insulin responses after two consecutive meals containing rapeseed oil, sunflower oil, or palm oil with or without glucose at the first meal. *British Journal of Nutrition* **82**, 97–104.

Potts JL, Humphreys SM, Coppack SW, Fisher RM, Gibbons GF & Frayn KN (1994) Fasting plasma triacylglycerol concentrations predict adverse changes in lipoprotein metabolism after a normal meal. *British Journal of Nutrition* **72**, 101–109.

Princen HMG, van Duyvenvoorde W, Buytenhek R, Van der Laarse A, van Poppel G, Leuven JAG & van Hinsberg VWM (1995) Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arteriosclerosis, Thrombosis, and Vascular Biology* **15**, 325–333.

Princen HMG, van Poppel G, Vogelezang C, Buytenhek R & Kok FJ (1992) Supplementation with vitamin E but not β-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. Effect of cigarette smoking. *Arteriosclerosis and Thrombosis* **12**, 554–562.

Rapp JH, Lespine A, Hamilton RL, Colyvas N, Chaumeton AH, Tweedie Hardman J, Kotite L, Kunitake ST, Havel RJ & Kane JP (1994) Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arteriosclerosis and Thrombosis* **14**, 1767–1774.

Reaven PD, Grasse BJ & Tribble DL (1994) Effects of linoleate-enriched and oleate-enriched diets in combination with α-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans. *Arteriosclerosis and Thrombosis* **14**, 557–566.

Reaven PD, Khouw A, Beltz WF, Parthasarathy S & Witztum JL (1993*a*) Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin E but not by β-carotene. *Arteriosclerosis and Thrombosis* **13**, 590–600.

Reaven PD, Parthasarathy S, Grasse BJ, Miller E, Steinberg D & Witztum JL (1993) Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. *Journal of Clinical Investigations* **91**, 668–676.

Roche HM, Zampelas A, Knapper JM, Webb D, Brooks C, Jackson KG, Wright JW, Gould BJ, Kafatos A, Gibney MJ & Williams CM (1998) Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *American Journal of Clinical Nutrition* **68**, 552–560.

Rumsey SC, Galeano NF, Arad Y & Demacker PNM (1992) Cryopreservation with sucrose maintains normal physical and biological properties of human plasma low density lipoproteins. *Journal of Lipid Research* **33**, 1551–1561.

Simpson HS, Williamson CM, Olivecrona T, Pringle S, Maclean J, Lorimer AR, Bonnefous F, Bogaievsky Y, Packard CJ & Shepherd J (1990) Postprandial lipemia, fenofibrate and coronary artery disease. *Atherosclerosis* **85**, 193–202.

Sorensen NS, Marckmann P, Høy C-E, Duyvenvoorde W & Princen HMG (1998) Effect of fish-oil-enriched margarine on plasma lipids, low-density-lipoprotein particle composition, size, and susceptibility to oxidation. *American Journal of Clinical Nutrition* **68**, 235–241.

Tinker LF, Parks EJ, Behr SR, Schneeman BO & Davis PA (1999) (n-3) fatty acid supplementation in moderately hyper-triglyceridemic adults changes postprandial lipid and apolipo-protein B responses to a standardized test meal. *Journal of Nutrition* **129**, 1126–1134.

Traber MG & Kayden HJ (1989) Preferetial incorporation of α-tocopherol vs gamma-tocopherol in human lipoproteins. *American Journal of Clinical Nutrition* **49**, 517–526.

Tribble DL, Thiel PM, van den Berg JJM & Krauss RM (1995) Differing α-tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL. *Arteriosclerosis, Thrombosis, and Vascular Biology* **15**, 2025–2031.

Trichopoulou A, Katsouyanni K & Gnardellis C (1993) The traditional Greek diet. *European Journal of Clinical Nutrition* **47**, S76–S81.

Turpeinen AM, Alfthan G, Valsta L, Hietanen E, Salonen JT, Schunk H, Nyyssönen K & Mutanen M (1995) Plasma and lipoprotein lipid peroxidation in humans on sunflower and rapeseed oil diets. *Lipids* **30**, 485–492.

Uiterwaal CS, Grobbee DE, Witteman JC, van Stiphout WA, Krauss XH, Havekes LM, de Bruijn AM, van Tol A & Hofman A (1994) Postprandial triglyceride response in young adult men and familial risk for coronary atherosclerosis [see comments]. *Annual Internal Medicine* **121**, 576–583.

Weintraub MS, Zechner R, Brown A, Eisenberg S & Breslow JL (1988) Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. *Journal of Clinical Investigations* **82**, 1884–1893.

Whitmann SC, Sawyez CG, Miller DB, Wolfe BM & Huff MW (1998) Oxidized type IV hypertriglyceridemic VLDL-remnants cause greater macrophage cholesteryl ester accumulation than oxidized LDL. *Journal of Lipid Research* **39**, 1008–1020.

Winklerhofer-Roob BM, Ziouzenkova O, Puhl H, Ellenmunter H, Greiner P, Müller G, van't Hof MA, Esterbauer H & Shmerling DH (1995) Impaired resistance to oxidation of low density lipoprotein in cystic fibrosis: improvement during vitamine E supplementation. *Free Radical Biology and Medicine* **19**, 725–733.

Zampelas A, Roche H, Knapper JME, Jackson KG, Tornaritis M, Hatzis C, Gibney MJ, Kafatos A, Gould BJ, Wright J & Williams CM (1998) Differences in postprandial lipaemic response between Northern and Southern Europeans. *Atherosclerosis* **139**, 83–93.

Zilversmit DB (1979) Atherogenesis: a postprandial phenomenon. *Circulation* **60**, 473–485.