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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(Received 22 June 2001 – Revised 14 December 2001 – Accepted 20 January 2002)

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 atherosclerotic plaques (Chung et al. 1994). In addition, TAG enriched apolipoprotein (apo) B-containing lipoproteins have been isolated from human atherosclerotic 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

Abbreviations: apo, apolipoprotein; CM, chylomicron; MUFA, monounsaturated fatty acids; OO, olive oil; PUFA, polyunsaturated fatty acids; RO, rapeseed oil; SFA, saturated fatty acid; SO, sunflower-seed oil; TAG, triacylglycerol.

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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/m². 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 1·49 (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>
<thead>
<tr>
<th>Table 1. Composition of the three experimental diets enriched in rapeseed oil, olive oil or sunflower-seed oil (% of energy)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapeseed oil</strong></td>
</tr>
<tr>
<td>Protein*</td>
</tr>
<tr>
<td>Fat*</td>
</tr>
<tr>
<td>Carbohydrate*</td>
</tr>
<tr>
<td>Vitamin E†</td>
</tr>
<tr>
<td>α-Tocopherol (α-TE/MJ)</td>
</tr>
<tr>
<td>α-Tocopherol (mg/MJ)</td>
</tr>
<tr>
<td>Fibre† (g/MJ)</td>
</tr>
</tbody>
</table>

* 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.
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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-turbidimetric 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 x 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 (Sf > 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
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–2-propanol–water (80:20:3:2, by vol.) (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 (SO) and sunflower-seed oil (RO)†

<table>
<thead>
<tr>
<th></th>
<th>RO</th>
<th>OO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Plasma TAG:</td>
<td>Fasting</td>
<td>0.68</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>1.91</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>CM-TAG:</td>
<td>AUC (mmol h/l)</td>
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<tr>
<td></td>
<td>Fasting</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>1.25</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>AUC (mmol h/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-TAG:</td>
<td>Fasting</td>
<td>0.32</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>0.41</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>AUC (mmol h/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-TAG:</td>
<td>Fasting</td>
<td>0.33</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>HDL-TAG:</td>
<td>Fasting</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>0.14</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>AUC (mmol h/l)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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 ($P<0.0001$).

**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 ($P<0.0001$). The content of PUFA in LDL was highest after SO and lowest after the OO diet ($P<0.0001$), while the relative content of $\alpha$-linolenic acid was higher after the RO diet compared with the SO and OO diets ($P<0.0001$). 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 ($P<0.0001$). 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 ($P<0.0001$). The SO diet resulted in the highest and the OO diet in the lowest content of PUFA in LDL ($P<0.0001$). The SO diet with the highest content of linoleic acid resulted in LDL with the highest content of this fatty acid ($P=0.0002$), 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 ($P<0.0001$).

**Vitamin content.** The content of vitamin E ($\alpha$-, $\delta$-, and $\gamma$-tocopherol) and of $\alpha$- and $\beta$-carotene in the LDL particles was determined (Table 8). The content of $\gamma$-tocopherol in LDL was significantly higher after the RO diet compared with the OO and SO diets ($P<0.0001$). No differences were found between the diets with respect to the content of $\delta$-tocopherol, $\alpha$-tocopherol, or $\alpha$-carotene in LDL in the fasting state. Postprandially, the content of $\alpha$-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 $\delta$-tocopherol, $\gamma$-tocopherol, $\alpha$-carotene, or $\beta$-carotene in LDL. The postprandial $\alpha$-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 ($r=0.83, P<0.0001$) and RO in the postprandial state ($r=0.83, P<0.0001$).

**Discussion**

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>RO</th>
<th>OO</th>
<th>SO</th>
<th>RO</th>
<th>OO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>23.3a 0.6</td>
<td>25.2b 0.5</td>
<td>23.4a 0.7</td>
<td>21.4 1.2</td>
<td>23.8 0.8</td>
<td>23.3 0.3</td>
</tr>
<tr>
<td>18:0</td>
<td>7.8 0.6</td>
<td>7.5 0.4</td>
<td>8.9 0.6</td>
<td>7.4a 1.2</td>
<td>7.0a 0.6</td>
<td>9.0b 0.3</td>
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<td>18:1</td>
<td>18.9a 0.3</td>
<td>23.9b 0.5</td>
<td>11.4a 0.3</td>
<td>21.7a 2.5</td>
<td>23.6a 0.5</td>
<td>13.8b 0.4</td>
</tr>
<tr>
<td>18:2</td>
<td>28.1a 1.6</td>
<td>23.3b 0.7</td>
<td>37.6c 1.7</td>
<td>29.1a 2.2</td>
<td>26.2a 1.3</td>
<td>34.5b 0.6</td>
</tr>
<tr>
<td>18:3</td>
<td>1.2a 0.1</td>
<td>3.0b 0.0</td>
<td>0.2b 0.0</td>
<td>1.9b 0.5</td>
<td>0.7b 0.0</td>
<td>0.8b 0.1</td>
</tr>
<tr>
<td>SFA</td>
<td>34.4</td>
<td>35.7</td>
<td>35.7</td>
<td>31.4</td>
<td>33.5</td>
<td>35.4</td>
</tr>
<tr>
<td>MUFA</td>
<td>24.3a 0.4</td>
<td>28.7b 0.5</td>
<td>15.2a 0.4</td>
<td>26.8a 2.6</td>
<td>28.3a 0.5</td>
<td>18.0b 0.4</td>
</tr>
<tr>
<td>PUFA</td>
<td>41.3a 1.2</td>
<td>35.7b 0.5</td>
<td>49.1a 1.2</td>
<td>42.0a 1.4</td>
<td>38.1b 1.0</td>
<td>46.7b 0.6</td>
</tr>
<tr>
<td>Unsaturation index</td>
<td>132.7a 1.8</td>
<td>124.7b 1.6</td>
<td>137.2a 1.9</td>
<td>134.8a 1.8</td>
<td>128.0b 2.4</td>
<td>135.9a 1.9</td>
</tr>
</tbody>
</table>

* 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).
‡ Mean values within a row with unlike superscript letters were significantly different (P<0.01).

### 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)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fasting</th>
<th>Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>23.3a 0.6</td>
<td>21.4 1.2</td>
</tr>
<tr>
<td>18:0</td>
<td>7.8 0.6</td>
<td>7.4a 1.2</td>
</tr>
<tr>
<td>18:1</td>
<td>18.9a 0.3</td>
<td>21.7a 2.5</td>
</tr>
<tr>
<td>18:2</td>
<td>28.1a 1.6</td>
<td>29.1a 2.2</td>
</tr>
<tr>
<td>18:3</td>
<td>1.2a 0.1</td>
<td>1.9b 0.5</td>
</tr>
<tr>
<td>SFA</td>
<td>34.4</td>
<td>31.4</td>
</tr>
<tr>
<td>MUFA</td>
<td>24.3a 0.4</td>
<td>26.8a 2.6</td>
</tr>
<tr>
<td>PUFA</td>
<td>41.3a 1.2</td>
<td>42.0a 1.4</td>
</tr>
<tr>
<td>Unsaturation index</td>
<td>132.7a 1.8</td>
<td>134.8a 1.8</td>
</tr>
</tbody>
</table>

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

* 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.
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 cannot 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 (Bonnano 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.

**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*

<table>
<thead>
<tr>
<th></th>
<th>RO</th>
<th>OO</th>
<th>SO</th>
<th>RO</th>
<th>OO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
</tbody>
</table>

*a,b,c Mean values within a row, within fasting and postprandial 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).
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

The authors would like to thank Lena Andersen, Kirsten Nielsen, Klara Jørgensen, Kirsten Bryde Rasmussen, Kitt S. Hoffman and Kira H. B. Larsen for their excellent technical assistance.

References


