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Original Communication

A diet containing rapeseed oil-based fats does not increase lipid peroxidation in humans when compared to a diet rich in saturated fatty acids

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Objective: To compare the effects of a rapeseed oil-based diet containing an increased proportion of easily oxidised polyunsaturated fatty acids such as α-linolenic acid with a diet rich in saturated fatty acids on the degree of lipid peroxidation in the human body.
Design: A randomised cross-over study.
Subjects and interventions: Nineteen healthy moderately hyperlipidemic subjects (six women and 13 men, age 50 ± 8 y and body mass index (BMI) 24.5 ± 2.6 kg/m^2) were given a rapeseed oil-based diet (RO) and a control diet (SAT) rich in saturated fatty acids during two consecutive 4 week periods separated by a 4 week wash-out period. Biomarkers of lipid peroxidation and antioxidants were analysed in plasma and urine.
Results: No significant differences in plasma or urinary levels of free 8-iso-prostaglandin $F_{2\alpha}$, plasma total 8-iso-prostaglandin $F_{2\alpha}$ plasma hydroperoxides or plasma malondialdehyde were observed between the RO and SAT diets ($P = 0.14-0.95$). A higher concentration of serum γ-tocopherol was detected after the RO diet compared to the SAT diet ($P < 0.001$), whereas the serum α-tocopherol concentration and plasma antioxidative capacity did not differ between the two test diets. The total cholesterol, LDL cholesterol and LDL/HDL ratio were lower after the RO diet compared to the SAT diet ($P < 0.001$), while HDL cholesterol and total triglyceride levels were similar after the two diets.
Conclusion: These results suggest that a rapeseed oil-based diet rich in α-linolenic acid does not seem to increase the degree of lipid peroxidation in plasma and urine compared to a diet rich in saturated fats. This is possibly due to a sufficient content of antioxidants in the rapeseed oil diet to increase circulating concentrations of antioxidants that may protect unsaturated fatty acids from oxidation.
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Introduction

It is well established that a high dietary intake of saturated fatty acids (SAFAs) is associated with high levels of total serum cholesterol and with an increased risk of developing coronary heart disease (CHD, Mensink & Katan, 1992; Caggiula & Mustad, 1997). However, epidemiological studies investigating effects of dietary monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) on the outcome of CHD are less conclusive (Caggiula & Mustad, 1997). Several intervention studies have demonstrated that the lipoprotein profile could be improved by substituting MUFA and PUFA for SAFA (McDonald *et al*, 1989; Wardlaw *et al*, 1991; Valsta

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Contributors: ES organised the dietary intervention, analysed hydroperoxides and total F_2-isoprostanes, co-ordinated data and drafted the manuscript. I-BG was involved in the study design and supervised the dietary intervention. SB performed the analyses of free F_2-isoprostanes. CN did the analyses of antioxidative capacity. AT performed the analyses of thromboxanes. LB was involved in the study design and statistical analyses. BV was involved in the study design and provided advice during all stages of the study. All authors were involved in editing the manuscript.
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et al, 1992; Nydahl *et al*, 1994). However, intervention studies with PUFA-rich diets have also shown that the susceptibility of lipoproteins to oxidation was increased and that very high intakes of PUFAs may carry non-favourable effects on the development of atherosclerosis (Tsimikas & Reaven, 1998; Eritsland, 2000). The oxidisability of fatty acids is considered to be dependent on the degree of unsaturation (Wagner *et al*, 1994; Porter *et al*, 1995). Therefore, dietary recommendations regarding fat intake and quality include a maximum level of the PUFA intake. Recent Nordic nutrition recommendations recommend a diet with a fat content that should not exceed 30 energy percent (E%), an intake of SAFA of less than 10 E%, a PUFA intake between 5–10 E% and the rest from MUFA (Sandström *et al*, 1996).

There has been a pronounced change in the type of edible fats used in many countries, eg in northern Europe and Canada, with an increased consumption of low-erucic acid rapeseed oil-based fats (Sanders, 2000). This development may lead to an increased dietary intake of MUFAs and PUFAs, especially α-linolenic acid (18:3 n-3). Several intervention studies with rapeseed oil-rich diets have shown favourable effects on the blood lipid profile in healthy volunteers (McDonald *et al*, 1989; Wardlaw *et al*, 1991; Valsta *et al*, 1992; Nydahl *et al*, 1994) and in hyperlipidemic patients (Lichtenstein *et al*, 1993; Gustafsson *et al*, 1994; Nydahl *et al*, 1995). In these studies, serum levels of total and low-density lipoprotein (LDL) cholesterol were reduced, while serum levels of triglycerides and high-density lipoprotein (HDL) cholesterol remained unchanged.

Even though an increased intake of rapeseed oil may favourably alter the serum cholesterol profile, the same diet may also potentially increase the degree of lipid peroxidation in the body. The oxidisability of fatty acids is considered to be directly dependent on their degree of unsaturation (Wagner *et al*, 1994; Porter *et al*, 1995). Rapeseed oil contains a high content of oleic acid (18:1) and linoleic acid (18:2 n-6) but also considerable amounts of α-linolenic acid, which is an easily oxidised fatty acid. It is well established that α-linolenic acid is partly metabolised in the body into very-long-chain n-3 PUFAs, which are also highly susceptible to peroxidation. Simultaneously, rapeseed oil is rich in both α- and γ-tocopherol. The degree of lipid peroxidation is largely dependent on the amount of unsaturated fatty acids on the one hand, and antioxidants present for protection on the other. However, it is not yet established how a rapeseed oil-based diet might influence this balance *in vivo*.

The primary aim of this study was to compare the effects of a rapeseed oil-based diet composed according to present dietary recommendations with a control diet rich in SAFAs on biomarkers of lipid peroxidation and antioxidants in plasma and urine. F_2-isoprostanes, hydroperoxides and malondialdehyde were measured as biomarkers of lipid peroxidation, and vitamin E and antioxidative capacity were used as biomarkers of antioxidants in the circulation. A secondary aim was to evaluate dietary effects on the serum lipid profile, glucose and insulin concentration and platelet aggregation.

Materials and methods

Study design

The study was conducted with a randomised cross-over design. Two consecutive 4 week diet periods were separated by a 4 week washout period. The two diets compared were a rapeseed oil-based diet (RO) rich in MUFAs and PUFAs, especially α-linolenic acid, and a control diet (SAT) with a high proportion of SAFAs. Subjects were randomised to start with either of the two diets and were blinded to the type of diet they were following. Measurements were performed after randomisation (baseline) and after each diet period. The design of the study was approved by the Ethics Committee of the Faculty of Medicine at Uppsala University, and all subjects gave their informed consent before entering the study.

Subjects

Nineteen healthy subjects (six female/13 male) aged 50 ± 8 y with normal or moderately increased body weight and blood lipids participated in the study. Baseline characteristics of the subjects are shown in Table 1. Subjects were recruited via posters in nearby companies. Eligible for the study were men (age 30–65) and postmenopausal women (age 50–65) with serum cholesterol 5.4–8.0 mmol/l, serum triglycerides 1.3–5.0 mmol/l, fasting blood glucose 3.0–6.5 mmol/l, diastolic blood pressure < 95 mmHg and body mass index (BMI) < 30 kg/m^2. Exclusion criteria were treatment for coronary heart, liver, kidney and thyroid disease, diabetes mellitus, hypertension, oestrogen replacement therapy and regular acetyl salicylic acid medication. The subjects completed a questionnaire about their medical background, dietary, smoking and physical activity habits. Four subjects who had taken low-dose vitamin and mineral supplements were asked to stop 2 weeks before entering the study. Two subjects were smokers. All subjects were asked to abstain from acetyl salicylic acid during the diet periods and to maintain their habitual lifestyle during the study.

Diets

The fat quality of the two diets (RO and SAT) was controlled by supplying fat products for cooking, spread and dressings, and food items based on the fats tested. The subjects were free living and were given dietary advice on

Table 1 Clinical characteristics of the subjects at baseline

	Means ± s.d.
Women/men	6/13
Age (y)	50 ± 8
Body weight (kg)	76.5 ± 12.5
BMI (kg/m^2)	24.5 ± 2.6
Serum cholesterol (mmol/l)	6.19 ± 1.02
Serum triglycerides (mmol/l)	1.40 ± 0.61
Fasting plasma glucose (mmol/l)	4.96 ± 0.29
Supine systolic blood pressure (mmHg)	119 ± 11
Supine diastolic blood pressure (mmHg)	72 ± 16

how to prepare their food and how to avoid fats from other dietary sources such as fatty dairy and meat products to assure good adherence to the diet. The amount of fat to be provided in the intervention was planned to contribute to approximately 76% of the total daily fat intake. The intake of α-linolenic acid (18:3 n-3) in the RO diet was planned to be about 9.2 g/day and in the SAT diet 1.1 g/day.

The diets were estimated to contain 37 E% fat, 12 E% protein and 50 E% of carbohydrates. The composition of the RO diet was aimed to represent a normal mixed rapeseed oil-based diet composed according to dietary recommendations (Sandström *et al*, 1996), but with a fat content corresponding to the average intake of a Swedish population (Becker, 1999). The control diet contained saturated fat products instead of rapeseed oil-based fat products. Free amounts of cooking fat (RO diet = rapeseed oil-based liquid margarine; SAT = butter), table margarine (80% fat; RO = rapseed oil-based; SAT = specially prepared without rapeseed oil) and oil (RO = rapseed oil; SAT = olive oil) to use for cooking, spread and dressings were supplied weekly. Ten different lunch meals (five/week), wholemeal bread (three/day) and muffins (one/day) prepared with the fats tested were also supplied weekly. The specially prepared table margarine for the SAT diet and ice-cream made from rapeseed oil for the RO diet were produced in pilot plants by Van den Bergh Foods AB (Helsingborg, Sweden) and Carlshamn Mejeri AB (Karlshamn, Sweden), respectively. Commercial qualities of all other cooking fats, table margarines, oils and ice-creams were purchased on the market or obtained from Carlshamn Mejeri AB (Karlshamn, Sweden). Two energy levels (9 and 12 MJ) were prepared to be able to adjust for individual energy requirements and avoid weight changes during the study period. Energy requirements for each subject were calculated using an estimate of 146 kJ/kg body weight (30 kcal/kg body weight) and the nearest energy level was chosen. The body weight of the subjects was checked once a week. If there was a tendency for a change in body weight, dietary advice regarding the use of the fat products was given and the energy level could be changed. Double portions including the planned daily intake of supplied food items and fat products were prepared on the 12 MJ energy level to give an estimate of the fat quality of the two diets. Table 2 shows the fatty acid composition determined in a sample pooled from 10 different double portions including each of the 10 different lunch meals.

Dietary intake was monitored using 3 day weighed food records (two week days and one weekend day) on five occasions, one at baseline and two during each diet period. The dietary food records were analysed by using the software program Stor MATs 4.0 (Rudans Lättdata, Västerås, Sweden) based on a food database from the Swedish National Food Administration (PC-Kost 1996, SLV, Uppsala, Sweden). Data on the specially prepared fats and food items supplied to the participants were entered into the food database and used in the analyses of the dietary records. The compliance to the two diets was also controlled by analyses of the fatty acid compositions in serum phospholipids (PL) and cholesterol esters (CE) before and after the diet periods.

Table 2 Relative fatty acid composition (% of total fatty acids) of the daily supply of food items and fat products from analyses of double portions of 10 pooled days of the rapeseed oil-based diet (RO) and the saturated diet (SAT)

Fatty acids	*RO*	*SAT*
8:0	0.51 ± 0.11	1.24 ± 0.28
10:0	0.59 ± 0.13	2.79 ± 0.41
12:0	3.57 ± 0.64	6.40 ± 0.68
14:0	1.63 ± 0.28	8.99 ± 0.55
16:0	12.2 ± 1.22	25.4 ± 0.65
16:1 n-7	0.37 ± 0.07	1.19 ± 0.05
18:0	3.14 ± 0.33	8.61 ± 0.47
18:1 n-9	50.1 ± 1.63	31.0 ± 1.11
18:2 n-6	19.8 ± 0.57	13.2 ± 0.71
18:3 n-3	7.77 ± 0.49	0.90 ± 0.11
20:4 n-6	0.12 ± 0.06	0.14 ± 0.03
20:5 n-3	0.23 ± 0.07	0.21 ± 0.10
22:6 n-3	0.49 ± 0.23	0.54 ± 0.20

Data are means ± s.d.

Blood and urine sampling

Venous blood samples were withdrawn in the morning after an overnight fast. No smoking or heavy physical activity was allowed in the morning before sample collection. Blood samples were immediately placed on ice and all serum and plasma samples were stored at −70°C within 1 h after sample collection until analysis.

At the end of each dietary period, three consecutive 24 h urine samples were collected in a special aliquot cup (Daisho Co. Ltd, Osaka, Japan). Each 24 h sample was stored in a refrigerator until all three urine samples were collected. The three urine samples were then pooled and stored at −70°C until analysis. Urinary analyses were adjusted for the creatinine concentration measured with an autoanalyser (Elan autoanalyser, Eppendorf, Germany) using a commercial kit (Merck, Germany) and a self-made calibrator (10 mmol/l creatinine in distilled water).

Biomarkers of lipid peroxidation

Free 8-iso-prostaglandin $F_{2\alpha}$. Plasma and urinary samples were analysed for free 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) by a newly developed radioimmunoassay (Basu, 1998). In brief, an antibody was raised in rabbits by immunisation with 8-iso-$PGF_{2\alpha}$ coupled to bovine serum albumin at the carboxylic acid by the 1,1′-carbonyldiimmidazole method. The cross-reactivity of the antibody with 8-iso-15-keto-13, 14-dihydro-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13, 14-dihydro-$PGF_{2\alpha}$, TXB_2, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$ was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%, respectively. Unextracted samples were used in the analyses. The detection limit of the assay was about 23 pmol/l.

Total 8-iso-prostaglandin $F_{2\alpha}$. Plasma samples were analysed for total (sum of free and esterified) 8-iso-$PGF_{2\alpha}$ as

described elsewhere (Nourooz-Zadeh *et al*, 1995). Briefly, the samples were incubated with 0.5 ml of aqueous KOH (1 mol/l) to release esterifed F_2-isoprostanes, pH of the samples was re-adjusted to 2 adding 0.5 ml of HCl (1 mol/l) and $PGF_{2\alpha}$-d_4 was added as the internal standard. F_2-isoprostanes were then isolated by a solid-phase extraction on a C_{18} cartridge and an NH_4 cartridge. F_2-isoprostanes as pentafluorobenzyl ester/trimethyl ether derivates were analysed by gas chromatography (GC) on a Hewlett-Packard 5890 GC (Bracknell, UK) linked to a VG70SEQ mass spectrometer (MS, Fisons Instruments, Manchester, UK) using electron capture negative ion chemical ionisation (NICI) with ammonia reagent gas. Quantitative analysis was carried out by selective ion monitoring (SIM) of the carboxylate anion [M-181]-at m/z 569 and 573 for the F_2-isoprostanes and $PGF_{2\alpha}$-d_4 (internal standard), respectively.

Hydroperoxides. Total plasma hydroperoxide concentrations were measured using the ferrous oxidation in xylenol orange, version 2 (FOX2) assay as described previously by Södergren *et al* (1998). In brief, for each sample, aliquots of plasma were both incubated (30 min, 20–25°C) in triphenylphosphine to remove hydroperoxides to generate a blank and in methanol to generate a test sample. Another incubation (30 min, 20–25°C) with a FOX2 reagent containing the ferric ion indicator xylenol orange was performed. After centrifugation, the absorbance of the supernatant was determined at 560 nm in an Ultraspec 2000 spectrophotometer (Amersham Pharmacia Biotech, Uppsala, Sweden). The hydroperoxide concentration of each sample was calculated from the difference of the absorbance of the blank and test samples using an extinction coefficient of $3.86\times10^4\,M^{-1}\,cm^{-1}$.

Malondialdehyde. Levels of malondialdehyde (MDA) in plasma samples were determined using HPLC with fluorescence detection as described by Öhrvall *et al* (1994). A thiobarbituric acid reaction was carried out in a boiling water bath for 60 min. The malondialdehyde–thiobarbituric acid complex was extracted with methanol. MDA was separated on an HPLC column (Lichrospher 100 RP_{18}, 250×4 mm) and fluorescence was measured with an exitation wavelength of 532 nm and an emission wavelength of 553 nm.

Biomarkers of antioxidants

Tocopherols. Serum α- and γ-tocopherols were assayed using HPLC with fluorescence detection as described by Öhrvall *et al* (1993). The levels of serum tocopherols were adjusted for the sum of serum cholesterol and triglyceride concentrations as suggested by Thurnham *et al* (1986).

Antioxidative capacity. Plasma antioxidative capacity was determined by an enhanced chemiluminescent assay as described by Öhrvall *et al* (1997). The tocopherol analogue trolox (Aldrich Chemie, Steinheim, Germany) was used as a standard and the antioxidative capacity is expressed as trolox equivalents/1.

Thromboxane A_2

11-Dehydro-thromboxane B_2, a stable metabolite of thromboxane A_2 was analysed in urine by a modified enzyme immunoassay (EIA) as described earlier by Freese and Mutanen (1997). Commercial reagents were purchased from Cayman Chemical Co, USA. All samples were analysed in duplicate on the same plate.

Clinical characteristics and biochemical analyses

Body weight was measured on a digital scale with an accuracy of 0.1 kg and height was measured to the nearest cm. The BMI was calculated as the ratio of body weight (kg) to height squared (m^2). The fatty acid composition in serum PL, serum CE and the supplied food items were determined by gas-liquid chromatography (GLC) as described earlier by Boberg *et al* (1985). Very low-density lipoproteins (VLDL), LDL, HDL were isolated by a combination of preparative ultracentrifugation (Havel *et al*, 1955) and precipitation with a sodium phosphotungstate and magnesium chloride solution (Seigler & Wu, 1981). Cholesterol and triglyceride concentrations in serum and the isolated lipoprotein fractions were measured by enzymatic methods using the IL Test Cholesterol Triander's method 181618-10 and the IL Test Triglyceride Enzymatic-Colorimetric method 181610-60 in a Monarch 2000 centrifugal analyser (Instrumentation Laboratories, Lexington, MA, USA). The concentrations of serum apolipoprotein (apo) A-1 and apo B were determined by immunoturbidimetry (Orion Diagnostica, Espoo, Finland) in a Monarch apparatus. Lipoprotein (a) (Lp (a)) was measured by a Pharmacia apo (a) radioimmunoassay (Pharmacia, Uppsala, Sweden). One unit/l of apo (a) corresponds to 0.7 mg/l Lp (a). Serum free fatty acids (FFA) were analysed with an enzymatic colorimetric method using a commercial kit (994-75409, Wako Chemical, Neuss, Germany) modified for use in a Monarch apparatus. Plasma glucose concentrations were measured in a Beckman Glucose analyser 2 (Beckman Instruments, Fullerton, CA, USA). Plasma insulin concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) performed in an ES 300 automatic analyser (Boehringer Mannheim, Germany).

Statistical analysis

The analyses take into account the cross-over design of the experiment, the scales and the distribution of variables. All variables were continuous and on an interval scale. Variables with skewed distributions (Shapiro Wilk's W-test < 0.95) were log-transformed. An analysis of variance model (ANOVA) with factors for intervention, subject and time was used. Results are expressed as least square means with s.d. Values obtained after the two test diets were compared with each other and with the baseline value. A test for carry-over effects was used according to Jones and Kenward (1989). If the carry-over test was significant, only data from the first diet period was used for comparisons of the two test diets. Variables with carry-over effect were 11-dehydro-TXB_2, total cholesterol and HDL cholesterol. Differences between and within diets are presented with

P-values. For variables used to evaluate the primary aim, results are also presented with 95% confidence intervals (95% CI) for differences between diets. For log-transformed variables, CI are ratios of the geometric least square means of the two test diets (95% CI ratio). All tests were two-tailed and the significance level was 0.05. The statistical analyses were performed using the software Statistical Analysis System (SAS Institute, Cary, NC, USA).

Results

Body weight, dietary intake and fatty acid composition in serum phospholipids

All subjects completed both diet periods and the supplied fats and food items were well accepted. There were no significant changes of body weight within the groups or between the two diets during the study (baseline 76.5 ± 12.5 kg; RO 76.0 ± 12.5 kg; and SAT 76.3 ± 12.7 kg). The average daily dietary intake of nutrients calculated from the 3 day dietary records are shown in Table 3. There were no differences in total energy intake, E% from fat, protein or carbohydrates between the RO and SAT diet. The E% from fat in both diets were close to the values in the planned diets, whereas the E% from protein was slightly higher and the E% from carbohydrates was slightly lower than planned. The reported intake of vitamin E expressed as α-tocopherol equivalents (α-TE) was higher after the RO diet compared to the SAT diet. The dietary fat quality was also reflected in the fatty acid composition in serum PL (Table 4). The proportions of α-linolenic (18:3 n-3), eicosapentaenoic (20:5 n-3) and oleic (18:1 n-9) acids were all higher after the RO diet compared to the SAT diet.

Table 3 Average daily dietary intake during the rapeseed oil-based diet (RO) and the saturated diet (SAT) calculated from two pooled 3 day weighed food records

Nutrients	*RO*	*SAT*	P-*value*
Energy (MJ)	8.9 ± 1.8	9.7 ± 2.1	0.07
Fat (E%)	35.9 ± 4.3	36.2 ± 3.7	0.70
SAFA (E%)	10.3 ± 1.7	17.6 ± 2.1	< 0.001
MUFA (E%)	15.7 ± 2.3	10.9 ± 1.3	< 0.001
PUFA (E%)	7.5 ± 1.0	4.6 ± 0.8	< 0.001
18:2 n-6 (E%)	5.4 ± 0.7	4.0 ± 0.7	< 0.001
18:3 n-3 (E%)	1.8 ± 0.3	0.4 ± 0.1	< 0.001
Protein (E%)	14.8 ± 2.0	14.4 ± 2.0	0.19
Carbohydrates (E%)	47.3 ± 5.3	47.5 ± 4.1	0.86
Alcohol (E%)	2.0 ± 1.7	1.9 ± 1.9	0.57
Cholesterol (mg)	281 ± 98	364 ± 101	0.008
Dietary fibre (g)	17.1 ± 5.9	18.9 ± 6.4	0.11
Vitamin E (mg α-TE)	15.3 ± 2.8	11.5 ± 2.3	< 0.001
Ascorbic acid (mg)	80 ± 43	87 ± 57	0.77
β-Carotene (mg)	2 ± 1	2 ± 1	0.36
Selenium (μg)	35 ± 8	36 ± 12	0.89

Data are means ± s.d.; $n = 19$. *P*-values are for difference between the RO and SAT diets.
E% = energy percent. α-TE = alpha-tocopherol equivalents.

Table 4 Relative fatty acid composition (%) of serum phospholipids at baseline and after the rapeseed oil-based diet (RO) and the saturated diet (SAT)

Fatty acids	*Baseline*	*RO*	*SAT*	P-*value*
14:0	0.52 ± 0.14	0.42 ± 0.06***	0.55 ± 0.09	< 0.001
15:0	0.21 ± 0.05	0.18 ± 0.04**	0.23 ± 0.03	< 0.001
16:0	29.6 ± 1.51	29.5 ± 1.25	29.8 ± 1.24	0.12
16:1 n-7	0.73 ± 0.18	0.57 ± 0.15***	0.68 ± 0.16	< 0.001
17:0	0.44 ± 0.07	0.41 ± 0.06*	0.43 ± 0.06	0.11
18:0	14.0 ± 0.77	14.0 ± 1.03	14.3 ± 0.79	0.049
18:1 n-9	13.9 ± 2.15	13.7 ± 1.23	12.8 ± 1.78***	0.002
18:2 n-6	21.4 ± 1.11	22.0 ± 1.52	22.5 ± 1.76**	0.18
18:3 n-3	0.53 ± 0.27	0.57 ± 0.12	0.45 ± 0.22*	< 0.001
20:3 n-6	3.05 ± 0.60	2.82 ± 0.60*	3.15 ± 0.51	0.004
20:4 n-6	8.23 ± 0.91	8.12 ± 1.04	8.14 ± 1.25	0.88
20:5 n-3	1.62 ± 0.41	1.79 ± 0.45	1.37 ± 0.37*	< 0.001
22:5 n-3	1.14 ± 0.16	1.13 ± 0.16	1.10 ± 0.18	0.37
22:6 n-3	4.65 ± 1.02	4.75 ± 0.75	4.48 ± 0.78	0.075
Sum n-3	7.94 ± 1.21	8.24 ± 1.01	7.40 ± 1.00*	0.001
Sum n-6	32.7 ± 1.23	33.0 ± 0.76	33.8 ± 1.19**	0.017
PI[a]	87.5 ± 5.51	88.6 ± 4.75	86.40 ± 5.06	0.03

Data are means ± s.d.; $n = 19$. *P*-values are for difference between the RO and SAT diets.
*$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$, difference between test diet and baseline.
[a]PI = peroxidation index = (dienoic fatty acids × 1) + (trienoic fatty acids × 2) + (tetraenoic fatty acids × 3) + (pentaenoic fatty acids × 4) + (hexaenoic fatty acids × 5).

On the contrary, the proportions of the SAFAs myristic (14:0), pentadecanoic (15:0) and stearic (18:0) acids as well as the proportions of palmitoleic (16:1 n-7) and dihomo-γ-linolenic (20:3 n-6) acids were higher after the SAT diet compared to the RO diet. The relative proportion of palmitic (16:0), heptadecanoic (17:0), linoleic (18:2 n-6), arachidonic (20:4 n-6) and docosapentaenoic (22:5 n-3) acids were similar after both diets. Furthermore, the sum of n-3 fatty acids and the peroxidation index (PI) were higher, whereas the sum of n-6 fatty acids was lower after the RO diet in comparison to the SAT diet.

Effects on biomarkers of lipid peroxidation, antioxidants and thromboxane A_2

No difference in the levels of plasma free 8-iso-$PGF_{2\alpha}$ (95% CI ratio 0.94 − 1.46), total 8-iso-$PGF_{2\alpha}$ (95% CI ratio 0.56 − 1.36), malondialdehyde (95% CI ratio 0.98 − 1.18) or hydroperoxides (95% CI − 0.81 − 0.86) were detected between the RO and SAT diets (Table 5). In the urine, the level of free 8-iso-$PGF_{2\alpha}$ did not differ between the two test diets (95% CI ratio 0.91 − 1.27). The level of serum γ-tocopherol was higher after the RO diet compared to the SAT diet (95% CI ratio 1.62 − 2.18), whereas no differences in the levels of serum α-tocopherol (95% CI ratio 0.98 − 1.09) and the antioxidative capacity (95% CI-6.8 − 54.8) in plasma were detected between the two diets. The levels of urinary 11-dehydro-thromboxane B_2 did not differ between the RO and the SAT diets (95% CI ratio 0.46 − 1.29).

Table 5 Levels of biomarkers of lipid peroxidation, antioxidants and a thromboxane (TX) A_2 metabolite in plasma, serum and urine at baseline and after the rapeseed oil-based diet (RO) and the saturated diet (SAT)

	Baseline	*RO*	*SAT*	P-*value*
*Lipid peroxidation—plasma (*n = *19)*				
Free 8-iso-$PGF_{2\alpha}$ (pmol/l)	111 ± 59	125 ± 70	111 ± 63	0.17
Total 8-iso-$PGF_{2\alpha}$ (pmol/l)[a]	740 ± 530	580 ± 640	530 ± 340	0.55
Hydroperoxides (μmol/l)	6.73 ± 1.59	6.69 ± 1.92	6.66 ± 2.63	0.95
Malondialdehyde (μmol/l)	0.69 ± 0.10	0.66 ± 0.15	0.61 ± 0.10*	0.14
*Lipid peroxidation—urine (*n = *17)*				
Free 8-iso-$PGF_{2\alpha}$ (ng/mg creatinine)		3.31 ± 1.33	3.10 ± 1.44	0.39
*Antioxidants—serum and plasma (*n-*19)*				
α-Tocopherol (μmol/mmol)[b]	4.20 ± 0.64	4.24 ± 0.45	4.09 ± 0.36	0.20
γ-Tocopherol (μmol/mmol)[b]	0.34 ± 0.14	0.42 ± 0.09**	0.23 ± 0.06***	< 0.001
AOC (μmol trolox equivalents/l)[c]	526.3 ± 87.5	546.0 ± 93.6	522.0 ± 89.8	0.13
*TXA_2 metabolite—urine (*n = *17)*				
11-dehydro-TXB_2 (ng/mg creatinine)[d]		1390 ± 980	2230 ± 1850	0.34

Data are means ± s.d. *P*-values are for difference between the RO and SAT diets.
AOC = antioxidative capacity.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, difference between test diet and baseline.
[a]$n = 14-17$. [b]Lipid adjusted. [c]Without lipid adjustment. [d]Variable with carry-over effect; data are from the first diet period only; $n = 10$ and nine for the RO and SAT diet, respectively.

Effects on metabolic variables

The serum cholesterol, LDL cholesterol, LDL/HDL ratio as well as the serum concentration of apo B was lower after the RO diet compared to the SAT diet (Table 6). The concentrations of serum triglyceride, VLDL cholesterol and triglyceride, HDL cholesterol and triglyceride, apo Al and Lp (a) were similar after the two diets. Fasting plasma glucose was lower after the RO diet in comparison to the SAT diet, while plasma insulin and FFA did not differ between the two diets.

Discussion

Over the past two decades there has been an expansion of the production of low-erucic acid rapeseed oil (canola oil). Rapeseed oil is rich in unsaturated fatty acids and has been shown to have beneficial nutritional effects but little is known about the risk of increased lipid peroxidation. From a public health perspective it is important to investigate the effects of a diet with rapeseed oil-based fats and consequently a high intake of MUFAs and PUFAs, especially

Table 6 Levels of lipids, lipoproteins, apolipoproteins and free fatty acids in serum and glucose and insulin in plasma at baseline after the rapeseed oil-based diet (RO) and the saturated diet (SAT)

	Baseline	*RO*	*SAT*	P-*value*
Total cholesterol (mmol/l)[a]	6.59 ± 1.02/5.73 ± 0.85	5.85 ± 0.97***	6.05 ± 1.14	< 0.001
Total triglycerides (mmol/l)	1.40 ± 0.61	1.24 ± 0.65	1.43 ± 0.96	0.18
VLDL cholesterol (mmol/l)	0.47 ± 0.26	0.39 ± 0.27	0.49 ± 0.46	0.64
VLDL triglycerides (mmol/l)	0.90 ± 0.62	0.79 ± 0.62	0.97 ± 0.92	0.39
LDL cholesterol (mmol/l)	4.31 ± 0.94	3.85 ± 0.81***	4.33 ± 1.10	< 0.001
LDL triglycerides (mmol/l)	0.39 ± 0.10	0.35 ± 0.09**	0.36 ± 0.08*	0.31
HDL cholesterol (mmol/l)[a]	1.55 ± 0.40/1.17 ± 0.27	1.41 ± 0.30*	1.15 ± 0.22	0.26
HDL triglycerides (mmol/l)	0.097 ± 0.04	0.074 ± 0.03*	0.075 ± 0.04*	0.67
LDL/HDL ratio	3.34 ± 0.97	2.99 ± 0.80**	3.47 ± 1.09	< 0.001
Apo A1	1.38 ± 0.19	1.37 ± 0.16	1.37 ± 0.18	0.96
Apo B	1.01 ± 0.15	0.95 ± 0.20	1.04 ± 0.19	0.012
Lp (a)	196 ± 182	205 ± 190	191 ± 213	0.42
Free fatty acids (mmol/l)	0.42 ± 0.19	0.41 ± 0.19	0.38 ± 0.17	0.58
Fasting glucose (mmol/l)	4.96 ± 0.29	4.86 ± 0.35	5.03 ± 0.33	0.015
Fasting insulin (munits/l)	8.42 ± 3.17	9.82 ± 5.67	8.74 ± 4.63	0.24

Data are means ± s.d. $n = 19$. *P*-values are for difference between the RO and SAT diets.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, difference between test diet and baseline.
[a]Variable with carry-over effect; data are from the first diet period only; baseline corresponds to the RO and SAT diet, respectively (RO/SAT); $n = 10$ and nine for RO and SAT diet, respectively.

α-linolenic acid. When we compared a rapeseed oil-based diet with a control diet rich in saturated fats we found no differences in the biomarkers of lipid peroxidation and antioxidants, except for increased levels of serum γ-tocopherol after the RO diet compared to the SAT diet. These findings suggest that there is a balance between the content of antioxidants and PUFA in rapeseed oil-based fats when used in a natural mixed diet and that the amount of antioxidants naturally present is sufficient.

Dietary intake was monitored by weighed dietary records and there were considerable differences in fat quality between the RO and SAT diets. The two diets differed essentially in E% of α-linolenic acid (18:3 n-3) and in the amount of SAFA, MUFA, PUFA, oleic acid (18:2 n-6) as well as the content of cholesterol and vitamin E expressed as α-TE. To evaluate if reported energy intakes were plausible we used calculated physical activity levels (PALs) based on reported energy intakes and estimated basal metabolic rate. According to minimum cut-off limits for realistic energy intakes established by Goldberg *et al* (1991) the PAL values indicated under-reporting. PALs were generally low (RO 1.33 ± 0.31 and SAT 1.33 ± 0.29, $P = 0.57$) after both diets and did not differ significantly between the two diets, which indicates that the possible under-reporting during both diet periods was similar. The body weight of the subjects remained unchanged during both diets. The compliance to the diets was also monitored by the plasma fatty acid profile. The changes of the proportions of the different fatty acids in serum PL were similar to results achieved in earlier studies investigating rapeseed oil-rich diets (Gustafsson *et al*, 1994; Nydahl *et al*, 1994), with increased proportions of α-linolenic acid, eicosapentaenoic acid and oleic acid after the RO diet compared to the SAT diet. The fatty acid pentadecanoic acid (15:0) has been shown to be a marker for dietary intake of milk fat (Smedman *et al*, 1999). The higher proportion of 15:0 in the serum PL after the SAT diet may therefore indicate a higher intake of saturated fatty acids derived from milk fat in the SAT diet.

Many factors may influence the evaluation of the propensity of n-3 fatty acids to oxidation such as (a) how they are supplied and the amounts—as supplements or by the diet; (b) their chain length and number of double bounds—α-linolenic acid (18:3 n-3) or long chain n-3 fatty acids; and (c) how lipid peroxidation assays are performed—*in vitro* oxidation of LDL or *in vivo* indices. Dietary fatty acid composition largely determines the fatty acid composition of plasma lipoproteins, which influences the rate and extent of LDL peroxidation (Tsimikas & Reaven, 1998). Whereas SAFA- and MUFA-rich LDL particles are fairly resistant to oxidation, PUFA-rich LDL particles are more susceptible to lipid peroxidation *in vitro* (Bonanome *et al*, 1992; Abbey *et al*, 1993; Reaven *et al*, 1993). However, the measurement of lipid peroxidation *in vivo* is of particular interest, because the oxidative conditions that are used to determine oxidative susceptibility of LDL *in vitro* may not be relevant *in vivo* (Upston *et al*, 1999).

A number of techniques have been employed for the assessment of the degree of lipid peroxidation *in vivo*. However, many of these often have shortcomings and limitations (Halliwell & Gutteridge, 1999). The thiobarbituric acid (TBA) test for measuring malondialdehyde (MDA) is commonly used on ground of its simplicity, but has often been criticised for being non-specific and producing artefacts. MDA is generated as a degradation product from peroxidised lipids; however, it can also be generated during the acid heating step in the TBA reaction. Another problem is that other compounds (eg other aldehydes and carbohydrates) can react with TBA to form complexes that absorb at the same wavelength as the TBA–MDA complex. An approach to avoiding artefacts and increasing the specificity of the TBA test for assessing MDA levels is to combine HPLC separation and fluorescence as in the present study. Several methods have been developed to measure lipid hydroperoxides formed in the initial steps in the lipid peroxidation chain reaction. A problem regarding hydroperoxide measurements are the wide range of reported values when using different methods. The discovery of F_2-isoprostanes, specific end-products of arachidonic acid peroxidation, has been a major advance in the ability to assess lipid peroxidation *in vivo* (Roberts & Morrow, 1997; Lawson *et al*, 1999). Measurement of F_2-isoprostanes in body fluids is now considered to be a promising biomarker for measurement of lipid peroxidation *in vivo* because it can be specifically and accurately measured in normal biological samples, and the levels are increased under conditions of oxidative stress and modulated by antioxidants. Both total and free levels of F_2-isoprostanes were measured in this study, because they reflect different patterns of formation. F_2-isoprostanes are initially formed *in situ* from esterified arachidonic acid in PL and are then released in the free form into the circulation (Roberts & Morrow, 1997; Lawson *et al*, 1999). Selecting a single test to monitor lipid peroxidation can give misleading results. We have employed several techniques as an approach to evaluate the effects on the degree of *in vivo* lipid peroxidation.

A few studies have investigated effects of dietary fat quality on biomarkers of *in vivo* lipid peroxidation. The influence of a natural mixed rapeseed oil-based diet on lipid peroxidation was investigated in a study by Turpeinen *et al*, (1995). There was considerable disparity between *in vitro* and *in vivo* indices of lipid peroxidation. Plasma levels of MDA and conjugated dienes and the amount of TBARS and hydroperoxides in LDL were unchanged or slightly decreased, while the susceptibility of LDL to oxidation *in vitro* (lag time and time to maximum oxidation) was increased. We did not find any changes in the *in vivo* biomarkers of lipid peroxidation measured in this study (8-iso-$PGF_{2\alpha}$, hydroperoxides and MDA), which is in line with the *in vivo* findings of Turpeinen *et al* (1995). The relatively narrow CI indicates the sufficient power of this study to detect clinically significant differences. However, it should be noted that there is a large inter-individual variation in the degree of loss of detectable hydroperoxides

after storage at −70°C (Södergren *et al*, 1998) and that our results from the measurements of hydroperoxides in samples stored frozen should be interpreted cautiously. In contrast to the rapeseed oil-based diets reported above, diets containing a higher proportion of PUFA and less MUFA suggest an increased lipid peroxidation with increased urinary excretion of F_2-isoprostanes as measured by radioimmunoassay (Turpeinen *et al*, 1998), plasma TBARS formation (Berry *et al*, 1991), and urinary TBARS levels (Jenkinson *et al*, 1999) in healthy volunteers. Intervention studies with n-3 fatty acid enriched diets have also shown increases of plasma MDA levels (Nair *et al*, 1993; Gustafsson *et al*, 1996; Allard *et al*, 1997; Foulon *et al*, 1999), which could be prevented by increasing the intake of vitamin E (Nair *et al*, 1993; Gustafsson *et al*, 1998). However, another study with n-3 fatty acid supplementation indicated that the *in vivo* lipid peroxidation was not increased (Higdon *et al*, 2000). Differences in the antioxidant content of the diets together with differences in the degree of unsaturation and oxidisability of fatty acids in the diets may partly explain the discrepancy between lipid peroxidation outcomes in these studies. In our study, the sum of n-3 fatty acids as well as the PI were both higher after the RO diet compared to the SAT diet. Yet, no differences in the *in vivo* biomarkers of lipid peroxidation were found between the two diets, which may indicate that the antioxidant content of the RO diet was sufficient to protect the unsaturated fatty acids from oxidation.

In general, there are few studies on the effects of dietary fat composition and urinary excretion of thromboxane metabolites. Fish oils have quite consistently decreased excretion of thromboxane A_2 metabolites (Prakash *et al*, 1994). For other types of fatty acids the data is more inconsistent. In a study of Freese and Mutanen (1997) 11-dehydro-thromboxane B_2 excretion did not differ after α-linolenic acid or eicosapentaenoic/docosapentaenoic acid supplementation. We found no difference in the urinary levels of 11-dehydro-TXB_2 between the RO and the SAT diets.

The estimated intake of vitamin E from the dietary records revealed a greater intake of vitamin E expressed as α-tocopherol equivalents (α-TE) during the RO diet compared to the SAT diet (15.3 and 11.5 mg α-TE per day). These data corresponds to 13.9 and 11.0 mg α-tocopherol and approximately 14.1 and 5.6 mg γ-tocopherol per day during the RO and SAT diets, respectively. We found here that the level of γ-tocopherol analysed in serum was higher after the RO diet compared to the SAT diet, whereas the serum α-tocopherol levels did not differ between the two diets. This leads to a lower ratio of α- to γ-tocopherol after the RO diet compared to the SAT diet. A low γ-tocopherol concentration and a high α- to γ-tocopherol ratio has been reported in patients with CHD compared to controls (Öhrvall *et al*, 1996; Kontush *et al*, 1999) and in a population with a high incidence of CHD (Kristenson *et al*, 1997). These results suggest that a serum tocopherol profile with low γ-tocopherol levels and a high α- to γ-tocopherol ratio may be an indicator of an increased risk for CHD and that rapeseed oil-rich diets may help to increase the levels of γ-tocopherol in the body. Recently, the final results of the Lyon Diet Heart Study indicated that a Mediterranean diet rich in α-linolenic acid was beneficial in secondary prevention of CHD (de Lorgeril *et al*, 1999). Surprisingly, data from the same study also suggest that the Mediterranean diet protects against CHD through mechanisms independent of traditional CHD risk factors such as blood lipid profile, blood pressure and smoking. The content of antioxidants and α-linolenic acid of several plant foods in the Mediterranean diet have been suggested to be critical mediators of the beneficial effects of this diet (de Lorgeril *et al*, 1999; Trichopoulou *et al*, 1999). Their data further support possible positive effects of rapeseed oil with its high content of vitamin E and α-linolenic acid.

Effects on serum lipids, lipoproteins, and apolipoproteins are in line with earlier intervention studies with rapeseed oil (McDonald *et al*, 1989; Wardlaw *et al*, 1991; Valsta *et al*, 1992; Nydahl *et al*, 1994). Even with the relatively high fat content of the test diets, we found lower total and LDL cholesterol levels, apo B concentrations, and LDL/HDL ratios and no differences in HDL cholesterol levels after the RO diet compared to the SAT diet. Due to carry-over effects in serum total and HDL cholesterol, data on these variables are only presented from the first diet period. No differences in the other metabolic variables were observed, except for a significant lower fasting plasma glucose concentration on the RO diet compared to the SAT diet.

In conclusion, we investigated the effects of a rapeseed oil-rich diet, composed essentially according to present dietary recommendations, on *in vivo* biomarkers of lipid peroxidation. No significant differences in the biomarkers of lipid peroxidation and antioxidants were found, except for increased levels of serum γ-tocopherol after the RO diet. These findings suggest that a rapeseed oil-rich diet does not seem to increase the degree of lipid peroxidation in the body.

This could be explained by a sufficient content of antioxidants in rapeseed oil to protect the unsaturated fatty acids from oxidation and to improve the circulating levels of γ-tocopherol. These results also imply the need for future investigation on other potential beneficial effects of a rapeseed oil-based diet in addition to lipid lowering, eg through effects of high contents of α-linolenic acid and antioxidants or through effects on endothelial function.

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