

## Effects of a Monounsaturated Rapeseed Oil and a Polyunsaturated Sunflower Oil Diet on Lipoprotein Levels in Humans

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The effects of high oleic acid rapeseed oil compared with polyunsaturated fats on serum lipoprotein levels are largely unknown. Therefore, we fed 30 women and 29 men a baseline diet rich in saturated fat, which was followed by a diet rich in high oleic and low erucic acid rapeseed oil (total energy content of fat, 38%; saturates, 12.4%; monounsaturates, 16%; n-6 polyunsaturates, 6%; and n-3 polyunsaturates, 2%) and one rich in sunflower oil (total energy content of fat, 38%; saturates, 12.7%; monounsaturates, 10%; n-6 polyunsaturates, 13%; and n-3 polyunsaturates, 0%). The oils were incorporated into mixed natural diets that were dispensed in a random order for 3.5 weeks each in a blinded crossover design. The diet composition was confirmed by analysis of duplicate diets. Both test diets reduced serum total cholesterol (TC) and low density lipoprotein (LDL) cholesterol levels from baseline, the monounsaturated rapeseed oil diet more than the polyunsaturated sunflower oil diet (TC: -15% versus -12%,  $p < 0.01$ ; LDL cholesterol: -23% versus -17%,  $p < 0.01$ ). Very low density lipoprotein (VLDL) cholesterol and total, VLDL, and LDL triglyceride levels were lower during the sunflower oil diet compared with the rapeseed oil diet. Total high density lipoprotein (HDL) cholesterol levels remained unchanged by both diets. The consumption of rapeseed oil resulted in a more favorable HDL<sub>2</sub> to LDL cholesterol ratio ( $0.43 \pm 0.19$  versus  $0.39 \pm 0.18$ ,  $p < 0.01$ ) and an apolipoprotein A-I to B ratio ( $3.0 \pm 1.4$  versus  $2.4 \pm 1.6$ ,  $p < 0.001$ ) than did the sunflower oil. Our results suggest that substitution by high oleic acid rapeseed oil of saturated fats strongly affects LDL metabolism, whereas substitution by polyunsaturated sunflower oil affects both VLDL and HDL<sub>2</sub> in addition to LDL. (*Arteriosclerosis and Thrombosis* 1992;12:50-57)

It has previously been commonly accepted that dietary saturated fatty acids increase and polyunsaturated fatty acids decrease serum total cholesterol (TC) concentration, whereas monounsaturated fatty acids have no effect on serum lipoproteins in humans.<sup>1-4</sup> However, several recent studies have disputed the concept of a specific decline in serum cholesterol by polyunsaturates relative to monounsaturates.<sup>5-8</sup> Most of the studies that analyze the effects of dietary monounsaturated fatty acids in humans have used olive oil<sup>6,8-11</sup> or oleic acid-rich variants of sunflower<sup>5,13-15</sup> or safflower<sup>5,13-15</sup> oil as a source of monounsaturated fatty acids. None of these

are widely consumed in northwestern Europe or America. The major high oleic acid oil in these areas is the new low erucic acid rapeseed oil ("canola oil"), which in the United States is now one of the most widely used edible oils. Low erucic acid rapeseed oil differs from olive oil not only in its minor constituents such as squalene but also in its content of the n-3 polyunsaturated acid,  $\alpha$ -linolenic acid (C18:3, n-3). None of the previous studies have compared the effects of monounsaturate- and polyunsaturate-rich oils on the lipid composition of lipoproteins. Therefore, we compared the effects of a monounsaturated fat diet of rapeseed oil with those of a polyunsaturated fat diet of sunflower oil on serum lipoprotein concentrations in healthy men and women who were fed a baseline diet high in saturated fatty acids.

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### Methods

#### Subjects

The subject population comprised 59 healthy volunteers, 36 of whom were students, and the remaining 23 were university employees or were not connected with the university. There were 30 women and

TABLE 1. Characteristics of the Subjects at Screening

Characteristic	All (n=59)		Women (n=30)		Men (n=29)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Age (yr)	30±12	18–65	33±14	20–65	28±9	18–60
Body mass index (kg/m <sup>2</sup> )	22.7±3.2	17.8–34.9	22.9±3.6	17.8–34.9	22.5±2.6	18.7–29.2
Serum cholesterol (mmol/l)	5.03±0.89	3.20–7.40	5.23±0.95	3.75–7.40	4.82±0.77	3.20–6.30
Serum HDL cholesterol (mmol/l)	1.29±0.26	0.59–1.94	1.39±0.24	1.00–1.94	1.19±0.24	0.59–1.74
Serum triglycerides (mmol/l)	0.98±0.49	0.32–2.79	0.82±0.31	0.32–1.8	1.15±0.59	0.37–2.79

HDL, high density lipoprotein.

29 men in the study group. Subject age was 18–65 years, with a median age of 25 years. Exclusion criteria for the study were serum cholesterol >7.4 mmol/l, hypertension, anemia, glycosuria, or proteinuria. The baseline characteristics of the subjects are shown in Table 1. None of the subjects were daily smokers. Four women used oral contraceptives throughout the study. One man received erythromycin (1,200 mg/day for 10 days), and one man lost 2.6 kg during the first 5 weeks of the study. Exclusion of these two men did not affect the results obtained, so the results are presented for all the subjects.

The study protocol, which had been approved by the ethics committees of the National Public Health Institute and the Faculty of Forestry and Agriculture at the University of Helsinki, was carefully explained to the volunteers, who then gave their written consent. Excluding free food they received no payment. The subjects were asked not to change their smoking habits or alcohol consumption during the study. The amount of alcohol consumed was recorded in diaries. In addition, the subjects were asked not to change their patterns of physical activity.

#### Diets and Design

The experiment lasted 63 days. During the first 2 weeks all the subjects received a baseline diet rich in saturated fatty acids. Then the two test diets were given in a crossover design; 30 subjects received the sunflower oil diet for 25 days followed by the rapeseed oil diet for 25 days (group 1), and the other 29 subjects were fed the same diets in reverse order (group 2) (Figure 1). Neither the subjects nor the staff who analyzed the blood samples were aware of the order of the test diets. At the end of the experiment 51% of the subjects wrongly guessed the order of the test diets, showing that the blinding had been successful.

The test oils were very low erucic acid ( $\approx 0.5\%$ ), high oleic acid Finnish turnip rapeseed oil (*Brassica rapa* subsp. *oleifera* DC; the breakdown of its fatty acids was as follows: 5% saturates, 58% monounsaturates, 24% n-6 polyunsaturates, and 13% n-3 polyunsaturates), which is equivalent to the so-called canola oil,<sup>16</sup> and high linoleic acid sunflower oil (*Helianthus annuus*, which has a fatty acid content of 12% saturates, 23% monounsaturates, 65% n-6 polyunsaturates, and only traces of n-3 polyunsaturates). Both were provided by the Finnish Unilever Com-

pany Paasivaara, Helsinki, Finland. Each diet consisted of conventional mixed solid foods. Menus for lunch and dinner were changed daily over a 3-week period, whereas weekly rotating menus were used for breakfasts. Both test diets included a special margarine that contained 68 g test oil/100 g margarine, specially prepared bread (11 g test oil/100 g bread), salad dressing (31 g test oil/100 g dressing), and ice cream (7 g test oil/100 g ice cream). Special buns, cakes, pies, and pancakes were prepared with the test oils or the special margarines in our research kitchen. Rapeseed oil contributed 63% of the monounsaturated fatty acids in the rapeseed oil diet, and sunflower oil contributed 87% of the polyunsaturated fatty acids in the sunflower oil diet.

Every weekday the subjects came to the Department of Nutrition at noon to have their lunch and receive their food for the rest of the day and their breakfast for the next morning. Food for the whole weekend was provided each Friday. All foodstuffs were weighed for each participant. The subjects recorded in their diaries any leftover food. In addition to the food supplied, the subjects were allowed to freely choose 10% of their energy intake so long as the foods chosen were free from fat and cholesterol (e.g., sugar, sweets, alcoholic drinks, beverages, fruit, and vegetables). The subjects received a weekly newsletter that included tips and recipes for combining their selected foods with the eggs and margarine provided. They were asked to record in their diaries any signs of illness together with any medication

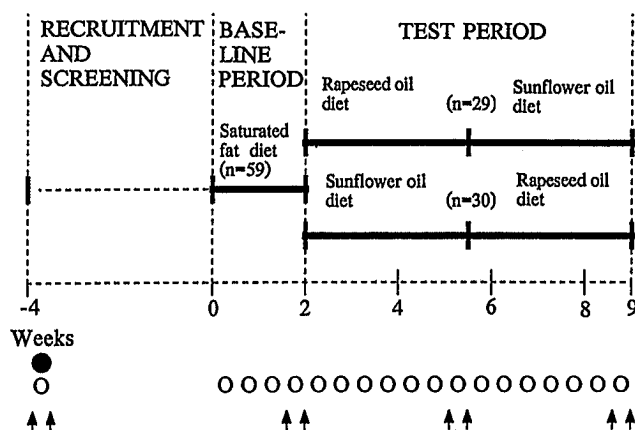


FIGURE 1. Chart showing study design. ●, Health and dietary questionnaire; ○, weight control; ↑, blood sampling.

used, the self-selected food items, the amount and type (filtered or boiled) of coffee consumed, and deviations from the diet. Body weight in light clothing was recorded biweekly, and energy intake was adjusted to avoid weight changes. The average weight changes during the diet periods were less than 0.3 kg.

Duplicate portions of each diet were collected daily, pooled for each diet period, and analyzed. The analyzed values were combined with the values calculated from the free-choice food (average, 9.8% of total daily energy), and those calculated for leftovers were subtracted (average, 0.2% of total daily energy).

#### Blood Sampling and Analysis

Fasting blood samples were taken on two separate days during the last week of each period (Figure 1). Serum was separated within 1 hour after venipuncture. Total cholesterol was measured in both samples, and lipoprotein analysis was performed in one of these two samples. Samples for TC, high density lipoprotein (HDL) cholesterol, triglycerides, and lipoprotein analysis were stored at 4°C until assayed. Total cholesterol and HDL cholesterol (after precipitation of  $\beta$ -lipoproteins with dextran sulfate plus magnesium chloride) were determined enzymatically (CHOD-PAP, Boehringer Mannheim, Mannheim, FRG).<sup>17</sup> Triglyceride concentration was determined with the fully enzymatic method of Wahlefeld.<sup>18</sup> Phospholipids were assayed with the phospholipase D–cholinoxidase–peroxidase (PAP) method<sup>19</sup> by using commercial reagents (Wako Chemicals, GmbH, Nuess, FRG). Apolipoproteins (apo) A-I and B were quantified by immunoturbidimetry.<sup>20</sup> The samples for apo A-I and B determinations were prediluted with physiological saline (0.9% saline) just before the assays. The immunoturbidimetric reaction was done in phosphate-buffered saline (pH 7.35) that contained 5% polyethylene glycol 6000 and 0.01% Triton X-100 (apo A-I assay buffer) or 0.01% Tween-20 (apo B assay buffer). It is notable that detergents that are present in assay buffers will remove any intrinsic turbidity of a possibly lipemic serum without interfering with the antigen–antibody precipitation reaction. The accuracy and precision of the different laboratory methods were controlled by participating in external national and international quality control programs (Lab Quality, Helsinki, Finland, and World Health Organization, Collaborating Lipid Reference Center, Heidelberg, FRG). The mean interassay coefficients of variation were 1% for TC, 3.9% for HDL cholesterol, 4.3% for triglycerides, 6% for apo A-I, and 9% for apo B. Dietary compliance was checked by analyzing the fatty acid composition of the plasma phospholipids by a gas chromatographic method.<sup>21,22</sup> The subjects and the staff were not given the results of the blood analyses until the end of the study.

#### Isolation of Lipoproteins

Very low density lipoprotein (VLDL), low density lipoprotein (LDL), HDL<sub>2</sub>, and HDL<sub>3</sub> (bottom fraction) were isolated from fresh serum samples by ultracentrifugation at  $d < 1.006$  g/ml,  $1.006 < d < 1.063$

g/ml,  $1.063 < d < 1.125$  g/ml, and  $d > 1.125$  g/ml, respectively, according to the method of Havel et al.<sup>23</sup> Solution densities were adjusted with potassium bromide. All centrifugations were performed in the Type TFT 45.6 rotor (Kontron Instruments, Zürich, Switzerland) by using the Beckman Model L preparative ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.). The specified lipoprotein fractions after centrifugation were removed by a standard tubelisting technique. Lipoprotein fractions were stored at 4°C, and they were analyzed for different lipids within 2 days. This short storage time did not have any significant effect on serum total lipid or lipoprotein lipid levels.<sup>24</sup>

#### Statistical Analysis

Differences in the serum lipid values between the test diets were analyzed with repeated-measures analysis of variance for crossover designs (BMDP, program 5V, BMDP Statistical Software, Los Angeles).<sup>25</sup> The statistical tests were done for the whole group and for men and women separately. The differences in carryover effects were evaluated,<sup>26</sup> and they were considered significant at  $p < 0.10$ .<sup>27</sup> No evidence was found to suggest that the carryover effects between the diet treatment sequences were different, and the results of the two treatment sequences were combined. For the test diets changes in serum lipid, lipoprotein, and apolipoprotein concentrations and plasma phospholipid oleic acid to linoleic acid ratios from the last week of the baseline diet to the last week of a test diet were analyzed by using dependent Student's *t* tests. All the statistical tests used were two tailed.

#### Results

All the subjects successfully completed the study. The composition of the diets, which were based on the analysis of daily duplicate portions, is shown in Table 2. In the two test diets the intake of saturated fats was kept constant (12.4–12.7% of energy). The polyunsaturated to saturated fat ratios of the sunflower oil diet and the rapeseed oil diet were 1.0 and 0.6, respectively. The cholesterol content of the diets was kept constant (average, 315–360 mg/day) throughout all the stages. Individual energy intakes ranged from 7.0 to 15.1 MJ (1,670–3,610 kcal) on the baseline diet, from 8.0 to 14.1 MJ (about 1,910–3,370 kcal) on the sunflower oil-rich diet, and from 7.9 to 14.1 MJ (about 1,890–3,370 kcal) on the rapeseed oil diet. The average consumption of filtered coffee was  $260 \pm 190$  ml/day and of boiled coffee  $4 \pm 19$  ml/day, the latter being negligible in terms of effects on cholesterol levels.<sup>28</sup>

Dietary compliance was evaluated by monitoring the plasma phospholipid fatty acid composition. The mean ratio of oleic (18:1 n-9) to linoleic (18:2 n-6) acid in the plasma phospholipids was  $0.48 \pm 0.06$  and  $0.50 \pm 0.08$  for groups 1 (sunflower oil diet first) and 2 (rapeseed oil diet first), respectively, at the end of the saturated baseline diet, and was reduced to  $0.27 \pm 0.04$  during the

TABLE 2. Mean Daily Intake of Nutrients According to Duplicate-Portion Analysis Plus Calculated Contribution of Freely Selected Items

Nutrient	Baseline diet	Sunflower oil diet	Rapeseed oil diet
Energy (MJ)	10.1	10.6	10.5
Protein (% of energy)	14.9	14.5	15.1
Fat (% of energy)	35.8	37.7	37.8
Saturates	18.9	12.7	12.4
C12–C16 saturates*	12.7	8.0	8.1
Monounsaturates	11.0	10.2	16.2
Oleic acid (C18:1)	9.5	9.6	15.0
Erucic acid (C22:1)	0.1	0.1	0.2
Polyunsaturates	3.7	13.3	7.6
Linoleic acid (C18:2 n-6)	3.2	12.7	5.5
$\alpha$ -Linolenic acid (C18:3 n-3)	0.4	0.4	2.1
Cholesterol (mg/day)†	354	315	360
Stigmasterol (mg/day)†§	12	15	27
$\beta$ -Sitosterol (mg/day)†§	111	276	200
Carbohydrates (% of energy)	49.5	48.4	47.7
Monosaccharides and disaccharides‡	20.9	20.2	20.1
Polysaccharides	28.6	28.2	27.6
Dietary fiber (g/day)†§	30	30	29
Alcohol (% of energy)	1.0	1.0	1.1

\*Saturated fatty acids with chain lengths of 12, 14, or 16 carbon atoms.

†For a subject of average energy intake.

‡Including the natural sugars in fruit, milk, etc.

§Does not include small amounts from freely selected foods.

sunflower oil diet (group 1, period 1) ( $p < 0.001$ ) and increased to  $0.56 \pm 0.07$  during the rapeseed oil diet (group 2, period 1) ( $p < 0.01$ ).

The changes in serum cholesterol, triglycerides, phospholipids, lipoprotein fractions, and apolipopro-

teins are shown in Tables 3–5 for the whole study group and separately for women and men. The changes in TC, LDL cholesterol, HDL cholesterol, triglycerides, and apo A-I to B ratio associated with the saturated baseline diet and the vegetable oil-

TABLE 3. Serum Total, Lipoprotein Lipid, and Apolipoprotein Concentrations in 59 Subjects Who Consumed Polyunsaturated and Monounsaturated Fat Diets

Parameter	Baseline diet	Sunflower oil diet (polyunsaturated)	Rapeseed oil diet (monounsaturated)	Difference in sunflower oil vs. rapeseed oil diet
Cholesterol (mmol/l)				
Total	5.35 $\pm$ 0.98	4.62 $\pm$ 0.87	4.52 $\pm$ 0.80	†
VLDL	0.25 $\pm$ 0.17	0.18 $\pm$ 0.14	0.24 $\pm$ 0.25	*
LDL	3.17 $\pm$ 0.82	2.58 $\pm$ 0.70	2.41 $\pm$ 0.67	†
HDL, total	1.33 $\pm$ 0.28	1.34 $\pm$ 0.26	1.34 $\pm$ 0.26	NS
HDL <sub>2</sub>	0.98 $\pm$ 0.35	0.92 $\pm$ 0.33	0.95 $\pm$ 0.32	*
HDL <sub>3</sub>	0.55 $\pm$ 0.08	0.52 $\pm$ 0.10	0.51 $\pm$ 0.08	NS
Triglycerides (mmol/l)				
Total	0.88 $\pm$ 0.37	0.79 $\pm$ 0.37	0.85 $\pm$ 0.38	*
VLDL	0.40 $\pm$ 0.26	0.34 $\pm$ 0.22	0.47 $\pm$ 0.31	‡
LDL	0.26 $\pm$ 0.07	0.21 $\pm$ 0.07	0.23 $\pm$ 0.03	†
HDL	0.09 $\pm$ 0.03	0.07 $\pm$ 0.02	0.07 $\pm$ 0.02	NS
Phospholipids (mmol/l)	2.85 $\pm$ 0.45	2.50 $\pm$ 0.46	2.92 $\pm$ 0.60	‡
Apolipoprotein A-I (g/l)	1.50 $\pm$ 0.36	1.23 $\pm$ 0.24	1.45 $\pm$ 0.25	‡
Apolipoprotein B (g/l)	0.80 $\pm$ 0.36	0.62 $\pm$ 0.25	0.61 $\pm$ 0.31	NS
Apolipoprotein A-I/apolipoprotein B	2.23 $\pm$ 1.09	2.39 $\pm$ 1.6	3.00 $\pm$ 1.36	‡

Values are mean $\pm$ SD. VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

\* $p < 0.05$ , † $p < 0.01$ , ‡ $p < 0.001$ , and NS, not significant.

TABLE 4. Serum Total, Lipoprotein Lipid, and Apolipoprotein Concentrations in Women ( $n=30$ ) Who Consumed Polyunsaturated and Monounsaturated Fat Diets

Parameter	Baseline diet	Sunflower oil diet (polyunsaturated)	Rapeseed oil diet (monounsaturated)	Difference in sunflower oil vs. rapeseed oil diet
Cholesterol (mmol/l)				
Total	5.63±0.99	4.86±0.96	4.74±0.48	†
VLDL	0.22±0.16	0.15±0.09	0.19±0.14	†
LDL	3.24±0.89	2.72±0.75	2.52±0.71	*
HDL, total	1.43±0.27	1.45±0.21	1.44±0.24	NS
HDL <sub>2</sub>	1.15±0.34	1.09±0.28	1.12±0.28	NS
HDL <sub>3</sub>	0.53±0.07	0.50±0.09	0.51±0.09	NS
Triglycerides (mmol/l)				
Total	0.84±0.36	0.71±0.34	0.81±0.35	‡
VLDL	0.32±0.20	0.29±0.17	0.39±0.28	‡
LDL	0.27±0.08	0.22±0.07	0.24±0.07	*
HDL	0.09±0.03	0.08±0.02	0.08±0.02	NS
Phospholipids (mmol/l)				
Apolipoprotein A-I (g/l)	1.55±0.36	1.29±0.23	1.46±0.19	‡
Apolipoprotein B (g/l)	0.84±0.39	0.56±0.2	0.60±0.34	NS
Apolipoprotein A-I/apolipoprotein B	2.19±0.98	2.34±0.99	3.17±1.34	‡

Values are mean±SD. VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

\* $p<0.05$ , † $p<0.01$ , ‡ $p<0.001$ , and NS, not significant.

based diets in both test periods of the crossover study are shown in Figures 2 and 3.

Both test diets reduced serum LDL cholesterol concentration compared with the baseline diet. The LDL cholesterol level was 6% lower ( $p<0.01$ ) at the end of the rapeseed oil diet than at the end of the

sunflower oil diet. Total cholesterol levels were 15% lower at the end of the rapeseed oil diet and 12% lower at the end of the sunflower oil diet compared with their levels at the end of the saturated fat baseline diet ( $p<0.001$  for both comparisons). The difference in serum cholesterol between the two test

TABLE 5. Serum Total, Lipoprotein Lipid, and Apolipoprotein Concentrations in Men ( $n=29$ ) Who Consumed Polyunsaturated and Monounsaturated Diets

Parameter	Baseline diet	Sunflower oil diet (polyunsaturated)	Rapeseed oil diet (monounsaturated)	Difference in sunflower oil vs. rapeseed oil diet
Cholesterol (mmol/l)				
Total	5.04±0.88	4.38±0.71	4.30±0.70	NS
VLDL	0.29±0.16	0.20±0.18	0.30±0.31	NS
LDL	3.09±0.74	2.43±0.63	2.28±0.62	†
HDL, total	1.23±0.25	1.22±0.26	1.23±0.25	NS
HDL <sub>2</sub>	0.81±0.27	0.74±0.28	0.78±0.25	†
HDL <sub>3</sub>	0.58±0.09	0.53±0.11	0.51±0.07	NS
Triglycerides (mmol/l)				
Total	0.94±0.37	0.85±0.39	0.89±0.40	NS
VLDL	0.49±0.28	0.40±0.25	0.55±0.32	‡
LDL	0.26±0.08	0.21±0.07	0.23±0.03	NS
HDL	0.09±0.04	0.07±0.02	0.07±0.02	NS
Phospholipids (mmol/l)				
Apolipoprotein A-I (g/l)	2.70±0.38	2.35±0.41	2.91±0.65	‡
Apolipoprotein B (g/l)	1.44±0.36	1.16±0.23	1.45±0.31	‡
Apolipoprotein A-I/apolipoprotein B	0.77±0.33	0.61±0.22	0.66±0.32	NS
Apolipoprotein A-I/apolipoprotein B	2.27±1.22	2.44±2.08	2.81±1.37	NS

Values are mean±SD. VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

† $p<0.01$ , ‡ $p<0.001$ , and NS, not significant.

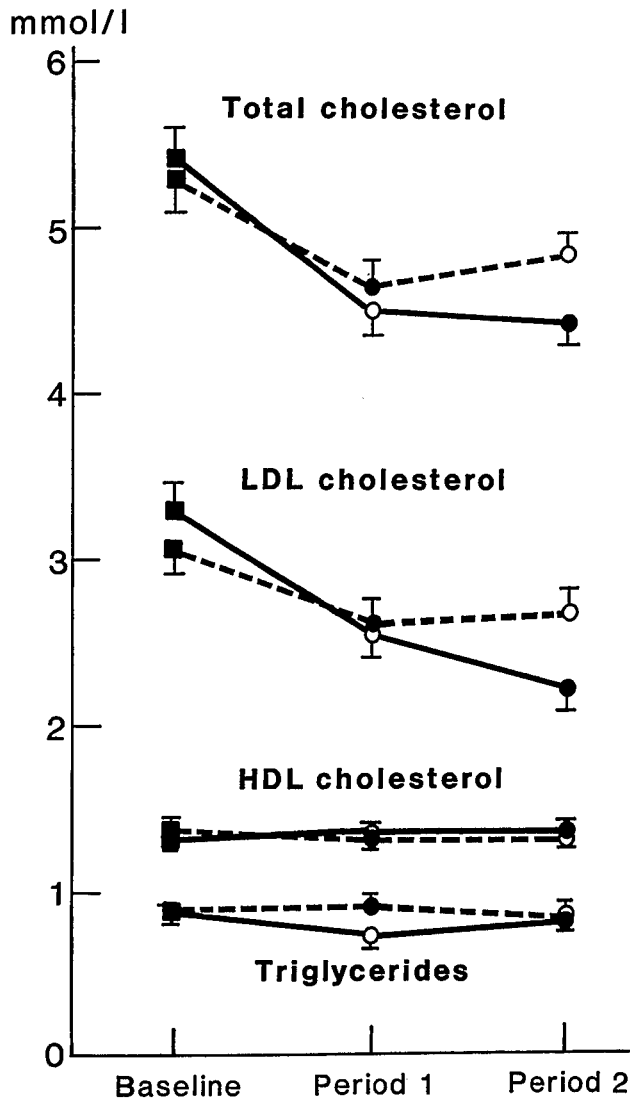


FIGURE 2. Graph showing serum total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride concentrations (mean  $\pm$  SEM) at the end of the diet periods in the two groups. ■, Saturated fat baseline diet; ○, polyunsaturated sunflower oil diet; ●, monounsaturated rapeseed oil diet.

diets was statistically significant ( $p < 0.01$ ) in the study group as a whole and for women ( $p < 0.01$ ) but not men (Tables 3–5) separately. Very low density lipoprotein cholesterol level was lower after the polyunsaturated fat diet than after the monounsaturated fat diet ( $p < 0.05$ ).

The test diets did not show any effects on HDL cholesterol level, as determined after precipitation with dextran sulfate and magnesium chloride. The HDL to TC ratio increased during both test diets, from a baseline value of 0.26 to 0.31 ( $p < 0.001$ ) for the rapeseed oil diet and from 0.26 to 0.30 ( $p < 0.001$ ) for the sunflower oil diet. The difference between the test diets was also significant ( $p < 0.05$ ). However, the HDL<sub>2</sub> cholesterol fraction was lower during the sunflower oil diet than during the rapeseed oil diet ( $p < 0.05$ ). The HDL<sub>2</sub> to LDL cholesterol ratio was

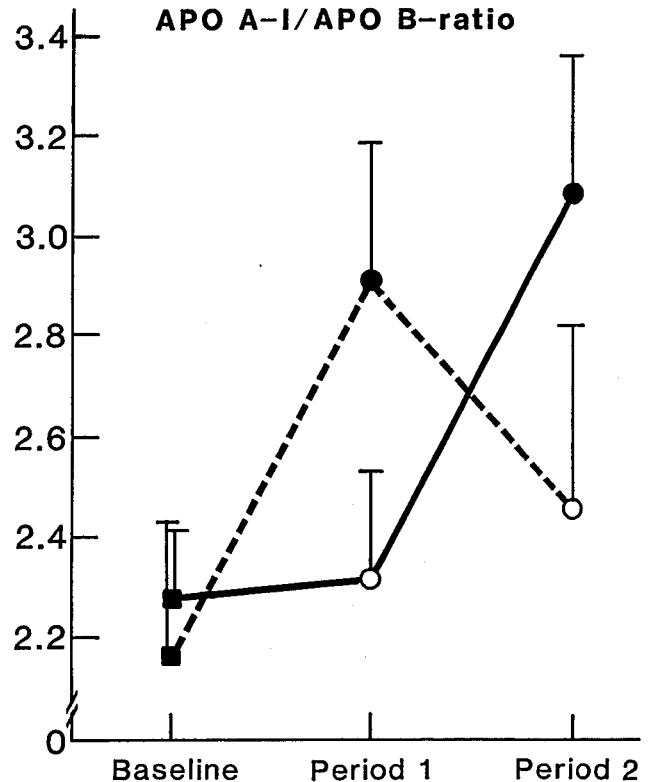


FIGURE 3. Graph showing serum apolipoprotein (apo) A-I to B ratio (mean  $\pm$  SEM) at the end of the diet periods in the two groups. ■, Saturated fat baseline diet; ○, polyunsaturated sunflower oil diet; ●, monounsaturated rapeseed oil diet.

higher ( $p < 0.01$ ) at the end of the rapeseed oil diet than at the end of the sunflower oil diet ( $0.43 \pm 0.19$  and  $0.39 \pm 0.18$ , respectively), but HDL<sub>3</sub> levels were similar during the two test diets.

The sunflower oil diet reduced total and VLDL triglyceride levels significantly compared with the rapeseed oil diet ( $p < 0.05$  and  $p < 0.001$ , respectively). Total phospholipid concentration was lower during the sunflower oil diet than during the rapeseed oil diet ( $p < 0.001$ ).

Apo A-I levels were significantly lower during the sunflower oil diet compared with the rapeseed oil diet. The effects on apo B levels of the test diets did not differ from each other. However, the ratio of apo A-I to apo B was significantly higher ( $p < 0.001$ ) after the rapeseed oil diet than after the sunflower oil diet, in which the ratios were  $3.0 \pm 1.4$  and  $2.4 \pm 1.6$ , respectively (Figure 3).

The differences between the test diets in TC, VLDL cholesterol, total triglyceride, and LDL triglyceride values were pronounced for women but statistically insignificant for men separately. Conversely, the effects of the test diets on LDL and HDL<sub>2</sub> cholesterol were more apparent in men than in women.

#### Discussion

The purpose of this carefully controlled diet study was to evaluate the effects of monounsaturated versus polyunsaturated fatty acids on serum lipoprotein and

apolipoprotein levels when consumed in moderate amounts. Previous reports, which were based on studies with highly different levels of fatty acid intake, have produced controversial results.<sup>2,3,5,6</sup> The researchers who performed the earlier studies were inclined to think that the monounsaturates in the diet would clearly be less effective than the polyunsaturates in reducing serum cholesterol levels, but recent studies have not shown much difference between the effects of monounsaturated and polyunsaturated fat diets on serum cholesterol levels. As a source of monounsaturates we used very low erucic acid ( $\approx 0.5\%$  of fatty acids) rapeseed oil that contained a slightly smaller amount of monoenes (58% versus 75%), less saturates (5% versus 10%), and considerably more n-3  $\alpha$ -linolenic acid (10–13% versus 0%) than olive oil, which has been used in most previous studies.

Serum TC level was reduced by both test diets compared with the baseline diet. The TC level was slightly but significantly lower after the rapeseed oil than after the sunflower oil diet when analyzed for the whole study group and for women separately. The results confirm earlier findings<sup>6,7</sup> that the widely used Keys's equations<sup>2,3</sup> do not correctly predict the effects of monounsaturated and polyunsaturated fatty acids on serum cholesterol levels.

The lowering effect of the high oleic acid rapeseed oil diet on LDL cholesterol was greater than that of the sunflower oil diet for both women and men. This kind of difference has not been previously reported. The  $\alpha$ -linolenic acid of the rapeseed oil diet (2.1% of energy versus 0.4% on the sunflower oil diet) was not studied separately in our design as such. How much, if any, of the observed effect on LDL cholesterol can be attributed to this fatty acid has not yet been determined. Although an  $\alpha$ -linolenic acid-enriched diet has been shown to differentially affect the lipid transfer protein activity compared with a linoleic acid-enriched diet in humans, these and other findings by Abbey et al<sup>29</sup> do not suggest that  $\alpha$ -linolenic acid might have a major effect on lipoprotein levels in the amounts ( $\approx 6$  g/day) ingested during the rapeseed oil diet in this study.

Total HDL cholesterol was unaffected by the diets, which supports previous findings that suggest that polyunsaturated fat diets do not lower HDL cholesterol levels when the amount of linoleic acid is moderate (i.e., less than 10–13% of total energy intake).<sup>6,30</sup> However, it contradicts the results obtained by Mattson and Grundy<sup>5</sup> and Shepherd et al,<sup>31</sup> who used diets with very high polyunsaturated to saturated fat ratios and extremely high intakes of linoleic acid (up to 29% of total energy intake). However, the analysis of HDL subfractions revealed a reduction of the HDL<sub>2</sub> cholesterol concentration, resulting in a lower HDL<sub>2</sub> to LDL ratio by the sunflower oil diet compared with the rapeseed oil diet. This is in contrast to the finding by Dreon et al<sup>8</sup> that HDL<sub>2</sub> cholesterol concentration was 50% higher for the polyunsaturated fat diet compared with the monounsaturated fat diet, which has thus far been obtained by using reduced fat diets.

Serum total and VLDL triglycerides were lower with the sunflower oil diet compared with the rapeseed oil diet. A similar effect with sunflower oil has been found in some<sup>6,32,33</sup> but not in other<sup>34,35</sup> previous studies, whereas monounsaturated fat diets have been reported to lower<sup>10,11</sup> or have no effect<sup>6,36</sup> on serum total triglyceride values. The triglyceride-reducing effect of the sunflower oil diet would be beneficial if serum triglycerides are considered to be an independent risk factor for coronary heart disease.<sup>37,38</sup>

The lower apo A-I levels after the sunflower oil diet that were found in this study agree with findings by some<sup>6,12,31</sup> but not all<sup>9,13</sup> studies. It may result from a reduction in the synthesis rate of apo A-I rather than from a change in its fractional rate of catabolism.<sup>31</sup> Monounsaturated fat diets have been reported to increase apo A-I levels,<sup>39</sup> to reduce this tendency in men,<sup>6</sup> or to not have any effect at all<sup>13</sup> compared with a baseline diet high in saturated fat.

The reduction of apo B by both the sunflower and rapeseed oil diets compared with a saturated fat diet agrees with the reduction of LDL concentrations by both test diets and accords with the results of other studies.<sup>6,12,13</sup> However, in contrast to LDL cholesterol, apo B levels were similar during the two test diets. The differences in apo A-I and apo B levels and the calculated apo A-I to B ratio between the test diet groups accord with the respective differences in HDL<sub>2</sub> and LDL cholesterol levels and the HDL<sub>2</sub> to LDL ratio.

The present study shows that the reduction of TC concentration by the rapeseed oil diet entirely resulted from a reduction in the concentration of the LDL fraction. In addition, the sunflower oil diet reduced LDL cholesterol concentration, but in this case VLDL and HDL<sub>2</sub> cholesterol were affected as well. This indicates that sunflower oil and rapeseed oil have different effects on the metabolism of VLDL and HDL. More work is required to elucidate the effects of the unique fatty acid composition of rapeseed oil on factors that regulate lipoprotein lipid levels (e.g., lipid transfer reactions, lipolytic enzymes).

In conclusion, the results suggest that both rapeseed oil and sunflower oil effectively lower serum cholesterol levels when substituted for milk fat and that low erucic acid rapeseed oil produces a more beneficial effect than sunflower oil on serum HDL<sub>2</sub> to LDL cholesterol and apo A-I to B ratios. This effect probably results from the high oleic acid content of rapeseed oil, but the possible role of  $\alpha$ -linolenic acid should also be considered in future studies. This study further shows that the sunflower oil and rapeseed oil diets, although both are effective in lowering serum TC and LDL cholesterol levels in healthy subjects, still have different effects on lipoprotein and apolipoprotein metabolism. High oleic acid rapeseed oil specifically and strongly affects LDL metabolism, whereas sunflower oil affects the metabolism of both VLDL and HDL<sub>2</sub> in addition to LDL.

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