Replacement of dietary saturated fat with monounsaturated fat: effect on atherogenesis in cholesterol-fed rabbits clamped at the same plasma cholesterol level

BY LARS B. NIELSEN[1], PER LETH-ESPENSEN[1], BØRGE G. NORDESTGAARD[2], ELINE FOGED[1], KNUD KJELDSEN[1] AND STEEN STENDER[3]*

[1] *Department of Clinical Biochemistry, Rigshospitalet,* [2] *Department of Clinical Chemistry, Herlev Hospital and* [3] *Department of Clinical Chemistry, Køge Hospital, University of Copenhagen, DK-4600 Køge, Denmark*

(*Received 2 September 1994 – Revised 5 January 1995 – Accepted 1 February 1995*)

The aim was to compare the effect on atherogenesis of dietary monounsaturated and saturated fatty acids in cholesterol-clamped rabbits. To obtain an average plasma cholesterol concentration of 20 mmol/l in each rabbit during the 13-week cholesterol-feeding period, dietary cholesterol was adjusted weekly. The amount of fat fed daily was 10 g per rabbit in Expts A (*n* 23), C (*n* 36), and D (*n* 58) and 5 g per rabbit in Expt B (*n* 24). The source of monounsaturated fatty acids was olive oil in all four experiments. The source of saturated fatty acids was butter in Expt A, lard in Expt B, coconut oil in Expt C, and butter or lard in Expt D. Generally, olive oil-fed groups received more cholesterol and tended to have more cholesterol in VLDL and less in LDL compared with groups receiving saturated fat. Analysis of variance of the combined results of all four experiments showed that, in comparison with saturated fat, olive oil lowered aortic cholesterol by 13 (−9–30, 95% confidence interval) % in the aortic arch, and by 10 (−10–26) % in the thoracic aorta, which was not significant. In the comparison with olive oil, no differences in effects on aortic cholesterol content were detected between butter, lard and coconut oil. These findings do not support the view that replacement of dietary saturated fat with olive oil has a major impact on the development of atherosclerosis in addition to that accounted for by changes in plasma cholesterol levels.

Atherosclerosis: Cholesterol: Fatty acids

Food rich in saturated fatty acids is a major contributing factor for hypercholesterolaemia in affluent societies. Since hypercholesterolaemia is a causal risk factor for atherosclerosis and, thus, responsible for a large part of cardiovascular disease and death in Western countries, a reduced intake of saturated fatty acids has been recommended for these populations (Carleton *et al.* 1991; International Task Force for Prevention of Coronary Heart Disease, 1992). *n*-6 Polyunsaturated fatty acids, when substituted for saturated or monounsaturated fatty acids, lower plasma cholesterol (Keys *et al.* 1957; Hegsted *et al.* 1965). Therefore, it was recommended initially that the intake of *n*-6 polyunsaturated fatty acids should be increased to balance partly the lower intake of saturated fatty acids (Consensus Conference, 1985). However, replacement of saturated fatty acids by monounsaturated fatty acids also lowers plasma cholesterol but does not lower HDL-cholesterol to the same extent as polyunsaturated fatty acids (Grundy, 1987; International Task Force for Prevention of Coronary Heart Disease, 1992; Mensink & Katan, 1992). More recently therefore, recommendations on fat intake have favoured monounsaturated

* For reprints.

over *n*-6 polyunsaturated fatty acids (Carleton *et al.* 1991; International Task Force for Prevention of Coronary Heart Disease, 1992).

Knowledge on the effect of monounsaturated fatty acids on atherosclerosis and coronary heart disease is sparse. In the Seven Countries Study (Keys, 1980), male subjects from Crete, Greece, had an incidence of coronary heart disease of only 2–5% of that of subjects from Eastern Finland and The Netherlands, despite a similar intake of total fat. In Crete, however, the major fat intake was olive oil rich in monounsaturated fatty acids, whereas male subjects from Finland and The Netherlands mainly ate food rich in saturated fat. Notably, serum cholesterol in male subjects from Crete was only 10–20% lower than that of male subjects from East Finland and The Netherlands, suggesting that monounsaturated fatty acids compared with saturated fatty acids may protect against the development of atherosclerosis and coronary heart disease beyond that accounted for by the plasma cholesterol-lowering effect.

Development of atherosclerosis appears to involve oxidation of LDL (Steinberg *et al.* 1989) and, compared with polyunsaturated fatty acids, monounsaturated fatty acids at least protect LDL against oxidation (Pathasarathy *et al.* 1990; Berry *et al.* 1991; Scaccini *et al.* 1992). Therefore, an antioxidative mechanism could be involved in an anti-atherogenic effect of monounsaturated fatty acids not mediated by an effect on plasma cholesterol levels. Other mechanisms could also operate, but it is important to emphasize that the theory of an anti-atherogenic effect of olive oil compared with saturated fat in human subjects is mainly based on observational epidemiological data. In cholesterol-fed rabbits, coconut oil, when compared with olive oil, increased both aortic cholesterol and plasma cholesterol markedly (Van Heek & Zilversmit, 1988, 1990), whereas lard, when compared with safflower oil with a high content of oleic acid, increased plasma cholesterol but did not affect atherosclerosis severity in pigs (Royce *et al.* 1984). Therefore, at this stage it remains to be established whether olive oil, when compared with saturated fat, has a neutral effect on, promotes, or retards the development of atherosclerosis; certainly, it is not known what the direct effect of olive oil is on the arterial wall.

Previously we have used the cholesterol-clamped rabbit, an animal model in which plasma cholesterol is continuously adjusted to the same level in all rabbits, in studying the effects of dietary components on atherogenesis not mediated through differences in plasma cholesterol level (Leth-Espensen *et al.* 1988). In the present studies we have used this model to examine a possible non-cholesterol-mediated anti-atherogenic, or atherogenic, effect of monounsaturated fatty acids compared with saturated fatty acids. Commonly ingested oils or fats were used: olive oil was the source of monounsaturated fatty acids, whereas butter, lard and coconut oil were used as the sources of saturated fatty acids.

METHODS

Animals

A total of 141 male white rabbits of the Danish country strain (Statens Seruminstitut, Copenhagen, Denmark) weighing 2·5–3·5 kg were housed individually under controlled environmental conditions. All rabbits had free access to water and were fed on 100 g chow/d. The experimental protocols were in accordance with Danish regulations on animal experiments.

Dietary fat and cholesterol

In four experiments (A, B, C and D), rabbits were fed on either olive oil or a source of saturated fat: butter, lard or coconut oil. The fatty acid composition of these fats is shown

Table 1. *Fatty acid composition (g fatty acid/100 g fat) of olive oil, butter, lard, and coconut oil used as source of dietary fat (from Møller* et al. *1991)*

Dietary fat... Fatty acid	Olive oil	Butter	Lard	Coconut oil
Saturated				
4:0	—	2·8	—	—
6:0	—	1·8	—	0·6
8:0	—	1·2	—	7·5
10:0	—	2·6	—	6·0
12:0	0	3·2	0	44·6
14:0	0	9·1	1·5	16·8
16:0	11·5	24·3	24·7	8·2
18:0	2·2	8·3	14·4	2·8
Total	13·7	53·3	40·6	86·5
Monounsaturated				
14:1	—	1·2	—	—
16:1	1·0	1·8	2·3	5·8
18:1	68·8	18·3	37·5	—
Total	69·8	21·3	39·8	5·8
Polyunsaturated				
18:2	10·5	1·7	8·0	1·8
18:3	0·7	1·6	0·7	—
Total	11·2	3·3	8·7	1·8

in Table 1 (Møller *et al.* 1991). In Expts A, C and D each rabbit received 10 g fat/d and in Expt B 5 g fat/d. In Expt A comparing olive oil (oleum olivae ph Dan 48; Mecobenzon, Copenhagen, Denmark; *n* 11) with butter (Enigheden, Copenhagen, Denmark; *n* 12), and Expt B comparing olive oil (*n* 12) with lard (FDB, Viby, Denmark; *n* 12), a stock of cholesterol-enriched chow (10 g/kg) was prepared as follows. Cholesterol (CH-USP; Sigma, Copenhagen, Denmark) was dissolved in diethyl ether and added to standard rabbit chow from Superfoss Korn A/S (Boserup, Faxe, Denmark). The Superfoss chow contained (g/kg): protein 140, fibre 150 and fat 30. Subsequently, the diethyl ether was allowed to evaporate and cholesterol-enriched chow was mixed with cholesterol-free chow daily to obtain the desired amount of cholesterol for each rabbit. Olive oil was kept in brown-glass bottles under N_2 at room temperature and mixed with the chow daily. Butter and lard were melted before the fats were mixed with the chow.

In Expt C, comparing olive oil (*n* 12 + 12) with coconut oil (*n* 12) (Oleum cocus ph Dan 80; Mecobenzon, Copenhagen, Denmark), cholesterol-enriched chows for one group of rabbits receiving olive oil and the group receiving coconut oil were prepared weekly by dissolving cholesterol in heated oil before mixing it with Altromin rabbit chow (Altromin 2113; Altromin, Lage, Germany). Altromin rabbit chow contained (g/kg): protein 150, fibre 200 and fat 35. In Expt C a third group of rabbits received an olive oil- and cholesterol-enriched chow prepared using the diethyl ether method described for Expts A and B, but the standard chow was Altromin chow instead of Superfoss Korn A/S chow. There was no statistically significant difference in aortic cholesterol content between the two olive oil groups in the present experiment, therefore the data from these two groups were pooled.

In Expt D, comparing olive oil (*n* 19) with butter (*n* 19) and lard (*n* 20), cholesterol-enriched chow was prepared weekly by dissolving cholesterol in heated oil or fat and mixing it with Altromin rabbit chow as in Expt C.

Experimental design

The four experiments were conducted using the same protocol. Rabbits were allocated to groups on the basis of body weights and plasma concentrations of triacylglycerols, total cholesterol and HDL-cholesterol, to obtain similar starting levels of these variables in olive oil- and saturated-fat-fed groups. The rabbits received fat-enriched chow without cholesterol for 1 week, and then fat- and cholesterol-enriched chow for 13 weeks. Based on determinations of plasma cholesterol concentrations at least once per week, the amount of dietary cholesterol for each rabbit was adjusted in order to clamp plasma cholesterol concentrations at an average of 20 mmol/l during the cholesterol-feeding period; this plasma cholesterol level was chosen so that detectable atherosclerosis would develop within the 13-week experimental period.

At the end of the experiment each rabbit received an intravenous injection of pentobarbital solution (50 g/l; 40–100 mg/kg). The thoracic cavity was opened and the aorta from the heart to the diaphragm was excised and freed of adventitia. The vessel was opened longitudinally, rinsed with saline (9 g NaCl/l) and divided into aortic arch and thoracic aorta at the level of the first intercostal arteries. The surface area was outlined on graph paper before the intima–inner media was stripped from the outer media. Tissues were kept at −20° until analysed.

Analysis

Cholesterol in aortic tissues was determined using the Liebermann–Burchard method (Ness *et al.* 1964) after extraction of lipids with chloroform–methanol (2:1, v/v; Folch *et al.* 1957). Aortic protein was determined using the method of Lowry *et al.* (1951) after digestion of the residue with NaOH (5 mol/l).

VLDL, IDL, LDL and HDL were separated by adjusting plasma samples to densities of 1·006, 1·019 and 1·063 g/ml followed by ultracentrifugation in a 40·3 or a 50·3 Ti Beckman Rotor (RAMCON A/S, Copenhagen, Denmark). Cholesterol and triacylglycerol concentrations in plasma and lipoprotein fractions were determined using enzymic kits from Boehringer Mannheim (Mannheim, Germany).

Statistical analysis

To render the distribution of data near-normal in the statistical analysis, logarithmic transformation was used for aortic cholesterol content and plasma and VLDL-triacylglycerol concentrations: the distribution of plasma triacylglycerol concentrations is skewed in humans as well (Carlsson, 1960). For each experiment, differences between mean values for rabbits receiving olive oil and those receiving saturated fat were analysed using Student's *t* test.

To examine the overall difference in aortic cholesterol content between rabbits receiving olive oil and those receiving a saturated fat, a two-way analysis of variance (ANOVA) was performed in which the type of fat (olive oil or saturated fat) and the Expt were the classifying variables. In these analyses, group means were weighted by the number of rabbits and inverse empirical variances using the statistical program S-PLUS (Statsci Europe, 1993).

Univariate and stepwise multiple linear-regression analyses were performed using the MINITAB program (Minitab Inc, 1991). These analyses tested type of fat (olive oil or saturated fat), Expt (A, B, C or D), amount of dietary cholesterol, and plasma total, VLDL-+IDL-, LDL- and HDL-cholesterol and -triacylglycerol as predictors for aortic cholesterol content; since IDL was not separated but measured together with VLDL in Expts A and B, VLDL+IDL were combined in these statistical analyses.

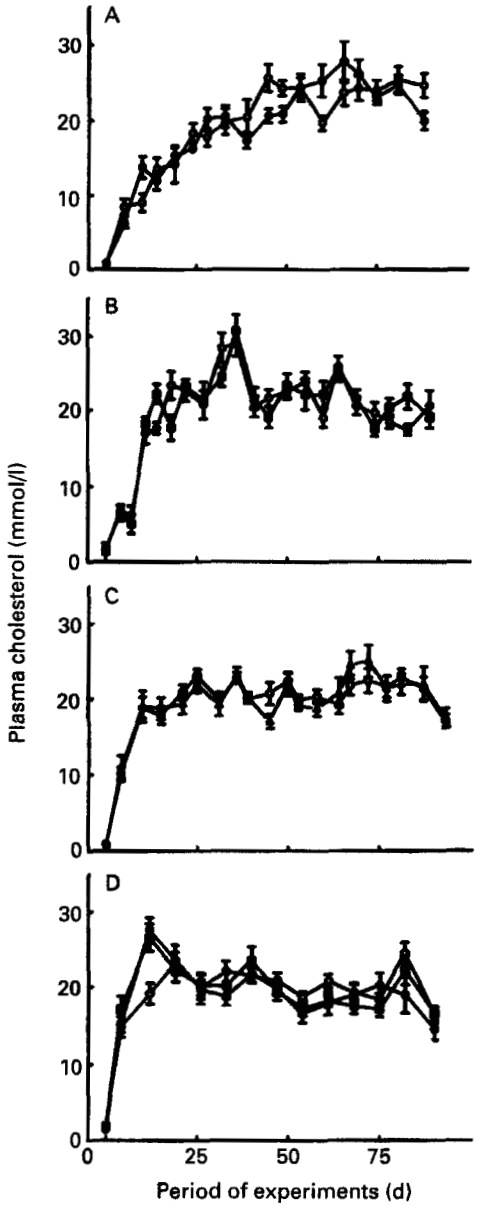

Fig. 1. Plasma cholesterol concentrations during the cholesterol-feeding period in four experiments (A–D). The amount of cholesterol fed to each rabbit was adjusted at least once weekly to obtain similar plasma cholesterol concentrations in olive oil-fed (○; *n* 11 Expt A, *n* 12 Expt B, *n* 24 Expt C, *n* 19 Expt D) and saturated fat-fed groups. Values are means with their standard errors represented by vertical bars. (●), Butter (*n* 12 Expt A, *n* 19 Expt D); (■), lard (*n* 12 Expt B, *n* 20 Expt D) (▲), coconut oil (*n* 12). For details of diets and procedures, see pp. 510–513 and Table 1.

Aortic cholesterol values were not normally distributed and, therefore, are presented as geometric means: a 95% CI was constructed as mean and 1·96 × standard error for logarithmic-transformed data, followed by back transformation (Altman, 1991). All other values are given as means with their standard errors. All *P* values are two-tailed.

Table 2. *Mean plasma cholesterol and triacylglycerols, dietary cholesterol, and aortic cholesterol in cholesterol-clamped rabbits fed on olive oil or saturated fat for 13 weeks*†

(Values are means with their standard errors, except aortic cholesterol contents which are expressed as geometric means and 95% CI)

Expt...	A				B				C				D					
	Olive oil		Butter		Olive oil		Lard		Olive oil		Coconut oil		Olive oil		Butter		Lard	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	11		12		12		12		24		12		19		19		20	
Mean plasma cholesterol‡ (mmol/l)	19·5	0·2	19·4	0·3	20·0	0·1	20·2	0·3	19·9	0·1	19·5	0·1	19·8	0·1	19·4	0·3	19·6	0·1
Mean plasma triacylglycerols‡ (mmol/l)	1·1	0·1	1·6	0·3	1·1	0·1	1·1	0·1	1·1	0·1	1·7*	0·2	1·9	0·1	2·2	0·2	2·1	0·2
Dietary cholesterol (g per rabbit)	36	3	33	3	39	2	40	4	35	3	25**	2	31	3	25	3	25**	2
Aortic cholesterol (nmol/mg protein)																		
Aortic arch																		
Mean	125		162		209		339		59		65		68		64		86	
95% CI	70–222		86–307		120–369		218–526		49–71		48–89		53–88		49–85		64–117	
Thoracic aorta																		
Mean	28		53		51		74		25		28		27		28		31	
95% CI	21–37		29–97		30–87		(38–147)		22–29		24–32		20–38		24–32		23–42	

† For details of diets and procedures, see pp. 510–513 and Table 1.

‡ Mean plasma cholesterol and triacylglycerols were calculated from the area under the plasma concentration *v.* time curves during the cholesterol-feeding period.

* $P < 0{\cdot}05$, ** $P < 0{\cdot}001$.

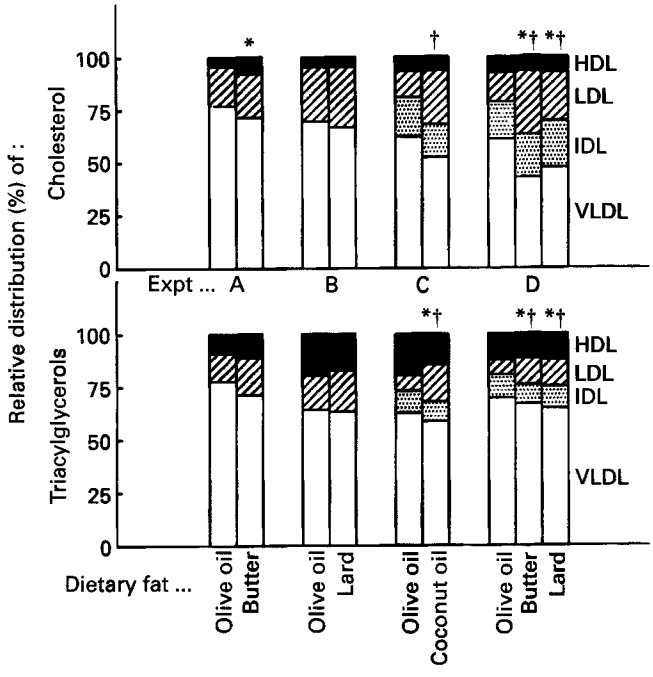

Fig. 2. Relative distribution of cholesterol and triacylglycerols among lipoproteins in rabbits. In each of the four experiments (A–D), lipoproteins were determined twice. Values are means for these two measurements at days 32–39 and 64–80. (■), HDL; (▨), LDL; (▩), IDL; (□), VLDL. Mean values for VLDL were significantly lower than those for the olive oil-fed group: * $P < 0{\cdot}05$. Mean values for LDL were significantly higher than those for the olive oil-fed group: † $P < 0{\cdot}05$. For details of diets and procedures, see pp. 510–513 and Table 1.

RESULTS

Dietary cholesterol and plasma lipids

By weekly adjustments of the amount of cholesterol fed to each rabbit, similar plasma cholesterol concentrations were obtained in rabbits receiving olive oil and in those receiving a source of saturated fat (Fig. 1). To achieve this, coconut oil-fed rabbits received significantly less cholesterol than olive oil-fed rabbits (Expt C) and in one of two experiments lard-fed rabbits received significantly less cholesterol than olive oil-fed rabbits (Expt D; Table 2). The gain in body weight was similar in rabbits receiving olive oil and rabbits receiving saturated fat in all experiments (data not shown).

Mean plasma triacylglycerol concentration was significantly lower in rabbits receiving olive oil than in those receiving coconut oil (Expt C), whereas there was no significant difference between olive oil and butter or lard in Expts A, B or D (Table 2).

Rabbits receiving olive oil had significantly less cholesterol and triacylglycerol in LDL compared with rabbits receiving coconut oil in Expt C, and lard or butter in Expt D (Fig. 2). In these experiments the lower LDL level was balanced by an increase in VLDL-cholesterol and -triacylglycerol; VLDL-cholesterol was increased also in the olive oil-fed group compared with the butter-fed group in Expt A.

Aortic cholesterol

In three of the four experiments the geometric mean cholesterol contents of both the aortic arch and the thoracic aorta were nominally lower in the olive oil-fed groups compared with the saturated-fat-fed groups (Fig. 3). However, using Student's *t* test there was no

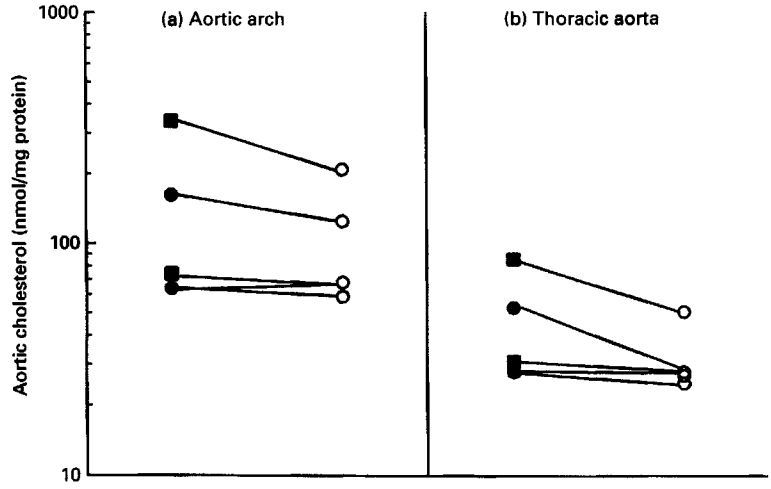

Fig. 3. Geometric mean aortic cholesterol content in groups of rabbits receiving olive oil (○) or saturated fat (butter (●), lard (■), coconut oil (▲)) in four experiments (A–D). For details of diets and procedures, see pp. 510–513 and Table 1.

statistically significant difference in aortic cholesterol content between rabbits receiving olive oil and those receiving saturated fat in any of the four experiments.

All four experiments were combined using a two-way ANOVA to test the independent impact of type of fat as well as Expt on aortic cholesterol content. In this analysis there was also no significant difference between olive oil and saturated fat: the reduction in aortic cholesterol content (geometric mean) by olive oil compared with saturated fat was 13 (−9–30, 95% confidence interval) % in the aortic arch and 10 (−10–26) % in the thoracic aorta ($P = 0{\cdot}23$ for the aortic arch and $P = 0{\cdot}24$ for the thoracic aorta). No differences between butter, lard, and coconut oil were detected in these statistical analyses. There was, however, a highly significant effect of Expt on aortic cholesterol content: in olive oil-fed rabbits, aortic cholesterol content was highest in Expts A and B compared with Expts C and D (Fig. 3).

To take into account differences between rabbits in lipoprotein profile and amount of dietary cholesterol ingested, univariate as well as multiple linear-regression analyses were performed: lipoprotein-cholesterol concentration, lipoprotein-triacylglycerol concentration, dietary cholesterol, type of fat and Expt were examined as predictors for aortic cholesterol content. In these analyses, type of fat was again not significantly associated with aortic cholesterol content. On multivariate analysis combining all four experiments the only significant predictor of aortic cholesterol content was Expt. Notably, using univariate or multiple linear-regression analysis there was no significant relationship between the amount of dietary cholesterol ingested by a rabbit and the aortic cholesterol content.

DISCUSSION

Monounsaturated fatty acids were previously regarded as neutral with respect to plasma cholesterol concentration (Keys *et al.* 1957; Hegsted *et al.* 1965; Grundy, 1987), although recently a plasma cholesterol-lowering effect of monounsaturated fatty acids has become evident (Mensink & Katan, 1992). In humans, no randomized intervention studies on the effect of monounsaturated fatty acids on the development of atherosclerosis and coronary heart disease are available. Nevertheless, epidemiological evidence suggests that mono-

unsaturated fatty acids, primarily in olive oil, may protect against coronary heart disease and that this effect cannot be accounted for by the plasma cholesterol-lowering impact alone (Keys, 1980).

An anti-atherogenic potential for monounsaturated fat compared with saturated fat is supported by studies in rats (comparing tristearin with triolein; Renaud, 1968), rabbits (comparing olive oil with coconut oil; Van Heek & Zilversmit, 1988, 1990), and C57BL/6J mice (comparing seven diets with different monounsaturated and saturated fat contents; Nishina *et al.* 1993). In these studies monounsaturated fat, when compared with saturated fat, reduced plasma cholesterol as well as atherosclerosis severity. On the other hand, a study in pigs (comparing safflower oil containing 700 g oleic acid/kg with lard; Royce *et al.* 1984) found no difference in atherosclerosis severity between the two groups, despite the fact that pigs fed on monounsaturated fat had a lower plasma cholesterol concentration than did pigs fed on saturated fat. Also, in another study using cholesterol-fed rabbits (comparing butter with butter + olive oil; Renaud & Gautheron, 1975) there were no differences in atherosclerosis between animals fed on monounsaturated + saturated fat or saturated fat alone; plasma cholesterol was similar in the two groups. To determine the anti-atherogenic effect of dietary fats which exceeds the effect on plasma cholesterol, Kritchevsky *et al.* (1984) and Kritchevsky & Tepper (1967) have suggested using a 'relative atherogenic effect' derived by dividing the extent of atherosclerotic lesion by the plasma cholesterol concentration; in cholesterol-fed rabbits, olive oil has a lower relative atherogenic effect than peanut (*Arachis hypogaea* L.) oil (Kritchevsky *et al.* 1984) and unheated maize oil (Kritchevsky & Tepper, 1967). Leth-Espensen *et al.* (1988) have used an alternative model, i.e. the cholesterol-clamped rabbit, to study the effect of dietary fat on atherogenesis independent of changes in total plasma cholesterol concentration. Using this model the presence, compared with the absence, of olive oil protected against atherosclerosis (Leth-Espensen *et al.* 1988), whereas there was no difference in atherosclerosis severity between olive oil- and margarine-fed rabbits (Mortensen *et al.* 1992). In the present studies the cholesterol-clamped rabbit has been used to investigate whether replacement of saturated fat with monounsaturated fat protects against atherosclerosis independently of the effect which might be mediated via a reduced plasma cholesterol level; plasma cholesterol in each of 141 rabbits fed on either olive oil or a source of saturated fatty acids was clamped at 20 mmol/l. Then the relative effect of dietary fats on atherogenesis directly at the arterial wall could be studied. Although hepatic lipoprotein metabolism is influenced by cholesterol feeding in the rabbit (Kovanen *et al.* 1981), transport of LDL from plasma into the arterial wall (Wiklund *et al.* 1985), as well as uptake of lipoproteins by scavenger-receptors on macrophages and then formation of foam cells (Schwartz *et al.* 1989), presumably are not down-regulated by cholesterol feeding: the cholesterol-clamped rabbit, therefore, appears useful in studying the development of atherosclerosis, although it may be less useful for studies on hepatic cholesterol metabolism.

In accordance with a plasma cholesterol-lowering effect for monounsaturated fatty acids, compared with saturated fatty acids (Grundy, 1987), olive oil-fed rabbits received significantly more cholesterol than did rabbits fed on coconut oil and lard (in one of two experiments). There was no significant difference in aortic cholesterol content between cholesterol-clamped rabbits receiving olive oil and those receiving saturated fat, but there was a trend toward less cholesterol accumulation in olive oil-fed rabbits. Since post-prandial lipoproteins may be involved in atherogenesis (Zilversmit, 1979), it can be speculated that the tendency towards a higher intake of cholesterol in some of the olive oil-fed groups compared with coconut oil-and lard-fed groups accelerated aortic cholesterol accumulation and, thus, counteracted a possible anti-atherosclerotic effect of olive oil. Furthermore, an increased absorption of cholesterol auto-oxidation products could have occurred in these olive oil-fed rabbits (Peng *et al.* 1987). Such products may have damaged

the aortic endothelium (Peng *et al.* 1986), which in turn could have led to excess atherosclerosis. However, on univariate linear-regression analysis, intake of dietary cholesterol was not a predictor of aortic cholesterol content in any of the four experiments, and there was also no relationship between the two variables on multiple linear-regression analysis when the four experiments were combined.

Although the same experimental protocol was used, with similar plasma cholesterol levels for the same period of time, there was a highly significant difference in aortic cholesterol contents between experiments. In Expts A and B, where the rabbits developed the most severe atherosclerosis, the standard chow was different from that used in Expts C and D. However, in a later study using the same protocol as in the present experiments there was no difference in aortic cholesterol accumulation between two groups (n 2×15 rabbits) of otherwise similar cholesterol- and fat-fed rabbits receiving either the chow used in Expts A and B or the chow used in Expts C and D. Helin *et al.* (1969) suggested that the susceptibility to atherosclerosis may vary with time of the year: rabbits are more resistant to atherosclerosis during the hair-shedding period than during the rest of the year. However, Expts A and D, in which the rabbits exhibited highly different levels of atherosclerosis, were performed during the same time of the year (mid-March–mid-June) with a 1 year interval. Thus, factors other than the basic chow or time of the year, e.g. batch of rabbits, are therefore more probable explanations for the different levels of atherosclerosis severity between Expts A + B and Expts C + D. Nevertheless, this finding emphasizes that when the effects of dietary factors, drugs etc. are examined in animal studies, it is essential that the various groups of animals are studied simultaneously, as was the case in the present experiments.

The plasma triacylglycerol concentration was lower in olive oil-fed rabbits when compared with that of saturated-fat-fed rabbits, although the difference was only significant when compared with that for coconut oil-fed rabbits (Expt C). The mechanism for the different effects of olive oil and coconut oil on plasma triacylglycerols may be related to a different effect of the two types of fat on lipoprotein lipase (*EC* 3.1.1.34) activity (Van Heek & Zilversmit, 1990), but hepatic lipase activity or non-enzymic elimination pathways, also, may be important (Broewer *et al.* 1993). Van Heek & Zilversmit (1988) have shown that in cholesterol- and coconut oil-fed rabbits, on multiple linear-regression analysis, aortic cholesterol accumulation is inversely related to serum triacylglycerol concentration. However, in the previously described study the average serum triacylglycerol levels in the coconut oil-fed rabbits were up to 8·9 mmol/l. This is more than five times higher than that in the coconut oil-fed rabbits from the present study, and in the present study there was no association between aortic cholesterol content and plasma total triacylglycerols, on univariate or multiple linear-regression analysis in coconut oil-fed rabbits alone or in all rabbits pooled. It is considered unlikely, therefore, that the difference in plasma triacylglycerol concentration between olive oil- and saturated-fat-fed rabbits has affected significantly the present findings on aortic cholesterol content.

Rabbits receiving olive oil tended to have lower LDL-cholesterol and -triacylglycerol levels than did rabbits receiving saturated fat. This suggests that saturated fat, when compared with olive oil, decreases LDL-receptor activity in cholesterol-fed rabbits. Similar observations have been reported in human subjects (Grundy, 1987). Since apolipoprotein B in LDL was not determined, it cannot be excluded, however, that the lowering of LDL lipids was due to lipid depletion rather than a reduction in number of LDL particles. The lower LDL-cholesterol level was balanced by a higher VLDL-cholesterol level since plasma cholesterol was clamped at 20 mmol/l. If β-VLDL is more atherogenic than LDL in cholesterol-fed rabbits, the different effect of olive oil and saturated fat on lipoprotein distribution could have counteracted a possible protective effect of olive oil on the arterial

wall. Nevertheless, as well as dietary cholesterol and total plasma triacylglycerol, neither VLDL- nor LDL-cholesterol and -triacylglycerol levels were associated with aortic cholesterol content in the regression analysis.

During the last decade monounsaturated fatty acids have gained interest as an alternative to *n*-6 polyunsaturated fatty acids as a substitute for saturated fat in the Western diet: monounsaturated fatty acids in contrast to polyunsaturated fatty acids do not lower HDL-cholesterol markedly (Grundy, 1987; Mensink & Katan, 1992), and, importantly have proved their long-term safety in Mediterranean countries. The inverse relationship between cohort death rates and intake of monounsaturated fatty acids in the Seven Countries Study (Keys, 1980; Keys *et al.* 1986) and the possible protection of LDL against oxidation by dietary monounsaturated fatty acids (Pathasarathy *et al.* 1990; Berry *et al.* 1991; Scaccini *et al.* 1992) opens the possibility that olive oil can protect against development of atherosclerosis independently of plasma cholesterol levels, i.e. directly at the arterial wall. Also, different sources of dietary fat may affect the turnover time of post-prandial lipoproteins differently (Broewer *et al.* 1993), which could be relevant in atherogenesis (Zilversmit, 1979).

We compared the effect of olive oil and saturated fats on atherosclerosis in the cholesterol-clamped rabbit where the dominant arterial lesion is the fatty streak, characterized by foam cells and cholesterol accumulation. The results suggest that replacement of dietary saturated fats with olive oil does not have a major effect on early atherogenesis over and above that associated with changes in plasma cholesterol levels. In the present studies the severity of atherosclerosis was assessed from the aortic cholesterol content and, thus, it cannot be excluded that olive oil and saturated fat have different effects on other variables related to atherogenesis, such as thrombosis, platelet function etc. However, since there was a trend towards less atherosclerosis in the rabbits fed on olive oil compared with those fed on saturated fat, it is unlikely that olive oil promotes atherosclerosis directly at the arterial wall.

The authors thank Peter Dalgaard, MS, PhD, Department of Biostatistics, University of Copenhagen, Denmark for help in the statistical analysis. Hanne Damm, Karen Rasmussen and Kirsten Krall provided excellent technical assistance. P.L.-E. was supported by the Danish Assurandørassocitetet, B.G.N. by the Danish Heart Foundation and the Danish foundation Lægeforeningens forskningsfond.

REFERENCES

Altman, D. G. (1991). *Practical Statistics for Medical Research*, pp. 177–228. London: Chapman and Hall.

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

Broewer, C. B., de Bruin, T. W. A., Jansen, H. & Erkelens, D. W. (1993). Different clearance of intravenously administered olive oil and soybean-oil emulsions: role of hepatic lipase. *American Journal of Clinical Nutrition* **57**, 533–539.

Carleton, R. A., Dwyer, J., Finberg, L., Goodman, D. S., Grundy, S. M., Havas, S., Hunter, G. T., Kritchevsky, D., Laver, R. M., Luepker, R. V., Ramirez, A. G., Horn, L. V., Stason, W. B. & Stokes, J. (1991). Report on the expert panel on population strategies for blood cholesterol reduction. A statement from the National Cholesterol Education Program, National Heart, Lung, and Blood Institute, National Institutes of Health. *Circulation* **83**, 2154–2232.

Carlsson, L. A. (1960). Serum lipids in men with myocardial infarction. *Acta Medica Scandinavica* **167**, 399–413.

Consensus Conference (1985). Lowering blood cholesterol to prevent heart disease. *JAMA* **253**, 2080–2086.

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

Grundy, S. M. (1987). Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. *American Journal of Clinical Nutrition* **45**, 1168–1175.

Hegsted, D. M., McGandy, R. B., Myers, M. L. & State, F. J. (1965). Quantitative effects of dietary fat on serum cholesterol in man. *American Journal of Clinical Nutrition* **17**, 281–295.

Helin, P., Lorenzen, I., Garbarsch, C. & Matthiessen, M. E. (1969). Relative immunity to arteriosclerosis in rabbits during the hair-shedding period. *Journal of Atherosclerosis Research* **10**, 359–369.

International Task Force for Prevention of Coronary Heart Disease (1992). Recommendations of the European Atherosclerosis Society. Prevention of coronary heart disease: scientific background and new clinical guidelines. *Nutrition, Metabolism and Cardiovascular Diseases* **2**, 113–156.

Keys, A. (1980). *A Multivariate Analysis of Death and Coronary Heart Disease*, pp. 248–262. Cambridge, Mass.: Harvard University Press.

Keys, A., Andersen, J. T. & Grande, F. (1957). Prediction of serum-cholesterol responses of man to changes in fats in the diet. *Lancet* **2**, 959–966.

Keys, A., Menotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic, B. S., Dontas, A. S., Fidanza, F., Keys, M. H., Kromhout, D., Nedeljkovic, S., Punsar, S., Seccareccia, F. & Toshima, H. (1986). The diet and 15-year death rate in seven countries study. *American Journal of Epidemiology* **124**, 903–915.

Kovanen, P. T., Brown, M. S., Basu, S. K., Bilheimer, D. W. & Goldstein, J. L. (1981). Saturation and suppression of hepatic lipoprotein receptors: a mechanism for the hypercholesterolemia of cholesterol-fed rabbits. *Proceedings of the National Academy of Sciences, USA* **78**, 1396–1400.

Kritchevsky, D. & Tepper, S. A. (1967). Cholesterol vehicle in experimental atherosclerosis. Part 9. Comparison of heated corn oil and heated olive oil. *Journal of Atherosclerosis Research* **7**, 647–651.

Kritchevsky, D., Tepper, S. A., Klurfeld, D. M., Vesselinovitch, D. & Wissler, R. W. (1984). Experimental atherosclerosis in rabbits fed cholesterol-free diets. Part 12. Comparison of peanut and olive oils. *Atherosclerosis* **50**, 253–259.

Leth-Espensen, P., Stender, S., Ravn, H. & Kjeldsen, K. (1988). Antiatherogenic effect of olive and corn oils in cholesterol-fed rabbits with the same plasma cholesterol levels. *Arteriosclerosis* **8**, 281–287.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Mensink, R. P. & Katan, M. B. (1992). Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arteriosclerosis and Thrombosis* **12**, 911–919.

Minitab Inc. (1991). *Minitab PC version* 8. State College, PA, USA: Minitab Inc.

Mortensen, A., Espensen, P. L., Hansen, B. F. & Ibsen, P. (1992). The influence of dietary olive oil and margarine on aortic cholesterol accumulation in cholesterol-fed rabbits maintained at similar plasma cholesterol. *Atherosclerosis* **96**, 159–170.

Møller, A., Saxholt, E. & Mikkelsen, B. E. (1991). *Food Composition Tables – Amino acids, Carbohydrates, and Fatty Acids in Danish Food.* Copenhagen, Denmark: Storkøkkenet, Levnedsmiddelstyrelsen.

Ness, A. T., Pastewka, J. V. & Peacock, A. C. (1964). Evaluation of a recently reported stable Liebermann-Burchard reagent and its use for the direct determination of serum total cholesterol. *Clinica Chimica Acta* **10**, 229–237.

Nishina, P. M., Lowe, S., Verstuyft, J., Naggert, J. K., Kuypers, F. A. & Paigen, B. (1993). Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *Journal of Lipid Research* **34**, 1413–1422.

Pathasarathy, S., Kheo, J. C., Miller, E., Barnett, J., Witztum, J. L. & Steinberg, D. (1990). Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. *Proceedings of the National Academy of Sciences, USA* **87**, 3894–3898.

Peng, S.-K., Phillips, G. A., Xia, G.-Z. & Morin, R. J. (1987). Transport of cholesterol autooxidant products in rabbit lipoproteins. *Atherosclerosis* **64**, 1–6.

Peng, S.-K., Taylor, C. B., Hill, J. C. & Morin, R. J. (1986). Cholesterol oxidation derivates and arterial endothelial damage. *Atherosclerosis* **62**, 91–104.

Renaud, S. (1968). Thrombogenicity and atherogenicity of dietary fatty acids in rat. *Journal of Atherosclerosis Research* **8**, 625–636.

Renaud, S. & Gautheron, P. (1975). Influence of dietary fats on atherosclerosis, coagulation, and platelet phospholipids in rabbits. *Atherosclerosis* **21**, 115–124.

Royce, S. M., Holmes, R. P., Takagi, T. & Kummerow, F. A. (1984). The influence of dietary isomeric and saturated fatty acids on atherosclerosis and eicosanoid synthesis in swine. *American Journal of Clinical Nutrition* **39**, 215–222.

Scaccini, C., Nardini, M., D'Aquino, M., Gentili, V., DiFelice, M. & Tomassi, G. (1992). Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. *Journal of Lipid Research* **33**, 627–633.

Schwartz, C. J., Kelley, J. L., Nerem, R. M., Sprague, E. A., Rozec, M. M., Valente, A. J., Edwards, E. H., Prasad, A. R., Kerbacher, J. J. & Logan, S. A. (1989). Pathophysiology of the atherogenic process. *American Journal of Cardiology* **64**, 23G–30G.

Statsci Europe (1993). *S-Plus Version* 3·2. Oxford: Statsci Europe.

Steinberg, D., Pathasarathy, S., Carew, T., Kheo, J. C. & Witztum, J. L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increases its atherogenicity. *New England Journal of Medicine* **320**, 915–924.

Van Heek, M. & Zilversmit, D. B. (1988). Evidence for an inverse relation between plasma triglyceride and aortic cholesterol in the coconut oil/cholesterol-fed rabbit. *Atherosclerosis* **71**, 185–192.
Van Heek, M. & Zilversmit, D. B. (1990). Postprandial lipemia and lipoprotein lipase in the rabbit are modified by olive and coconut oil. *Arteriosclerosis* **10**, 421–429.
Wiklund, O., Carew, T. E. & Steinberg, D. (1985). Role of the low density lipoprotein receptor in penetration of low density lipoprotein into rabbit aortic wall. *Arteriosclerosis* **5**, 135–141.
Zilversmit, D. B. (1979). Atherogenesis: a postprandial phenomenon. *Circulation* **60**, 473–485.

Printed in Great Britain