Comparison of the effects of fish oil and olive oil on blood lipids and aortic atherosclerosis in Watanabe heritable hyperlipidaemic rabbits

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To compare the effects of fish oil and olive oil on the development of atherosclerosis in Watanabe heritable hyperlipidaemic (WHHL) rabbits, 6-week-old animals were given a daily dose (1·5 ml/kg body weight) of fish oil (*n* 10) or olive oil (*n* 10) by oral administration for 16 weeks. Plasma cholesterol and triacylglycerols were measured once monthly, and their concentrations in lipoproteins, together with susceptibility of LDL to oxidation were measured *in vitro* at the termination of the experiment. Aortic atherosclerosis was quantified biochemically and microscopically. After 4 weeks of treatment, and throughout the study thereafter, blood lipids were significantly ($P < 0.05$) lower in the fish-oil group than in the olive-oil group (cholesterol: 17·0 *v.* 30·3 mmol/l, triacylglycerols 2·97 *v.* 6·25 mmol/l, at termination). In the fish-oil group cholesterol was significantly lower in intermediate-density lipoproteins (2·69 *v.* 6·76 mmol/l) and VLDL (3·36 *v.* 11·51 mmol/l). Triacylglycerol levels of intermediate-density lipoproteins and VLDL in the fish-oil group were also significantly lower when compared with the olive-oil group (0·54 *v* 1·36 mmol/l and 0·92 *v.* 2·87 mmol/l respectively). No group differences were recorded for LDL- and HDL-cholesterol or triacylglycerol levels. A significantly higher oxidation of LDL was recorded 1 h after exposure to $CuSO_4$ in the fish-oil group when compared with the olive-oil group (0·465 *v.* 0·202, arbitrary units). The following indicators of athero-sclerosis development were significantly lower in the fish-oil group than in the olive-oil group: the cholesterol content (mg/g tissue) in the ascending aorta (29·8 *v.* 48·9), the intima:media value (4·81 *v.* 18·24) and the area of intima (0·10 *v.* 0·57 mm^2) in the thoracic aorta. It was concluded that fish-oil treatment decreased blood lipids and the development of aortic atherosclerosis in WHHL rabbits when compared with olive-oil treatment.

WHHL rabbit: Fish oil: Olive oil: Atherosclerosis

Fish oils and olive oil are of interest for dietary prevention of human CHD (Department of Health, 1994). According to the results of population studies, fish oils containing long-chain *n*-3 polyunsaturated fatty acids (PUFA) are considered to have a beneficial effect on the development of atherosclerosis and lipid metabolism (Dyerberg *et al.* 1978; Krombout *et al.* 1985). These results have been supported by other results from studies in healthy human subjects (Haglund *et al.* 1990; Tsai & Lu, 1997) and patients (Harris, 1989; Subbaiah *et al.* 1989; Bairati *et al.* 1992; Hau

et al. 1996). However, no improvement of human coronary atherosclerosis has been reported after long-term treatment with fish oil (Sacks *et al.* 1995). Olive oil, which is rich in monounsaturated fatty acids (MUFA) has been considered to be less atherogenic than other types of fat which contain more saturated fatty acids (SFA) since epidemiological studies have shown that Mediterranean populations, which consume diets rich in olive oil, have a lower risk of CHD than people in the northern part of Europe or the USA in spite of a comparable fat intake (about 40 % energy) (Keys,

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1970; Grundy *et al.* 1982). The results from epidemiological studies have been supported by results from studies in human subjects (Trevisan *et al.* 1990; Mensink & Katan, 1992). At present, MUFA are considered to be a useful substitute for SFA due to their beneficial effect, which is the lowering of plasma LDL but not HDL concentrations.

The effects of fish oil and olive oil on blood lipids and atherosclerosis have been extensively studied in animal models for atherosclerosis and the findings are diverse. In accordance with some human studies several animal studies also indicate beneficial effects of *n*-3 PUFA on experimental atherosclerosis in different animal models such as domestic swine (Weiner *et al.* 1986; Groot *et al.* 1989), monkeys (Davis *et al.* 1987) and mice (Renier *et al.* 1993). Furthermore, fish oils have been demonstrated to have hypolipidaemic and/or antiatherogenic effects in cholesterol-fed (Zhu *et al.* 1990) and homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbits (Clubb *et al.* 1989; Lichtenstein & Chobanian, 1990). However, negative results have also been reported in different animal models, such as the cholesterol-fed rabbit (Thiery & Siedel, 1987; Campos *et al.* 1989), WHHL rabbit (Rich *et al.* 1989), quail (Chamberlain *et al.* 1991), rat (Russell *et al.* 1991) and domestic swine (Barbeau *et al.* 1997). Therefore, at this stage it remains to be established whether fish oil has any ameliorating effect on the development of atherosclerosis. The effect of olive oil in animals has been studied in comparison with other fats. Olive oil has been reported to have a lower relative atherogenic effect than unheated maize oil (Kritchevsky & Tepper, 1967) or peanut oil (Kritchevsky *et al.* 1984) in the cholesterol-fed rabbit. Furthermore, the antiatherogenic potential of olive oil compared with coconut oil has been demonstrated in cholesterol-fed rabbits (Van Heek & Zilversmit, 1988, 1990) and compared with seven diets with different MUFA and SFA contents in mice (Nishina *et al.* 1993). However, when olive oil was used as a reference fat in cholesterol-fed, so called cholesterol-clamped rabbits, its effect on atherosclerosis severity was no different from that of maize oil (Leth-Espensen *et al.* 1988), margarine (Mortensen *et al.* 1992), butter, lard or coconut oil (Nielsen *et al.* 1995).

In most animal models for atherosclerosis, hypercholesterolaemia is produced exogenously, when the normal mechanisms of lipoprotein clearance are overwhelmed by large amounts of dietary cholesterol. In contrast, human hypercholesterolaemia is caused by genetic or acquired abnormalities in the synthesis or degradation of plasma lipoproteins which transport endogenous cholesterol between body tissues. Since dietary and endogenous cholesterol are transported by different plasma lipoproteins, the analogy between the cholesterol-fed model and the human disease is imperfect (Goldstein *et al.* 1983). In contrast to many animal models for atherosclerosis the homozygous WHHL rabbit develops endogenous hypercholesterolaemia due to a genetic defect (a deficiency in LDL receptors) which is analogous to the genetic defect in human familial hypercholesterolaemia (FH). WHHL rabbits fed on a standard diet develop hypercholesterolaemia like that in patients with FH, with pure elevation of the lipoproteins that carry endogenous cholesterol, and atherosclerosis which is prominent within 1 year and which bears a morphological resemblance to the human disease (Goldstein *et al.* 1983; Barter, 1994; Hansen *et al.* 1994). However, in contrast to patients with FH the WHHL rabbit also has elevated levels of plasma triacylglycerols (Mortensen & Frandsen, 1997; Nordestgaard *et al.* 1997) as rabbit LDL has a relatively high triacylglycerol content in comparison with human LDL; therefore, an elevation in LDL in WHHL rabbits causes not only hypercholesterolaemia but also hypertriacylglycerolaemia (Goldstein *et al.* 1983). Furthermore, some other differences originating in the metabolic differences between the two species occur, such as lower activity of hepatic lipase (*EC* 3.1.1.3) and lower conversion of VLDL to LDL, and higher activity of the cholesterol ester transfer protein and paraoxonase in rabbits than in man (Brealey *et al.* 1980; Ha & Barter, 1982; Havel *et al.* 1989). Despite the species differences the homozygous WHHL rabbit appears to be an appropriate model for human FH with respect to altered lipoprotein levels and deficiency of LDL receptors (Havel *et al.* 1989). In addition, the homozygous WHHL rabbit might be an adequate model to study the role of gene–nutrient interaction in the development and prevention of atherosclerosis (Clarke & Abraham, 1992; Wilson *et al.* 1992).

In the present study we decided to use the homozygous WHHL rabbit to study the effects of fish oil on hypercholesterolaemia and the development of spontaneous atherosclerosis in comparison with those of olive oil. The reason for using the olive oil group instead of an untreated control group was to ensure that any difference in blood lipids and atherosclerosis between the fish oil group and the reference group would not originate in a difference in energy and fat intake. This particular animal model seemed to us appropriate because the hypercholesterolaemia in this model, due to its endogenous origin, mimics human hypercholesterolaemia more closely than the hypercholesterolaemia in cholesterol-fed animal models. Furthermore, the disease in WHHL rabbits can directly be attributed to a genetic disorder similar to that found in human subjects. In addition, this model permits the study of hypercholesterolaemia, hypertriacylglycerolaemia and atherosclerosis at the same time (Lichtenstein & Chobanian, 1990). The endpoints in this study included monitoring of changes in blood lipids, evaluation of aortic atherosclerosis by biochemical and histological methods, and examination of LDL susceptibility to *in vitro* oxidation.

Materials and methods

Animals, housing and clinical observations

Twenty-two homozygous WHHL rabbits of both sexes, 6 weeks old, with a mean total plasma cholesterol level of 24·0 (SD 3·3) mmol/l at 4 weeks of age, from our own breeding colony (derived from parent generation obtained from Professor Jansen, University of Leiden, The Netherlands, with permission from Dr Y. Watanabe), were housed individually in stainless steel cages under controlled environmental conditions (temperature 18 ± 2°, relative humidity 55±5 %, 12 h light–dark cycle, air changed 10 times/h) and observed at least twice daily for any abnormalities in clinical condition.

Experimental procedure

The rabbits were allocated to two groups each containing eleven individuals. The initial plasma cholesterol level and body weight were similar in the two groups. During the study period of 16 weeks, the rabbits received daily by oral administration 1·5 ml/kg body weight of fish oil (kindly donated by Lube A/S, Hadsund, Denmark; peroxide value 1·6 mmol/kg oil according to the producer) (group 1) or olive oil (oleum olivae ph Dan 48; Nomeco A/S, Copenhagen, Denmark) (group 2). Since fish oil is known to be susceptible to atmospheric oxidation, the administration of both oils by intubation was chosen instead of mixing the oils with the pelleted diet, which would have been consumed over a period of several hours. Furthermore, to prevent the oxidation of the fish oil during storage it was provided in brown glass bottles containing sufficient for 1 d, and all the portions were kept frozen at −15°. Olive oil was kept at +15° in brown glass bottles under N_2. Analyses of fatty acid composition of both oils used in this study, as well as analysis of the cholesterol content in the fish oil, were performed at the Institute for Food Research and Nutrition, Danish Veterinary and Food Administration according to the method of Ovesen *et al.* (1996) (Table 1). The rabbits were given 100 g/d per rabbit of a standard diet, Altromin 2113 (g/kg: crude protein 150, crude fat 35, crude fibre 195, ash 80, moisture 120 and nitrogen-free extract 420; metabolizable energy 9·7 MJ/kg, data provided by manufacturer) obtained from Altromin GmbH u., Co KG, Lage, Germany. They had free access to tap water. The feed intake was recorded daily and body weight weekly. After 16 weeks of treatment, the rabbits were killed by intravenous injection of pentobarbital (100 mg/kg) into an ear vein. A midline incision was made, the caval vein was transected for exsanguination and the central arterial system was perfused with about 500 ml saline (9 g NaCl/l) through a cannula inserted into the left ventricle. The lungs, heart and the entire aorta to 10 mm distal to bifurcation of the iliac arteries were isolated and the adventitial fat was removed. Two cross-sections of the unopened aorta were taken for histological examination, one from the ascending aorta just above the aortic valves, and one from the thoracic aorta at the level of the first intercostal arteries. The aortic rings were fixed in 40 ml/l buffered formalin and were later processed for histological examination: six to eight serial sections from each sample were stained with elastic haematoxylin–eosin and elastic van Gieson stains respectively. The remaining parts of the ascending and thoracic aorta were opened longitudinally and divided into the intima–inner media and the outer media. The tissues from intima–inner media of the ascending and thoracic aorta were then weighed and stored at −20° until biochemical analysis.

During the study no difference was recorded in the feed intake between the two groups as all the rabbits finished their feed rations every day. There was no difference in body weight between the two groups during the study. The body weight increased from 1·0 (SD 0·2) kg and 1·1 (SD 0·2) kg at the start of the study to 2·7 (SD 0·2) kg and 2·8 (SD 0·1) kg at the termination in fish-oil and olive-oil groups respectively. No effect of treatment on the clinical appearance was observed in any of the rabbits. However, one rabbit in each group was lost during the study due to accidental dosing into the lungs instead of the stomach using the peroral intubation. Thus, the study included two groups of ten rabbits (seven females and three males) each.

Plasma lipids

The plasma cholesterol and triacylglycerol levels were measured before dosing and once monthly thereafter; their concentrations in lipoproteins were measured at termination of the experiment. Blood samples were collected from the marginal ear vein of unanaesthetized animals fasted overnight, into tubes containing potassium EDTA, and the plasma was isolated after centrifugation at 2000 ***g*** for 10 min. The concentrations of plasma cholesterol and plasma triacylglycerols were determined enzymically (CHOD-PAP, Boehringer Mannheim, Mannheim, Germany, and UNIMATE, Hoffmann–La Roche, Grenzach-Wyhlen, Germany respectively). Plasma lipoproteins were separated by density gradient ultracentrifugation by the method of Terpstra *et al.* (1981). The density ranges of the isolated fractions were: VLDL $\rho < 1·0063$ g/ml, intermediate-density lipoprotein $1·0063 < \rho < 1·019$ g/ml, LDL $1·019 < \rho < 1·063$ g/ml, HDL $1·063 < \rho < 1·21$ g/ml. The concentrations of cholesterol and triacylglycerols in each fraction were determined as previously described.

Chemical oxidation of LDL by $CuSO_4$

Blood samples for measurement of the susceptibility of LDL to oxidation were collected in week 16 of the study. The LDL fraction was isolated by ultracentrifugational flotation as described by Mackness & Durrington (1992). Following dialysis, the LDL fraction was diluted with

Table 1. Fatty acid composition of fish oil and olive oil (g/100 g total fatty acids)

Fatty acid	Fish oil*	Olive oil
14:0	1·1	–
16:0	4·3	10·9
16:1	2·0	0·7
18:0	3·7	2·5
18:1	8·5	75·1
18:1n-7	3·1	1·7
18:2	1·2	8·1
18:3n-3	0·3	0·42
18:4n-3	2·4	–
20:0	0·4	0·3
20:1	1·8	0·2
20:2	0·2	–
20:3	0·2	–
20:4	0·3	–
20:4n-3	1·3	–
20:5n-3	33·3	–
22:1n-11	2·9	–
22:1n-9	0·3	–
22:5n-3	4·4	–
22:6n-3	21·4	–
24:1	1·5	–
Sum of others	5·4	
Total fatty acids	100·0	100·0

–, Not detectable in the sample.
* Cholesterol content in fish oil: 530 mg/100 g.

phosphate-buffered saline and adjusted to a final concentration of 40 μM-CuSO$_4$ (Roma *et al.* 1992), incubated for 24 h at 37°, samples taken at 0, 1, 4, 7 and 24 h, the oxidation stopped by addition of butylated hydroxytoluene (final concentration 40 μM) and immediately cooled on ice. The kinetics of the LDL oxidation were followed by continuously monitoring the change of 234 nm diene absorption in a u.v.-PC spectrometer (UV-PC Shimatzo Kyoto, Japan) (Esterbauer *et al.* 1989). The content of the protein in all LDL samples was equal.

Microscopic examination

Three types of lesions were recorded in the sections from aortic samples: (1) fatty streaks as subintimal accumulations of foam cells, (2) fibrous plaques as intimal fibrous thickening with occasional foam cells and (3) advanced lesions as intimal thickenings with many foam cells and/or cholesterol crystals, usually localized in deep-seated pools (Hansen *et al.* 1994). Quantitation of atherosclerotic lesions was performed by point-counting. Using a projective device (Ocular Periplan 12·5/20, Wild Leitz, Wetzlar, Germany) the microscopic picture of the aorta was transferred to a grid with regularly spaced points (point-grid). Degree of magnification and size of the point-grid were kept constant. Quantitation was always performed in two to four serial sections and the mean value reported. The number of points covering the intima and points covering the media were recorded. The quantitative evaluation of atherosclerosis in the ascending and thoracic aorta was performed in two different ways: (1) the intima : media ratio (I:M) and (2) the area of intima in mm^2 (Hansen *et al.* 1994). The calculation of area of the intima was based on knowledge of both the exact degree of magnification of the microscopic picture and the exact distance between points in the grid. In our estimation 1 mm of aortic tissue was magnified to 2·1 cm on the grid where points were regularly placed 1 cm apart. According to the formula 1 point $= 1 \times 1/2 \cdot 1 \times$

$2 \cdot 1 = 0 \cdot 227$ (Gundersen *et al.* 1988) the quantitation recorded in points could be converted to mm^2.

Biochemical evaluation

Aortic intima–inner media from the ascending and thoracic aorta were minced with scissors and the lipids were extracted for 24 h with chloroform–methanol (1:1, v/v). Lipids in the supernatant fraction and proteins in the precipitate were separated by the methods of Folch *et al.* (1957). Total cholesterol in the supernatant fraction was determined by the Liebermann–Burchard method after saponification (Ness *et al.* 1964). The results were expressed as mg total cholesterol/g wet tissue.

Statistical methods

The results for body weight, plasma lipids, chemical oxidation of LDL, I:M, the area of intima and cholesterol content in aortic tissue were analysed by Student's *t* test, as the data were normally distributed. The changes (type of lesions) in thoracic aorta were analysed by Fisher's exact test. Values of $P < 0 \cdot 05$ were considered statistically significant. All statistical analyses were performed using Statistical Analysis System (SAS) software (release 6.11, 1996; SAS Institute Inc., Cary, NC, USA).

Results

Plasma lipids

The results are summarized in Table 2. Compared with olive oil the fish-oil treatment resulted in significant decreases in plasma cholesterol and triacylglycerol levels. In the fish-oil group the concentrations of cholesterol and triacylglycerols were significantly lower in intermediate-density lipo- proteins and VLDL when compared with the olive-oil group. No significant differences were recorded in

Table 2. Blood lipids (mmol/l) in homozygous Watanabe heritable hyperlipidaemic rabbits given a daily dose of fish oil or olive oil for 16 weeks†
(Mean values and standard deviations for ten rabbits per group)

		Week of treatment				Concentration in lipoproteins in week 16			
	0	4	8	12	16	HDL	LDL	IDL	VLDL
Cholesterol‡									
Fish oil: Mean	21·2	15·9*	16·5*	18·7*	17·0*	0·28	9·61	2·69*	3·36*
SD	2·2	2·6	2·6	3·5	1·8	0·12	2·33	0·59	0·78
Olive oil: Mean	22·8	25·1	26·7	29·4	30·3	0·32	11·42	6·76	11·51
SD	1·9	4·6	5·6	5·4	5·6	0·07	3·09	2·55	4·04
Triacylglycerols§									
Fish oil: Mean	6·89	2·97*	2·69*	3·38*	2·97*	0·12	1·41	0·54*	0·92*
SD	3·97	0·88	0·44	0·61	0·68	0·04	0·25	0·24	0·36
Olive oil: Mean	6·85	4·72	4·32	5·97	6·25	0·11	1·71	1·36	2·87
SD	4·45	0·98	1·22	1·46	2·37	0·02	0·58	0·78	1·94

IDL, intermediate-density lipoproteins.
Mean values were significantly different from those for olive oil, *$P < 0 \cdot 05$.
† For details of oils and procedures, see Table 1 and pp. 566–567.
‡ Mean recovery of cholesterol, 96·2 (SD 5·7)%.
§ Mean recovery of triacylglycerols, 98·9 (SD 6·3)%.

LDL-cholesterol and LDL-triacylglycerols between the groups. The concentrations of cholesterol and triacylglycerols in HDL were comparable in both groups.

LDL susceptibility to oxidation

The results for chemical oxidation of LDL are presented in Table 3. A significantly higher LDL oxidation was recorded in the fish-oil group when compared with that in the olive-oil group 1 h after exposure to $CuSO_4$. At longer incubation times, the extent of LDL oxidation was similar for the two groups.

Quantitative biochemical and quantitative and qualitative microscopic evaluation of aortic atherosclerosis

The results are shown in Table 4. Significantly less deposition of cholesterol was found in the ascending aorta in the fish-oil group when compared with the olive-oil group. Histological quantitative evaluation demonstrated significantly lower severity of atherosclerotic lesions calculated as I:M and recorded as area of intima in thoracic aorta of WHHL rabbits from the fish-oil group when compared with the olive-oil group. No significant difference in type of atheromatous lesions in the thoracic aorta was found between the groups. However, no atherosclerotic lesions were demonstrated in the thoracic aorta in two rabbits from the fish-oil group, while the atherosclerotic lesions were demonstrated in all the rabbits from the olive-oil group.

Table 3. Oxidation of LDL isolated from rabbits given a daily dose of fish oil or olive oil, after exposure to copper sulfate (arbitrary units)

(Mean values for ten samples)

Time after exposure (h) ...	1	4	7	24
Fish oil	0·456*	0·431	0·780	0·857
Olive oil	0·202	0·434	0·797	0·754

Mean value was significantly different from that for olive oil, *$P < 0.05$.

Discussion

The quantitative biochemical and microscopical methods for estimation of aortic atherosclerosis used in the present study were chosen based on our evaluation of different quantitative methods in a study on spontaneous development of atherosclerosis in WHHL rabbits (Hansen *et al.* 1994): (1) macroscopic estimation of aortic lesions by inspection with the naked eye or point-counting, (2) microscopic estimation of aortic lesions as I:M and area of intima, and (3) biochemical determination of aortic cholesterol content. In aorta, a highly statistically significant correlation between these methods was found especially in the ascending and thoracic parts. The measurement of cholesterol content seemed the best to demonstrate the differences in degree of aortic lesions between the different parts of the aorta. The I:M value in the thoracic aorta was found to be the best microscopic quantitative variable to evaluate the progression of aortic atherosclerosis with age. Furthermore, all correlations between I:M value and area of intima in aorta and in the right coronary artery and percentage stenosis of the left coronary artery were statistically highly significant. For these reasons the cholesterol content, I:M value and area of intima in the ascending and thoracic aorta were chosen as the pathoanatomical end-points in the present study.

In the present study, the fish-oil treatment significantly reduced total plasma cholesterol and triacylglycerol levels and their concentrations in VLDL and intermediate-density lipoprotein fractions when compared with the olive-oil treatment. Both reduction of total cholesterol and triacylglycerols and their content in VLDL and intermediate-density lipoproteins could explain the lower aortic atherosclerosis in the fish-oil group when compared with the olive-oil group (Juhel *et al.* 1997; Nordestgaard *et al.* 1997). Our findings of significant reductions in the concentrations of cholesterol and triacylglycerols in VLDL and intermediate-density lipoproteins in response to fish oil

Table 4. Quantitative biochemical and quantitative and qualitative microscopical evaluation of aortic atherosclerosis in homozygous Watanabe heritable hyperlipidaemic rabbits given a daily dose of fish oil or olive oil for 16 weeks†

(Mean values and standard deviations for ten rabbits per group)

	Fish oil		Olive oil	
	Mean	SD	Mean	SD
Cholesterol content (mg/g tissue)				
Ascending aorta	29·81*	15·77	48·88	24·69
Thoracic aorta	22·99	18·70	28·68	12·34
Intima : media				
Ascending aorta	8·08	5·71	15·30	12·25
Thoracic aorta	4·81*	5·05	18·24	15·70
Area of intima (mm^2)				
Ascending aorta	0·41	0·31	0·82	0·64
Thoracic aorta	0·10*	0·09	0·57	0·61
Prevalence and type of lesions in thoracic aorta				
No lesion	2		0	
Fatty streak	5		6	
Fibrous plaque	2		2	
Advanced lesion	1		2	

Mean values were significantly different from those for olive oil, *$P < 0.05$.
† For details of oils and procedures, see Table 1 and pp. 566–567.

are in accordance with the reports of Clubb *et al.* (1989) and Lichtenstein & Chobanian (1990) on the hypolipidaemic effect of fish oil in homozygous WHHL rabbits when compared with untreated controls. Furthermore, our findings of a stronger hypolipidaemic effect of fish oil compared with the olive oil in WHHL rabbits is in accordance with the finding of a stronger hypolipidaemic effect of fish oil compared with olive oil in rats of the atherosclerosis prone JCR:LA corpulent strain, a spontaneous animal model for cardiovascular disease (Russell *et al.* 1991).

In clinical practice an increase in HDL-cholesterol level is regarded as a marker of a possible antiatherogenic effect of a therapeutic diet or a dietary supplement. In the present study a lower degree of aortic atherosclerosis was not accompanied by an increase in the HDL-cholesterol in the fish-oil group when compared with the olive-oil group. In fact, the levels of HDL-cholesterol were comparable in the two groups. This suggests that the effect on HDL-cholesterol may be of minor importance when assessing the antiatherogenic effect of fish oil in the WHHL rabbit. This could possibly apply to other animal models, as the prevention of atherosclerosis by fish oil in non-human primates was accompanied by a reduction in HDL-cholesterol (Davis *et al.* 1987).

The fatty acid composition of LDL is influenced by dietary fatty acids (Parthasarathy *et al.* 1990; Harats *et al.* 1991; Reaven *et al.* 1991; Tripodi *et al.* 1991; Berry *et al.* 1992; Nenseter *et al.* 1992). The modification of LDL by oxidation of their unsaturated lipid components is believed to play a significant role in the development of atherosclerosis (Steinberg *et al.* 1989; Steinberg, 1992; Parthasarathy *et al.* 1992; Ross, 1993). In the present study, a significantly higher oxidation of LDL was recorded 1 h after incubation with $CuSO_4$ in the fish-oil group when compared with the olive-oil group in accordance with reports on a high resistance to oxidation of oleate-rich LDL particles (Parthasarathy *et al.* 1990; Reaven *et al.* 1991; Berry *et al.* 1992; Visioli *et al.* 1995; Wiseman *et al.* 1996). This indicates that the effect of *n*-3 PUFA in LDL is to increase the susceptibility to oxidative stress when compared with that of MUFA. Despite the higher susceptibility of LDL to *in vitro* oxidation a lower degree of atherosclerosis was found in the fish-oil group when compared with the olive-oil group. This may suggest that the oxidative susceptibility of LDL *in vitro* does not necessarily reflect the biological processes *in vivo*, where competition between the oxidative and protective processes may depend on both the composition of unsaturated fatty acids and total plasma antioxidant levels. The latter seems to be supported by a report of Whitman *et al.* (1994) on fish-oil-treated pigs. It should be further pointed out that paraoxonase activity is high in rabbits compared with human subjects or other species (Brealey *et al.* 1980). As this HDL-associated enzyme may protect LDL against oxidative modifications (Mackness *et al.* 1993), the LDL susceptibility to *in vitro* oxidation may be less of a determinant factor for *in vivo* development of atherosclerosis in the rabbit compared with man.

Several studies have been conducted to investigate the effect of fish oil on experimental or spontaneous atherosclerosis in rabbits. However, none of these studies compared the atherogenic effect of fish oil with those of other types of fat. In the cholesterol-fed rabbit, Zhu *et al.*

(1990) reported a beneficial effect of fish oil on regression of experimental atherosclerosis. Thiery & Siedel (1987) reported aggravation of experimental atherosclerosis. Campos *et al.* (1989) reported no effect of fish oil on the experimental atherosclerosis when compared with untreated controls. In homozygous WHHL rabbits, treatment with fish oil for 1 year had no effect on the plasma lipids and aortic atherosclerosis estimated by percentage surface area involved or an index of total lesion volume when compared with those in untreated controls (Rich *et al.* 1989). Supplementation of the diet with fish oil for 5 months in homozygous WHHL rabbits resulted in a reduction of plasma triacylglycerol and total, VLDL-, LDL- and HDL-cholesterol levels in females and VLDL-cholesterol levels in males, but no difference was found in aortic atherosclerosis between the treated and untreated groups by morphometry of lipid-positive areas of the aortas or by comparison of intimal thickness (Clubb *et al.* 1989). Supplementation of the diet with fish oil for 6 months *v.* the standard diet resulted in significant decreases in plasma triacylglycerol and cholesterol levels and a significant decrease in aortic atherosclerosis in the descending and abdominal parts of the aorta expressed as the cholesterol content in the aortic tissue, but not as a surface area covered by plaques (Lichtenstein & Chobanian, 1990). The different findings on aortic atherosclerosis in the last two studies in homozygous WHHL rabbits may indicate that the choice of methods for evaluation of aortic atherosclerosis may be crucial for detection of the antiatherogenic effects of tested compounds.

A direct comparison of the effect of fish oil on spontaneous atherosclerosis in the homozygous WHHL rabbits in the present study and in the three studies mentioned earlier is not possible because of different study designs. However, two of these studies demonstrated a significant hypolipidaemic effect of fish-oil treatment (Clubb *et al.* 1989; Lichtenstein & Chobanian, 1990) and one of them an ameliorating effect on cholesterol accumulation in the aorta (Lichtenstein & Chobanian, 1990). The present study demonstrated a hypolipidaemic effect of fish oil compared with olive oil. Furthermore, the results of the present study indicate that the hypolipidaemic effect of fish oil resulted in retardation of the spontaneous atherosclerosis in young WHHL rabbits. The latter is based on the fact that a significantly lower degree of atherosclerosis was recorded in the ascending and thoracic parts of the aorta from the fish-oil-treated group when compared with the olive-oil group.

However, the relevance of this study for people with FH is highly speculative. One should be cautious in extrapolating from the results in WHHL rabbits in the present study to the human situation because of differences in lipid metabolism between the two species (Ha & Barter, 1982; Havel *et al.* 1989) and because of the higher activity of paraoxonase in the rabbit than in man (Brealey *et al.* 1980), which may reduce LDL oxidation *in vivo* (Mackness *et al.* 1993), which is believed to be an essential stage in atherogenesis.

In conclusion, lower plasma lipids and degree of aortic atherosclerosis were recorded in the fish-oil-treated group when compared with the olive-oil-treated group. This suggests a beneficial effect of *n*-3 PUFA on spontaneous hyperlipidaemia and atherosclerosis in the homozygous WHHL rabbit model. Additionally, the differences in

LDL susceptibility to *in vitro* oxidation might not necessarily be reflective of the atherogenic processes *in vivo*.

Acknowledgements

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