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Extravascular lipid deposition and morphology of atherosclerosis in heterozygous WHHL rabbits fed vegetable (n-6) and marine (n-3) oils

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Introduction

The rabbit has been widely used in atherosclerosis research as this species easily develops atherosclerotic lesions in aorta when fed cholesterol. During the last 10 years the homozygous Watanabe heritable hyperlipidemic (WHHL) rabbit has been widely used as a model for familial hypercholesterolemia as this rabbit develops spontaneous atherosclerosis with many similarities to the atherosclerosis in man (*Buja et al.* 1983, *Fischer Hansen et al.* 1994, *Rosenfeld et al.* 1987a, b, *Watanabe* 1980). The heterozygous WHHL rabbit develops only minimal spontaneous atherosclerosis, which is not observed until the age of approximately 2 years (*Atkinson et al.* 1989, *Esper et al.* 1993, *Fischer Hansen et al.* 1994). The development of atherosclerotic changes in younger heterozygous WHHL rabbits can be obtained by cholesterol feeding as in other normolipidemic rabbits. *Atkinson et al.* (1989) reported that the histological picture of aortic atherosclerosis in heterozygous WHHL rabbits fed 1 % cholesterol resembled aortic atherosclerotic lesions in homozygous WHHL rabbits, and in humans to a greater extent than these lesions in NZW rabbits fed the same amount dietary cholesterol. Furthermore, the cholesterol-fed heterozygous WHHL rabbit was proposed as a new rabbit model which 1) to a greater extent simulates human population than homozygous WHHL rabbit, since humans with the heterozygous familial hypercholesterolemia outnumber those with the homozygous form, and 2) complies better than cholesterol-fed NZW rabbit with the request on morphologic resemblance between atherosclerotic lesions in animal model and in humans.

The aim of this study was to test the heterozygous WHHL rabbit fed 1 % cholesterol as a model for investigations of atherogenic effect of dietary fats. For this purpose 1) the blood lipids were recorded, and 2) the evaluation of the degree and extent of atherosclerotic changes in aorta, epicardial coronary and pulmonary arteries, and of extravascular lipid infiltration in various organs were performed.

As one of the test fats a marine oil was chosen, because fish oils with large quantities of (n-3) long chain polyunsaturated fatty acids are considered to be antiatherogenic due to their hypolipidemic effect demonstrated in healthy volunteers and patients (*Bairati et al.* 1992, *Friday et al.* 1991, *Haglund et al.* 1990, *Harris* 1989, *Subbaiah et al.* 1989), population studies (*Dyerberg et al.* 1978) and in animal studies (*Groot et al.* 1989, *Weiner et al.* 1986, *Zhu et al.* 1990). As another test fat a vegetable oil with large quantities of (n-6) polyunsaturated fatty acids was chosen since these polyunsaturated fatty acids are also known for their plasma cholesterol lowering effects (*Friday et al.* 1991, *Katan & Mensink* 1993, *Nordøy & Goodnight* 1990). To exclude that any difference in the effect on blood lipids and experimental atherosclerosis between marine and vegetable oils could derive from the difference in the contents of saturated or mono-

saturated fatty acids, the contents of saturated and monoic acids in both oils were balanced.

Materials and methods

Animals

Twentytwo heterozygous WHHL rabbits of both sexes, 8–9.5 months old, with plasma cholesterol levels of 0.95 mmol/l ± 0.53 (mean ± SD), were obtained from own breeding colony established from parent generation obtained with the permission from dr. Y. Watanabe from Professor Jansen, University of Leiden, The Netherlands. The rabbits were housed individually in steel cages under controlled environmental conditions (temperature 18° ± 2° C, the relative humidity 55 ± 5 %, 12/12 hours light/dark cycle, air changed 10 times/h).

Diet

Three different powdered diets were used (fat content 3.5 %): standard diet Altromin 2110, and two standard diet concentrates containing the nutrients of the standard diet allowing for addition of 5 % and 10 % by weight of fat respectively (Altromin 2110 standard, 741.2110 95 % and 742-2110 90 %). The powdered diets were used to produce pellets with 0 and 1.11 % (w/w) cholesterol (Sigma C 8503) respectively. The vegetable oil contained 50 % palm kernel oil (Palmotex, Aarhus Oliefabrik A/S, Denmark), 40 % grape kernel oil (FDB, Viby, Denmark), and 10 % olive oil (FDB, Viby, Denmark). The marine oil contained 60 % tobis oil (Technological Laboratory, Danish Ministry of Fisheries), 20 % grape kernel oil and 20 % palm kernel oil (Table 1). In order to avoid oxidation of the added oils the diets with both cholesterol and the added oils were prepared daily by pouring the oils over pellets and mixing thoroughly. Both oils were kept frozen at – 15° C in portions for one day use. Pellets for all three groups were stored at + 15° C.

Table 1. Fatty acid composition (%) in vegetable and marine oils.

Fatty acid	Vegetable oil (Group II)	Marine oil (Group III)
C 12:0	0.00	0.12
C 14:0	0.43	4.13
C 14:1	0.00	0.30
C 16:0	25.07	20.05
C 16:1	0.25	7.33
C 18:0	5.10	3.58
C 18:1	35.27	19.86
C 18:2	32.86	17.43
C 18:3	0.61	1.12
C 18:4	0.00	2.52
C 20:1	0.00	2.70
C 20:2	0.00	0.18
C 20:4 n-6	0.00	0.30
C 20:4 n-3	0.00	0.30
C 20:5	0.00	6.30
C 22:1	0.00	4.50
C 22:3	0.00	0.24
C 24:1	0.00	0.48
C 22:5	0.00	0.36
C 22:6	0.00	5.76
Total n-3	0.61	16.60
Total n-6	32.86	17.73
Total saturated acids	30.59	27.88
Total monoenes	35.52	35.18

Experimental design

The rabbits were randomized in three groups: group I of eight (three females and five males), and groups II and III of seven rabbits (four females and three males) each. Each group received the specific diet 100 g per rabbit daily: group I cholesterol enriched standard diet, groups II and III cholesterol enriched diet added vegetable or marine oils respectively, during 14 weeks. During the first 5 weeks the dose of cholesterol was increased by 0.2 % and fats by 2 % per week up to 1 respectively 10 %. The dose of the fats in group II and III was reduced to 5 % from the sixth week. The rabbits were fed once daily before 9:30 h or after collection of the blood samples from the marginal ear vein of unanesthetized animals. The body weight was recorded weekly and feed intake

daily. The total plasma cholesterol was determined prior to and during the dosing period at week 4, 6, 8, 9, and 15 (prior to euthanasia), the plasma lipoproteins prior to and in week 4, and the plasma triglycerides in week 4, 6, 8, 9, 11, and 15. The concentrations of plasma cholesterol and plasma triglycerides were determined enzymatically (CHOD-PAP, GPO-PAP, Boehringer Mannheim, Germany). 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, LDL $1.019 < \rho < 1.063$, HDL $1.063 < \rho$. The concentration of cholesterol in each fraction was determined as above.

Separation of blood cells and determination of their fatty acid composition

In week 4, 10 ml blood was collected from an ear vein of fasted animals and stabilized with EDTA and diluted with saline 1:1. Five ml of the diluted blood sample were carefully placed on 4 ml of Lymphoprep™ (Nycomed Pharma A/S, Oslo, Norway) which had a density on 1.077 ± 0.001 g/ml and centrifuged for 20 minutes at 200 g_{av}. The top fraction containing the platelets and the middle fraction with the lymphocytes were isolated. The erythrocytes were isolated from the bottom fraction by further centrifugation at 400 g_{av} for 10 minutes and washed three times with saline. The platelets were harvested from the platelet rich plasma by centrifugation at 600 g_{av} for 20 minutes and washed 3 times with saline. The middle fraction containing the lymphocytes was diluted with saline 1:3, placed on 3 ml of Lymphoprep™, and centrifuged 15 minutes at 200 g_{av}. The lymphocyte fraction was isolated and the lymphocytes were harvested by centrifugation at 220 g_{av} for 10 minutes and washed 3 times with saline. The lipids of the erythrocytes, lymphocytes and platelets were extracted according to *Folch et al.* (1957). Methyl esters of total fatty acids were prepared (*Høy & Hølmer* 1988) and were analyzed by gas liquid chromatography on a 5890 series II Hewlett Packard gas chromatograph (Hewlett Packard, PA, USA) equipped with a 30 m SP™ 2380 fused silica capillary column with an internal diameter of 0.32 mm (Supelco, Inc., PA, USA). On-column injection was employed. Carrier gas was helium and the initial flow was 1 ml/min. Initial oven temperature programming at 40° C/min to 140° C and 4° C/min to 200° C, which was maintained for 20 min. Authentic standards (Sigma Inc., St. Louis, MO, USA) were used for peak identification. The amounts of individual fatty acids were calculated as a relative weight percentage of the identified fatty acids, which was more than 95 % of the total peak area.

Pathological examination

At the end of the experiment (week 15), the rabbits were euthanized by intravenous injection of 5 % pentobarbital solution. The entire aorta, heart, lungs, liver, spleen, intestines, kidneys, testes or ovaries were removed. From the longitudinally opened aorta transverse sections for microscopic examination were taken from the ascending, thoracic, diaphragmatic and abdominal part. The heart was sliced transversely and 4 slices of left and right ventricular myocardium were processed for microscopy. The distal epicardial coronary arteries were evaluated in these sections. Pulmonary arteries were evaluated in hilar and peripheral lung sections. Atherosclerotic lesions were recorded as 1: fibrous plaques, i.e. discrete fibrous intimal thickenings, 2: fibrous plaques with a few foam cells and 3: advanced lesions, i.e. intimal thickenings with closely packed foam cells, eventually deep separated necrotic pools and/or cholesterol crystals (*Fischer Hansen et al.* 1994). The extravascular lipid infiltrations were evaluated semiquantitatively (slight, pronounced) in the myocardium, lungs, kidneys, intestines, liver, spleen, mesenterial lymph nodes and in internal genitalia. All sections were stained by

elastic hematoxylin-eosine and elastic van-Gieson stains. Microscopic examination was performed blindly.

Statistical analysis

The results of feed intake were analyzed by analysis of variance followed by Dunnett's test. The results of body weight, total plasma cholesterol, triglycerides and lipoproteins were analyzed by analysis of variance followed by Duncan's test. The effects were considered significant for p values less than 0.05. All statistical analysis were performed using Statistical Analysis system (SAS) software (SAS Institute Inc., release 6.03, 1988).

Results

Body weight and feed intake

The mean body weight was 3.66 kg ± 0.45 (SD), 3.65 kg ± 0.31 and 3.78 kg ± 0.28 at the beginning of the study and 3.36 kg ± 0.33, 3.28 kg ± 0.26 and 3.22 kg ± 0.08 at the termination in group I, II, and III respectively. No significant difference in body weight was found between the groups during the experiment.

The mean daily feed intake was 95 g ± 4 (SD), 87 g ± 9 and 75 g ± 15 in group I, II, and III respectively. During the experiment, a significant difference in feed intake was recorded in group II in the first week, and in group III from the fourth to the tenth week when compared to group I ($p < 0.05$).

As a consequence of the decreased feed intake two males from group III were removed from the experiment after 7 weeks (their organs are excluded from the histological evaluation), and one female in group II was euthanized after 11 weeks. The organs from this female and from a female from group III which died unexpectively in week 12 (the cause of death was myocardial infarction) are included in the histological examination.

Blood lipids

The data for blood lipids for males and females in each group were pooled as no sex differences were observed on experimental diet. One percent cholesterol in the diet caused pronounced hypercholesterolemia which was significantly enhanced by addition of oils. The increase in total cholesterol was especially reflected in the increase in the very low density lipoprotein (VLDL) fraction (Table 2). The plasma cholesterol (Fig. 1) and triglycerides (Fig. 2) levels were comparable between the two oil fed groups apart from a transitory lowering in the marine oil fed rabbits.

Table 2. Cholesterol concentration (mmol/l ± SEM) in plasma lipoproteins in heterozygous WHHL rabbits on diet with cholesterol (group I), cholesterol and vegetable oil (group II), and cholesterol and marine oil (group III).

Fraction	Week	Group I	Group II	Group III
VLDL	0	0.18 ± 0.05	0.24 ± 0.07	0.09 ± 0.03
	4	33.0 ± 3.9	52.8 ± 2.3*◆	38.5 ± 1.8
LDL	0	0.31 ± 0.11	0.28 ± 0.12	0.14 ± 0.05
	4	0.72 ± 0.26	1.45 ± 0.15	4.42 ± 0.59*◆
HDL	0	0.45 ± 0.09	0.51 ± 0.08	0.57 ± 0.13
	4	1.09 − 0.18	1.88 ± 0.36*	1.40 ± 0.10

LDL: low density lipoprotein.
HDL: high density lipoprotein.
Mean recovery of cholesterol (%): 102.8 ± 8.9 (SD).
* = $p < 0.05$ compared to group I.
◆ = $p < 0.05$ difference between group II and III.

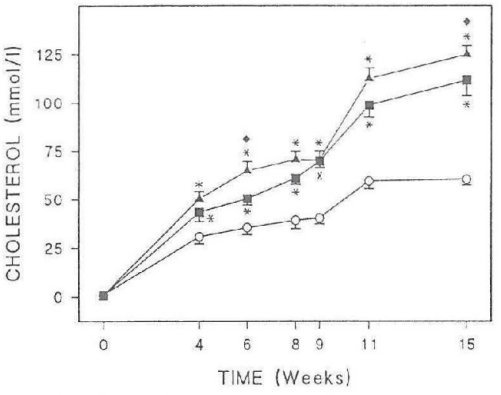

Fig. 1. Total plasma cholesterol concentration (mean ± SEM) of heterozygous WHHL rabbits on diet with cholesterol (group I: ○-○), cholesterol and vegetable oil (group II: ▲-▲), and cholesterol and marine oil (group III: ■-■).* $p<0.05$ compared to group I, ◆$p<0.05$ group II compared to group III.

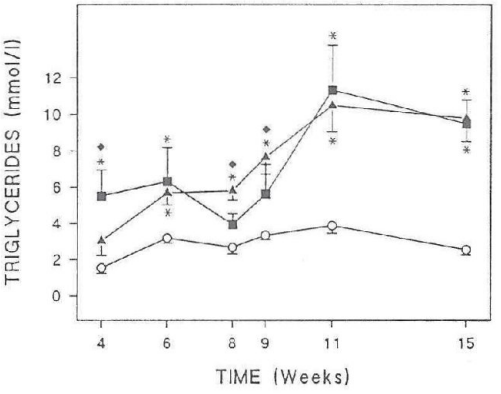

Fig. 2. Triglycerides concentration (mean ± SEM) of heterozygous WHHL rabbits on diet with cholesterol (group I: ○-○), cholesterol and vegetable oil (group II: ▲-▲), and cholesterol and marine oil (group III: ■-■).* $p<0.05$ compared to group I, ◆$p<0.05$ group II compared to group III.

Blood cells fatty acid composition

The fatty acid composition of blood cells reflected the differences in the fatty acid composition in the vegetable and marine oils (Table 3). In group III in all 3 types of blood cells the overall content of (n-3) polyunsaturated fatty acids was higher than in the two other groups, due to increase in 20:5 (n-3), 22:5 (n-3) and 22:6 (n-3) acids. This increase was compensated by a decrease in content of 20:4 (n-6) and 18:2 (n-6) acids in all types of blood cells. The lower content of (n-6) polyunsaturated fatty acids in lymphocytes in group III was additionally compensated by higher content of monoenes, when compared with the two other groups.

Histological examination

Atherosclerotic changes

Aorta. All animals had atherosclerotic lesions but both the type and peripheral extension of lesions varied between groups (Fig. 3). Lesions were less pronounced in group I compared to groups II and III. The prevalence of advanced lesions and the periferal extension of lesions were highest in group III. In all groups, the atherosclerotic lesions were most pronounced in the ascending aorta.

Coronary arteries. The epicardial arteries were evaluated in their more peripheral parts as seen in epicardial fat on the transverse slices of left and right ventricular myocardium. Discrete eccentric fibrous plaques were seen in 1 animal in groups I and II and in 3 animals in group III. Acute myocardial infarction of the papillary muscles of the left ventricle was seen in 2 of 8 animals in group I and in 2 of 5 animals in group III.

Pulmonary arteries. Atherosclerotic lesions in the hilar pulmonary arteries were demonstrated in all 3 groups but especially in group I. The morphology of lesions, however, differed between groups (Fig. 3). The lesions were most severe in group I (Fig. 5a) and less severe in group III.

Renal arteries. Discrete fibrous plaques were seen in the hilar arteries in 1 or 2 animals in each group.

Extravascular lipid infiltration

Myocardium. Myocardial infiltration with foam cells was seen in all animals in groups I and II, but was never seen in group III (Fig.

Table 3. Fatty acid composition (% w/w) in blood cells of heterozygous WHHL rabbits during the fourth week on diet with cholesterol (group I), cholesterol and vegetable oil (group II), and cholesterol and marine oil (group III).

	Group I			Group II			Group III		
Fatty acid	Erythrocytes (n=8)	Lymphocytes (n=7)	Platelets (n=6)	Erythrocytes (n=7)	Lymphocytes (n=7)	Platelets (n=6)	Erythrocytes (n=7)	Lymphocytes (n=5)	Platelets (n=7)
C 12:0	0.00	0.36	0.33	0.00	0.45	0.25	0.00	0.55	0.23
C 14:0	0.43	0.90	0.80	0.41	1.22	0.71	0.42	1.91	1.21
C 16:0	23.57	15.99	14.64	22.25	15.77	14.04	23.37	18.07	18.52
C 16:1	0.75	1.19	0.67	0.60	1.64	0.40	0.71	2.24	0.83
C 18:0	16.99	19.44	22.84	17.53	16.72	21.13	17.72	13.48	19.11
C 18:1	14.75	18.55	17.38	14.16	26.46	20.86	12.22	28.41	17.20
C 18:2	33.48	23.28	24.27	34.13	22.80	26.23	26.61	16.18	21.39
C 18:3	1.73	2.18	1.32	1.58	1.67	0.90	1.78	1.58	1.43
C 20:0	0.00	0.14	0.07	0.01	0.00	0.03	0.02	0.04	0.00
C 20:1	0.72	0.51	0.14	0.65	0.11	0.11	0.73	0.74	0.29
C 20:3 (n-6)	0.31	1.49	1.22	0.27	0.84	0.32	0.23	0.88	0.76
C 20:4 (n-6)	5.02	12.42	12.45	5.05	10.12	11.16	4.75	5.83	7.49
C 20:5 (n-3)	0.02	0.32	0.25	0.10	0.24	0.26	3.16	4.16	5.30
C 22:1	0.22	0.00	0.00	0.51	0.00	0.00	0.88	0.00	0.00
C 22:4 (n-6)	0.58	0.00	0.00	0.57	0.00	0.00	0.32	0.00	0.00
C 22:5 (n-6)	0.18	0.00	0.00	0.41	0.00	0.00	0.43	0.00	0.00
C 22:5 (n-3)	0.92	1.93	1.44	1.02	1.29	1.49	2.00	2.55	1.67
C 22:6 (n-3)	0.28	0.32	0.29	0.59	0.26	0.40	4.40	3.03	2.96
C 24:1	0.00	0.00	0.56	0.00	0.00	0.47	0.00	0.00	0.09
Total n-3	1.43	2.57	1.98	1.71	1.79	2.13	9.56	9.74	9.93
Total n-6	41.31	39.38	39.26	42.02	35.43	38.61	34.12	24.47	31.07
Total saturates	41.05	37.79	40.01	40.36	34.57	37.43	41.77	34.42	40.59
Total monoenes	16.43	20.26	18.75	15.91	28.20	21.83	14.54	31.38	18.41
Total poly-unsaturates	42.74	41.95	41.24	43.73	37.22	40.74	43.69	34.21	40.78

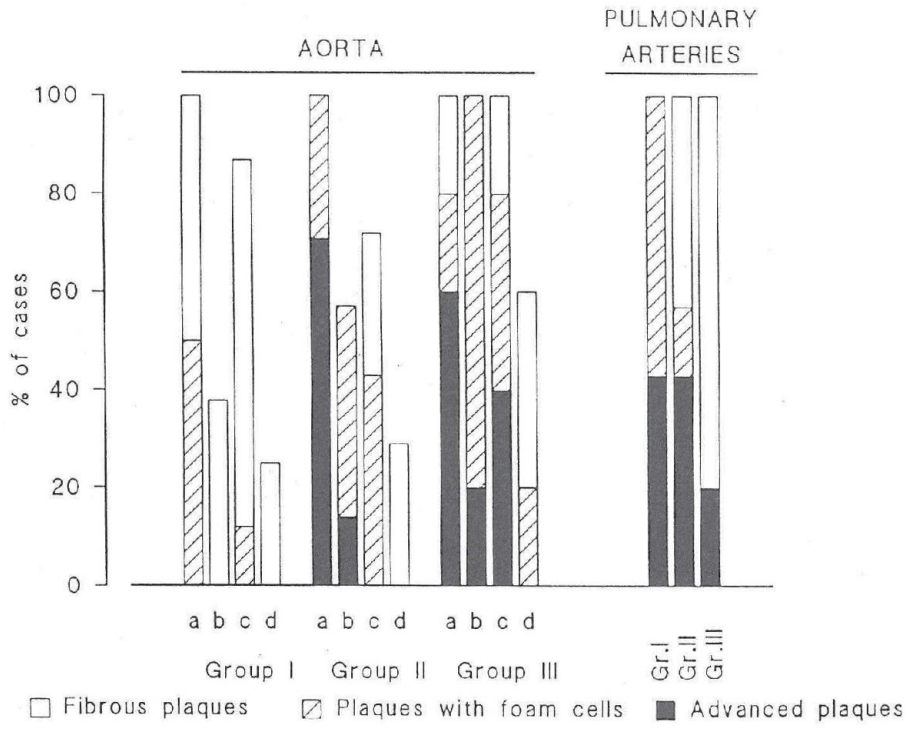

Fig. 3. Morphology and peripheral extension of aortic atherosclerosis estimated at four defined levels of the vessel: a - ascending aorta, b - thoracic aorta, c - diaphragmatic aorta, d - abdominal aorta, and morphology of atherosclerosis in pulmonary arteries.

4, 6). A semiquantitative estimation demonstrated that animals in group II had the most pronounced infiltrates.

Lungs. A remarkable deposition of foam cells was demonstrated in the interstitial lung tissue in group II (Fig. 5b) and III but never in group I (Fig. 4).

Kidneys. Infiltration with foam cells in renal interstitial stroma was seen in all three groups and to almost the same extent (Fig. 4). The slightest infiltrations were seen as stripes between the distal tubules and collecting tubules. The more pronounced infiltrates had an infarction-like configuration and clefts after cholesterol crystals were seen between foam cells.

Intestines. Foam cells were demonstrated in the intestinal mucosa in all three groups but especially in group III (Fig. 4). In this group the foam cells were seen throughout the lamina propria and in some cases also infiltrating the muscularis mucosae (Fig. 7).

Liver. (Fig. 4). A slight lipid infiltration in hepatocytes was seen in 4 of 8 animals in group I and 2 of 7 animals in group II. All animals in group III had very pronounced and diffuse lipid infiltration.

Other tissues. In all animals in all three groups a heavy infiltration with foam cells was seen in the spleen, in the mesenterial lymph nodes and in the ovaries. Foam cells were never demonstrated in the testes.

Discussion

Body weight and feed intake

The minor body weight loss in all three groups could be attributed to the decreased feed intake. The decreased feed intake in

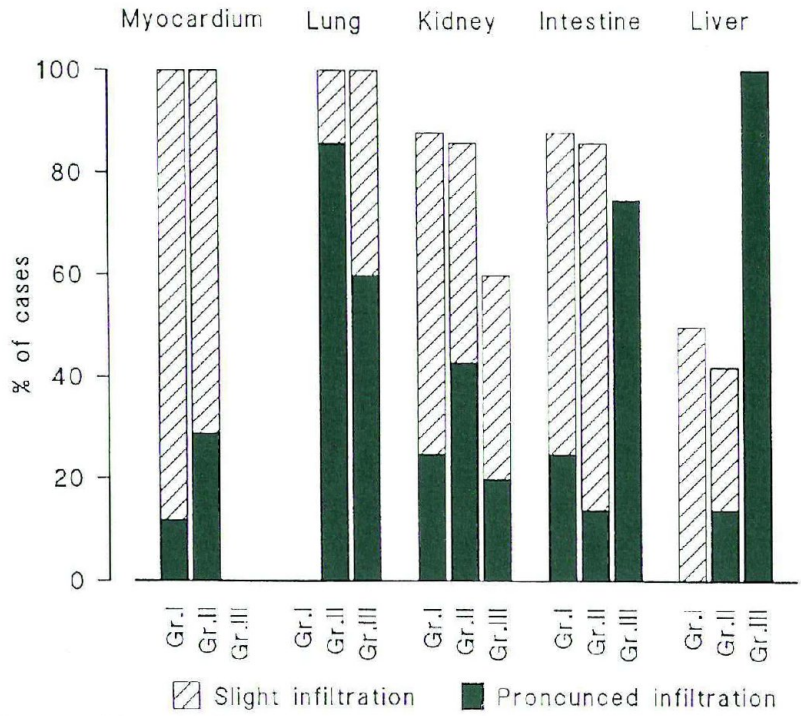

Fig. 4. Infiltration of foam cells in myocardium, lung, kidney, intestinal mucosa and liver – a semiquantitative assessment.

week 1 was interpreted as a consequence of palatability problems when changing from the standard to the test diets. The decreased feed intake in all groups but especially in the marine oil group in week 5 indicates the reduced palatability of the diet with high content of cholesterol (0.8–1 %) and fats (8–10 %). The reduced feed acceptance or rejection of the diet added fish oils, as well as decreased body weight or erratic fluctuations in body weight towards the end of the experimental period have previously been reported (*Verschuren et al.* 1990). Furthermore, the decreased feed intake in the present study could be related to the significant increase in plasma cholesterol. The short lasting feed rejection in cholesterol-fed rabbits with high plasma cholesterol has been observed previously in our laboratory (*Mortensen et al.* 1992).

Blood lipids

The recorded increase in plasma lipids due to cholesterol feeding (group I) is in accordance with that previously reported (*Atkinson et al.* 1989). The additional increase of blood lipids due to oil supplementation to the cholesterol diet corresponds with the general statement that a high fat diet has a hyperlipidemic effect when compared to a low fat diet but the magnitude of this increase has been surprising. Such a massive hypercholesterolemia is considered as a disadvantage in a model to study the effect of dietary factors on atherosclerosis as it may mask the effect of the test compounds on blood lipids. Indeed, it can not be excluded that this was the reason for no difference in concentration of plasma lipids in the two oil groups. The observation that the increase in plasma cholesterol in all groups was especi-

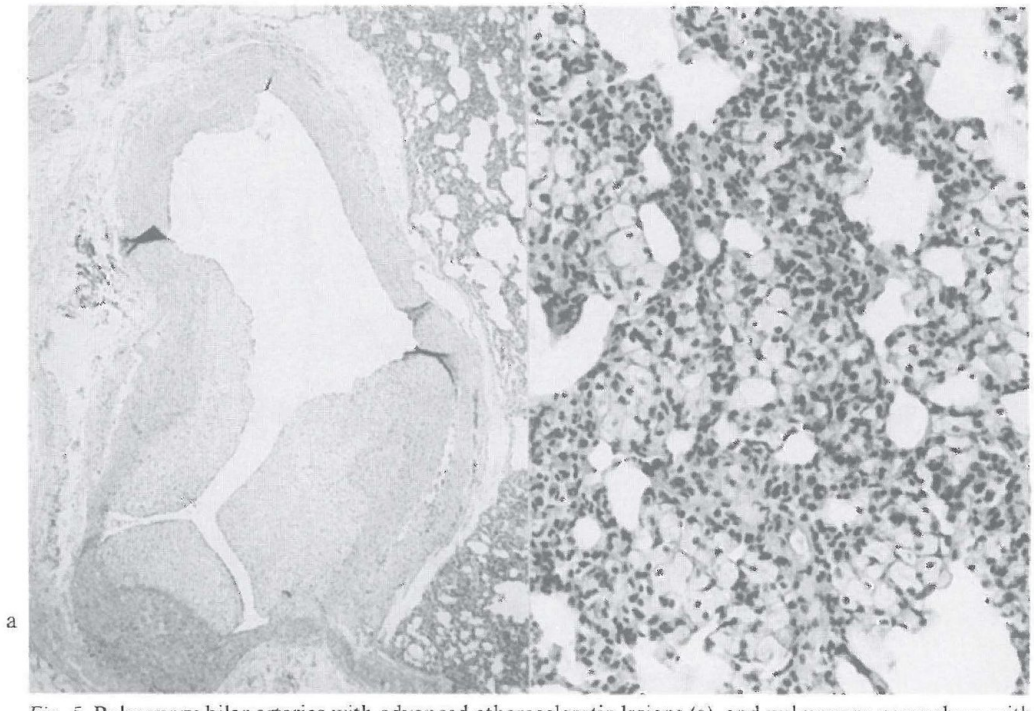

Fig. 5. Pulmonary hilar arteries with advanced atherosclerotic lesions (a), and pulmonary parenchym with pronounced infiltration of foam cells (b). Elastica van Gieson, × 25 (a) and × 100 (b).

ally reflected in the increase of VLDL is in accordance with previous reports on cholesterol-fed heterozygous WHHL rabbits (*Atkinson et al.* 1989) and other species (*Daugherty et al.* 1995, *Grundy et al.* 1982).

Incorporation of n-3 fatty acids into blood cells

The incorporation of n-3 fatty acids into blood cells of the rabbits on marine oil diet in this study is in accordance with the previous reports on the incorporation of n-3 fatty acids into platelets of homozygous WHHL rabbits on diet supplemented with Menhaden oil (*Clubb et al.* 1989) and on fatty acid patterns in plasma and platelets of homozygous WHHL rabbits feed diet with Maxepa oil (*Lichtenstein & Chobanian* 1990).

Experimental atherosclerosis in cholesterol-fed heterozygous WHHL rabbit

The morphology of atherosclerotic lesions in group I in the present study is comparable to the findings of *Atkinson et al.* (1989). However, the reported focal calcium deposition in atherosclerotic plaques was never seen in the present study. The morphology of the atherosclerotic lesions caused by 1 % cholesterol seemed to us similar to lesions reported in other studies with cholesterol-fed rabbits receiving lower doses of cholesterol (*Haarbo et al.* 1991, *Mortensen et al.* 1992) and to the changes seen in 6 months old homozygous WHHL rabbits (*Fischer Hansen et al.* 1994).

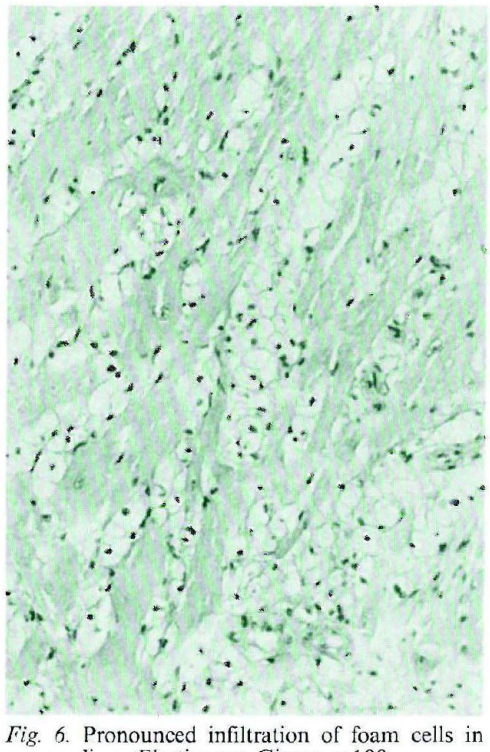

Fig. 6. Pronounced infiltration of foam cells in myocardium. Elastica van Gieson, × 100.

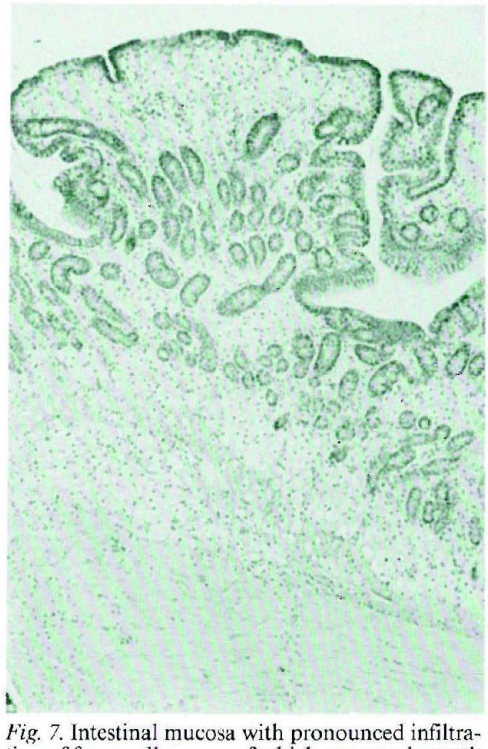

Fig. 7. Intestinal mucosa with pronounced infiltration of foam cells, some of which are seen beneath the muscularis (see arrow). Elastica van Gieson, × 25.

The effect of vegetable and marine oils on experimental atherosclerosis

The added oils aggravated the aortic and coronary atherosclerosis caused by the dietary cholesterol. This can be explained by the significant difference in blood lipids between the cholesterol group and the cholesterol and oils fed groups. The marine oil was more atherogenic considering the aortic and coronary atherosclerosis. No explanation can be given for less severe atherosclerotic changes in pulmonary arteries in the marine oil group compared to the two other groups.

The reports on the influence of fish oils on either experimental atherosclerosis in cholesterol-fed rabbits (*Campos et al.* 1989, *Thiery & Siedel* 1987, *Zhu et al.* 1990) or spontaneous atherosclerosis in homozygous WHHL rabbits (*Clubb et al.* 1989, *Lichtenstein & Chobanian* 1990, *Rich et al.* 1989) are contradictory. The findings on the aortic atherosclerosis in this study are in accordance with the findings of *Thiery & Siedel* 1987), who reported the enhancement of the aortic atherosclerosis by fish oil addition to the 1.5 % cholesterol diet in NZW rabits.

Extravascular foam cell infiltrations in non vascular tissues

Extravascular lipid deposits are the evidence of the cholesterol overload and indicate that lower doses of dietary cholesterol should be used to avoid the disturbed cholesterol metabolism which is a disadvantage in an animal model for atherosclerosis research. The differences in the occurrence and severity of the foam cell infiltration in the parenchymatous organs between the groups indicates

that the fatty acid composition of dietary fats affects the degree of fatty infiltration in various organs. The most notable finding was the lack of infiltration in the myocardium and simultaneous pronounced infiltration of the liver in the marine oil group. This has to the authors knowledge not previously been reported and the explanation of this remains obscure.

In conclusion, this study demonstrates that 1 % cholesterol-fed rabbit is not a suitable model for dietary studies in atherosclerosis research (1) due to a massive hypercholesterolemia which is severely aggravated by the test oils, and which may mask any effect of the test compounds on blood lipids, and (2) due to disturbed cholesterol metabolism manifested by extravascular lipid deposition in various organs. Both findings indicate that lower doses of cholesterol should be used when the cholesterol-fed heterozygous WHHL rabbit is chosen to study the effect of various fats on blood lipids and development of arterial atherosclerosis in the future. Concerning the effect of the vegetable and marine oils on experimental atherosclerosis in this study, the results demonstrate that (1) the marine oil seemed to protect against the development of atherosclerosis in pulmonary arteries, (2) it prevented lipid accumulation in the myocardium but was associated with the severe lipid infiltration in the liver, and (3) it was more atherogenic than the vegetable oil based on the morphological appearance of the aortic and coronary atherosclerosis.

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Summary

The aim of this experiment was to test the cholesterol-fed heterozugous WHHL rabbit as a model for investigation of atherogenicity of different fats. Twentytwo rabbits of both sexes, 8–9.5 months old were randomized in 3 groups, and fed 100 g diet daily: cholesterol enriched standard diet (group I, n=8), cholesterol enriched diet with added vegetable (group II, n=7), or marine (group III, n=7) oils during 14 weeks. The vegetable oil (n-6 = 33 %, n-3 less than 1 %) and a marine oil (n-6 = 18 %, n-3 = 17 %) were adjusted to contain equal amounts of saturated and monounsaturated fatty acids. One percent cholesterol in the diet caused a pronounced hypercholesterolemia which was significantly enhanced by addition of oils. The increase in total cholesterol was especially reflected in the increase in the VLDL concentration. The blood cholesterol and triglyceride levels were comparable between the two oil fed groups apart from a transitory lowering in the marine oil fed rabbits. The atherosclerotic lesions caused by 1 % cholesterol in the diet were fibrous plaques and plaques with foam cells. The added oils aggravated the atherosclerosis caused by cholesterol. Based on morphological appearance of the aortic and coronary atherosclerosis the marine oil was more atherogenic than the vegetable oil. In pulmonary arteries, however, the less severe atherosclerotic changes were found in the marine oil group. In this group no lipid infiltrations were seen in the myocardium but very severe infiltrations were seen in the liver. In the vegetable oil group these infiltrations were severe in the myocardium and less pronounced in the liver. The massive hypercholesterolemia and extravascular lipid deposition in different parenchymatous organs suggest that lower doses of dietary cholesterol should be used when the cholesterol-fed heterozygous WHHL rabbit is chosen to study the effect of various fats on blood lipids and development of atherosclerosis.

Sammendrag

Formålet med dette forsøg var at afprøve den kolesterol-fodrede heterozygote WHHL kanin som dyremodel ved undersøgelser af den atherogene effekt af forskellige fedtstoffer. Toogtyve kaniner af begge køn, 8–9,5 måneder gamle, blev tilfældigt fordelt i 3 hold og tildelt 100 g foder dagligt: Kolesterolberiget standard foder (hold I, n=8), kolesterolberiget foder tilsat vegetabilsk olie (hold II, n=7), eller marin olie (hold III, n=7). Dyrene blev fodret i 14 uger. Både den vegetabilske olie (n-6 = 33 %, n-3 mindre end 1 %) og den marine olie (n-6 = 18 %, n-3 = 17 %) blev justeret til at indeholde samme mængde af mættede og monoumættede fedtsyrer. Én procent kolesterol i foderet forårsagede stærk hyperkolesterolæmi som blev signifikant forstærket af tilsætningen af olierne i foderet. Stigningen i total kolesterol blev specielt afspejlet i stigningen i VLDL-koncentrationen. Niveauerne af blodkolesterol og triglycerider var sammenlignelige hos alle tre grupper med undtagelse af en kortvarig sænkning hos kaninerne som fik den marine olie. De atherosklerotiske

læsioner forårsaget af 1 % kolesterol i foderet var fibrøse plaques og plaques med skumceller. De tilsatte olier skærpede atherosklerosen forårsaget af kolesterol alene. Baseret på det morfologiske udseende af atherosklerosen i aorta og koronararterierne var den marine olie mere atherogen end den vegetabilske olie. På den anden side blev den svageste atherosklerose i lungearterierne set i holdet med den marine olie. I dette hold sås ingen lipidinfiltrationer i myocardiet, men der sås stærke infiltrationer i leveren. I holdet tildelt den vegetabilske olie var lipidinfiltrationer heftige i myocardiet og lavere i leveren. Den massive hyperkolesterolæmi og de extravasculære lipidaflejringer i diverse parenkymatøse organer tyder på at mindre doser af kolesterol i foderet skal anvendes når den kolesterolfodrede heterozygote WHHL kanin vælges til at undersøge effekten af diverse fedtstoffer på blodlipider og udviklingen af atherosklerose.

Uhteenveto / K. Pelkonen

Tutkimuksen tarkoituksena oli selvittää miten kolesterolia ruuassa saavat heterotsygoottiset WHHL-kanit soveltuvat malliksi ravinnossa olevien eri rasvojen atherogeenisyyden selvittämisessä. Kokeessa käytettiin 22 kania, kumpaakin sukupuolta, ikä 8–9.5 kk, satunnaistettuna kolmeen ryhmään. Kukin kani sai päivässä 100 g rehua: kolesterolirehua, kolesterolirehua johon oli lisätty kasvisöljyä, ja kolesterolirehua johon oli lisätty marine-öljyseosta. Rehut oli säädetty sisältämään sama määrä tyydyttyneitä ja monotyydyttämättömiä rasvahappoja. 1 % kolesterolia rehussa aiheutti huomattavan hyperkolesterolemian, jota öljyjen lisäys merkittävästi nosti. Kokonaiskolesterolin lisääntyminen näkyi erityisesti VLDL-nousuna. Öljyä saavillaryhmillä veren triglyseridien nousu ja kolesterolimäärät olivat samanlaisia, sillä erolla että marineöljyseosta saavissa kanaissa havaittiin ohimenevä lasku. 1 % kolesterolilisän aiheuttamat leesiot ilmenivät fibroottisina kolesteroliplakkeina ja vaahtosoluplakkeina. Öljyt pahensivat kolesterolin aiheuttamaa ateroskleroosia. Marine-öljyseos oli aterogeenisempää kuin kasviöljy, lukuunottamatta keuhkovaltimoita, joissa tilanne oli päinvastainen. Marine-öljyseosryhmässä ei sydänlihaksessa ollut rasvainfiltraatioita, mutta kylläkin maksassa. Kasviöljyhmässä nämä löydökset olivat toisin päin. Massiivinen hyperkolesterolemia ja ekstravaskulaarisen rasvan keräytyminen eri parenkyymielimiin antaa aiheen suositella alle 1 % kolesterolipitoisuutta ravinnossa, kun käytetään heterotsygoottista WHHL-kania mallina selvitettäessä eri rasvojen vaikutusta veren rasvamääriin ja ateroskleroosin kehittymiseen.

References

Atkinson JB, RL Hoover, KK Berry & LL Swift: Cholesterol-fed heterozygous Watanabe heritable hyperlipidemic rabbits: a new model for atherosclerosis. Atherosclerosis 1989, *78*, 123–136.

Bairati I, L Roy & F Meyer: Effects of a fish oil supplement on blood pressure and serum lipids in patients treated for coronary artery disease. Can. J. Cardiol. 1992, *8*, 41–46.

Buja LM, T Kita, JL Goldstein, Y Watanabe & MS Brown: Cellular pathology of progressive atherosclerosis in the WHHL rabbit. An animal model of familial hypercholesterolemia. Arteriosclerosis 1983, *3*, 84–101.

Campos CT, VN Michalek, JP Matts & H Buchwald: Dietary marine oil supplementation fails to affect cholesterol metabolism or inhibit atherosclerosis in rabbits with diet-induced hypercholesterolemia. Surgery 1989, *106*, 177–184.

Clubb FJ, Schmitz JM, Butler MM, Buja M, JT Willerson & WB Campbell: Effect of dietary omega-3 fatty acid on serum lipids, platelet function, and atherosclerosis in Watanabe heritable hyperlipidemic rabbits. Arteriosclerosis 1989, *9*, 529–537.

Daugherty A, LG Lange, BE Sobel & G Schonfeld: Aortic accumulation and plasma clearance of VLDL and HDL: effect of diet-induced hypercholesterolemia in rabbits. J. Lipid Res. 1985, *26*, 955–963.

Dyerberg J, HO Bang, E Stoffersen, S Moncada & JR Vane: Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 1978, *2*, 117–119.

Esper E, EK Chan & H Buchwald: Natural history of atherosclerosis and hyperlipidemia in heterozygous WHHL (WHHL-Hh) rabbits. I. The effect of aging and gender on plasma lipids and lipoproteins. J. Lab. Clin. Med. 1993, *121*, 97–102.

Fischer Hansen B, A Mortensen, J Fischer Hansen, P Ibsen, H Frandsen & BC Nordestgaard: Atheroschlerosis in Watanabe heritable hyperlipidemic rabbits. Evaluation by macroscopic, microscopic and biochemical methods and comparison of atherosclerosis variables. APMIS 1994, *102*, 177–190.

Folch J, M Lees & GH Sloane-Stanley: A simple method for the isolation and purification of take lipids from animal tissues. J. Biol. Chem. 1957, *226*, 497–509.

Friday KE, RA Failor, MT Childs & EL Bierman: Effects of n-3 and n-6 fatty acid-enriched diets on plasma lipoproteins and apolipoproteins in heterozygous familial hypercholesterolemia. Arteriosclerosis and Thrombosis 1991, *11*, 47–54.

Groot PHE, LM Scheek, ML Dubelaar, PD Verdouw, JM Hartog & JK Lamers: Effects of diets supplemented with lard fat or mackerel oil on plasma lipoprotein lipid concentrations and lipase activities in domestic swine. Atherosclerosis 1989, *77,* 1–6.

Grundy SM, D Bilheimer, H Blackburn, W Brown, PO Kwiterovich, F Mattson, G Schonfeld & WH Weidman: Rationale of the diet-heart statement of the American Heart Association. Report of Nutrition Committee. Circulation 1982, *65,* 839A–854A.

Haarbo J, B Fischer Hansen & C Christiansen: Hormone replacement therapy prevents coronary artery disease in ovariectomized cholesterol-fed rabbits. APMIS 1991, *99,* 721–727.

Haglund O, R Wallin, R Luostarinen & T Saldeen: Effects of a new fluid fish oil concentrate, Eskimo-3, on triglycerides, cholesterol, fibrinogen and blood pressure. J. Intern. Med. 1990, *227,* 347–353.

Harris WS: Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J. Lipid Res. 1989, *30,* 785–807.

Høy C-E & G Hølmer: Dietary linoleic acid and the fatty acid profiles in rat fed partially hydrogenated marine oils. Lipids 1988, *23,* 973–980.

Katan MB & RP Mensink: Dietary fat quality and serum lipoproteins – an update. Scand. J. Nutr. 1993, *37,* 52–54.

Lichtenstein AH & AV Chobanian: Effect of fish oil on atherogenesis in Watanabe heritable hyperlipidemic rabbit. Arteriosclerosis 1990, *10,* 597–606.

Mortensen A, PL Espersen, B Fischer Hansen & P Ibsen: The influence of dietary olive oil and margarine on aortic cholesterol accumulation in cholesterol-fed rabbits maintained at similar plasma cholesterol level. Atherosclerosis 1992, *96,* 159–190.

Nordøy A & SH Goodnight: Dietary lipids and thrombosis. Relationship to atherosclerosis. Arteriosclerosis 1990, *10,* 149–163.

Rich S, FJ Miller, S Charous, HR Davis, P Shanks, S Glagov & WEM Lands: Development of atherosclerosis in genetically hyperlipidemic rabbits during chronic fish-oil ingestion. Arteriosclerosis 1989, *9,* 189–194.

Rosenfeld ME, T Tsukada, AM Grown & R Ross: Fatty streak initiation in Watanabe heritable hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits. Arteriosclerosis 1987, *7,* 9–23.

Rosenfeld ME, T Tsukada, A Chait, E Bierman, AM Gown & R Ross: Fatty streak expansion and maturation in Watanabe heritable hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits. Arteriosclerosis 1987, *7,* 24–34.

Subbaiah PV, MH Davidson, MC Ritter, W Buchanan & JD Bagdade: Effects of dietary supplementation with marine lipid concentrate on the plasma lipoprotein composition of hypercholesterolemic patients. Atherosclerosis 1989, *79,* 157–166.

Terpstra AHM, CDH Woodward & FJ Sanchez-munic: Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. Anal. Bioch. 1981, *111,* 149–157.

Thiery J & D Seidel: Fish oil feeding results in enhancement of cholesterol-induced atherosclerosis in rabbits. Atherosclerosis 1987, *63,* 53–56.

Verschuren PM, UMT Houtmuller & JL Zevenbergen: Evaluation of vitamin E requirement and food palatability in rabbits fed a purified diet with a high fish oil content. Lab. Amin. 1990, *24,* 164–171.

Watanabe Y: Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit). Incidence and development of atherosclerosis and xanthoma. Atherosclerosis 1980, *36,* 261–268.

Weiner BH, IS Ockene, PH Levine, HF Cuénoud, M Ficher, BF Johnson, AS Daoud, J Jarmolych, D Hosmer, MH Johnson, A Natale, C Vaudreuil & JJ Hoogasian: Inhibition of atherosclerosis by cod-liver oil in a hyperlipidemic swine model. N. Engl. J. Med. 1986, *315,* 841–846.

Zhu, B-Q, RE Sievers, WM Isenberg, DL Smith & WW Parmley: Regression of atherosclerosis in cholesterol-fed rabbits: effects of fish oil and verapamil. J. Am. Coll. Cardiol. 1990, *15,* 231–237.