JACC Vol. 15, No. 1
January 1990:231–7

Regression of Atherosclerosis in Cholesterol-Fed Rabbits: Effects of Fish Oil and Verapamil

BO-QING ZHU, MD, RICHARD E. SIEVERS, BS, WILLIAM M. ISENBERG, PHD, DONALD L. SMITH, PHD,* WILLIAM W. PARMLEY, MD, FACC

San Francisco and Palo Alto, California

Previous studies have shown that either fish oil or verapamil can attenuate the development of atherosclerosis in the lipid-fed rabbit. The present study was designed to evaluate the individual and combined effects of these two interventions on regression.

Seventy New Zealand rabbits in seven groups (10 each) were fed a 0.3% cholesterol diet for 10 weeks. Control group C10 was then killed. Control group C20 was fed a 0.3% cholesterol diet and the other five groups were fed a normal diet for an additional 10 weeks. Group F in three treated groups received 2 ml/day of fish oil (Proto-Chol, eicosapentaenoic acid, 180 mg/ml and docosahexaenoic acid, 120 mg/ml) by gavage. Group V received verapamil, 2 g/1,000 ml drinking water, and group FV received both fish oil and verapamil for an additional 10 weeks. Group CF (control for fish oil) received 2 ml/day of water by gavage and group CV (control for verapamil) received water without gavage for an additional 10 weeks.

The percent of aortic and pulmonary atherosclerosis was measured by planimetry of sudanophilic lesions. The percent of aortic lesions in the four control groups (C20, C10, CF and CV) was 57 ± 22, 40 ± 15, 40 ± 14 and 33 ± 25%, respectively. The fish oil or verapamil groups (F, V, FV) showed a significant reduction in aortic lesions: 15 ± 17%, $p < 0.05$; 16 ± 12%, $p < 0.05$; and 26 ± 24%, p = NS, respectively. The area of pulmonary artery lesions was significantly higher in the control group (CF, 24 ± 9%) than in group F (11 ± 9%, $p < 0.05$), group V (12 ± 9%, $p < 0.05$) and group FV (17 ± 14%, p = NS).

These data demonstrate that either fish oil or verapamil can decrease atherosclerosis in cholesterol-fed rabbits placed on a normal diet. However, there was no additive effect of fish oil and verapamil. Although not statistically significant, there was a suggestive antagonistic effect between fish oil and verapamil.

(J Am Coll Cardiol 1990;15:231–7)

We have previously shown (1–3) that either fish oil or verapamil could attenuate the development of aortic and pulmonary atherosclerosis in cholesterol-fed rabbits. The mechanisms of suppression of atherosclerosis are probably different for these two interventions.

Dietary fish oil contains large quantities of polyunsaturated omega-3 fatty acids (EPA and DHA). These may attenuate the development of atherosclerosis by hypotriglyceridemic or hypocholesterolemic effects (4,5), by reduction of platelet aggregation leading to a prolonged bleeding time (1,5,6), by diminishing cytotoxic, phagocytic and chemotactic properties of monocytic cells and neutrophils and by inhibiting the utilization of arachidonic acid (6).

Verapamil presumably suppresses atherosclerosis in cholesterol-fed animals by reducing calcium influx into cells. This is inferred from the fact that all calcium entry blocking agents appear to have this same beneficial effect (2,7). These drugs exert their effects without altering cholesterol levels (8) and independent of any blood pressure-lowering effect (9).

There are few data concerning regression of atherosclerosis by use of dietary fish oil or verapamil. The present study was designed to evaluate the individual and combined effects of fish oil and verapamil on regression of atherosclerosis in the cholesterol-fed rabbit.

Methods

Protocol. Seventy New Zealand white male rabbits (2.8 to 3.4 kg) were randomly separated into seven groups (10 each) and fed a high cholesterol diet for 10 weeks. The

From the Cardiovascular Division, Department of Medicine and the Cardiovascular Research Institute, University of California, San Francisco and *Syntex, Palo Alto, California. This work was supported in part by the George D. Smith Fund, San Francisco and presented in part at the 38th Annual Scientific Session of the American College of Cardiology, Anaheim, California, March 1989.

Manuscript received April 10, 1989; revised manuscript received June 16, 1989, accepted July 7, 1989.

Address for reprints: William W. Parmley, MD, 1186-Moffitt Hospital, University of California, San Francisco, San Francisco, California 94143.

0735-1097/90/$3.50

Table 1. Serum Lipid Levels (mg/dl) in the Different Rabbit Groups (n = 70)

Group	Cholesterol			Triglycerides			HDL Cholesterol		
	Before Diet	10 Weeks	20 Weeks	Before Diet	10 Weeks	20 Weeks	Before Diet	10 Weeks	20 Weeks
CF	38 ± 6	824 ± 641	53 ± 95	48 ± 9	56 ± 58	24 ± 19	20 ± 4	32 ± 17	14 ± 8
F	164 ± 126	983 ± 348	38 ± 18	129 ± 38	115 ± 100	117 ± 72	45 ± 13	33 ± 11	16 ± 7
CV	43 ± 14	921 ± 585	47 ± 33	84 ± 42	51 ± 42	34 ± 21	21 ± 8	28 ± 24	16 ± 10
V	74 ± 20	1,049 ± 333	233 ± 127	161 ± 55	130 ± 91	111 ± 68	46 ± 10	48 ± 24	14 ± 1
C10	59 ± 14	1,010 ± 486		70 ± 31	128 ± 243		28 ± 7	33 ± 19	
FV	120 ± 97	1,057 ± 351	138 ± 70	124 ± 35	50 ± 36	66 ± 24	34 ± 9	36 ± 16	12 ± 6
C20	128 ± 137	1,388 ± 649	1,748 ± 986	231 ± 186	139 ± 108	341 ± 271	24 ± 8	33 ± 14	13 ± 8

C10 and C20 = control groups fed high cholesterol diet for 10 and 20 weeks, respectively; CF and CV = control groups for fish oil and verapamil, respectively; F = fish oil group; FV = combined fish oil and verapamil group; V = verapamil group.

cholesterol diet (Ziegler Bros., Inc.) contained 3% soybean oil and 0.3% cholesterol by weight. After 10 weeks on the diet, one control group (C10) was then killed. Control group C20 was fed a 0.3% cholesterol diet for another 10 weeks. The other five groups were switched to a normal diet for the last 10 weeks. In addition to the normal diet, group F received 2 ml/day of fish oil (Proto-Chol mix, Banner Gelatin Products Corp, E. R. Squibb and Sons) for an additional 10 weeks. One milliliter of fish oil contained 1,000 mg of marine lipid concentrate (180 mg eicosapentaenoic acid [EPA] and 120 mg docosahexaenoic acid [DHA]; 35% of the fatty acid was omega-3); vitamin E, 1 IU; cholesterol, 5.3 mg and no vitamin A or D. The fish oil was administered daily by intrabuccal gavage. Group V received verapamil in the drinking water (2 g/1,000 ml) with honey added to taste, and group FV received both fish oil and verapamil for an additional 10 weeks. The verapamil drinking water was changed three times a week to assure potency. Group CF (control for the fish oil group) received 2 ml/day of drinking water by gavage and group CV (control for the verapamil group) received the drinking water without gavage for an additional 10 weeks. All animal procedures were carried out according to the National Institutes of Health guidelines.

Laboratory methods. Weight and food consumption of the rabbits were measured at the beginning of the experiment and at 5 week intervals thereafter. All rabbits ate an average of 147 g of feed each day. After fasting 12 h, blood samples were obtained at the beginning of the experiment and at 5 week intervals for calcium and lipid determinations. Total serum cholesterol and triglyceride levels were determined by automated enzymatic methods (Enzymatic Cholesterol Reagent, Trig-Gpo Reagent, Behring Diagnosis; Dacos Coulter Electronics, Inc.). High density lipoprotein (HDL) cholesterol concentrations were measured after precipitation of other lipoprotein classes with dextran and magnesium ions (Fluorescence Light Scattering Multistat III Plus, instrumentation laboratory microcentrifugal analyzer).

11-Dehydro-thromboxane B_2 (thromboxane A_2 metabolite) in groups C20, F, V and FV was measured on plasma samples with the use of radioimmunoassay kits (Amersham). 6-Keto-prostaglandin F-1 alpha (prostacyclin metabolite) was measured in plasma samples with the use of direct radioimmunoassay kits (Biotecx Laboratories). Serum levels of verapamil and norverapamil in groups F and FV were measured by high pressure liquid chromatography (Bioscience Laboratories) at death.

Determination of bleeding times was performed by warming the rabbit ear for 5 min in a normal saline bath maintained at a temperature of 37°C. The ear was then removed from the bath and a standard prick was made into the ear avoiding any macroscopically obvious vessels. The incised ear was then immediately replaced into the saline bath, which was gently agitated with a magnetic stirrer. The bleeding time was taken as the time required for blood flow in the incision to cease. Each result represents the mean of two determinations.

Morphologic studies. Rabbits were killed by an intravenous injection of sodium pentobarbital, 30 mg/kg body weight. The chest was opened and the aorta was dissected from its origin at the aortic valve to 2 cm beyond the bifurcation of the internal iliac arteries. The pulmonary artery was dissected from its origin at the pulmonary valve to the hila beyond the bifurcation. The vasculature was opened longitudinally, pinned endothelial side up to a cork board and placed in a normal saline bath. The aorta and pulmonary artery were fixed in 10% formalin, stained with Sudan IV and photographed. The stained lesions of the aorta and pulmonary artery surface were estimated quantitatively by planimetry of the sudanophilia in the photographs. The planimetric determinations were performed without knowledge of other data and average values were calculated from two determinations.

Statistical analysis. Data were subjected to an analysis of variance and, when appropriate, pairwise comparisons were made with use of the Dunnett or Student-Newman-Keul test. Student's paired *t* test was used to compare the bleeding time before and after dietary fish oil or verapamil supplementation. A log transformation was used for cholesterol, triglyceride, HDL cholesterol, 11-dehydro-thromboxane B_2, 6-keto-prostaglandin F-1 alpha and the ratio of 6-keto-prostaglandin F-1 alpha to 11-dehydro-thromboxane B_2 val-

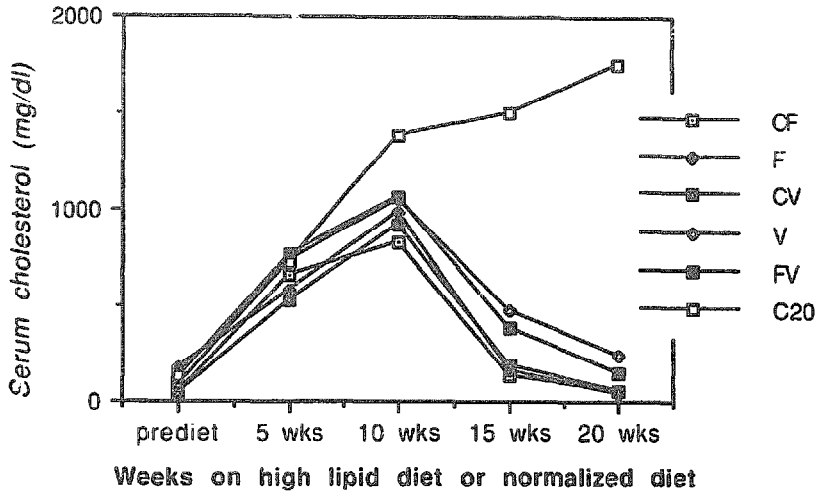

Figure 1. Average serum cholesterol levels in each rabbit group during the control period and at 5 week intervals. C10 = control group fed a high cholesterol diet for 10 weeks; C20 = control group fed a high cholesterol diet for 20 weeks; CF = control group for fish oil; CV = control group for verapamil; F = fish oil group; FV = combined fish oil and verapamil group; V = verapamil group.

ues. The text and tables list data as the mean value ± 1 SD. Statistical significance was assumed at a p value <0.05.

Multivariate analysis (multiple linear regression) was used to make aortic lesions (%) the dependent variable and place pulmonary lesions (%), cholesterol levels, percent of cholesterol changes (20 weeks versus 10 weeks) and fish oil or verapamil treatment in the regression equation. Data were processed by a Microvax computer.

Results

Response to Diet and Drugs

Serum lipids (Table 1). There was s similar body weight gain in all seven groups of rabbits throughout the 20 week period. The average body weight before diet and at 10 and 20 weeks was 3.1 ± 0.5, 4.4 ± 0.5 and 4.9 ± 0.6 kg, respectively. Figure 1 shows the similar time course of the increase and decrease in serum cholesterol in all groups. The average serum cholesterol at the end of 10 weeks in all seven groups was 1,033 mg/dl. After the rabbits resumed a normal diet, the serum cholesterol level in group F was lower than the levels in group V and FV (38 ± 18 versus 233 ± 127, 138 ± 70 mg/dl; $p < 0.05$); but there was no significant difference in comparison with levels in control groups CF and CV (53 ± 95, 47 ± 33 mg/dl, respectively; p = NS). The percent decrease in cholesterol (20 weeks versus 10 weeks) in group V was smaller than that in groups F, FV, CF and CV (75% versus 96%, 86%, 93% and 95%; $p < 0.05$, respectively). Although serum triglyceride levels at the end of 20 weeks in the three treated groups (F, V, FV) were higher than those in the two control groups (CF, CV) (117 ± 72, 111 ± 68, 66 ± 24 versus 24 ± 19, 34 ± 21, mg/dl, respectively; $p < 0.05$), there were no significant differences in the percent triglyceride changes (20 weeks versus 10 weeks) in these five groups. There were no significant differences in the final serum HDL cholesterol levels and the percent change in HDL cholesterol.

Changes in bleeding time (Table 2). There was a significant decrease in the four control groups (CF, CV, C10, C20) after feeding a high cholesterol diet (47 ± 8 versus 56 ± 9 s, $p < 0.05$; 44 ± 10 versus 71 ± 16 s, $p < 0.01$; 51 ± 11 versus 69 ± 21 s, $p < 0.05$; and 52 ± 19 versus 75 ± 13 s, $p < 0.01$, respectively). There was a significant increase in groups F and FV (86 ± 21 versus 54 ± 22 s, $p < 0.01$; 102 ± 49 versus 62 ± 19 s, $p < 0.05$, respectively). However, there was a significant decrease in group V (46 ± 18 versus 76 ± 18 s, $p < 0.01$). After the rabbits resumed a normal diet, only group V showed a significant decrease in percent bleeding time (23%, 20 weeks versus 10 weeks).

Plasma and serum biochemical and drug levels (Tables 3 to 6). The levels of plasma 11-dehydro-thromboxane B_2 increased in groups F, V, FV and C20 at 10 weeks and decreased slightly at 20 weeks (Table 3). However, there were no significant differences at 10 weeks or 20 weeks in these four groups. The levels of plasma 6-keto-prostaglandin F-1 alpha (pg/ml) were increased in all seven groups at 10 weeks and decreased at 20 weeks (Table 4). The levels in the three treated groups (F, V, FV) were higher than those in the three control groups (CF, CV, C10) at 10 weeks ($p < 0.05$). The level in group V was lower than that in the other four groups (F, FV, CF, CV) at 20 weeks ($p < 0.05$). The

Table 2. Average Bleeding Time (s) in the Seven Rabbit Groups

Group	Control	10 Weeks	20 Weeks
CF	56 ± 9	47 ± 8*	68 ± 14
F	54 ± 22	52 ± 14	86 ± 21†
CV	71 ± 16	44 ± 10†	66 ± 9
V	76 ± 18	64 ± 21	46 ± 18†
C10	69 ± 21	51 ± 11*	
FV	62 ± 19	58 ± 15	102 ± 49*
C20	75 ± 13	52 ± 19†	54 ± 16†

*$p < 0.05$ or †$p < 0.01$ compared with control values. Abbreviations as in Table 1.

Table 3. 11-Dehydro-Thromboxane B_2 (ng/ml) Levels in the Four Rabbit Groups

Group	Before Diet	5 Weeks	10 Weeks	15 Weeks	20 Weeks
C20	135 ± 77	200 ± 88	163 ± 159	159 ± 104	150 ± 131
F	43 ± 24	215 ± 163	204 ± 110	203 ± 92	179 ± 37
V		102 ± 49	139 ± 76	111 ± 53	126 ± 74
FV	84 ± 113	117 ± 66	210 ± 125	196 ± 114	199 ± 100

No statistical differences when compared with the control group at 10 or 20 weeks. Abbreviations as in Table 1.

prostaglandin F/thromboxane B_2 ratio (Table 5) in the three treated groups was lower than that in control group C20. The ratio in group V was lower than that in group F and FV at 20 weeks. Serum calcium levels for all seven groups did not change throughout the 20 weeks. The average serum calcium level before diet and at 10 weeks and 20 weeks was 13.4 ± 0.7, 13.7 ± 0.7 and 13.2 ± 0.5 mg/dl, respectively. The levels of serum verapamil and norverapamil in groups V and FV were in the therapeutic range (Table 6).

Morphologic studies. Figure 2 shows the percent of total aortic and pulmonary artery surface area exhibiting sudanophilia for each of the seven groups. The percent of aorta stained in group F and group V was less than that in control groups CF, C10 and C20 (15 ± 17 and 16 ± 12% versus 40 ± 14, 40 ± 15 and 57 ± 22%; $p < 0.05$, $p < 0.01$, respectively). The percent of aorta stained in group FV was 26 ± 24%; however, there was no significant difference between this value and that of the control groups except group C20. In control group CF (with gavage), the percent of aorta stained was a little higher than that in group CV without gavage (40 ± 14 versus 33 ± 25%, p = NS). The percent of pulmonary artery surface area stained in group F and V was less than that in the control groups CF and C20 (11 ± 9 and 12 ± 9 versus 24 ± 9 and 37 ± 11%; $p < 0.05$, $p < 0.01$, respectively). There were no significant differences among groups FV, C10 and CV (17 ± 14, 20 ± 7, 19 ± 14%, respectively).

The average serum cholesterol levels at 20 weeks for each group are plotted in Figure 3 versus the percent of aorta covered by lesions. The higher mean cholesterol level in group V than that of other groups (F, CF and CV; $p < 0.05$) may have countered to some degree the beneficial effects of fish oil so that the extent of lesions in group FV was greater than that in group F or V. Multiple linear regression analysis showed that there were no significant independent predictors of protection except fish oil or verapamil treatment. There was a positive correlation between the percent of lesions in the aorta and in the pulmonary artery for all 70 rabbits ($y = 6.72 + 0.41x$, $R_2 = 0.52$, $r = 0.72$, $p < 0.0001$) (Fig. 4). There was no correlation between the percent of lesions in the aorta and changes in cholesterol in all seven groups.

Discussion

Progression and regression of atherosclerosis. These have been demonstrated in several animal species including rabbits, chickens, rats, dogs, pigeons, nonhuman primates and human beings after withdrawal of cholesterol from the diet, as well as after the administration of certain substances (10,11). Regression has been characterized by shrinkage of plaques, arrest of cell proliferation, decreased numbers of cells and necrotic foci, lessened concentrations of cholesterol esters, free cholesterol and elastin, decreased activity of certain acyl esterases and altered composition of glycosaminoglycans (12). When the plasma cholesterol is lowered below about 150 mg/dl, lipid is mobilized from lesions and regression gradually occurs. After a prolonged period of low plasma cholesterol, cholesterol esters and foam cells disappear and crystalline cholesterol gradually dissolves, leading to true regression (13).

The present study attempted to determine whether lipid lesions induced by a high lipid diet regressed after rabbits were placed on 1) a normal diet (C); 2) a normal diet with fish oil (F); 3) a normal diet with verapamil (V); and 4) a normal diet with fish oil and verapamil (FV). After 10 weeks on a high cholesterol diet, conversion to a normal diet for 10 more weeks (groups CF and CV) did not cause regression of lipid

Table 4. 6-Keto-Prostaglandin F-1 Alpha Levels (pg/ml) in the Seven Rabbit Groups

Group	Before Diet	5 Weeks	10 Weeks	15 Weeks	20 Weeks
CF	582 ± 115	760 ± 330	889 ± 581	572 ± 130	615 ± 323
F	3,805 ± 7,914	15,443 ± 16,355	26,971 ± 13,676	1,226 ± 2,661	11,387 ± 15,592
CV	975 ± 305	949 ± 505	1,140 ± 807	630 ± 129	458 ± 69
V		19,510 ± 21,405	27,080 ± 14,501	13,658 ± 17,147	241 ± 258
C10	989 ± 556	918 ± 333	1,871 ± 1,815		
FV	3,567 ± 9,145	11,884 ± 13,755	20,997 ± 16,933	852 ± 625	10,127 ± 16,060
C20	8,127 ± 12,254	8,307 ± 10,992	21,128 ± 13,877	27,721 ± 10,496	27,597 ± 15,956

Abbreviations as in Table 1.

Table 5. Prostaglandin F-1/Thromboxane B_2 Ratio/11 in the Four Rabbit Groups

Group	Before Diet	10 Weeks	20 Weeks
F	0.06 ± 0.11	0.17 ± 0.11	0.08 ± 0.13*
V		0.38 ± 0.53	0.003 ± 0.004*
FV	0.05 ± 0.09	0.14 ± 0.12	0.08 ± 0.11*
C20	0.08 ± 0.13	0.23 ± 0.23	0.55 ± 0.60

*$p < 0.05$ compared with value in the control group. Abbreviations as in Table 1.

lesions. The groups that received fish oil or verapamil from weeks 11 to 20 along with a normal diet (groups F and V) showed significantly reduced lesions in the aorta and pulmonary artery. These results demonstrate that either fish oil or verapamil together with a normal diet can decrease atherosclerosis induced by a high cholesterol diet in rabbits.

Mechanism of antiatherogenic effect by verapamil. The precise mechanisms for these findings remain unclear. Although verapamil, nifedipine, nicardipine, isradipine and diltiazem are distinctly different calcium channel blocking agents, their similar effects on experimental atherosclerosis support the idea that the mechanism is related to their ability to reduce intracellular levels of calcium (14,15). Nicardipine, like nifedipine, did not significantly alter low density lipoprotein (LDL) cholesterol or HDL cholesterol (14). Verapamil can inhibit collagen-induced platelet aggregation (15). Our previous study (3) demonstrated the ability of verapamil and a normal diet to halt the progression of aortic plaque in experimental atherosclerosis in rabbits. In the present study, the levels of serum verapamil in groups V and FV were higher than levels in our previous study (137 ± 39 and 82 ± 34 versus 41 ± 35 ng/ml; $p < 0.01$ respectively). In addition, the time course of developing atherosclerosis was only 10 weeks in the present study compared with 12 weeks in the previous study (3). The longer a cholesterol diet is fed to rabbits (2 to 6 months), the greater the possibility that regression may no longer occur (12). Thus, the ability to decrease experimental atherosclerosis may be a time-dependent phenomenon.

Beneficial and adverse effects of fish oil. Both experimental (16–18) and clinical studies (19–21) demonstrate that dietary fish oil can inhibit atherosclerosis. Fish oil has also been shown to decrease the restenosis rate after coronary angioplasty (19,20) and also to be beneficial in various

Table 6. Serum Verapamil Levels (μg/liter) in the Two Rabbit Groups

Group	Verapamil	Norverapamil
V	137 ± 39	122 ± 45
FV	82 ± 34	62 ± 53
Therapeutic range	100 to 600	

Abbreviations as in Table 1.

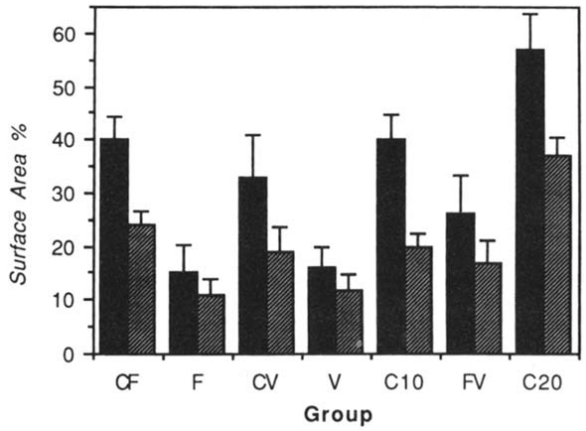

Figure 2. Percent of aortic (left columns) and pulmonary artery (right columns) surface areas covered by atherosclerotic lesions for each group. Comparison of means in aorta: group F or V versus CF and C10, $p < 0.05$; group F or V versus C20, $p < 0.01$; group CF versus C10, p = NS. Comparison of means in pulmonary artery: group F or V versus CF and C20, $p < 0.05$, 0.01, respectively; group FV versus C10, p = NS. Abbreviations as in Figure 1.

thrombotic disorders (22). Our previous study (1) suggested that the beneficial effect of fish oil was unrelated to changes in plasma lipids, including serum cholesterol levels. Some studies (17,18) also showed that fish oil had an antiatherogenic effect that was not mediated through changes in total cholesterol plasma concentration. The present data on regression are consistent with this conclusion when group F (on fish oil) is compared with its control group (Fig. 3). On the other hand, some studies (23–25) have shown that fish oil supplements can increase LDL cholesterol and may have a potentially adverse effect in hyperlipoproteinemic patients. These data emphasize the need for careful monitoring of plasma lipoproteins during fish oil supplementation in relation to their long-term benefits (26). Some other side effects should also be considered apart from adverse lipid changes. Other effects that have been reported include bleeding, vitamin E deficiency and, with some preparations, vitamin A and D toxicity (27).

The appropriate dose of fish oil to maximize benefit is unclear. We used 2 ml/day fish oil, which showed the greatest inhibition of atherosclerosis in our previous study (1), 2 ml/day of fish oil contains 360 mg EPA and 240 mg DHA. Some data suggest that a relatively small amount of fish oil can have beneficial effects (26). For example, subjects with traditionally low fish consumption receive only 0.25 to 0.90 g EPA/day (28). These low doses of EPA can prolong bleeding time and inhibit platelet aggregation (28,29). Therefore, low doses of a fish lipid concentrate can favorably affect the risk profile for coronary heart disease in healthy volunteers (29) and avoid some adverse effects caused by large doses of fish oil.

Antithrombotic effect of fish oil. The present data confirmed that fish oil can prolong bleeding time significantly,

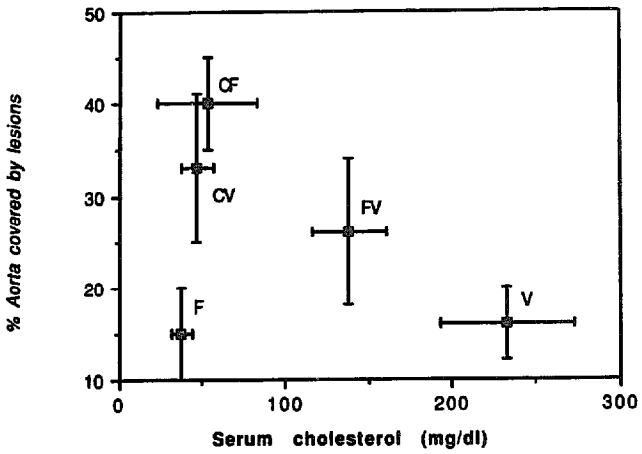

Figure 3. Average serum cholesterol at 20 weeks versus the percent of aortic surface covered by lesions for five rabbit groups. Abbreviations as in Figure 1.

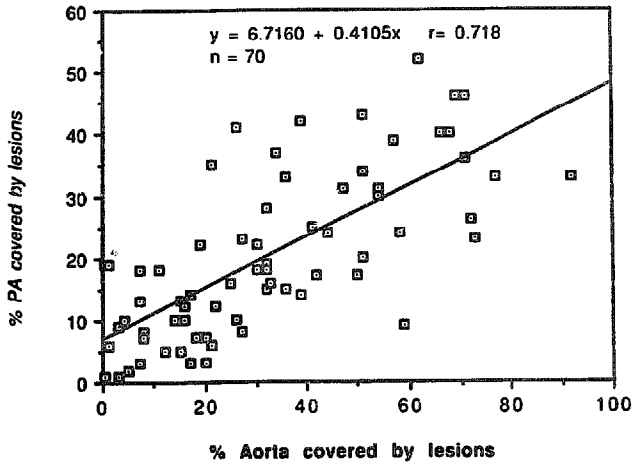

Figure 4. Relation between percent of aorta and pulmonary artery (PA) covered by lesions in all 70 rabbits ($r = 0.72$, $p < 0.0001$; $y = 6.72 + 0.41x$).

presumably because of its antiplatelet effects. However, there were no significant differences in 11-dehydrothromboxane B_2 at 10 or 20 weeks when compared with that of the control group. Some investigators (30–32) proposed that the antithrombotic action of EPA (10, −30, −50 mg/kg per day) may be partially related to its effects on platelet aggregability and prostacyclin generation by the vessel wall; but the major mechanism remains unclear. We measured 6-keto-prostaglandin F-1 alpha in plasma as a potential marker of endothelial prostacyclin production. Results showed that the prostaglandin F/thromboxane B_2 ratio was lower in the treated groups as compared with the control group (C20); the values in the verapamil group were the lowest. One study (33) reported that rats fed sheep fat displayed the highest values for prostaglandin 12/thromboxane B_2 ratio, whereas rats fed tuna fish oil showed the lowest, which may be because of the synthesis of thromboxane A_3. Because thromboxane A_3 lacks biologic activity compared with thromboxane A_2, the 'biologically relevant' prostacyclin/thromboxane B_2 ratio in the fish oil group may have been higher than the measured value indicates.

Antiatherogenic effect of fish oil. Recent data (34,35) showed that n-3 fatty acids may interfere in several ways with the atherosclerotic process. Fish oil may have an antiatheromatous effect by 1) increasing the formation of prostacyclin, thus making the endothelial surface less thrombogenic; 2) blocking the production of thromboxane, thus allowing fewer or smaller platelet thrombi to form in the vessel wall; 3) reducing the amount of platelet-derived growth factor released, thus diminishing intimal hyperplasia and the accumulation of foam cells; and 4) reducing the level of leukotriene B_4, thus decreasing the inflammatory response at the site of injury to the vessel wall. Reduced vasoconstriction and enhanced relaxation may be mechanisms for improved postischemic reperfusion in rats fed fish oil (36). Dietary supplementation with cod-liver oil facilitates endothelium-dependent relaxation and inhibits endothelium-dependent contraction in porcine coronary arteries (37).

No additive effect of fish oil and verapamil. Our present study showed that there was no additive effect of fish oil and verapamil. There was a suggestive antagonistic effect between the two even though both had an antiatherogenic effect; however, the mechanisms are unclear at this time. It is of note that verapamil shortened bleeding time, whereas fish oil prolonged it. The combined fish oil-verapamil (FV) group had a bleeding time similar to that of the fish oil group (F). The values of prostaglandin F and the ratio of prostaglandin F/thromboxane B_2 at 20 weeks in verapamil groups were lower than those of the fish oil group. Additional studies may shed light on this suggestive antagonistic effect.

We thank Robert Appleyard, MD for expert help in the statistical analysis; Knoll Pharmaceutical Co. for providing the verapamil; and Banner Gelatin Products Corp., E. R. Squibb and Sons for providing the fish oil (Proto-Chol) for the study.

References

1. Zhu, B-Q, Smith DL, Sievers RE, Isenberg WM, Parmley WW. Inhibition of atherosclerosis by fish oil in cholesterol-fed rabbits. J Am Coll Cardiol 1988;12:1073–8.
2. Rouleau J-L, Parmley WW, Stevens J, et al. Verapamil suppresses atherosclerosis in cholesterol-fed rabbits. J Am Coll Cardiol 1983;1:1453–60.
3. Sievers RE, Rashid T, Garrett J, Blumlein SL, Parmley WW. Verapamil and diet halt progression of atherosclerosis in cholesterol fed rabbits. Cardiovasc Drugs Ther 1987;1:65–9.
4. Davis HR, Bridenstine RT, Vesselinovitch D, Wissler RW. Fish oil inhibits development of atherosclerosis in rhesus monkeys. Arteriosclerosis 1987;7:441–9.

5. Chamberlain JG, Belton C. Effects of long term consumption of fish oil (Maxepa) on serum lipids and arterial ultrastructure in Japanese quail. Atherosclerosis 1987;68:95–103.

6. Goerig M, Habenicht A, Schettler G. Atherosclerosis and fish oil. Cardiovasc Drugs Ther 1988;2:281–2.

7. Parmley WW, Blumlein SL, Sievers RE. Modification of experimental atherosclerosis by calcium-channel blockers. Am J Cardiol 1985;55:165B–71B.

8. Henry PD. Atherosclerosis, calcium, and calcium antagonists. Circulation 1985;72:456–9.

9. Blumlein SL, Sievers RE, Kidd P, Parmley WW. Mechanism of protection from atherosclerosis by verapamil in the cholesterol-fed rabbit. Am J Cardiol 1984;54:884–9.

10. Malinow MR. Experimental models of atherosclerosis regression. Atherosclerosis 1983;48:105–18.

11. Kramer JR, Kitazume H, Proudfit WL, Matsuda Y, Williams GW, Sones FM Jr. Progression and regression of coronary atherosclerosis: relation to risk factors. Am Heart J 1983;105:134–44.

12. Malinow MR. Atherosclerosis: progression, regression, and resolution. Am Heart J 1984;108:1523–37.

13. Small DM. Progression and regression of atherosclerosis lesions: insight from lipid physical biochemistry. Arteriosclerosis 1988;8:103–29.

14. Willis AL, Nagel B, Churchill V, et al. Antiatherosclerotic effects of nicardipine and nifedipine in cholesterol-fed rabbits. Arteriosclerosis 1985;5:250–5.

15. Nomoto A, Hirosumi J, Sekiguchi C, Mutoh S, Yamaguchi I, Aoki H. Antiatherogenic activity of FR 34235 (nilvadipine), a new potent calcium antagonist: effect of cuff-induced intimal thickening of rabbit carotid artery. Atherosclerosis 1987;64:255–61.

16. Lichtenstein AH, Chobanian AV. Fish oil feeding inhibits aortic atherosclerosis in the Watanabe hereditable hyperlipidemic rabbit (abstr). Circulation 1988;78(suppl II):II-573.

17. Leth-Espensen P, Stender S, Foged E, Kjeldsen K. Antiatherogenic effect of cod liver oil compared with lard in equi-cholesterolemic rabbits (abstr). Circulation 1988;78(suppl II):II-573.

18. Sarris GE, Fann JI, Handen CE, et al. Mechanism of fish oil inhibition of canine vein graft arteriosclerosis (abstr). Circulation 1988;78(suppl II):II-234.

19. Milner MR, Gallino RA, Leffingwell A, Pichard AD, Rosenberg J, Lindsay J. High dose omega-3 fatty acid supplementation reduces clinical restenosis after coronary angioplasty (abstr). Circulation 1988:78(suppl II):II-634.

20. Dehmer GJ, Popma JJ, van den Berg EK, et al. Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids. N Engl J Med 1988;319:733–40.

21. von Schacky C. Prophylaxis of atherosclerosis with marine omega-3 fatty acids: a comprehensive strategy. Ann Intern Med 1987;107:890–9.

22. Tamura Y, Hirai A, Terano T, Yoshida S, Takenaga M, Kitagawa H. Anti-thrombotic and anti-atherogenic action of eicosapentaenoic acid. Jpn Circ J 1987;51:471–7.

23. Reis GJ, Boucher TM, Sipperly ME, McCabe CH, Silvermen DI, Pasternak RC. Fish oil supplements raise LDL cholesterol: results of a blinded, placebo-controlled trial in patients with coronary disease (abstr). Circulation 1988;78(suppl II):II-385.

24. Zucker ML, Bilyeu DS, Helmkamp GM, Harris WS, Dujovne CA. Effects of dietary fish oil on platelet function and plasma lipids in hyperlipoproteinemic and normal subjects. Atherosclerosis 1988;73:13–22.

25. Demke DM, Peters GR, Linet OI, Metzler CM, Klott KA. Effects of a fish oil concentrate in patients with hypercholesterolemia. Atherosclerosis 1988;70:73–80.

26. Harris WS, Dujovne CA, Zucker M, Johnson B. Effects of a low saturated fat, low cholesterol fish oil supplement in hypertriglyceridemic patients: a placebo-controlled trial. Ann Intern Med 1988;109:465–70.

27. Yetiv JZ. Clinical applications of fish oils. JAMA 1988;260:665–70.

28. Simonsen T, Nordoy A, Sjunneskog C, Lyngmo V. The effect of cod liver oil in two populations with low and high intake of dietary fish. Acta Med Scand 1988;223:491–8.

29. Terres W, Schmidt A, Kupper W, Bleifeld W. Antiaggregatory and lipid lowering effect of a low dose of a fish lipid concentrate (abstr). Circulation 1988;78(suppl II):II-573.

30. Yamaguchi K, Mizota M, Hashizume H, Kumagai A. Antiatherogenic action of eicosapentaenoic acid (EPA) in multiple oral doses. Prostaglandins Leukotrienes Med 1987;28:25–43.

31. Herald PM, Kinsella JE. Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trial. Am J Clin Nutr 1986;43:566–98.

32. DeCaterina R, Giannessi D, Mazzone A, et al. Vascular prostacyclin is increased in patients taking eicosapentaenoic acid prior to coronary bypass surgery (abstr). Circulation 1988;78(suppl II):II-325.

33. Abeywardena MY, McLennan PL, Charnock JS. Long-term saturated fat supplementation in the rat causes an increase in PG12/TXB2 ratio of platelet and vessel wall compared to n-3 and n-6 dietary fatty acids. Atherosclerosis 1987;66:181–9.

34. Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. N Engl J Med 1988;318:549–57.

35. Bolton-Smith C, Gibney MJ, Gallagher PJ, Jewell R, Hillier K. Effect of polyunsaturated fatty acids of the n-3 and n-6 series on lipid composition and eicosanoid synthesis of platelets and aorta and on immunological induction of atherosclerosis in rabbits. Atherosclerosis 1988;72:29–35.

36. Malis CD, Varadarajan GS, Force T, Weber PC, Leaf A, Bonventre JV. Effects of dietary fish oils on vascular contractility subsequent to anoxia (abstr). Circulation 1988;78(suppl II):II-216.

37. Shinokawa H, Lam JYT, Chesebro JH, Bowie EJW, Vanhoutte PM. Effects of dietary supplementation with cod-liver oil on endothelium-dependent responses in porcine coronary arteries. Circulation 1987;76:898–905.