

N-3 Fatty Acids from Fish Oil

Effects on Plasma Lipoproteins and Hypertriglyceridemic Patients^a

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In the early 1970s, epidemiologic studies conducted in Greenland Eskimos by Bang and Dyerberg led to the hypothesis that marine oils rich in n-3 fatty acids have antiatherogenic properties.^{1,2} This hypothesis was derived from the observation that coronary artery disease was uncommon among Eskimos despite their distinctively high intake of animal fat. Paradoxically, the Danes who also ate a high animal fat diet exhibited much higher rates of coronary artery disease. The dietary fat of the Eskimos was different. It came from the sea (fish, seal, and whale) and contained large quantities of the n-3 fatty acids, eicosapentaenoic acid (EPA), 20:5(n-3)^c and docosahexaenoic acid (DHA), 22:6(n-3). Conversely, the Danish fat intake was largely saturated, from land animals. Eskimos also had lower plasma lipid levels (both cholesterol and triglyceride) and increased bleeding times. From these initial observations, literally hundreds of studies with n-3 fatty acids were and are being carried out. (See recent reviews in refs. 3-6.)

Besides Eskimos, other populations in Japan and Holland that consume more n-3 fatty acids from fish and sea mammals have less coronary heart disease,⁷ and fish oil fed to monkeys and pigs greatly retards the development of experimental atherosclerosis.^{8,9} Furthermore, human experiments also show anticoronary effects after fish oil feeding, particularly hypolipidemic actions and decreased platelet aggregation.^{10,11}

Even more basic factors in the pathogenesis of atherosclerosis are affected by n-3 fatty acids. These include inhibition of intimal hyperplasia in autologous vein grafts in dogs,¹² the decrease in endothelial cell production of platelet-derived growth factor-like protein,¹³ and increased activity of endothelium-derived relaxation factor.¹⁴ There are even hints that the restenosis rate after coronary angiography is lessened by fish oil feeding,¹⁵⁻¹⁷ but evidence from some other quarters is conflicting.¹⁸ One large scale clinical trial in Wales showed both a significant reduction in the

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^cFatty acid nomenclature: first number indicates length of carbon chain; second number, followed by colon, specifies number of double bonds; third number, after n, gives number of carbons before first double bond, counting from methyl end of the fatty acid chain. N can be used interchangeably with omega.

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coronary death rate and a lower mortality in men with known coronary disease consuming fish or taking fish oil.¹⁹

It has been known for more than two decades that the highly polyunsaturated fish oils had a hypolipidemic effect.³ However, until recently the hypolipidemic action of fish oils was considered in the same category as that of the plasma lipid-lowering effect occurring from vegetable oils rich in the n-6 fatty acid, linoleic acid. No distinction was made between possible differences of action in these two categories of edible oils despite their different kinds of polyunsaturated fatty acids. Fish oils contain little of the n-6 fatty acid, linoleic, and large quantities of the n-3 fatty acids, EPA and DHA.

In the experiments to be described, the hypolipidemic actions of fish oil *versus* polyunsaturated vegetable oils are compared to elucidate the metabolic basis for the specific hypolipidemic actions of fish oil.

STUDIES IN NORMAL SUBJECTS

Twelve healthy adults (six men and six women) participated in the study.²⁰ Plasma cholesterol levels at the time of entry ranged from 132–259 mg/dl (mean 192 ± 41), and triglyceride (TG) levels ranged from 41–162 mg/dl (mean 85 ± 36). The study was carried out in the Clinical Research Center of the Oregon Health Sciences University, and all meals were prepared by the metabolic kitchen staff.

Diets. Three diets that differed only in fatty acid composition were fed in random order for 4 weeks each: a "saturated" control diet, a salmon oil diet containing considerable amounts of n-3 fatty acids, and a vegetable oil diet high in n-6 fatty acids. Each diet provided 40% of the calories as fat, 15% as protein, and 45% as carbohydrate (CHO) and about 500 mg/day of cholesterol. The control diet was designed to simulate the fatty acid composition of a typical American diet and contained cocoa butter and peanut oil. The vegetable oil diet was identical to the control diet, except that a mixture of safflower and corn oils provided the dietary fat. The salmon diet contained salmon fillets and salmon oil. Salmon was chosen as the source of n-3 fatty acids because of its accessibility in the Pacific Northwest, its exceptionally good taste, and its high fat content (approximately 15% by weight). Salmon also contains relatively high proportions of n-3 fatty acids.

Plasma Lipid and Lipoprotein Levels. Plasma levels of cholesterol fell from 188 to 162 mg/dl during the salmon oil diet *versus* the control diet ($p < 0.001$). Similarly, VLDL cholesterol levels fell from 13 to 8 mg/dl ($p < 0.001$), whereas LDL decreased from 128 to 108 mg/dl ($p < 0.005$). High-density lipoprotein cholesterol levels were not affected by the salmon oil diet. The changes in plasma triglyceride were most striking, from 76 to 50 mg/dl. The polyunsaturated vegetable oils decreased plasma cholesterol similarly, but they did not affect VLDL and triglyceride levels.

The most striking finding of this study was the ability of salmon oil to lower plasma triglyceride and VLDL levels, as well as plasma cholesterol and LDL levels, in normolipidemic subjects. Although the hypocholesterolemic effect of marine oils was a consistent finding in studies of the 1950s, its hypotriglyceridemic action was not appreciated.^{21–24} In recent studies, fish oil feeding has invariably led to lower triglyceride levels.^{25,26} To date, however, no other reported study using diets with controlled fatty acid compositions has compared fish oil with both polyunsaturated vegetable oils and saturated fats in the same individuals. Our finding that, of the three fats tested, only salmon oil was hypotriglyceridemic indicates that its polyunsat-

urated n-3 fatty acids have different metabolic effects from those of n-6 fatty acids from vegetable oils.

STUDIES IN HYPERLIPIDEMIC PATIENTS

Because of the hypolipidemic effect of n-3 fatty acids in normal subjects, it seemed reasonable to test their effects in hyperlipidemic patients.²⁷ The two groups of hyperlipidemic patients selected for study were characterized by hypertriglyceridemia, because depression of plasma triglyceride and VLDL appeared to be a unique effect of n-3 fatty acids from fish oil. Overproduction of VLDL has been a characteristic feature of most patients with hypertriglyceridemia.²⁸

Twenty hypertriglyceridemic patients (8 men and 12 women) volunteered for the study. Ten patients presented with increased levels of both VLDL and LDL, consistent with the type II-b phenotype, according to established criteria.²⁹ Mean plasma lipid levels at the time of entry were 337 mg/dl for cholesterol and 355 mg/dl for triglyceride. Clinically, many of these patients had familial combined hyperlipidemia, a disorder characterized by a strong disposition to the development of coronary heart disease and by overproduction of lipoproteins, particularly VLDL.²⁹ All patients had normal thyroid, renal, and hepatic function. The genotype was familial combined hyperlipidemia in seven patients and was unknown in three patients.

The other 10 patients had apparent type V hyperlipidemia, characterized by increased chylomicrons and greatly increased VLDL levels in the fasting state. Mean plasma lipid levels at entry were 514 mg/dl for cholesterol and 2,874 mg/dl for triglyceride. The type V phenotype is characterized by both overproduction of VLDL and impaired clearance of the remnants of chylomicron and VLDL metabolism. Some type V patients belong to families with familial combined hyperlipidemia. Clinically, type V patients have the "chylomicronemia" syndrome characterized by episodes of abdominal pain from enlargement of abdominal viscera (hepatomegaly and splenomegaly) and by episodes of acute pancreatitis. These patients also have eruptive xanthomas, neuropathy, and lipemia retinalis. Although LDL levels in type V patients are low, the presence of atherogenic remnant particles predisposes them to the development of atherosclerotic complications, including coronary heart disease.

Four of these patients had concomitant, non-insulin-dependent diabetes mellitus, and two had adult-onset, insulin-dependent diabetes mellitus. Their insulin doses and diabetic control remained constant throughout the study despite the salmon oil. The genotype diagnosis for eight type V patients was indeterminate. One had familial combined hyperlipidemia. Another was later discovered to have type III or dysbetalipoproteinemia (homozygous for apoprotein apo E₂).

Special care was taken to make certain that the patients were in steady-state conditions before entry. Steady state was defined as constancy of body weight and diet and absence of any residual hypolipidemic drug effect. Most patients had not been receiving any hypolipidemic drugs just before the study. In patients previously given drugs, these were discontinued, and plasma lipid levels were monitored until pre-drug levels were attained.

Two different control diets were used for the two groups of hypertriglyceridemic patients depending on the phenotype of hyperlipidemia. Type II-b patients received their usual low cholesterol (100 mg/day), low fat (20-30% of total calories) diet. Subsequent dietary periods for type II-b patients consisted of a fish oil diet for 4

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weeks, followed in some patients by a 4-week period of a diet high in a vegetable oil containing a predominance of n-6 fatty acids. Both of these diets were balanced for cholesterol content (approximately 250 mg/day) and contained 30% of calories as fat. The diets in all periods were eucaloric so that the subjects neither gained nor lost weight.

For type V patients, the control diet consisted of a very low fat diet (5%) to lower plasma triglyceride levels maximally.³⁰ The next dietary interval contained fish oil at 20% or 30% of total calories. Finally, a polyunsaturated vegetable oil diet was also provided, which contained 20–30% of total calories as fat, and 200–300 mg of cholesterol per day. Both the fish oil and the vegetable oil diets were initially used cautiously in type V patients to minimize the risk of hepatosplenomegaly, abdominal pain, and acute pancreatitis.

TABLE 1. Plasma Lipid and Lipoprotein Levels in Type IIb Patients Fed Diets Rich in Fish Oil ($n = 10$)

Subject	Cholesterol (mg/dl)							
	Total		VLDL		LDL		HDL	
	Control	Fish Diet	Control	Fish Diet	Control	Fish Diet	Control	Fish Diet
1	286	201	92	21	162	153	32	22
2	280	184	107	24	140	142	31	33
3	296	196	75	20	180	141	38	38
4	279	214	47	7	208	188	41	37
5	241	175	32	8	188	147	33	33
6	394	308	47	11	256	288	48	26
7	402	321	92	30	277	268	33	15
8	347	301	26	24	261	224	68	55
9	447	287	55	16	343	236	38	44
10	263	182	47	13	182	148	47	34
$\bar{X} \pm SD$	324 ± 69	236 ± 60	62 ± 28	17 ± 8	220 ± 63	194 ± 56	41 ± 11	34 ± 11
Change		-88		-45		-26		-7
Significance		$p < 0.001$		$p < 0.001$		$p < 0.05$		$p < 0.05$

\bar{X} = mean value.

The salmon oil diet provided about 20 g/day of n-3 fatty acids for a 2,600-kcal intake, with 30% of the total calories as fat. The commercial fish oil preparation supplied 30 g of n-3 fatty acids under similar circumstances. On the other hand, the vegetable oil diet provided about 47 g of the n-6 polyunsaturated fatty acid, linoleic acid. Thus, the fish oil diets actually provided 43–64% less total polyunsaturated fatty acids gram for gram than did the vegetable oil diet.

Plasma Lipid and Lipoprotein Levels. The fish oil diet decreased the plasma LDL-cholesterol levels in type II-b patients by 26 mg/dl (TABLE 1). Of individual changes in lipoprotein cholesterol, the decline in VLDL cholesterol was most striking, but LDL and HDL cholesterol also decreased. Plasma triglyceride changes were even greater than cholesterol changes with the fish oil diet, decreasing from 334 to 118 mg/dl. This decrease occurred largely because of the change in VLDL triglyceride, which was lowered from 216 to 55 mg/dl (TABLE 2).

TABLE 2. Plasma Lipid and Lipoprotein Levels in Type II-b Patients Fed Diets Rich in Fish Oil ($n = 10$)

Subject	Triglyceride					
	Total		VLDL		LDL	
	Control	Fish Diet	Control	Fish Diet	Control	Fish Diet
1	393	157	352	78	70	73
2	462	130	399	71	44	63
3	420	156	314	91	41	47
4	333	103	149	34	80	73
5	185	99	114	99	64	69
6	293	104	142	42	106	63
7	458	137
8	169	112	102	27	50	50
9	319	79	180	20	200	77
10	314	100	207	35	107	54
$\bar{X} \pm SD$	334 ± 108	118 ± 26	216 ± 112	55 ± 30	85 ± 50	63 ± 11
Change		-206		-161		-22
Significance		$p < 0.001$		$p < 0.005$		NS

\bar{X} = mean value.

The highly polyunsaturated vegetable oil diet led to less marked plasma cholesterol and triglyceride lowering in type II-b patients, in contrast to the effects of fish oil, because the vegetable oil diet had much less effect on VLDL cholesterol and triglyceride. LDL values were similar, but in contrast, HDL cholesterol was higher after the vegetable oil diet. Plasma apolipoprotein changes reflected the lipoprotein lipid changes. In type II-b patients, significant reductions occurred in apo B and C-III levels in the fish oil period, which paralleled the declines in LDL and VLDL levels.

In type V patients, effects of the fish oil diet were even more striking (FIG. 1 and 2; TABLE 3). With consumption of the very low fat control diet, the initial plasma lipid levels declined considerably but still remained greatly elevated. Many of these patients still had milky-appearing plasma, with chylomicrons present in the fasting state. The first change to occur in these patients after the fish oil diet was the virtual disappearance of fasting chylomicronemia, which had been present in five of the patients. Triglyceride content of these chylomicron fractions declined from a mean value of 443 ± 210 to 22 ± 31 mg/dl. During the fish oil diet, total plasma triglyceride decreased from a control value of 1,353 to 281 mg/dl, a drop of 79% (FIG. 1). VLDL triglyceride decreased similarly, from 1,087 to 167 mg/dl. Plasma cholesterol levels declined into the normal range after the fish diet, from 373 to 207 mg/dl (FIG. 2). Most of this total plasma cholesterol decrease occurred as a result of marked changes in the amount of VLDL cholesterol, which decreased from 270 to 70 mg/dl. Of interest was the 48% concomitant rise in LDL cholesterol from the low value of 84 to 125 mg/dl. Apolipoprotein levels changed to reflect altered lipoprotein lipid levels. Apo A-1 levels did not change, whereas apo B, C-III, and E all decreased significantly.

When the n-6-rich vegetable oil replaced the fish oil in the diets of eight type V patients, all patients had increases in plasma triglyceride levels within 3-4 days (TABLE 3). After 10-14 days of vegetable oil feeding, mean plasma triglyceride values rose 198%, and VLDL triglyceride increased from 171 to 550 mg/dl. Plasma cholesterol also increased, from 195 to 264 mg/dl. LDL-cholesterol levels, on the

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contrary, were decreased 28% by the vegetable oil diet, another indication that the metabolic abnormality of the type V phenotype was worsening. Because of enhanced hypertriglyceridemia and the risk of the development of abdominal pain typical of this type V disorder, the vegetable oil feeding period was discontinued prematurely in all type V patients.

SUMMARY AND IMPLICATIONS OF STUDIES IN HYPERTRIGLYCERIDEMIC PATIENTS

In the 20 hypertriglyceridemic patients, fish oil incorporated in the diet led to an even more profound hypolipidemic effect than had been observed in normal subjects. Plasma triglyceride levels decreased in each of the 20 patients, a 79% decrease in type V patients and a 64% decrease in type II-b patients; plasma cholesterol levels decreased 45% and 27%, respectively. In the 12 normal subjects previously investigated,²⁰ decreases were smaller for plasma triglyceride (38%) and much smaller for plasma cholesterol (14%). Apparently, the greater the hypertriglyceridemia, the

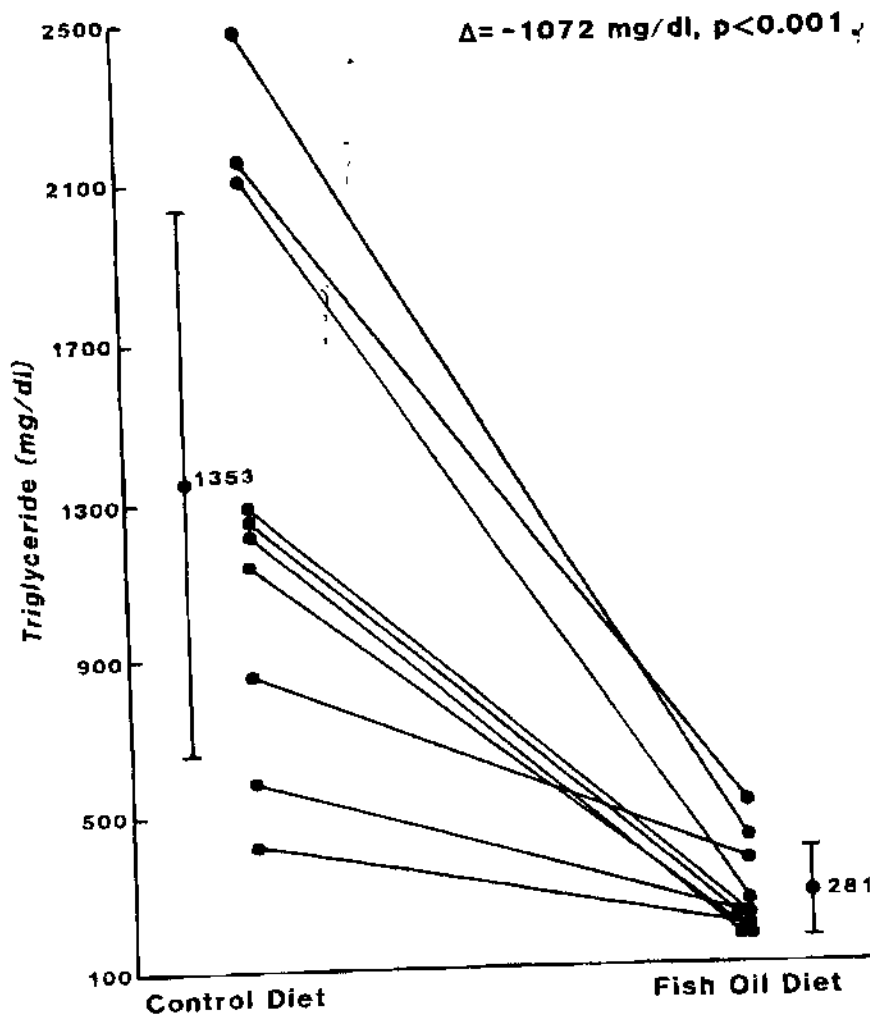


FIGURE 1. Changes in plasma triglyceride levels in the 10 type V patients: control diet vs fish oil diet. To convert triglyceride from milligrams per deciliter to millimoles per liter, multiply by 0.0113.

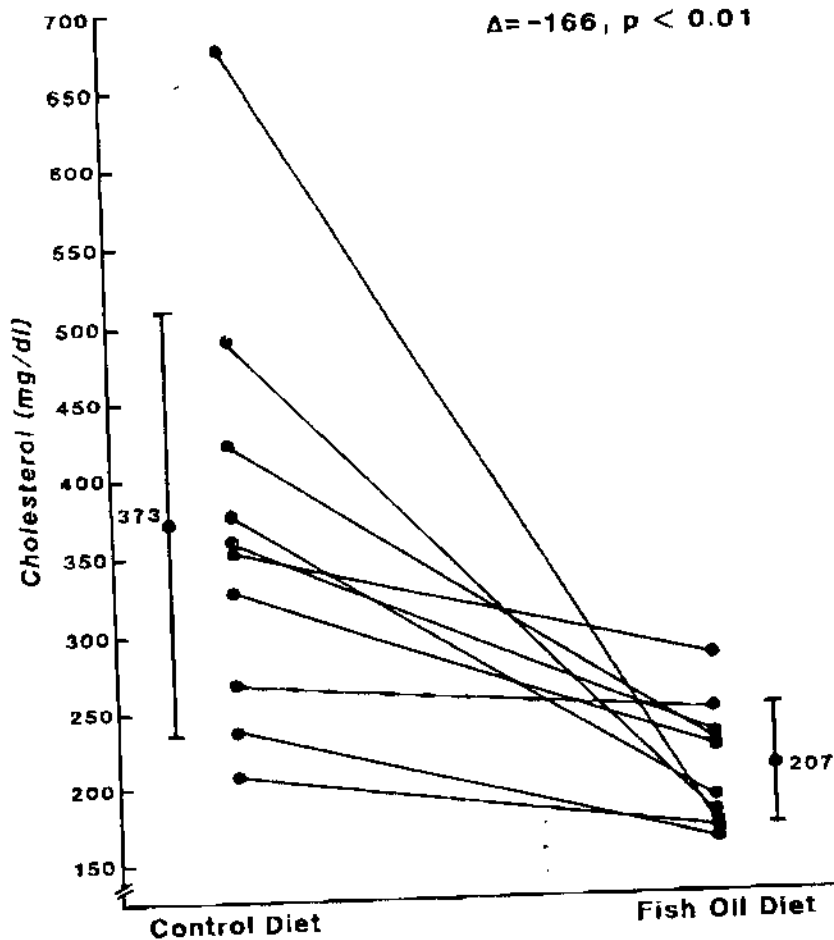


FIGURE 2. Changes in plasma cholesterol levels in the 10 type V patients: control diet vs fish oil diet. To convert cholesterol from milligrams per deciliter to millimoles per liter, multiply by 0.026.

greater the reductions brought about by dietary fish oil—in plasma lipids and especially in VLDL.

These results may have considerable therapeutic importance for patients with severe and moderate hypertriglyceridemia. The only dietary treatment to date for severely hypertriglyceridemic type V patients has been the very severe and therapeutically difficult restriction of dietary fat to between 5% and 10% of total calories in an effort to approach normal plasma triglyceride levels.³⁰ Americans find this possible to do on a short-term basis, but very difficult on a long-term basis because they are accustomed to eating higher quantities of fat, that is, approximately 40% of total calories. Hitherto, all fatty foods were contraindicated in type V hyperlipidemia. The findings of this study suggest that some fatty and even high cholesterol foods (i.e., fish or even shellfish) containing marine n-3 fatty acids are appropriate for ingestion and may further lower triglyceride over and above that which results from the very low fat diet.

A fatty fish such as salmon, containing 10–15% fat by weight, was previously contraindicated in the dietary prescription of patients with severe hypertriglyceridemia. Our results indicate that such fish may be consumed with therapeutic benefit. This applies not only to the type V patient but also to patients with types II-b and IV

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hypertriglyceridemia who have elevated VLDL levels. The commonly consumed white fish are lower in total fat content (1-2%) and contain a high percentage of total fatty acids as n-3 fatty acids (up to 48%). TABLE 4 indicates the fat and n-3 fatty acid content of various fish, shellfish, and fish oil. Thus, both low and high fat fish may have therapeutic benefit when consumed by hypertriglyceridemic patients. The shellfish that have a higher cholesterol content may also be consumed by the type V patient, because of their low fat content and high concentration of n-3 fatty acids.

The question of whether fish oils *per se* should be used as dietary supplements in the treatment of hypertriglyceridemia remains uncertain. The large doses of fish oil used in the present studies were selected to produce a maximal effect and at this point in time should be regarded as experimental. However, we suggest that doses of fish oil from 8-15 g per day or higher have definite therapeutic benefits.^{4-6,31} Future studies will be required to establish the minimal efficacious amounts of fish oils and fish for optimal hypolipidemic effects in hyperlipidemic patients.

Other studies in familial combined hyperlipidemia and in type IV hyperlipidemia showed increases in LDL and apo B, while plasma VLDL and triglyceride values were declining.^{6,31,32} Such LDL increases have also occurred in type IV patients given the drug gemfibrozil. Perhaps this is an expected physiologic action when hypertriglyceridemia is being corrected. If LDL levels become abnormally high after either drugs or fish oil, further therapy of the LDL specifically is warranted.

Effects on Chylomicrons. With regard to the reduction in chylomicron levels that occurred in type V hypertriglyceridemic patients, we and others observed that fish oils markedly decreased the usual chylomicronemia that follows fatty meals.³³ In other words, fat tolerance was greatly improved (FIG. 3). This improvement could result from diminished absorption, slower synthesis, and slower entry of chylomicrons into the circulation or, alternatively, from a more rapid removal of the chylomicrons that do appear in the circulation. There is no evidence of diminished absorption, and fat balance studies did not show increased fat excretion in stools after dietary periods enriched with fish oil.³⁰ Whether reduced chylomicron production or enhanced removal of chylomicrons is responsible has not yet been examined.

TABLE 3. Plasma Lipid Changes in Eight Patients with the Type V Phenotype, after the Control, Vegetable Oil, and Fish Oil Diets^a

Plasma Lipids (mg/dl)	Diet			p Value	
	Control	Fish Oil	Vegetable Oil	Control vs Fish Oil	Fish vs Vegetable Oil
Total cholesterol	377 ± 155	195 ± 31	264 ± 97	<0.01	NS
Total triglyceride	1,432 ± 750	272 ± 120	841 ± 514	<0.01	<0.05
VLDL cholesterol	251 ± 148	74 ± 67	216 ± 219	<0.05	<0.05
VLDL triglyceride	1,249 ± 681	171 ± 119	550 ± 360	<0.01	NS
LDL cholesterol	77 ± 55	110 ± 34	79 ± 30	<0.05	<0.05
LDL triglyceride	70 ± 34	65 ± 18	71 ± 31	NS	NS
HDL cholesterol	31 ± 7	35 ± 12	31 ± 11	NS	NS

^aValues are means ± SD. To convert cholesterol and triglycerides values from milligrams per deciliter to millimoles per liter, multiply by 0.026 and 0.0113, respectively. NS denotes not significant. No significant differences were noted between the control and vegetable oil diets.

TABLE 4. Fat and Omega-3 Fatty Acid Content of Fish and Fish Oils^a
(100 g., edible portion, raw)

Fish	Fat (g)	Omega-3 Fatty Acids ^b (g)
Fish		
Anchovy, European	4.8	1.4
Bass, striped	2.3	0.8
Bluefish	6.5	1.2
Carp	5.6	0.3
Catfish, channel	4.3	0.3
Cod, Atlantic	0.7	0.3
Cod, Pacific	0.6	0.2
Flounder, unspecified	1.0	0.2
Haddock	0.7	0.2
Halibut, Pacific	2.3	0.4
Herring, Atlantic	9.0	1.6
Herring, Pacific	13.9	1.7
Mackerel, Atlantic	13.9	2.5
Mullet, unspecified	4.4	1.1
Ocean perch	1.6	0.2
Pike, Walleye	1.2	0.3
Pompano, Florida	9.5	0.6
Sablefish	15.3	1.4
Salmon, Atlantic	5.4	1.2
Salmon, Chinook	10.4	1.4
Salmon, pink	3.4	1.0
Salmon, Sockeye	8.6	1.2
Sardines, in sardine oil ^c	15.5	3.3
Shark	1.9	0.5
Snapper, red	1.2	0.2
Sole	1.2	0.1
Sturgeon	3.3	0.3
Swordfish	2.1	0.2
Trout, brook	2.7	0.4
Trout, lake	9.7	1.6
Trout, rainbow	3.4	0.5
Tuna	2.5	0.5
Crustaceans		
Crab, Alaska King	0.8	0.3
Crab, Dungeness	1.0	0.3
Crayfish, unspecified	1.4	0.1
Lobster, Northern	0.9	0.2
Shrimp, unspecified	1.1	0.3
Mollusks		
Abalone, New Zealand	1.0	tr
Clam, hardshell	0.6	tr
Clam, littleneck	0.8	tr
Mussel, blue	2.2	0.5
Octopus, common	1.0	0.2
Oyster, Pacific	2.3	0.6
Scallop, unspecified	0.8	0.2
Squid, unspecified	1.1	0.3
Fish oils		
Cod liver oil	100.0	18.5
Herring oil	100.0	11.4
MaxEPA ^{lmc}	100.0	29.4
Promega ^{lmd}	100.0	44.2
Salmon oil	100.0	19.9

^aTaken from ref. 48.

^b20:5 plus 22:6.

^cAnalysis by the Atherosclerosis Research Laboratory, 1987.

^dConcentrated fish body oils.

MECHANISM OF THE HYPOLIPIDEMIC EFFECTS OF FISH OIL

Two further experiments have determined how n-3 fatty acids exerted their effects to decrease the levels of plasma triglyceride and cholesterol: (1) the inhibition by fish oil of the usual hypertriglyceridemia that inevitably results when a high carbohydrate (CHO) diet is suddenly fed to humans; and (2) the effects of fish oil on apo B, VLDL, and LDL production rates and turnovers.

The well-known phenomenon of CHO-induced hypertriglyceridemia is a physiologic response. In this model, VLDL triglyceride synthesis is stimulated as dietary CHO intake abruptly increases. Increased VLDL synthesis leads to hypertriglyceridemia, which may persist for many weeks.³⁴⁻³⁷ If n-3 fatty acids do inhibit VLDL synthesis, then the usual CHO-induced hypertriglyceridemia should not occur when fish oil is incorporated into the high CHO diet.

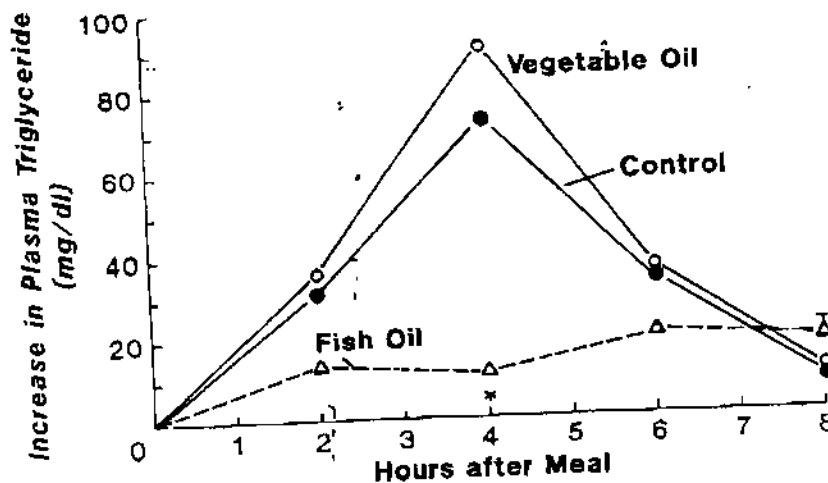


FIGURE 3. The increase in plasma triglyceride levels after ingestion of 50 g of fat. Saturated fat test meal given during the saturated fat diet (*closed circles*); vegetable oil test meal given during the vegetable oil diet (*open circles*); salmon oil test meal given during the salmon oil diet (*triangles*). Mean fasting triglyceride levels were 72 ± 19 , 76 ± 37 , and 46 ± 11 mg/dl before the saturated fat, vegetable oil, and salmon oil test meals, respectively, were administered.

Seven mildly hypertriglyceridemic, but otherwise healthy subjects (aged 22–54 years) were fed three different experimental diets. Each was composed of a liquid formula plus three bran muffins per day to supply fiber. The baseline diet contained 45% of calories from CHO. The two high carbohydrate (high CHO) diets (control and fish oil) contained 15%, 10%, and 75% of calories as fat, protein, and CHO, respectively. In the baseline and high CHO control diets, a blend of peanut oil and cocoa butter provided the fat, which was replaced by fish oil, in the form of a commercially available marine lipid concentrate, in the high CHO fish oil diet. The total amount of fish oil consumed per day was 50 g (in a 3,000-kcal diet), equivalent to approximately 3.3 tablespoons of oil. This amount provided 8.5 g of EPA and 5.5 g of DHA.

The three experimental diets were fed in three different sequences in the Clinical Research Center (FIG. 4). In the first sequence, the high CHO control diet preceded the high CHO fish oil diet (FIG. 4A). In the second sequence, the high CHO diet was given for 20 days instead of 10 in order to demonstrate that the hypertriglyceridemia

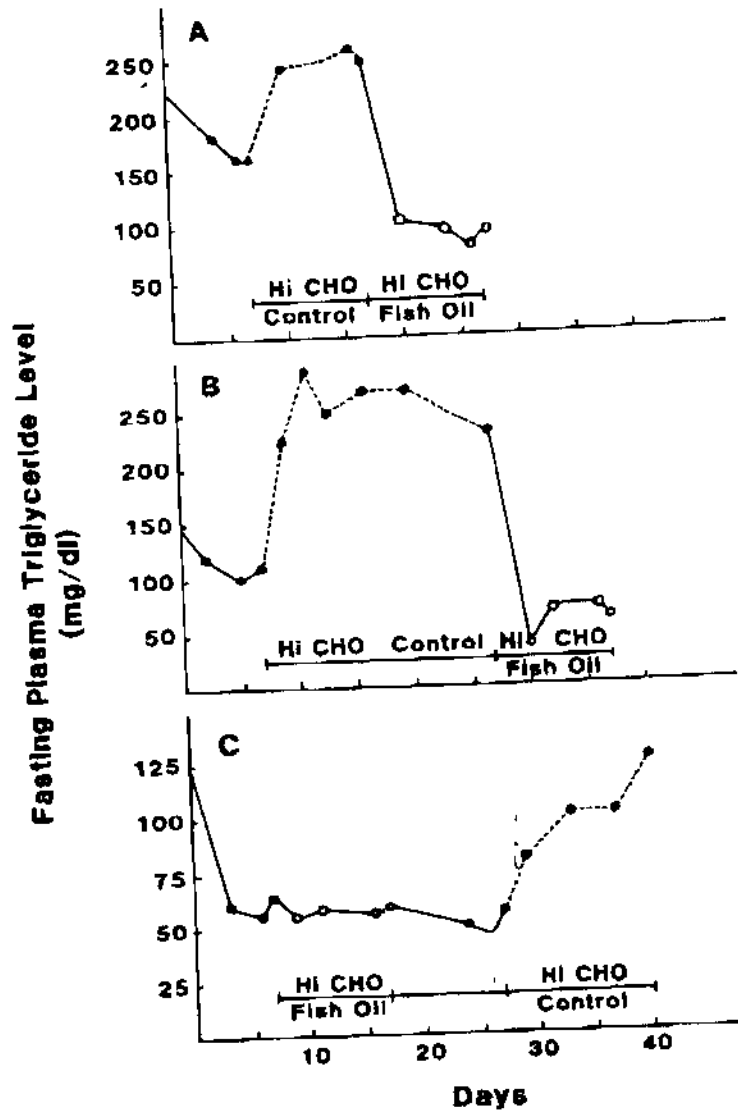


FIGURE 4. Effects of the baseline diet and the control and fish oil diets on plasma triglyceride levels in three subjects. We see the reversal of carbohydrate-induced hypertriglyceridemia by dietary fish oil (A), the persistence of hypertriglyceridemia (throughout 20 days) and the subsequent reversal by fish oil (B), and the prevention of carbohydrate-induced hypertriglyceridemia by fish oil (C).

did not spontaneously resolve after the first 10 days. It was then followed by the fish oil diet (FIG. 4B). In the third sequence, the fish oil was first fed with the high CHO diet for 25 days and then removed to permit the effects of the high CHO diet to be manifest for the next 15 days (FIG. 4C). Three subjects were studied with the first sequence, and two subjects each were studied with the second and third sequences.

In all seven subjects, the high CHO control diet increased the plasma triglyceride levels over the baseline diet, from 105 to 194 mg/dl. The magnitude of the CHO-induced hypertriglyceridemia correlated significantly with each individual's baseline triglyceride levels. The rise in plasma triglyceride levels was complete by day 5 and resulted almost entirely from an increase in the VLDL triglyceride fraction, which more than doubled during the control diet, from 69 to 156 mg/dl (FIG. 5). Although total plasma cholesterol levels did not change, VLDL cholesterol levels

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approximately doubled from 18 to 34 mg/dl, and HDL cholesterol was reduced from 49 to 41 mg/dl.

When the fat of the high CHO control diet was replaced isocalorically with fish oil, the elevated plasma triglyceride concentration was reduced from 194 to 75 mg/dl, a decrease of 61%. This decrease usually occurred within 3 days (FIG. 4A). Once again, changes in VLDL triglyceride levels were largely responsible for this effect (156 to 34 mg/dl). Total cholesterol levels decreased insignificantly during the high CHO fish oil diet, from 172 to 153 mg/dl, primarily because of the decrease in VLDL cholesterol levels (34 to 12 mg/dl).

When the period of CHO induction was prolonged from 10 to 20 days, the hypertriglyceridemia persisted and did not significantly decrease until the high CHO fish oil diet began (FIG. 4B). When the high CHO fish oil diet followed the baseline diet, the plasma triglyceride level did not rise, but the level increased when the high CHO control diet was fed subsequently (FIG. 4C). The high CHO control diet decreased the levels of apo B and increased apo C-III concentrations; apo A-1 and E levels did not change. The high CHO fish oil diet decreased apo A-1 and apo C-III levels; apo B and E concentrations did not change.

The incorporation of corn oil in place of fish oil into the high CHO regimen did not affect the induced hypertriglyceridemia. For the three subjects who participated in this study, triglyceride levels were as follows: baseline, 93 ± 23 mg/dl; high-CHO control, 196 ± 58 mg/dl; high CHO corn oil, 215 ± 90 mg/dl; and high CHO fish oil, 86 ± 10 mg/dl.

In this study, dietary fish oil not only prevented but also rapidly reversed the

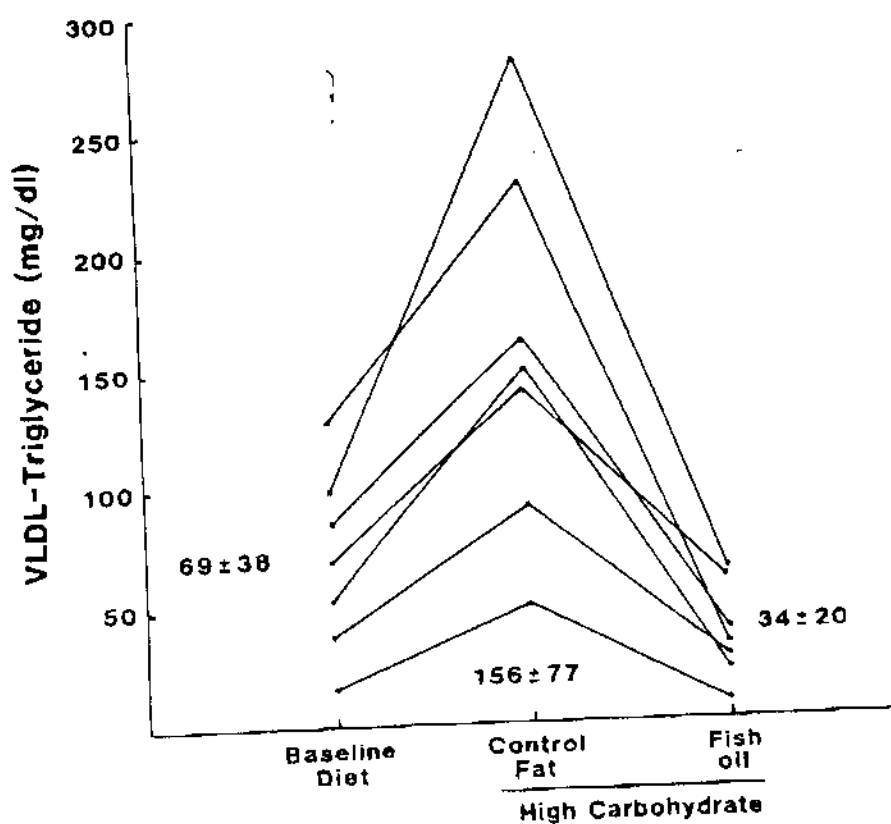


FIGURE 5. Effects of high carbohydrate control and fish oil diets on plasma VLDL triglyceride levels in the seven subjects.

dietary, CHO-induced elevations in plasma triglyceride and VLDL levels, whereas the n-6 fatty acid-rich corn oil had no effect at all. As the primary difference between corn oil and the commercial fish oil preparation is the type of polyunsaturated fatty acids present (corn, 57% 18:2 n-6; linoleic acid; the commercially available fish oil preparation, 32% n-3 fatty acids), the difference in effect was due to the n-3 fatty acids in the fish oil. This finding implied a probable inhibitory effect of n-3 fatty acids on hepatic VLDL production.

Turnover Studies. The hypothesis that n-3 fatty acids probably reduced VLDL levels by inhibiting VLDL synthesis was further supported by studies designed to elucidate further mechanisms in the hypotriglyceridemic effect of n-3 fatty acids. Dietary fish oil probably affected either the synthesis or the removal of VLDL. The rates of flux and turnover of VLDL triglyceride were measured after injection of ^3H -glycerol into persons studied under two dietary protocols, one containing fish oil and the other containing fats typical of the American diet. This technique permits the calculation of both synthetic and removal rates of VLDL.

Ten male subjects were selected on the basis of having a wide range of fasting plasma triglyceride concentrations, from 34 to 4,180 mg/dl, so that the hypothesis about the mechanism of action of dietary fish oils could be tested in subjects with greatly different pool sizes of plasma triglyceride. Liquid formula diets containing 15–20% fat, 65–75% CHO, and 10–15% protein were fed during both the control and the fish oil dietary periods. The two diets differed only in the type of fat they contained. In the control diet, a blend of cocoa butter and peanut oil (1:2) was incorporated into the formulas. The fish oil diet containing the commercial preparation was taken in three divided doses daily and was not mixed into the formulas. The principal difference between the two diets was the higher content of linoleic acid (18:2(n-6)) in the control diet and the presence of n-3 fatty acids in the fish oil diet. The former diet contained virtually no n-3 fatty acids, whereas the latter provided about 17 g/day of these highly polyunsaturated fatty acids.

The experimental diets were consumed for 3–5 weeks before the actual VLDL turnover procedure was conducted. This time was needed for the plasma triglyceride levels to stabilize, particularly in subjects whose triglyceride levels were above normal. Seven subjects consumed the control diet first, followed by the fish oil diet; in the remaining three, the order was reversed. The order in which the diets were administered did not affect the results.

The isocaloric substitution of fish oil for the control vegetable fat produced the expected significant reductions in the total and lipoprotein lipid levels in all 10 subjects. The mean values for the normal and hypertriglyceridemic groups are given in TABLE 5. Total cholesterol levels for all 10 subjects fell from 195 to 144 mg/dl, a reduction of 22%. Decreases in VLDL levels accounted for most of the decrease in plasma cholesterol (83 to 21 mg/dl). LDL cholesterol levels did not change significantly, whereas HDL cholesterol concentrations fell from 31 to 24 mg/dl. All of these changes were evident in both the normal and the hypertriglyceridemic groups (TABLE 4).

After the administration of ^3H -glycerol and its incorporation into the triglyceride of VLDL, the decay curves were analyzed by a computer model, so that VLDL synthesis and turnover could be calculated.³⁸ The incorporation of n-3 fatty acids into the diet caused a 72% decrease in the VLDL triglyceride pool size (11.4 to 3.2 g; $p < 0.025$) (TABLE 6). The decreased pool size was associated with a 45% reduction in the VLDL triglyceride synthetic rate (23 to 12.6 mg/kg IBW; $p < 0.005$) and a 45% decrease in the residence time of VLDL triglyceride in the plasma (5.8 to 3.2 hours; $p < 0.005$). The reciprocal of the residence time is the fractional catabolic rate (FCR)

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which was increased by 65% (0.23 to 0.38 hr⁻¹, $p < 0.005$). A significant rise in the cholesterol/triglyceride ratio in VLDL occurred during the fish oil interval (0.18 to 0.25; $p < 0.05$). Finally, the ratio of the fast to the slow synthetic pathways did not change with fish oil feeding. The same trends were seen in both normal and hypertriglyceridemic patients. Similar results were also found by a slightly different dietary plan and with the addition of I₁₂₅ apo B labeling.³⁹

Direct evidence that the hepatic synthesis of triglyceride and VLDL is suppressed by n-3 fatty acids from fish oil has been supplied by three *in vitro* studies of the perfused rat liver and of liver cells from rats and rabbits in primary culture.⁴⁰⁻⁴² In all studies, triglyceride synthesis was reduced. In one, enhanced ketone body production resulted; in the others, n-3 fatty acids were diverted from triglyceride synthesis into phospholipid synthesis. When the results of all of these studies are combined, evidence is strong that suppression of VLDL and triglyceride synthesis is a primary mechanism of n-3 acids to explain their hypolipidemic effects.

TABLE 5. Effects of Fish Oil^a on Plasma Lipid and Lipoprotein Levels (means \pm SD in mg/dl)

	Cholesterol				Triglyceride	
	Total	VLDL	LDL	HDL	Total	VLDL
Normal (n = 3)						
Control	151 \pm 0.5	14 \pm 6	106 \pm 23	39 \pm 7	102 \pm 34	62 \pm 34
Commercial fish oil preparation	129 \pm 9	3 \pm 2	90 \pm 14	40 \pm 9	48 \pm 10	13 \pm 3
Hyperlipidemic (n = 7)						
Control	213 \pm 36 ^b	113 \pm 56 ^c	79 \pm 36	27 \pm 5 ^c	581 \pm 255 ^c	542 \pm 257 ^c
Commercial fish oil preparation	147 \pm 42	29 \pm 15	108 \pm 59	16 \pm 5	194 \pm 74	118 \pm 71
Total (n = 10)						
Control	195 \pm 44 ^c	83 \pm 66 ^c	87 \pm 34	31 \pm 8 ^c	442 \pm 314 ^b	398 \pm 317 ^c
Commercial fish oil preparation	144 \pm 40	21 \pm 17	103 \pm 50	24 \pm 13	150 \pm 93	87 \pm 77

^aControl fats and commercial fish oil preparation were fed at 15–20% of total calories.

^b $p < 0.025$.

^c $p < 0.005$.

No superscript = not significant.

Many other studies throughout the world have confirmed the triglyceride-lowering action of fish oil even when it was administered as a supplement without any, or little, dietary control.⁶ However, in many of these experiments, LDL increased, particularly in patients with type IV hyperlipidemia but also in a few patients with familial combined hyperlipidemia.^{6,43,44} Apo-B concentrations were lowered in some of the metabolically controlled studies but increased somewhat in other studies.^{6,44,45}

Difficulty in interpreting the effects of fish oil in various hyperlipidemic patients has occurred because of vastly different experimental conditions. In some studies, fish oil was simply added as a supplement to the usual diet in doses of 8–16 g/day. In the control period a placebo oil such as olive oil or safflower oil was not always used. In other studies, the customary diet plus an appropriate placebo oil was used. Furthermore, various kinds of fish oil have been utilized, some containing a considerable amount of cholesterol and saturated fat. Newer fish oils have been much less

TABLE 6. Effects of Dietary Fish Oil on VLDL Metabolism (means \pm SD)

Subject	Diet	VLDL Lipids ^a		VLDL-TG		VLDL Triglyceride (TG) Synthesis		VLDL-TG FCR (hr ⁻¹)	Residence Time of VLDL-TG in Plasma (hr)	Fast/Slow Synthetic Pathway (ratio)
		TG ^a (mg/dl)	Chol/TG (ratio)	Pool Size (g)	(mg/hr)	(mg/kg IBW)				
Mean \pm SD	Control	398 \pm 317	0.18 \pm 0.03	11.4 \pm 10.2	1,685 \pm 1,073	23.0 \pm 14.3	0.23 \pm 0.12	5.8 \pm 3.4	2.3 \pm 0.79	
	Fish oil	87 \pm 77	0.25 \pm 0.08	3.2 \pm 2.5	918 \pm 563	12.6 \pm 7.5	0.38 \pm 0.16	3.2 \pm 1.6	2.4 \pm 1.34	
<i>p</i> value		<i>p</i> < 0.005	<i>p</i> < 0.05	<i>p</i> < 0.025	<i>p</i> < 0.005	<i>p</i> < 0.005	<i>p</i> < 0.005	<i>p</i> < 0.005	NS	
Percent change		-78%	+38%	-72%	-46%	-45%	+65%	-45%	+3%	
Normal values (n = 13) ⁴⁴		113 \pm 10	0.21 \pm 0.01	...	806 \pm 123	11.5 \pm 1.8	0.19 \pm 0.01	5.2 \pm 0.38	2.7 \pm 0.36	

^aRepresents the average VLDL-TG concentration during the 48-hour turnover study.

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saturated and have contained higher concentrations of n-3 fatty acids as well as very low levels of cholesterol. It is of interest that all life in the seas and in fresh water contain n-3 fatty acids. Fatty fish, such as sardines and salmon, are especially rich (TABLE 4).

Some conclusions have emerged from the wide variety of studies, most of which have not been metabolically controlled. Fish oil is most effective when administered at 6-30% of the total calories and when the diet is metabolically controlled. In these studies, LDL lowering as well as profound VLDL and triglyceride lowering has usually occurred in normal subjects and in a wide variety of hyperlipidemic states. In our experience this lowering of plasma cholesterol levels has occurred in patients with types V, II-a, II-b, III, and IV hyperlipidemia, with the most dramatic results occurring in type V patients who do not tolerate any other kind of dietary fat.^{10,11,45} In the literature and in our experience, HDL levels are not greatly affected by fish oil. Clearly the use of fish oil in hyperlipidemia must be individualized as to both use and dosage.

At lower dosages, when fish oil was used as a supplement, usually triglyceride lowering was observed, but paradoxically, in some studies, levels of cholesterol and LDL did not change after fish oil, and in other studies, some increases in LDL and apo-B occurred. Why plasma LDL and apo B at times increase after fish oil when at the same time the plasma VLDL and triglyceride decrease is a most challenging question⁶ and may relate to fundamental aspects of VLDL-LDL metabolism. Normally, LDL is derived from two sources, conversion from VLDL and direct synthesis from the liver. The catabolism of VLDL likewise occurs in two directions through IDL. IDL may be removed by the apo E receptor in the liver or converted to LDL. The experiments of Huff and Telford⁴⁶ suggest why in some instances fish oil might increase LDL. Turnover studies in the miniature pig revealed that fish oil feeding increased the proportion of VLDL being converted to LDL. Apparently, the n-3 fatty acids of fish oil produce a smaller VLDL particle that is more likely to be converted to LDL. In this pig study, however, LDL concentrations did not increase, because the direct synthesis of LDL was reduced more by fish oil than by the increase in LDL from VLDL. These pig studies await confirmation in humans. They do explain why LDL may increase in some humans fed fish oil; more VLDL is converted to LDL and direct LDL synthesis does not decrease, thus adding up to more LDL. LDL turnover studies have shown decreased production of LDL in normal humans given large amounts of salmon oil versus vegetable oil.⁴⁷ In this study, the plasma LDL decreased.

SUMMARY AND CONCLUSIONS

In the experimental studies reported in this review, dietary n-3 fatty acids from fish and fish oil had profound hypolipidemic effects in normal subjects and in hypertriglyceridemic patients with combined hyperlipidemia (type II-b) and types IV and V hyperlipidemia. In these carefully controlled metabolic experiments, dramatic reductions occurred in plasma triglycerides and to a lesser extent in plasma total cholesterol. Reductions in VLDL, chylomicrons, remnants, LDL, apo B, and apo E were also noted. HDL changes varied from subject to subject. These plasma lipoprotein changes occurred in subjects with non-insulin-dependent diabetes mellitus as well, without deterioration of diabetic control. Similar results are reported in two other papers in this volume. Fish oil did not cause deterioration of diabetic control.

Whereas the mechanism of the hypolipidemic action of the n-6 rich vegetable oils

containing linoleic acid such as corn or safflower oil still remains obscure, the mechanism of the hypolipidemic action of the n-3 fatty acids in fish oil is well documented. The synthesis of triglyceride and VLDL in the liver is greatly reduced by n-3 fatty acids. At the same time, the turnover of VLDL in plasma is shortened. In another study, LDL production was decreased.⁴⁷

Combined with other dietary manipulations, such as a reduction in saturated fat and dietary cholesterol, the use of n-3 fatty acids to treat hyperlipidemia, especially hypertriglyceridemia, appears to have a well-supported rationale. Fish oil combined with a low cholesterol, low saturated fat diet has been shown to produce complementary effects. Total plasma cholesterol and LDL cholesterol were lowered by the fish oil.⁴⁹ In most situations, the use of fish oil supplements should be regarded as pharmacologic therapy, particularly effective in severe hypertriglyceridemic states (e.g., chylomicronemia). However, a lifelong diet rich in fish may be protective against atherosclerosis as well. Further studies are required to delineate exact doses and precise indications for the use of fish oil in different types of hyperlipidemias and to differentiate the effects, if any, of the two major n-3 fatty acids in fish oil, EPA and DHA.

The hypolipidemic effects of n-3 fatty acids coupled with their known antithrombotic actions (secondary to changes in prostaglandin secretion, platelet function, inhibition of growth factors, and enhancement of endothelial-derived relaxation factor) appear to have an important potential role in the control of coronary heart disease and other atherosclerotic disorders. Moreover, fish oil may prevent the "chylomicronemia" syndrome of type V hyperlipidemia.

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