Effects of fish oil on VLDL triglyceride kinetics in humans[1]

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Abstract Dietary n-3 fatty acids (FAs) found in fish oils markedly lower plasma triglyceride (TG) and very low density lipoprotein (VLDL) levels in both normal and hypertriglyceridemic subjects. The present study examined the mechanism of this effect. Ten subjects with widely different plasma triglyceride levels (82 to 1002 mg/dl) were fed metabolically controlled diets containing 20% fat. The control diet contained a blend of cocoa butter and peanut oil (P/S = 0.8). The test diet contained fish oil (P/S = 1.1) and provided 10–17 g of n-3 FAs per day (depending on calorie intake). After 3 to 5 weeks of each diet, the kinetics of VLDL-TG were determined over a 48-h period after the injection of [^{3}H]glycerol. The fish oil diet reduced the VLDL-TG synthetic rate from 23 ± 14.3 (mean ± SD) to 12.6 ± 7.5 mg/h per kg ideal weight ($P<0.005$) and increased the fractional catabolic rate (FCR) for VLDL-TG from 0.23 ± 0.12 to 0.38 ± 0.16 h $^{-1}$ ($P<0.005$). At the same time, there was a 66% reduction of plasma triglyceride levels, resulting largely from a 78% decrease in VLDL-TG levels (398 ± 317 to 87 ± 77 mg/dl; $P<0.005$). There was a strong correlation (r = 0.83; $P<0.01$) between the change in synthetic rates and pool sizes, but there was no correlation (r = 0.24; NS) between changes in FCRs and pool sizes. The VLDL cholesterol: triglyceride ratio increased during the n-3 diet suggesting that smaller VLDL particles were present. These particles would be expected to leave the VLDL fraction more rapidly than larger particles producing a higher FCR. We conclude that the hypotriglyceridemic effect of fish oil appears to be caused primarily by an inhibition of very low density lipoprotein-triglyceride synthesis, but an additional, independent effect upon VLDL catabolsim cannot be ruled out.—**Harris, W. S., W. E. Connor, D. R. Illingworth, D. W. Rothrock, and D. M. Foster.** Effects of fish oil on VLDL triglyceride kinetics in humans. *J. Lipid Res.* 1990. **31:** 1549–1558.

Supplementary key words fractional catabolic rate • n-3 fatty acids

The primary effect of dietary fish oils rich in n-3 fatty acids is to lower total plasma and very low density lipoprotein (VLDL) triglyceride levels (1–3). This could result from an inhibition of triglyceride secretion, an enhancement of triglyceride removal, or a combination of these effects. Liver perfusion studies (4) and a variety of studies in cultured cells (5–12) have supported the hypothesis that n-3 fatty acids inhibit the synthesis of triglyceride, thereby reducing the rate of secretion of VLDL triglyceride from the liver. The kinetics of VLDL triglyceride in humans given n-3 fatty acids have been reported in three earlier trials (13–15). However, these studies were limited by either the failure to give n-3 fatty acids during the actual kinetic studies, the lack of a control period, dietary inconsistencies among subjects, or by examining very small numbers of patients with rare types of secondary hyperlipidemia.

The purpose of the current study was to examine the effects of dietary n-3 fatty acids on VLDL triglyceride kinetics in a relatively larger number of individuals who were given fish oil during the turnover study. Subjects with widely varying baseline triglyceride levels were purposely chosen in order to determine whether the mechanism(s) of triglyceride lowering by fish oil was influenced by the magnitude of the hypertriglyceridemia.

METHODS

Subjects

Ten male subjects participated in this study (**Table 1**) which was conducted in the Clinical Research Center of the Oregon Health Sciences University. They were selected on the basis of having a wide range of fasting

Abbreviations: FAs, fatty acids; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; FCR, fractional catabolic rate; TG, triglyceride; P/S, polyunsaturated to saturated fatty acid ratio; IBW, ideal body weight; IDL, intermediate density lipoproteins.

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TABLE 1. Description of subjects

Subject	Age	Height	Weight	IBW[a]	Plasma Lipid Levels at Entry	
					Cholesterol	Triglyceride
	yr	*cm*	*kg*			*mg/dl*
1	30	181	76	73.6	169	34
2	24	177	68	71.9	169	77
3	37	180	77	72.3	142	91
4	35	174	79	69.5	222	170
5	60	160	69	62.7	370	670
6	57	174	99	69.5	670	4180
7	61	180	77	72.7	235	1145
8	59	188	117	81.4	320	1396
9	65	173	78	68.6	300	859
10	34	185	89	79.1	201	414

[a]IBW is ideal body weight based upon Metropolitan Life Insurance Co. Tables, 1983.

plasma triglyceride concentrations, from 34 to 4180 mg/dl at entry, so that the mechanism of action of dietary fish oils could be studied in subjects with greatly different pool sizes of plasma triglyceride. Two of the hypertriglyceridemic subjects were obese and had adult-onset diabetes (#6 and #8) for which they required stable doses of insulin. Informed consent was obtained from each participant. The study protocol had been approved by the Committee on Human Research of the Oregon Health Sciences University.

Protocol

This study utilized a randomized, controlled, crossover design. The experimental diets were consumed for a period of 3–5 weeks before the actual VLDL turnover procedure was conducted. This provided time for the plasma triglyceride levels to stabilize, particularly in the subjects with severe hypertriglyceridemia. Seven subjects consumed the control diet first followed by the fish oil diet, and in the remaining three the order was reversed. The subjects returned to their home diets for at least 1 month before beginning the next dietary phase.

Diets

To compare the effects of dietary n-3 fatty acids on VLDL triglyceride metabolism, liquid formula diets containing 20% fat, 65% carbohydrate, and 15% protein were fed during both the control and the fish oil dietary periods. Three bran muffins per day were given to provide fiber. Protein was derived from calcium caseinate and nonfat dry milk, and the carbohydrate sources were Moducal^{R} (Mead Johnson, Evansville, IN), nonfat dry milk (lactose), and sucrose. The two diets differed only in the type of fat they contained. The control diet contained a blend of cocoa butter and peanut oil (1:2) which was incorporated into the formulas as described in detail previously (16). The n-3 fatty acid diet provided protein and carbohydrate as a liquid formula, but the oil component (MaxEPA^{R}, R. P. Scherer, Clearwater, FL) was given separately in three divided doses daily. It was not mixed into the formulas because of its strong flavor. The fatty acid composition of the two diets is presented in **Table 2**. 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 while the latter provided a daily intake of 10–17 g fatty acids (about 5% of total calorie intake). Saturated fatty acid intakes were similar. The cholesterol intake of each subject in each of the two diets was identical (110 mg/1000 kcal) and averaged about 275 mg/day. Cholesterol was derived from dried egg yolk and the fish oil. Weights were maintained by adjusting calorie intake.

VLDL triglyceride kinetic studies

The protocol utilized for the turnover studies was described previously by Zech et al. (17) Briefly, after 1

TABLE 2. Dietary fat composition

Source	Control	Fish Oil
	Cocoa Butter–Peanut Oil (1:2)	MaxEPA[Ra]
Fatty acid composition[b]		
Saturated	30	32
Monounsaturated	46	30
Polyunsaturated	24	36
18:2 n-6	22	3
20:5 n-3	0	16
22:6 n-3	0	10
Total n-3 fatty acids (g/day)	0	10–17[c]

[a]R. P. Scherer Co. (Clearwater, FL).
[b]Fatty acids (% of total) were determined by gas chromatography.
[c]Depending on calorie intake.

36-h pre-feeding period (see below), 300 μCi of [^{3}H]glycerol in sterile saline solution was injected intravenously through an indwelling catheter. Blood samples were drawn immediately before the injection and at 0.25, 1, 2, 3, 4, 5, 6, 8, 10, 12 h and every 3 h thereafter for a total of 48 h. Blood was collected into EDTA tubes (1 mg/ml) and spun to obtain the plasma which was subsequently centrifuged at density 1.006 g/ml for 11.5 h at 50,000 rpm in a Beckman 50.3 fixed angle rotor. The top 1.2 ml of each 6.5-ml ultracentrifuge tube was removed by tube slicing and the VLDL fraction contained therein was transferred immediately to 20 ml of isopropyl alcohol. The solution was mixed, about 2 g of zeolite mixture was added to bind the phospholipids, and the supernatant was removed for measurement of VLDL triglyceride mass by the AutoAnalyzer II (Technicon Instruments, Tarrytown, NY) (18) and radioactivity by Packard TriCarb Scintillation Counter (Downers Grove, IL). The specific activity (dpm/mg) of the VLDL triglyceride was then calculated. The fraction of the injected dose per ml of plasma was derived from the specific activity after idealizing plasma VLDL triglyceride values according to Zech et al. (17). Both specific activity and fraction of injected dose were plotted over time and produced virtually identical kinetic parameters.

In previous studies of VLDL kinetics (17), the total caloric intake was reduced to 60% of the eucalorit level by selectively removing all dietary fat (40% of calories) without changing the absolute quantities of carbohydrate and protein ingested. In our study, the total calories were also reduced by 40% beginning 36 h before the injection of the [^{3}H]glycerol. However, the amount of fat, carbohydrate, and protein was equally reduced in order to retain some n-3 fatty acids in the diet. The formula was given in divided doses every 3 h around the clock until the end of the turnover protocol (36 h pre-study plus 48 h of turnover study; a total of 84 h), and on this regime each subject consumed about 4 g of fat (containing 1.2 g of n-3 fatty acids during the fish oil period) with each of the 3-h feedings. These intakes of n-3 fatty acids were still adequate to maintain reduced plasma triglyceride levels (1).

Our decision to retain some n-3 fatty acids in the feeding protocol was based upon two preliminary studies. In the first, we sought to determine whether and how rapidly plasma triglyceride levels would change when the fish oil was completely removed from the diet. For this study, subject #10 consumed the eucaloric fish oil diet up to 36 h before the [^{3}H]glycerol injection, and fish oil was then removed from his diet. Plasma triglyceride levels in this subject increased by almost 80% (from 133 to 230 mg/dl) over the 48-h period of the turnover study, indicating that plasma triglycerides were clearly not in a steady state during his VLDL turnover study. Other trials have also documented the very rapid changes that occur in lipoprotein metabolism when fish oil is removed from the diet (19, 20). The results from subject #10 indicated that, in order to maintain stable triglyceride levels during the VLDL turnover study, n-3 fatty acids needed to be taken throughout the period of the turnover study. This subject was unable to continue with the study for personal reasons, leaving nine subjects for whom complete kinetic data were available.

Since n-3 fatty acids had to be included in the turnover diet, control fats also had to be fed during the control period. Therefore, we conducted a second preliminary trial to investigate the effects of the added fat on VLDL triglyceride kinetics. Subject #1 was studied once while consuming our modified low-fat (control) formula and then re-studied on a fat-free formula as used by Zech et al. (17). Both studies were performed while the subject was consuming the control background diet. Since the decay curves in both studies were very similar (**Fig. 1**) we proceeded with the modified dietary protocol for the remaining eight subjects.

Data analysis

The VLDL triglyceride turnover curves were analyzed using the multicompartmental models proposed by Zech et al. (17). The model was used to estimate the residence time of triglyceride in the plasma VLDL density range. The reciprocal of this value is the fractional catabolic rate (FCR) for VLDL triglyceride. The synthetic (or production) rate of VLDL triglyceride was then calculated as the product of the FCR times the pool size divided by the ideal body weight (IBW) (21). Ideal body weights were calculated from the Metropolitan Life Insurance Co. 1983 table, and plasma volume was calculated as (0.045 × IBW) + (0.01 × excess kg over IBW) according to Grundy et al. (22). The size of the VLDL triglyceride pool was calculated from the plasma concentration and the plasma volume.

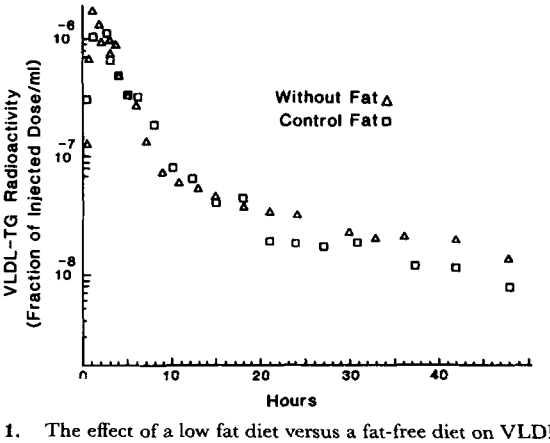

Fig. 1. The effect of a low fat diet versus a fat-free diet on VLDL triglyceride turnover curves. Subject #1 was studied once while receiving a low-fat diet (□) which provided about 4 g of fish oil every 3 h for 48 h, and once while consuming a fat-free diet (△). [^{3}H]Glycerol was injected at time zero and the radioactivity appearing in the VLDL triglyceride fraction was plotted over time.

The Zech model is characterized by two synthetic pathways for VLDL triglyceride from plasma glycerol, the so-called "fast" and "slow" pathways. Since the plasma glycerol pool size was not measured in these studies, no inferences can be drawn vis-à-vis the actual contribution that plasma glycerol made to VLDL triglyceride synthesis. We could, however, compare the relative ratios of flux through the fast and slow pathways to see whether the dietary intervention produced a relative change in either of these pathways. This was achieved by analyzing each of the two studies (fish oil and control) in a given individual simultaneously.

Statistical analysis

Because all subjects served as their own controls, the Student's *t*-test for paired observations was carried out according to Winer (23).

RESULTS

Plasma lipids and lipoproteins

The institution of the low fat, low cholesterol control diet caused plasma lipid levels to decrease substantially from entry values (compare Tables 1 and 3). This was particularly evident in patient #6 whose massive chylomicronemia resolved on the control diet leading to marked decreases in both cholesterol and triglyceride levels.

The isocaloric substitution of fish oil for the control vegetable fat produced significant reductions in the total and lipoprotein lipid levels in all ten subjects. Individual data for each subject is given in **Table 3**. Mean cholesterol levels for all ten subjects fell from 195 to 144 mg/dl (− 26%, $P<0.001$) and triglyceride levels were reduced from 442 to 150 mg/dl (− 66%, $P<0.025$). Decreases in total plasma cholesterol and triglyceride levels resulted

TABLE 3. Influence of dietary omega fatty acids upon plasma lipids and lipoproteins

		Cholesterol				Triglyceride	
Subject	Diet	Total	VLDL	LDL	HDL	Total	VLDL
		mg/dl				*mg/dl*	
1	Control	151	12	127	38	83	36
	Fish oil	129	<5	102	31	43	12
2	Control	151	9	108	33	82	49
	Fish oil	140	<5	92	49	42	16
3	Control	150	20	82	46	141	101
	Fish oil	118	<5	75	41	59	11
4	Control	157	41	94	24	326	293
	Fish oil	119	22	85	20	153	113
5	Control	238	78	133	33	383	337
	Fish oil	229	17	204	22	113	43
6	Control	216	97	100	20	616	545
	Fish oil	168	28	133	11	211	129
7	Control	212	117	76	28	714	644
	Fish oil	129	13	95	17	163	49
8	Control	220	108	85	24	745	698
	Fish oil	190	35	153	9	272	196
9	Control	274	223	35	33	1002	984
	Fish oil	129	57	56	13	312	225
10	Control	177	124	33	26	328	294
	Fish oil	92	28	31	22	133	74
Mean ± SD	Control	195 ± 44	83 ± 66	87 ± 34	31 ± 8	442 ± 314	398 ± 313
	Fish oil	144 ± 40	21 ± 17	103 ± 50	24 ± 13	150 ± 93	87 ± 77
Percentage change		− 26	− 75	+ 18	− 23	− 66	− 87
P value		0.001	0.005	NS	0.005	0.025	0.005

Values were determined 1-4 days before the VLDL turnover study began.

almost entirely from reductions in VLDL cholesterol (83 to 21 mg/dl), and VLDL triglyceride (398 to 87 mg/dl) ($P<0.005$, both). LDL cholesterol levels increased, but not significantly, whereas HDL cholesterol concentrations fell from 31 to 24 mg/dl ($P<0.005$).

VLDL triglyceride kinetics

The dietary regimes used during the turnover studies (60% of eucaloric levels) produced stable levels of plasma triglycerides. The only exception to this was a small mean decrease (370 to 311 mg/dl, $P<0.05$) which occurred between 0 and 12 h during the control diet. Between 12 and 48 h, triglyceride levels remained stable.

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 4**). This was accompanied by a significant rise in the cholesterol/triglyceride ratio in VLDL during the fish oil period (0.18 to 0.25, $P<0.05$) suggesting the presence of smaller particles. There was a 45% reduction in both the VLDL triglyceride synthetic rate (23 to 12.6 mg/h per kg IBW, $P<0.05$) and the residence time of triglycerides in the VLDL density fraction (5.8 to 3.2 h, $P<0.005$). The reciprocal of the residence time is the FCR which was increased by 65% (0.23 to 0.38 h^{-1}, $P<0.005$). The ratio of the fast to the slow synthetic pathways did not change with fish oil feeding. The same trends in each kinetic parameter were seen in both normal and hypertriglyceridemic subjects (**Table 5**). Mean triglyceride levels decreased by 69% and 72%, respectively, whereas VLDL triglyceride synthetic rates were reduced by 48% and 45% and the FCR for VLDL triglyceride increased 74% and 76%, respectively. Representative decay curves for labeled VLDL triglyceride for one patient (#7) on the control and fish oil enriched diets are shown in **Fig. 2**.

DISCUSSION

The object of this present investigation was to define the mechanism(s) responsible for the marked reduction in VLDL levels that occur during consumption of diets rich in n-3 fatty acids. In this study, n-3 fatty acids appeared to have two actions: a reduction in the rate at which triglycerides enter the VLDL density fraction and an increase in their fractional removal rate from that fraction. This dual effect was responsible for the remarkably rapid and extensive hypotriglyceridemic action which reduced plasma VLDL triglyceride levels by 72% in this trial and to similar extents in previous studies (1, 24–28). Accompanying the marked fall in VLDL levels was a tendency toward higher LDL cholesterol levels, as has been observed in other fish oil trials with hypotriglyceridemic patients (1), and in studies with hypotriglyceridemic drugs such as gemfibrozil (29). The increase was observed only in those patients with triglyceride levels over 350 mg/dl; LDL levels tended to decrease in those subjects with more normal triglyceride levels, confirming earlier observations (1). Although the mechanism of the LDL raising effect is not known, fish oil may enhance the conversion of VLDL to LDL in hypertriglyceridemic patients (30). Alternatively, fish oil treatment may have increased levels of the intermediate density lipoproteins (VLDL remnants) that would have been isolated with LDL in this study.

The inhibition of VLDL triglyceride synthesis by n-3 fatty acids receives support from several other studies. Nestel et al. (13) reported that dietary n-3 fatty acids lowered the secretion rates of both VLDL apolipoprotein B and VLDL triglyceride in humans. Carbohydrate-induced hypertriglyceridemia can be completely inhibited by the inclusion of n-3 fatty acids in the diet, again suggesting inhibition of VLDL triglyceride synthesis (19). Sanders et al. (14) found that 15 ml of fish oil given daily to four hyperlipidemic patients produced a significant decrease in VLDL triglyceride synthesis. Finally, the effects of fish oil supplementation on VLDL triglyceride kinetics in two patients with the rare disease lipodystrophic diabetes mellitus were recently reported by Stacpoole et al. (15). In one patient, the 20% fish oil diet lowered triglyceride levels from 2,835 to 1,576 mg/dl. This reduction was associated with a 50% reduction in synthetic rates and no change in FCR. Neither triglyceride levels nor kinetic parameters were affected by fish oil therapy in the other patient. In our study, all patients responded with changes in synthesis and FCR.

In primary cultures of isolated rabbit (7) and rat (5) hepatocytes, eicosapentaenoic acid specifically inhibited triglyceride synthesis, perhaps via inhibition of diacylglycerol acyltransferase (6) or phosphatidate phosphohydrolase (8). Perfused liver studies by Wong et al. (4) demonstrated that fish oil-fed rats produced less VLDL triglyceride and more ketones than control-fed animals. N-3 fatty acids from shellfish were found in inhibit fatty acid synthesis in rat livers (9) and in isolated rat hepatocytes (10). Further studies revealed that the mass of lipogenic enzymes (not their activity) appeared to be reduced (11). This was recently corroborated by Clarke and Armstrong (12) who reported that n-3 fatty acids reduced levels of mRNA for acetyl-CoA carboxylase in rat liver. Thus, evidence is available to support the view that n-3 fatty acids inhibit the synthesis of both fatty acids and triglycerides in the liver.

In addition to the decreased synthetic rates for VLDL triglyceride, we also found that fractional catabolic rates were significantly increased in every subject during the fish oil diet. Thus, the average triglyceride molecule remained in the plasma VLDL fraction for a shorter period of time during the fish oil feeding phase than during the control phase. Nestel et al. (13) also reported small increases in the FCR for VLDL triglyceride in two of three

TABLE 4. Effects of dietary fish oil on VLDL metabolism

Subject	Diet	Plasma Volume (dl)	VLDL Lipids TG[a] (mg/dl)	VLDL Lipids Chol/TG (ratio)	VLDL-TG Pool Size (g)	VLDL-Triglyceride Synthesis (mg/h)	VLDL-Triglyceride Synthesis (mg/kgIBW)	VLDL-TG FCR (h^{-1})	Residence time of VLDL-TG in Plasma (h)	Fast/Slow Synthetic Pathway (ratio)
1	Control	33.4	38	[b]	1.27	577	7.8	0.455	2.2	2.26
	Fish oil		14	[b]	0.47	275	3.7	0.588	1.7	1.02
2	Control	32.0	63	[b]	2.02	671	9.3	0.333	3.0	1.41
	Fish oil		26	[b]	0.83	347	4.8	0.417	2.4	2.58
3	Control	33.0	101	[b]	3.33	813	11.2	0.244	4.1	1.44
	Fish oil		22	[b]	0.73	454	6.3	0.625	1.6	1.33
4	Contol	32.2	144	0.14	4.64	1299	18.5	0.278	3.6	2.52
	Fish oil		59	0.20	1.90	826	11.9	0.435	2.3	0.66
5	Control	28.9	214	0.23	6.19	1286	20.5	0.208	4.8	3.67
	Fish oil		107	0.40	3.09	909	14.5	0.294	3.4	5.0
6	Control	37.6	378	0.18	14.21	2032	25.7	0.143	7.0	2.5
	Fish oil		91	0.22	3.42	1130	14.3	0.303	3.3	3.6
7	Control	33.2	574	0.18	19.06	3888	53.5	0.204	4.9	1.78
	Fish oil		150	0.27	4.98	2077	28.6	0.417	2.4	2.41
8	Control	40.2	704	0.16	28.30	2575	31.6	0.091	11.0	2.0
	Fish oil		198	0.18	7.96	1345	16.5	0.169	5.9	2.64
9	Control	31.8	750	0.23	23.85	2027	29.6	0.085	11.7	3.34
	Fish oil		158	0.25	5.02	899	13.1	0.179	5.6	2.4
Mean ± SD	Control		330 ± 282	0.18 ± 0.3	11.4 ± 10.2	1685 ± 1073	23.0 ± 14.3	0.23 ± 0.12	5.8 ± 3.4	2.3 ± 0.79
	Fish oil		92 ± 67	0.25 ± 0.08	3.2 ± 2.5	918 ± 563	12.6 ± 7.5	0.38 ± 0.16	3.2 ± 1.6	2.4 ± 1.34
P value			<0.005	<0.05	<0.025	<0.005	<0.005	<0.005	<0.005	NS
Percent change			−72%	+39%	−72%	−46%	−45%	+65%	−45%	+3%
Normal values (n = 13) (16)			113 ± 10	0.21 ± 0.01		806 ± 123	11.5 ± 1.8	0.19 ± 0.01	5.2 ± 0.38	2.7 ± 0.36

[a]Represents the average VLDL-TG concentration during the 48-h turnover study.
[b]Values of VLDL cholesterol were too low to measure accurately.

TABLE 5. Effects of dietary fish oil on VLDL triglyceride (TG) kinetics: normals versus hypertriglyceridemic patients[a]

VLDL Parameter	Normal (n = 3)			Hypertriglyceridemic (n = 6)		
	Control	Fish Oil	% Change	Control	Fish Oil	% Change
Triglyceride (mg/dl)	67 ± 32	21 ± 6	−69	461 ± 254	127 ± 51	−72
TG pool size (g)	2.2 ± 1.04	0.68 ± 0.2	−69	16.04 ± 9.5	4.4 ± 2.11	−73
TG synthetic rate (mg/h)	687 ± 119	359 ± 90	−48	2185 ± 969	1198 ± 471	−45
TG synthetic rate (mg/h/kg IBW)	9.4 ± 1.7	4.9 ± 1.3	−48	29.9 ± 12.6	16.5 ± 6.1	−45
TG FCR (h^{-1})	0.31 ± 0.11	0.54 ± 0.11	+74	0.17 ± 0.08	0.3 ± 0.11	+76
TG residence time (h)	5.4 ± 1.5	3.3 ± 0.6	−39	12.2 ± 5.8	6.5 ± 2.7	−47
Ratio of fast/slow synthetic pathways	1.7 ± 0.5	1.6 ± 0.8	−6	2.6 ± 0.7	2.8 ± 1.4	+8

[a]Subjects 1, 2, and 3 were considered normal; 4–9 were considered hypertriglyceridemic.
Values were determined as average TG values over the 48 h of the VLDL turnover study.

normolipidemic subjects and in both of the hypertriglyceridemic patients they studied. FCRs for VLDL apoprotein B were increased in the normolipidemic subjects, but not in the two hyperlipidemic patients. In contrast, Sanders et al. (14) reported no mean change in the FCR for VLDL triglyceride in four hypertriglyceridemic patients treated with 15 g of fish oil per day.

The difference between the results of these earlier trials and those noted in the present study may be attributed to several factors. In the present study, carefully controlled, liquid formula diets were fed, and all patients were studied on both dietary regimens in a randomized, crossover design. Perhaps most importantly, n-3 fatty acids were included in the diet during the turnover study in the present trial, but not in the others (13, 14). Once these agents are removed from the diet, triglyceride metabolism changes rapidly with plasma levels beginning to rise within 24 h (19, 20). Thus, even when plasma triglyceride levels were reported to be stable in previous studies, it is possible that hepatic triglyceride metabolism was in a nonsteady state.

Several factors may potentially contribute to the increase in FCR for VLDL-TG observed when the study subjects were on the fish oil-enriched diet. The increase was greatest in those subjects with lower triglyceride concentrations and was less pronounced in the patients with pre-existent hypertriglyceridemia (**Fig. 3**); this suggests that decreases in VLDL pool size (particularly at concentrations that are below those needed to saturate physiological removal mechanisms) per se may contribute to the increase in FCR.

The FCR increase could have resulted from enhanced clearance mechanisms (e.g., stimulation of lipoprotein lipase activity), or it could have been secondary to the secretion of smaller VLDL particles that would spend less time in the VLDL density fraction. With respect to the first possibility, we (31) and others (32) have shown that in vitro postheparin lipolytic activity was unaffected by fish oil consumption. In the rat, triglyceride-rich lipoproteins carrying fish oil were not hydrolyzed more quickly in vitro (33), nor were they removed more rapidly from the circulation in vivo than control particles (34). Nevertheless, Weintraub et al. (32) reported that chylomicrons isolated from volunteers after an oral fish (or vegetable) oil load were hydrolyzed by bovine lipoprotein lipase more rapidly than chylomicrons containing saturated fats. The significance of this in vitro observation is unclear, however, since an oral load of fish oil produces the same fat tolerance curves as control fats (31). Thus, although it appears unlikely that the presence of n-3 fatty acids in the VLDL triglycerides enhances their lipolysis in vivo, this possibility deserves further study.

The second potential explanation for the increased FCR in the fish oil phase is that fish oil causes the secretion of smaller, triglyceride-poor VLDL particles. The increase in the VLDL cholesterol-to-triglyceride ratio suggests that the fish oil diet was associated with smaller VLDL particles as also reported by Nestel et al. (13), Sanders et al. (14), and Inagaki and Harris (35). Such particles could leave the VLDL density range more rapid-

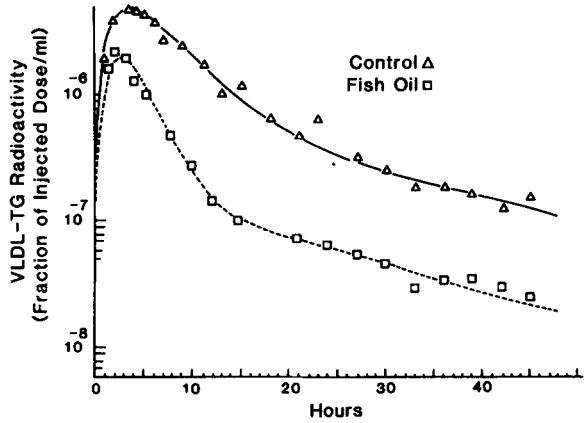

Fig. 2. VLDL triglyceride turnover curves for subject #7 during the control (△) and fish oil (□) diets. At time zero, 300 μCi of [^{3}H]glycerol was injected and the radioactivity subsequently isolated in the VLDL fraction was plotted over 48 h. The curves were constructed using the SAAM computer program.

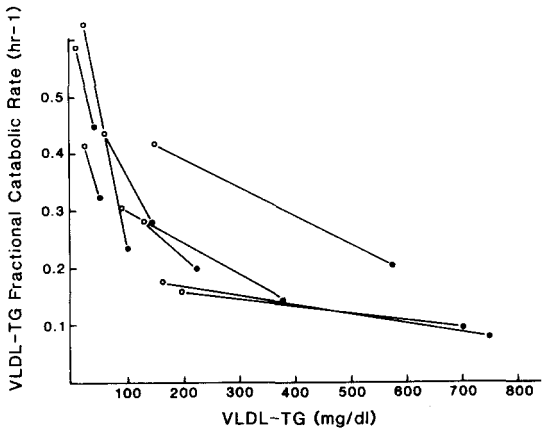

Fig. 3. Individual VLDL triglyceride levels and fractional catabolic rates before (●) and after (○) fish oil treatment.

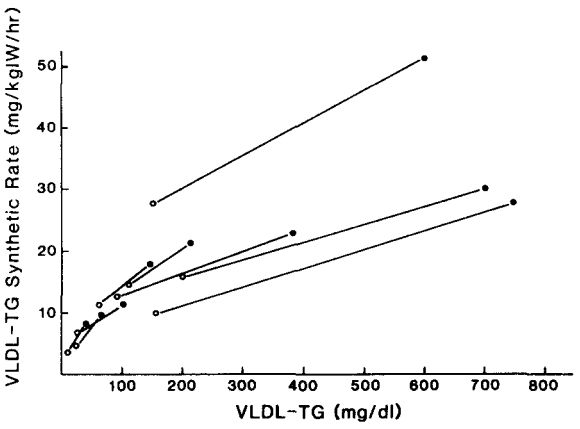

Fig. 5. Individual VLDL triglyceride levels and synthetic rates before (●) and after (○) fish oil treatment.

ly than larger particles, even with an unchanged lipolytic activity. The FCR is calculated as the reciprocal of the residence time of triglyceride in the VLDL fraction. If the size of a newly secreted VLDL particle is reduced by an intervention, then the amount of time that that particle would spend in the VLDL density fraction would likely be shorter than the time a larger particle would spend in that fraction (see **Fig. 4**). If this were the case, then the kinetics of one spectrum of VLDL particles was measured during the control phase while that of another spectrum was measured in the fish oil phase. This may have contributed to the decreased residence time, and thus increased the FCR.

Further support for the secretion of smaller VLDL particles comes from the finding the LDL levels tended to rise in our subjects during the fish oil phase. If smaller VLDL particles were being made, then it is possible that some of the these might float in the LDL density range (especially when LDL includes IDL as in this study). The apparent "independent" secretion of LDL in some fish oil-treated subjects was interpreted as the secretion of triglyceride-poor VLDL (13). Alternatively, smaller VLDL may be more readily converted into LDL than large VLDL (36). Huff and Telford (30) reported such an effect in miniature pigs fed fish oil.

Curves depicting how the VLDL-triglyceride pool size of each subject changed with production rate and FCR are given in **Fig. 5** and Fig. 3, respectively. These curves support the contention of Grundy and Vega (37) that there is a unique relationship between production rate and pool size for each individual, with some people efficiently shutting off production at high pool sizes and others less able to do this. It would appear that the hypertriglyceridemic patients experienced a smaller increase in FCR than did those with lower levels. The mean change in FCR for the four subjects with the highest triglyceride levels was 0.139; the four subjects with lowest levels, the FCR changes an average of 0.189.

An examination of the relationship between the change in plasma triglyceride pool size and the changes in synthetic rates (**Fig. 6**) and in FCR (**Fig. 7**) is revealing. There was a strong, positive correlation ($r = 0.83$; $P < 0.01$) between the change in synthetic rates and pool sizes,

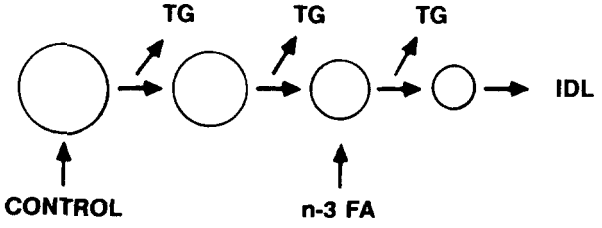

Fig. 4. The potential effects of n-3 fatty acids on VLDL particle size and the subsequent point of entry into the VLDL delipidation chain. Smaller VLDL particles would, of necessity, spend less time in the VLDL density range, thus producing shorter residence times. Since the FCR is the reciprocal of the residence time, the FCR would increase despite no change in the activity of lipolytic enzymes.

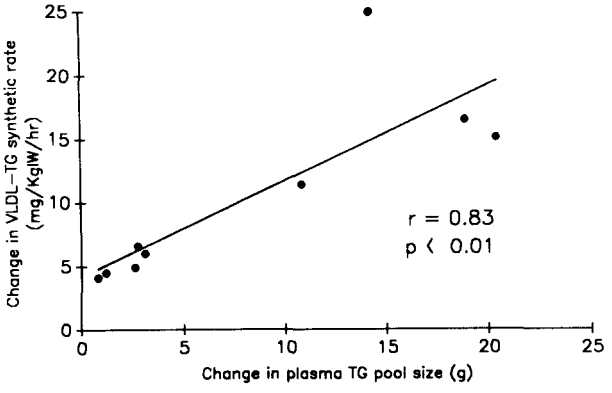

Fig. 6. The relationship between the change in VLDL triglyceride synthetic rate and pool size (n = 9). Both changes were calculated by subtracting the value on the fish oil diet from the control value (Table 4).

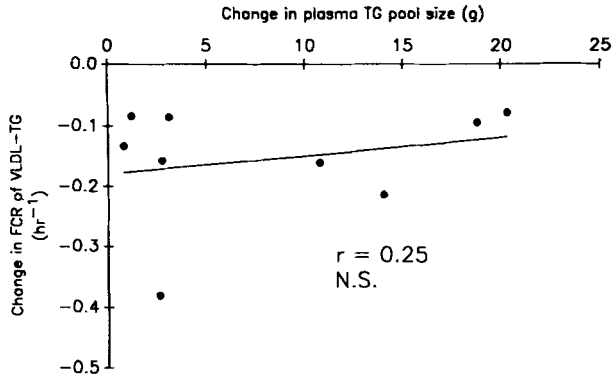

Fig. 7. The relationship between the change in VLDL triglyceride FCR and pool size (n = 9). Both changes were calculated by subtracting the value on the fish oil diet from the control value (Table 4).

but there was no correlation between changes in FCR and pool size (r = 0.24, NS). Thus, these data support the hypothesis that the primary effect of n-3 fatty acids was on triglyceride synthesis and not on catabolism.

HDL cholesterol levels were reduced by 23% in the fish oil period in this trial. This has been reported previously (19, 27, 38, 39), but it is not a consistent finding (1). The mechanism responsible for this reduction is unknown, but we speculate it may result from the decreased flux of triglyceride-rich lipoproteins. In support of this explanation, it has recently been shown that a diet rich in n-3 fatty acids produced significantly lower post-prandial chylomicron levels (31, 32). Because HDL arises, at least in part, from the surface components of triglyceride-rich lipoproteins (40), a reduction in triglyceride flux through both chylomicrons and VLDL may have contributed to the reduction in HDL cholesterol levels. The effects of fish oil on HDL metabolism will require further study, especially in view of the fact that when moderate doses of fish oils have been given, HDL cholesterol levels have been reported to increase (1, 15, 25, 26, 41, 42).

In conclusion, the reduction in VLDL triglyceride levels that occurred with the feeding of n-3 fatty acids to human subjects was mediated primarily by a reduction in VLDL-TG synthesis. This mechanism appeared to operate in all subjects regardless of baseline plasma triglyceride levels, age, or body weight. Nevertheless, an effect of fish oil on VLDL triglyceride removal rates cannot be excluded from these data.

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REFERENCES

1. Harris, W. S. 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *J. Lipid Res.* **30:** 785–807.
2. Goodnight, S. H., W. S. Harris, W. E. Connor, and D. R. Illingworth. 1982. Polyunsaturated fatty acids, hyperlipidemia and thrombosis. *Arteriosclerosis.* **2:** 87–113.
3. Phillipson, B. E., D. R. Rothrock, W. E. Connor, W. S. Harris, and D. R. Illingworth. 1985. Reductions of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N. Engl. J. Med.* **312:** 1210–1216.
4. Wong, S. H., P. J. Nestel, R. P. Trimble, G. B. Storer, R. J. Illman, and D. L. Topping. 1984. The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. *Biochim. Biophys. Acta.* **792:** 103–109.
5. Nossen, J. O., A. C. Rustan, S. H. Gloppestad, S. Malbakken, and C. A. Drevon. 1986. Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. *Biochim. Biophys. Acta.* **879:** 56–65.
6. Rustan, A. C., J.Ø. Nossen, E. N. Christiansen, and C. A. Drevon. 1988. EPA reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. *J. Lipid Res.* **29:** 1417–1426.
7. Benner, K. C., W. E. Connor, and A. Sasaki. 1985. Omega-3 fatty acid suppression of triglyceride synthesis and secretion by isolated rabbit hepatocytes. *Clin. Res.* **33:** 567A.
8. Marsh, J. B., D. L. Topping, and P. J. Nestel. 1987. Comparative effects of fish oil and carbohydrate on plasma lipids and hepatic activites of phosphatidate phosphohydrolase, diacylglycerol acyltransferase and neutral lipase activities in the rat. *Biochim. Biophys. Acta.* **922:** 239–243.
9. Iritani, N., K. Inoguchi, M. Endo, E. Fukuda, and M. Morita. 1980. Identification of shellfish fatty acids and their effects on lipogenic enzymes. *Biochim. Biophys. Acta.* **618:** 378–382.
10. Yang, Y. T., and M. A. Williams. 1978. Comparison of C-18, C-20, and C-22 unsaturated fatty acids in reducing fatty acid synthesis in isolated rat hepatocytes. *Biochim. Biophys. Acta.* **531:** 133–140.
11. Iritani, N., E. Fukuda, K. Inoguchi, M. Tsubosaka, and S. Tashiro. 1980. Reduction of lipogenic enzymes by shellfish triglycerides in rat liver. *J. Nutr.* **110:** 1664–1670.
12. Clarke, S. D., and M. K. Armstrong. 1988. Suppression of rat liver fatty acid synthetase mRNA level by dietary fish oil. *FASEB J.* **2:** A852.
13. Nestel, P. J., W. E. Connor, M. R. Reardon, S. Connor, S. Wong, and R. Boston. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin. Invest.* **74:** 72–89.
14. Sanders, T. A. B., D. R. Sullivan, J. Reeve, and G. R. Thompson. 1985. Triglyceride-lowering effect of marine

polyunsaturates in patients with hypertriglyceridemia. *Arteriosclerosis.* **5:** 459–465.
15. Stacpoole, P. W., J. Alig, L. L. Kilgore, C. M. Ayala, P. N. Herbert, L. A. Zech, and W. R. Fisher. 1988. Lipodystrophic diabetes mellitus. Investigations of lipoprotein metabolism and the effects of omega-3 fatty acid administration in two patients. *Metabolism.* **37:** 944–951.
16. Harris, W. S., and W. E. Connor. 1980. The effects of salmon oil upon plasma lipids, lipoproteins and triglyceride clearance. *Trans. Assoc. Am. Physicians.* **43:** 148–154.
17. Zech, L., S. M. Grundy, D. Steinberg, and M. Berman. 1979. Kinetic model for production and metabolism of very low density lipoprotein triglycerides: evidence for a slow production pathway and results for normolipidemic subjects. *J. Clin. Invest.* **63:** 1262–1273.
18. Manual of Laboratory Operations, Lipid Research Clinics Program. 1974. Vol. 1. Lipid and Lipoprotein Analysis. DHEW Publications No. (NIH) 75-628.
19. Harris, W. S., W. E. Connor, S. B. Inkeles, and D. R. Illingworth. 1984. Omega-3 fatty acids prevent carbohydrate-induced hypertriglyceridemia. *Metabolism.* **33:** 1016–1019.
20. Sztern, M., C. A. Dujovne, and W. S. Harris. 1989. Acute effects of fish oil on plasma triglyceride and low density lipoprotein levels. *Arteriosclerosis.* **9:** 766a.
21. Grundy, S. M., Y. H. I. Mok, L. Zech, D. Steinberg, and M. Berman. 1979. Transport of very low density lipoprotein triglycerides in varying degrees of obesity and hypertriglyceridemia. *J. Clin. Invest.* **63:** 1274–1283.
22. Grundy, S. M., Y. H. I. Mok, L. Zech, and M. Berman. 1981. Influence of nicotinic acid on metabolism of cholesterol and triglycerides in man. *J. Lipid Res.* **22:** 24–36.
23. Winer, I. D. 1971. Statistical Principles of Experimental Design. Second edition. McGraw-Hill, NY.
24. Boberg, M., B. Vessby, and I. Selinus. 1986. Effects of dietary supplementation with n-6 and n-3 long-chain polyunsaturated fatty acids on serum lipoproteins and platelet function in hypertriglyceridemic patients. *Acta Med. Scand.* **220:** 153–160.
25. Harris, W. S., C. A. Dujovne, M. L. Zucker, and B. E. Johnson. 1988. Effects of a low saturated fat, low cholesterol fish oil supplement in hypertriglyceridemic patients. *Ann. Int. Med.* **109:** 465–470.
26. Sullivan, D. R., T. A. B. Sanders, I. M. Trayner, and G. R. Thompson. 1986. Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. *Atherosclerosis.* **61:** 129–134.
27. Phillipson, B. E., D. R. Rothrock, W. E. Connor, W. S. Harris, and D. R. Illingworth. 1985. Reductions of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N. Engl. J. Med.* **312:** 1210–1216.
28. Demke, D. M., G. R. Peters, O. I. Linet, C. M. Metzler, and K. A. Klott. 1988. Effects of a fish oil concentrate in patients with hypercholesterolemia. *Atherosclerosis.* **70:** 73–80.
29. Manninen, V., M. O. Elo, M. H. Frick, K. Haapa, O. P. Heinonen, P. Heinsalmi, P. Helo, J. K. Huttunen, P. Kaitaniemi, P. Koskinen, et al. 1988. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. *JAMA.* **260:** 641–651.
30. Huff, M. W., and D. E. Telford. 1989. Dietary fish oil increases conversion of very low density lipoprotein apoprotein B to low density lipoprotein. *Arteriosclerosis.* **9:** 58–66.
31. Harris, W S., W. E Connor, N. Alam, and D. R. Illingworth. 1988. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. *J. Lipid Res.* **29:** 1451–1460.
32. Weintraub, M. S., R. Zechner, A. Brown, S. Eisenberg, and J. L. Breslow. 1988. Dietary polyunsaturated fats of the ω-6 and ω-3 series reduce postprandial lipoprotein levels. *J. Clin. Invest.* **82:** 1884–1893.
33. Chen, I. S., A. J. Sheppard, M. M. Cassidy, and G. V. Vahouny. 1987. In vitro clearance of chylomicron triglycerides containing (ω-3) eicosapentaenoate. *Atherosclerosis.* **65:** 193–198.
34. Chen, I. S., T. Le, S. Subramaniam, M. M. Cassidy, A. J. Sheppard, and G.V. Vahouny. 1987. Comparison of the clearances of serum chylomicron triglycerides enriched with eicosapentaenoic acid or oleic acid. *Lipids.* **22:** 318–321.
35. Inagaki, M., and W. S. Harris. 1990. Changes in lipoprotein composition in hypertriglyceridemic patients taking cholesterol-free fish oil supplements. *Atherosclerosis.* **82:** 237–246.
36. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and C. J. Shepherd. 1984. Metabolism of apolipoprotein B in large, triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
37. Grundy, S. M., and G. L. Vega. 1982. Are plasma triglyceride concentrations explained by saturation kinetics? *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 271–286.
38. Illingworth, D. R., W. S. Harris, and W. E. Connor. 1984. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in man. *Arteriosclerosis.* **4:** 270–275.
39. Nestel, P. J. 1986. Fish oil attenuates the cholesterol-induced rise in lipoprotein cholesterol. *Am. J. Clin. Nutr.* **43:** 752–757.
40. Patsch, J. R., J. B. Karlin, L. W. Scott, L. C. Smith, and A. M. Gotto. 1983. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of post-prandial lipemia. *Proc. Natl. Acad. Sci. USA.* **80:** 1449–1453.
41. Bruckner, G., P. Webb, L. Greenwell, C. Chow, and D. Richardson. 1987. Fish oil increases peripheral capillary blood cell velocity in humans. *Atherosclerosis.* **66:** 237–245.
42. Sanders, T. A. B., and F. Roshanai. 1983. The influence of different types of ω-3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clin. Sci.* **64:** 91–99.