

# Dietary Polyunsaturated Fats of the W-6 and W-3 Series Reduce Postprandial Lipoprotein Levels

## Chronic and Acute Effects of Fat Saturation on Postprandial Lipoprotein Metabolism

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### Abstract

The chronic and acute effects of different types of dietary fat on postprandial lipoprotein metabolism were studied in eight normolipidemic subjects. Each person was placed for 25 d on each of three isocaloric diets: a saturated fat (SFA), a w-6 polyunsaturated fat (w-6 PUFA) and a w-3 polyunsaturated fat (w-3 PUFA) diet. Two vitamin A-fat loading tests were done on each diet. The concentrations in total plasma and chylomicron ( $S_f > 1,000$ ) and nonchylomicron ( $S_f < 1,000$ ) fractions of retinyl palmitate (RP) were measured for 12 h postprandially. Compared with the SFA diet, the w-6 PUFA diet reduced chylomicron and nonchylomicron RP levels 56 and 38%, respectively, and the w-3 PUFA diet reduced these levels 67 and 53%, respectively. On further analysis, the main determinant of postprandial lipoprotein levels was the type of fat that was chronically fed, which appeared to mediate its effect by changing the concentration of the endogenous competitor for the system that catabolizes triglyceride-rich lipoproteins. However, there was a significant effect of the acute dietary fat load, which appeared to be due to a differential susceptibility to lipolysis of chylomicrons produced by SFA as opposed to PUFA fat loads. The levels of postprandial lipoproteins are determined by the interaction of these chronic and acute effects.

### Introduction

Epidemiological evidence suggests that the replacement of saturated fatty acids (SFA)<sup>1</sup> in the diet by polyunsaturated fatty acids (PUFA) reduces the incidence of coronary heart disease (1–3). Recently, studies of Greenland Eskimos (4, 5) and men from the Netherlands (6) indicated that polyunsaturates of the w-3 fatty acid series, which are enriched in some types of fish oil, may be even more beneficial than polyunsaturates of the w-6 fatty acid series, commonly found in vegetable oils. Isoca-

loric substitution of w-6 PUFA for SFA has been shown to lower total and LDL cholesterol levels (7–10), but effects on triglyceride, VLDL, and HDL cholesterol levels have been inconsistent (8–13). The epidemiological studies mentioned above have stimulated studies of the effects of w-3 PUFA on fasting plasma lipid and lipoprotein levels. The w-3 PUFA when substituted for SFA also lower LDL cholesterol. However, in normolipidemic and especially in hypertriglyceridemic subjects, fish oil enriched in w-3 PUFA substantially lowers triglyceride and VLDL cholesterol levels (14–20).

Thus far, most studies have dealt with the effects of dietary fat on fasting lipid and lipoprotein levels. However, most of our lives are spent in the postprandial state, during which time the vessel wall is exposed to postprandial lipoproteins. It has been suggested by Zilversmit that these lipoproteins may be particularly atherogenic, as they are metabolized on the endothelial surface of large arteries and their cholesterol becomes incorporated into the artery wall, where it may stimulate formation of atherosclerotic lesions (21–23). In type III hyperlipoproteinemia, accumulation of postprandial lipoproteins, due to delayed clearance, is associated with severe premature atherosclerotic disease (24, 25). Therefore, the effects of dietary fat on coronary heart disease could be exerted not only by altering fasting lipoprotein levels, but also by influencing postprandial lipoprotein levels.

This study was designed to determine the chronic and acute effects of dietary SFA, w-6 and w-3 PUFA on postprandial lipoprotein levels. To measure these levels, we have used the vitamin A-fat loading test, which specifically labels intestinally derived lipoproteins, chylomicrons and their remnants, with retinyl palmitate (26–33). Once secreted into plasma, chylomicron metabolism involves a two-step process that includes hydrolyzing the triglycerides of the newly formed particles by lipoprotein lipase (34–36) and, subsequently, liver uptake of the chylomicron remnant (37, 38) by receptor-mediated recognition of apolipoprotein E on the particle's surface (39–43). As we have shown in our previous studies (32, 33), the vitamin A-fat loading test can be used to follow both chylomicron and chylomicron remnant metabolism. Our results indicate that the substitution of w-6 and even more so w-3 PUFA for SFA in the diet dramatically reduces postprandial lipoprotein levels. The major effect was due to the type of fat that was chronically fed, but a significant effect of the acute fat load was also found.

### Methods

**Subjects.** Eight healthy, normolipidemic adult men were admitted to the general Clinical Research Center at The Rockefeller University in New York. Their ages ranged from 19 to 37 yr. The subjects' characteristics, including age, sex, apo E phenotype, height, weight, body

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1. Abbreviations used in this paper: CHM, chloroform/heptane/methane solution; HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; PUFA, polyunsaturated fat; RP, retinyl palmitate; SFA, saturated fat.

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Table I. Subject Characteristics

Subjects	Apo E					Fasting lipids and lipoproteins				
	Age	Phenotype	Weight	Height	Body mass index	TC	TG	VLDL-C	LDL-C	HDL-C
	yr		kg	cm	kg/m <sup>2</sup>			mg/dl		
1	30	3/3	61.8	172	20.9	149	51	12	78	59
2	37	3/3	86.7	178	27.3	228	96	31	153	44
3	22	3/3	81.3	181	24.6	150	93	21	76	53
4	33	3/2	77.0	175	24.8	161	83	26	89	46
5	22	3/3	77.0	171	26.6	185	69	20	123	42
6	31	3/3	68.3	169	23.9	228	100	32	146	50
7	19	3/3	75.6	176	24.4	166	68	14	118	34
8	31	3/3	64.9	170	22.4	162	60	29	92	41
Mean	28		74.1	174	23.3	179	78	23	109	46
SD	6		8	4	2	32	18	7	30	8

mass index, and ad lib. lipid and lipoprotein levels, are listed in Table I. They had no evidence of cardiac, hepatic, renal, or endocrine diseases and were on no medication.

**Study design.** The study design is shown in Fig. 1 A. The subjects were put on three different metabolic isocaloric diets: a SFA diet, a w-6 PUFA diet, and a fish oil-enriched diet called the w-3 PUFA diet. Each diet was fed for 25 d. There was a 5-7-d ad lib. period between the metabolic diets. To adjust for possible diet order effects, the w-6 PUFA diet was assigned to the middle diet period, and the first and third diet periods were randomized between the SFA diet and the w-3 PUFA diet. Diets consisted of natural foods whose composition had been determined by the USDA and listed in the Handbook 8 food tables (44). For variety, 2-d rotating menus were developed for each diet period. The caloric requirement for each subject was initially estimated using the Harris-Benedict equation (45), and adjustments were made, when necessary, to maintain a steady weight during the entire period of study. Subjects were asked to eat all of the food served to them and maintain their physical activity at a constant rate.

Blood was drawn to measure postheparin lipase activities on the 16th day of each diet. On the 21st day of each diet, a vitamin A-fat loading test was done with an acute fat load similar in composition to the chronically fed diet. On the 25th day of each diet period, a second vitamin A-fat loading test was done with an acute fat load, which in the case of the chronic SFA diet was similar to the w-6 PUFA diet and in the cases of the chronic w-6 and w-3 PUFA diets was similar to the SFA diet. Five blood samples were drawn after a 12-h fast in the last week of each diet period on days 20, 21, 23, 24, and 25. These were analyzed for lipid, lipoprotein, and apolipoprotein levels. Fasting blood samples were also analyzed for glucose, insulin, HgbA1C levels, and platelet count. 2-h postprandial insulin levels were measured during each fat tolerance test.

**Diet composition.** Each diet consisted of 42% of calories from fat, 43% from carbohydrate, and 15% from protein and contained 200 mg cholesterol/1,000 cal (Fig. 1 B). In addition, each diet had its monounsaturated fatty acid content fixed at 26-28% of dietary fat. The SFA diet had a P/S ratio of 0.07, the w-6 PUFA diet had a P/S ratio of 1.4, and the w-3 PUFA diet had a P/S ratio of 1.39. In the w-3 PUFA diet, 30% of the fat was derived from fish oil. The fish oil used was a deodorized preparation of Menhaden oil that was kindly given to us by Zapata Haynie Corporation, Reedville, VA. It contained 25% w-3 PUFA, 27% monounsaturated fatty acids, 15% w-6 PUFA, 33% SFA, and 353 mg/dl cholesterol. The w-3 PUFA diet provided 3.5 g of w-3 PUFA/1,000 cal, which contributed 3.2% of the total caloric intake.

**Vitamin A fat-loading test.** After an overnight 12-h fast, subjects were given a fatty meal plus 60,000 U of aqueous vitamin A/m<sup>2</sup> body surface. Vitamin A (50,000 U/m Aquasol A) was purchased from Armour Pharmaceutical Co., Kankakee, IL. The fatty meal contained

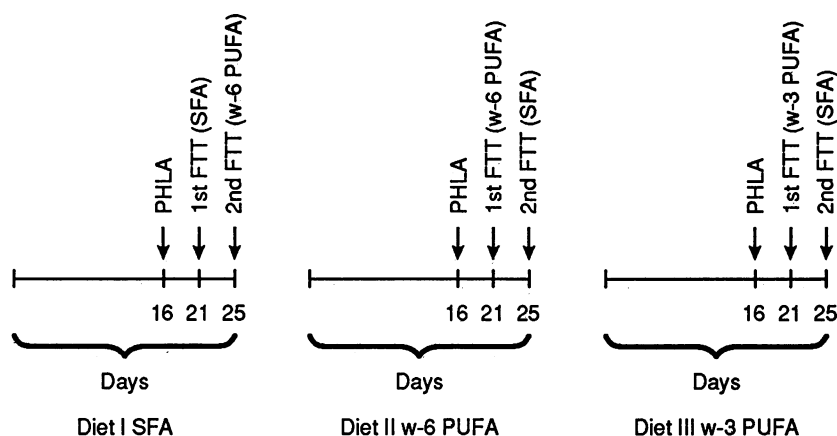
50 g of fat/m<sup>2</sup> body surface, consisting of 65% of calories as fat, 20% as carbohydrate, and 15% as protein (Fig. 1 C). It contained 600 mg cholesterol/1,000 cal. The P/S ratio, in the case of the SFA loading test, was 0.08, and in the w-6 and w-3 PUFA loading test, it was 1.38. In the latter case, 30% of the fat was derived from fish oil.

The fatty meal was given as scrambled eggs, cheese, bread, and a milkshake, in the case of the SFA loading test, and as turkey, bread, and a modified milkshake that included safflower oil or fish oil, in the case of the w-6 and w-3 PUFA loading tests, respectively. The meal was eaten in 10 min. Vitamin A was added to the milkshake. After the meal, subjects fasted for 12 h, but as much drinking water as desired was allowed. Blood samples were drawn before and every hour after the meal until 6 h, and then every 2 h until 12 h. The subjects tolerated the meal well, and no one had diarrhea or other symptoms of malabsorption.

**Preparation of blood samples for retinyl ester assay.** Venous blood was drawn from the forearm and transferred to a tube containing sodium EDTA. Samples were immediately centrifuged at 1,500 g for 15 min and 0.5 ml of plasma was stored wrapped in foil at -20°C for retinyl ester assay. Another 0.5 ml was stored at 4°C for triglyceride determinations. An aliquot of 2.5 ml of plasma was transferred into a ½ × 2 in. cellulose nitrate tube and overlaid with 2.5 ml sodium chloride solution (*d* = 1.006 g/ml). Tubes were subjected to preparative ultracentrifugation for 1.6 × 10<sup>6</sup> g/min in a rotor (SW-55, Beckman Instruments, Fullerton, CA) to float chylomicron particles of Sf > 1,000 (46-48). The chylomicron-containing supernatant was removed and brought to a total volume of 2 ml with saline. The infranatant was brought to a volume of 5 ml with saline. 0.5-ml aliquots of supernatant and infranatant were wrapped in foil and assayed for retinyl ester. Additional aliquots were assayed for triglyceride concentration. As discussed elsewhere, the procedure appears to separate a predominantly chylomicron population from a predominantly remnant population (32, 33).

**Retinyl ester assay.** The assays were carried out in subdued light with HPLC grade solvents. Retinyl acetate was added to the samples as an internal standard. The samples were then mixed with 4 ml ethanol, 5 ml hexane, and 4 ml water, with vortexing between each addition. Two phases were formed, and 4 ml of the upper (hexane) phase was removed and evaporated under nitrogen (49). The residue was dissolved in a small volume of benzene, and an aliquot was injected into an HPLC 5-μm ODS-18 radial compression column. 100% methanol was used as the mobile phase at a flow rate of 2 ml/min. The effluent was monitored at 340 nm, and the peak of retinyl palmitate (RP) was identified by comparison to the retention time of purified standard (Sigma Chemical Co., St. Louis, MO). In agreement with previous reports (30, 50), it was found that 75-80% of total plasma retinyl esters were accounted for by retinyl palmitate. In addition, the distribution of

### A. Study Design



### B. Fatty Meal Composition

	Saturated	Polyunsaturated	Fish oil
Protein	15%	15%	15%
COH	20%	20%	20%
Fat (50 g/m <sup>2</sup> )	65% { S 67% M 28% P 5%	65% { S 30% M 28% P 42%	65% { S 30% M 28% P 42% { 18% W3 82% W6
P/S ratio	0.08	1.38	1.38
Cholesterol (mg/1,000 cal)	600	600	600
Vitamin A (U/m <sup>2</sup> )	60,000	60,000	60,000

### C. Diet Composition

	Saturated	Polyunsaturated	Fish oil
Protein	15%	15%	15%
COH	43%	43%	43%
Fat	42% { S 67% M 28% P 5%	42% { S 30% M 28% P 42%	42% { S 31% M 26% P 43% { 20% W3 80% W6
P/S ratio	0.07	1.40	1.39
Cholesterol (mg/1,000 cal)	200	200	200

Figure 1. Study design, diet and fatty meal composition. In A, the sequence of diets, postheparin lipase tests (PHLA), and vitamin A-fat loading tests is described. (B) Composition of the three vitamin A fat loads. (C) Composition of each of the three diets.

retinyl esters remained constant throughout the study. The amounts of retinyl palmitate in plasma and lipoprotein fractions were quantitated by the area ratio method (51) using retinyl acetate as a reference (30).

**Lipid, lipoprotein, and apolipoprotein determinations.** Cholesterol and triglycerides were measured enzymatically using the reagents cholesterol 236691, triglyceride 126012 (Boehringer-Mannheim Biochemicals, Indianapolis, IN). Total and high-density lipoprotein (HDL) cholesterol measurements were standardized by the Lipid Standardization Program of the Center for Disease Control, Atlanta, GA. HDL cholesterol was determined after precipitation of whole plasma with dextran sulfate-magnesium (52). HDL plus LDL cholesterol was measured on the infranant after a 2-h spin in the airfuge (Beckman

Instruments) to float the VLDL (53). Appropriate corrections were made for dilution factors, and VLDL, LDL, and HDL cholesterol levels were calculated.

Three frozen aliquots of plasma obtained during the last week of each diet period were assayed for apo A-I and B levels by a sandwich ELISA. The assay plates were coated with antibody, either a monospecific polyclonal goat antibody to apo A-I (generously supplied by Dr. Peter Herbert, Miriam Hospital/Brown University, Providence, RI) or a rabbit antibody to apo B. After overnight incubation and washing, the plates were treated with ELISA-grade BSA to block nonspecific binding sites. Multiple dilutions of antigen were then applied and incubated for 2 h at 37°C. After rewashing, the plates were incubated

for 2 h with alkaline phosphatase-conjugated antibody. The plates were then rewashed, incubated with phosphatase substrate, and color development was read at 410 nm in a plate reader (Dynatech Laboratories, Alexandria, VA). Assays were standardized with a reference serum pool supplied by the Center for Disease Control and two frozen control sera, whose apolipoprotein values were determined by three independent laboratories: Brown University/Miriam Hospital, Providence, RI; the Lipid Metabolism Laboratory, USDA Human Nutrition Center on Aging, Boston, MA; and the Northwest Lipid Research Clinic, Seattle, WA. Assays were rejected when the control sera values were > 15% above or below their target value. To avoid potential bias from plate to plate variations, each assay plate contained one specimen from each of the three diets for the same subject.

**Postheparin plasma lipolytic activities.** Lipolytic activities were determined 1 wk before the vitamin A-fat loading test. Lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) were released into the circulation by intravenous heparin injection at a dose of 60 U/kg body weight. After 15 min, blood was drawn into tubes containing 4 mM EDTA. The plasma was immediately separated at 4°C by centrifugation at 2,700 rpm for 12 min and promptly frozen at -70°C. The assay itself is a modification of the method of Krauss (54) as recently described (32, 33). In short, 20 µl of postheparin plasma is added to freshly sonified substrate, containing radiolabeled and unlabeled triolein and Triton X-100 in a Tris HCl buffer (pH 8.6), mixed, and incubated for 20 min at 37°C. The reaction is stopped by a mixture of chloroform, methanol, and heptane, and FFA are extracted into a K<sub>2</sub>CO<sub>3</sub>/H<sub>3</sub>BO<sub>3</sub> buffer (pH 10). After separation, an aliquot of the aqueous phase is counted, and the total lipolytic activity is calculated as micromoles FFA liberated/milliliters plasma per hour. Another aliquot of 20 µl of postheparin plasma is incubated for 1 h at 4°C with 25 µl of rabbit antihuman LPL antiserum before the lipase assay described above. The antiserum was prepared in our laboratory by injecting rabbits with LPL purified from fresh human breast milk. This antiserum completely inhibited the activity of purified human breast milk lipoprotein lipase but had no effect on human hepatic lipase purified by heparin Sepharose chromatography from postheparin plasma. LPL activity is the difference between total and antiserum-inhibited activity. The latter is taken to be the HTGL activity.

**In vitro lipolysis assay.** To examine the susceptibility to lipolysis of the chylomicrons produced after each fat load, 2.5-ml aliquots of plasma drawn between 3 and 6 h after the acute fat load were subjected to preparative ultracentrifugation to isolate chylomicron particles of Sf > 1,000 (as described above). The chylomicrons were pooled and brought to a volume of 1 ml containing 1 mg of chylomicron triglyceride and a final concentration of 6% BSA, and 0.1 M Tris HCl, pH 8.6. These samples were incubated for 1 h at 37°C with a 10-µl aliquot of human milk lipoprotein lipase purified by heparin and phenyl Sepharose chromatography from fresh human breast milk. The preparation used had an activity of 3.6 µm fatty acids/ml per h determined by the assay for postheparin lipolytic activity, which uses an artificial substrate (see previous method). The amount of FFA liberated was measured by a modification of a colorimetric method using a copper reagent (55). 0.2 ml of the incubated chylomicrons or of a fatty acid standard (stearic acid; Sigma Chemical Co.) were mixed with 0.5 ml of a 0.05 M phosphate buffer (pH 6.2) and 2.5 ml of a chloroform/heptane/methanol solution 28:21:1 (CHM). The mixture was vortexed for 10 seconds and centrifuged for 5 min at 3,000 rpm. The upper layer was removed by vacuum suction. The lower layer was mixed with 1 ml of a copper reagent (triethanolamine [1 M]/Na citrate [0.51 M]/Cu(NO<sub>3</sub>)<sub>2</sub> [0.267 M], 9:1:10), vortexed for 10 s, and centrifuged for 5 min at 3,000 rpm. 1 ml from the upper phase was transferred to a test tube and 0.2 ml of 0.1% diethyldithiocarbamate in butanol was added. After vortexing and standing at room temperature for 15 min, a colorimetric determination at 440 nm was made. The LPL used in all the assays was from the same batch. It was stored at -20°C in small aliquots that were thawed once. The chylomicrons obtained from the two different fat loading tests done on each diet were assayed at the

same time. The conditions selected for the in vitro lipolysis assay were linear with time and independent of substrate concentration.

**Statistical analysis.** The differences between the three diet periods were analyzed for significance using analysis of variance and Tukey's multiple comparison test (56). The differences between the two vitamin A-fat loading tests done on each diet were analyzed for significance using the paired *t* test. Correlations between the measured variables and RP responses were calculated by linear regression analysis using the least squares method. Differences in the slopes and intercepts between the regression lines comparing triglycerides and RP responses for the various diets were analyzed for significance (56). Calculations were performed on the Rockefeller University Hospital Clinco system.

## Results

**Dietary effects on fasting lipid, lipoprotein, apolipoprotein, postheparin lipase, glucose, Hgbc AIC, insulin, and platelet count levels.** The subjects' mean weight, fasting lipid, and lipoprotein levels on the SFA, w-6 PUFA, and w-3 PUFA diets are shown in Table II. The weights of the subjects were stable during the three diet periods. All fasting plasma lipid and lipoprotein levels were highest on the SFA diet, intermediate on the w-6 PUFA diet, and lowest on the w-3 PUFA diet. Analysis of variance indicated a significant diet effect for total cholesterol, triglycerides, and VLDL cholesterol. Tukey's multiple comparison test indicated that the levels on the w-3 PUFA diet, but not the w-6 PUFA diet, were significantly different from those on the SFA diet. The values on the w-3 PUFA diet were not significantly different from the w-6 PUFA diet. We also compared values for the SFA and w-6 PUFA diets and SFA and w-3 PUFA diets by paired *t* test. Highly significant (*P* < 0.01) differences were found between SFA and w-6 PUFA diets for total cholesterol, triglycerides, and LDL cholesterol. Highly significant differences (*P* < 0.01) were also found between SFA and w-3 PUFA diets for total cholesterol, triglycerides, VLDL, LDL, and HDL cholesterol levels. Comparing the w-6 with the w-3 PUFA diets, significant differences (*P* < 0.01) were found for total cholesterol, triglycerides, and VLDL cholesterol levels. Finally, the w-3 PUFA diet lowered HDL cholesterol significantly (*P* < 0.01) when compared with the SFA diet.

The mean levels of the subjects' postheparin lipases, apo B and A-I, fasting glucose and Hgbc AIC, fasting and the increment in 2-h postprandial insulin levels, and platelet count on each of the diets are shown in Table III. Analysis of variance indicated a significant diet effect only on apo B and A-I and platelet count levels. Tukey's multiple comparison tests indi-

Table II. Effects of Dietary SFA and w-6 and w-3 PUFA on Fasting Lipids and Lipoproteins (n = 8)

Diet	Weight	TC*	TG*	VLDL-C*	LDL-C	HDL-C
	kg			mg/dl		
SFA	75.3±9	190±37	96±35	24±7	120±34	46±7
w-6 PUFA	75.6±8	162±37 <sup>††</sup>	75±23 <sup>††</sup>	19±5 <sup>†</sup>	100±33 <sup>†</sup>	42±5
w-3 PUFA	75.9±8	148±36 <sup>†††</sup>	55±17 <sup>†††</sup>	14±7 <sup>†††</sup>	95±34 <sup>†</sup>	39±5 <sup>†</sup>

\* Analysis of variance indicates a significant diet effect *P* < 0.05 for TC, TG, VLDL-C.

<sup>†</sup> *P* < 0.01 from SFA diet in a paired *t* test.

<sup>††</sup> *P* < 0.01 from w-3 PUFA diet in a paired *t* test.

<sup>†††</sup> Tukey's multiple comparison test indicates significant differences from the SFA diet *P* < 0.05.

<sup>††††</sup> *P* < 0.001 from SFA diet in a paired *t* test.

Table III. Effects of the Diets on Lipolytic Activity, Apo B, Apo AI, Glucose, HgbA1C, Insulin, and Platelet Number

Diet	Lipolytic activity				Glucose	HgbA1C	Fasting insulin		Platelet number
	LPL	HTGL	Apo B	Apo AI			Insulin	Increment*	
	$\mu\text{mol FFA/ml per h}$		mg/dl				$\mu\text{U/ml}$		
SFA	8.9 (3)	13.4 (6)	96 (17)	116 (16)	87 (7)	4.7 (0.2)	8 (6)	10 (9)	250 (28)
w-6 PUFA	9.3 (3)	13.6 (6)	77* (9)	105 (9)	89 (6)	4.9 (0.3)	14 (4.9)	25 (21)	239 (32)
w-3 PUFA	10.2 (3)	14.3 (7)	74* (8)	96* (11)	91 (7)	4.8 (0.5)	12 (3.3)	14 (7)	226* (17)

Significantly different from the SFA diet by Tukey's multiple comparison test: \* $P < 0.05$ . Values shown are means with standard deviations indicated below in parentheses. \* The increase in 2-h postprandial insulin levels over fasting levels.

ated that apo B levels were significantly lower on both w-6 and w-3 PUFA diets compared with the SFA diet. Apo A-I and platelet count levels were only significantly lower on the w-3 PUFA diet compared with the SFA diet.

**Dietary effects on postprandial lipoprotein levels.** As shown in Fig. 1 A, each subject underwent six different vitamin A-fat loading tests. This allowed us to separate the chronic from the acute dietary effects on postprandial lipoprotein levels. The chronic dietary effects can best be seen in Fig. 2. On the left are shown the RP concentration curves in the chylomicron and nonchylomicron fractions when the subjects were chronically fed SFA, w-6 PUFA, and w-3 PUFA diets and received an acute fat load of similar composition. It can be seen that postprandial lipoprotein levels were highest for SFA, intermediate for w-6 PUFA, and lowest for w-3 PUFA diets. As shown by the area calculated below the RP concentration curve in Table IV, compared with the SFA diet, the w-6 PUFA diet reduced

chylomicron and nonchylomicron levels 56 and 38%, respectively, and the w-3 PUFA diet reduced these levels 67 and 53%, respectively. Analysis of variance and Tukey's multiple comparison test showed all of these reductions to be significant. The differences between the w-6 and w-3 PUFA diet postprandial lipoprotein levels were not significant. In Fig. 3 are shown the chronic dietary effects on total plasma RP area for each of the eight subjects studied. As can be seen, there was a remarkable consistency in behavior from subject to subject.

To determine the effect of an identical acute fat load, the right panel in Fig. 2 shows the postprandial lipoprotein levels when the subjects were chronically fed SFA, w-6 PUFA, and w-3 PUFA diets and received an acute SFA load. The same qualitative pattern in postprandial lipoprotein levels, highest for SFA, intermediate for w-6 PUFA, and lowest for w-3 PUFA chronic diets, is seen with acute matching fat loads. As shown in Table IV, compared with the SFA diet, the w-6 PUFA diet reduced chylomicron and nonchylomicron levels 30 and 20%, respectively, and the w-3 PUFA diet reduced these levels 54 and 38%, respectively. Analysis of variance showed a significant chronic diet effect on both postprandial lipoprotein fractions, but Tukey's multiple comparison test indicated that the only significant difference was between the SFA and the w-3 PUFA diets.

Our study design also allowed us to examine the effects of an acute fat load on postprandial lipoprotein levels as shown in Fig. 4. On the left are shown the postprandial lipoprotein levels for the chronic SFA diet with both a SFA and an w-6 PUFA acute fat load. As can be seen and confirmed by quantitation in Table IV, there was no difference in either chylomicron or nonchylomicron levels between the two different acute fat loads. However, significant differences between acute fat loads were seen when subjects were chronically fed the w-6 and w-3 PUFA diets. In the middle of Fig. 4 are shown the postprandial lipoprotein levels for the chronic w-6 PUFA diet with both a SFA and an w-6 PUFA acute fat load. As shown in the figure and in Table IV, the chylomicron and nonchylomicron levels were reduced 38 and 22%, respectively, when comparing the SFA and w-6 PUFA acute fat loads. This difference was significant by paired  $t$  test ( $P < 0.01$ ). On the right of Fig. 4 are shown the postprandial lipoprotein levels for the chronic w-3 PUFA diet with both a SFA and an w-3 PUFA acute fatload. As shown in the figure and Table IV, the chylomicron and nonchylomicron levels were reduced 38 and 24%, respectively,

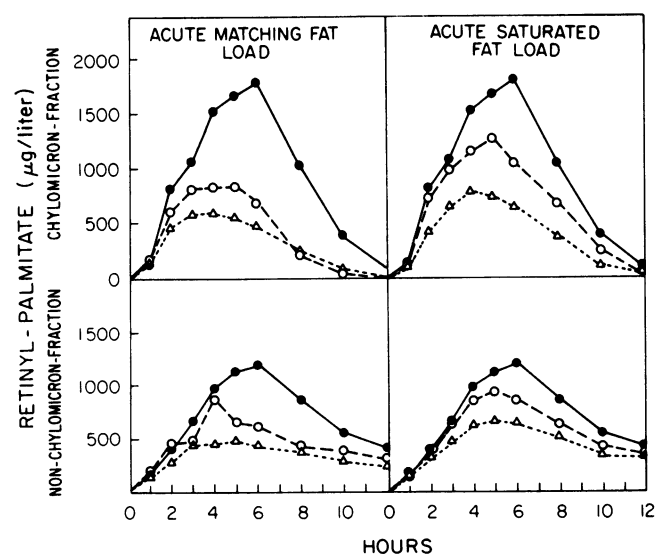


Figure 2. Chronic effects of SFA, w-6 PUFA, and w-3 PUFA diets on postprandial lipoprotein levels. (Left) Chylomicron and nonchylomicron RP responses on the SFA (●), w-6 PUFA (○), and w-3 PUFA (△) diets when an acute fat load of similar composition is given. (Right) Postprandial lipoprotein levels when subjects were on the same diets as in the left panel, but received an acute SFA load.

Table IV. Chronic and Acute Effects of Dietary Fat Saturation Levels on Postprandial Lipemia

Acute fat load	Area below RP curves ( $\mu\text{g/liter per h}$ )					
	SFA		w-6 PUFA		w-3 PUFA	
	Diet					
	SFA	w-6 PUFA	w-6 PUFA	SFA	w-3 PUFA	SFA
Chylomicron	10,956	10,154	4,787**	7,698	3,665**	5,039*
Fraction	(5,033)	(5,294)	(2,669)	(3,724)	(2,023)	(2,779)
Nonchylomicron	8,369	7,778	5,271 <sup>  </sup>	6,700	3,960**	5,218*
Fraction	(3,068)	(3,208)	(1,494)	(1,790)	(1,372)	(2,008)

\*  $P < 0.01$ , significantly different from the SFA diet-acute SFA load by Tukey's multiple comparison test. †  $P < 0.01$ , significantly different from the SFA load on the same diet by paired  $t$ -test. ‡  $P < 0.001$ , significantly different from the SFA diet-acute SFA load by Tukey's multiple comparison test. ||  $P < 0.05$ , significantly different from the SFA diet-acute SFA load by Tukey's multiple comparison test. Values shown are means with SD indicated below in parentheses.

when comparing the SFA and the w-3 PUFA acute fat loads. This difference was also significant by paired  $t$  test ( $P < 0.01$ ).

**Dietary effects on postprandial triglyceride levels.** To rule out the effect of w-6 and w-3 series fatty acids on retinol absorption as the explanation for the differences observed, we also analyzed the postprandial increment in triglyceride levels on the different diets. The increments in triglyceride concentrations over time, when the subjects were chronically fed SFA, w-6 and w-3 PUFA diets and received an acute fat load of similar composition, are shown in Fig. 5. The postprandial increments were highest for SFA, intermediate for w-6 PUFA and lowest for w-3 PUFA diets. Compared with the SFA diet, the w-6 PUFA diet reduced the area below the increment in triglyceride concentration curve 41% and the w-3 PUFA diet reduced it 68%. This is virtually identical to the results seen with the retinyl palmitate assay, which means that dietary effects on retinol absorption are not the explanation for our findings.

**Susceptibility of chylomicrons to in vitro lipolysis.** We next examined the susceptibility to lipolysis of chylomicrons produced after ingestion of different fat loads. This was done by isolating chylomicrons produced during each of the fat tolerance tests. We then incubated 1 mg of chylomicron triglyceride with a standard preparation of purified lipoprotein lipase

and measured the rate of FFA release, as described in Methods. The w-6 and w-3 PUFA-containing chylomicron were hydrolyzed faster than the SFA-containing chylomicrons. On average, chylomicrons obtained after loads of PUFA acids released  $0.163 \mu\text{m}$  fatty acids/h, whereas chylomicrons after loads of SFA released  $0.108 \mu\text{m/h}$  ( $P < 0.01$ ) (Table V). There was no effect of the chronic diet on the in vitro lipolysis of chylomicrons.

## Discussion

In this study, we measured the effect of an isocaloric substitution of w-6 and w-3 PUFA for SFA on fasting and postprandial lipoprotein levels. Both types of PUFA lowered total cholesterol, triglyceride, VLDL, and LDL cholesterol levels. In addition, the w-3 PUFA diet produced a significantly lower total cholesterol, triglycerides, and VLDL cholesterol compared with the w-6 PUFA diet. We also found that the w-3 PUFA diet lowered HDL cholesterol levels compared with the SFA diet. This is in agreement with other studies in the literature (7–20). Previous studies have found different effects of w-3 PUFA on HDL cholesterol levels, but this may be due to study design. In studies in which w-3 PUFA were substituted for SFA, HDL cholesterol levels fell (19, 57). However, when studies were done by adding fish-oil supplements to the diet, many of which contain cholesterol and SFA, this resulted in no change or an increase in HDL cholesterol (14–16, 20). The w-6 and w-3 PUFA diets also affected apo B and apo A-I levels. The changes observed were very compatible with the changes seen in lipoprotein levels.

Using the vitamin A-fat loading test, we now show that w-6 and w-3 PUFA diets dramatically reduce total postprandial lipoprotein levels 48 and 61%, respectively, compared with an SFA diet. Our study design allowed us to separate the chronic and acute effects of the type of fat fed on postprandial lipoprotein levels. To ascertain the effects of the type of fat chronically fed, in all three diet periods we performed a fat tolerance test with an acute SFA load. Even with the same acute fat load, the chronic feeding of w-6 and w-3 PUFA diets reduced total postprandial lipoprotein levels 36 and 47%, respectively, compared with the SFA diet. The differences between the w-6 and w-3 PUFA diets were not significant. To ascertain the effects of an acute fat load on postprandial lipoprotein levels, in all three

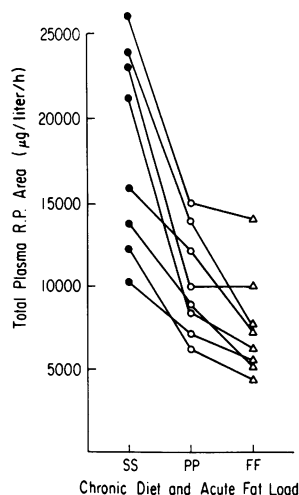


Figure 3. Individual response to a dietary fat load. The total plasma RP areas for each of the eight subjects are shown on the SFA diet with an acute SFA load (SS), on the w-6 diet with an acute w-6 PUFA load (PP), and on the w-3 diet with an acute w-3 PUFA load (FF).

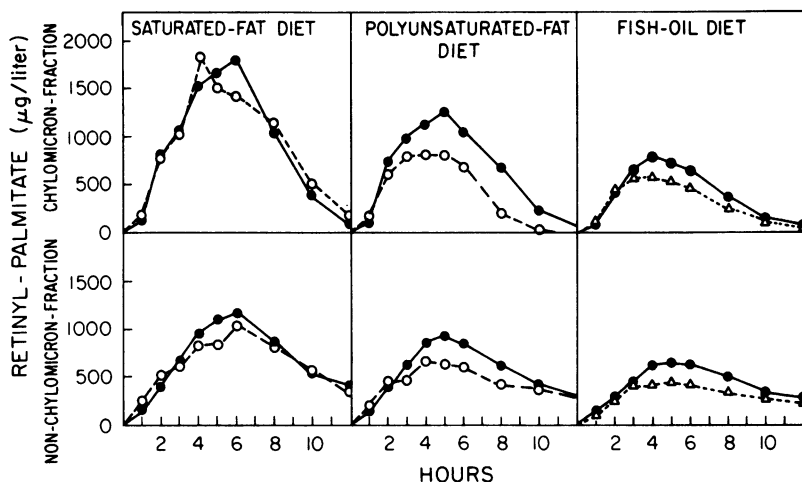


Figure 4. Acute effects of SFA, w-6 PUFA, and w-3 PUFA loads on postprandial lipoprotein levels. Chylomicron and nonchylomicron RP responses are shown when SFA (●) and w-6 PUFA (○) loads were given on an SFA diet (left), on a w-6 PUFA diet (middle) and when SFA and w-3 PUFA (Δ) loads were given on a w-3 PUFA diet (right). Some of the data are the same as in Fig. 2 for comparison purposes.

diets two fat tolerance tests were done. One of these always consisted of a SFA load, whereas the other was a PUFA load either of the w-6 or w-3 type. Significant differences in postprandial lipoprotein levels between the SFA and PUFA acute fat loads were seen only with the w-6 and w-3 PUFA diets. On the SFA diet both the SFA and the PUFA acute fat loads yielded identical plasma RP responses in spite of lower susceptibility of SFA chylomicrons to LPL *in vitro* (see below). In contrast, on the w-6 PUFA diet, the w-6 PUFA acute fat load lowered postprandial lipoprotein levels 30%, and on the w-3 PUFA diet, the w-3 PUFA acute fat load lowered them 26%, compared with the SFA acute fat load. Thus, the major effect of PUFA feeding on postprandial lipoprotein levels reflects the nature of the fat in the chronically fed diet. However, in this study on the PUFA diets we also demonstrate a significant effect of the acute fat load.

In previous studies, we showed that particles of  $S_f < 1,000$  behaved differently than those of  $S_f > 1,000$  in patients with the E2/2 phenotype and type III hyperlipoproteinemia and in normal subjects with the E3/2 phenotype (32, 33). We suggested that these differences reflect less efficient clearance of chylomicron remnants from the  $S_f < 1,000$  fraction, although that fraction also contains small chylomicrons and partially lipolyzed chylomicrons. In the present investigation, the fractions of  $S_f > 1,000$  and  $S_f < 1,000$  behaved similarly indicating that SFA, w-6 PUFA, and w-3 PUFA diets may not have a significant affect on chylomicron remnant clearance.

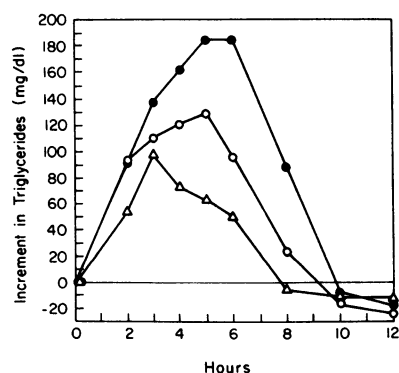


Figure 5. Dietary effects on postprandial triglyceride levels. The figure demonstrates increments in plasma triglyceride levels on the SFA (●), w-6 PUFA (○), and w-3 PUFA (Δ) diets when an acute fat load of similar composition is given.

In our study, we also attempted to understand the mechanism causing the different levels of postprandial lipoproteins after different types of chronic and acute fat loads. The differences observed are not due to differential absorption of retinol as recently shown in the rat by Groot (58) or of the chronic or acute fat loads as shown here and in previous studies (59–62). One explanation could be the differences in fasting triglyceride levels on the different diets. It is known that exogenous and endogenous triglyceride-rich lipoproteins compete for the same clearance mechanism (63). Previous studies of our own using the vitamin A-fat tolerance test (32) and of others using different methods have indeed shown high positive correlations between fasting triglyceride and postprandial lipoprotein levels (64–66). In this study, fasting triglyceride levels were highest on the SFA diet and lower on the PUFA diets. In Fig. 6, the chylomicron RP area is plotted against the fasting triglyceride levels for each of the fat tolerance tests. When all 48 studies were combined, the overall correlation is 0.82 with a  $P < 0.0001$ . This confirms the results of previous studies.

Fig. 6 also shows the correlation of chylomicron RP area and triglyceride levels for each of the six different types of studies. The linear regressions for the SFA, w-6 PUFA, and w-3 PUFA diets each with an acute SFA load are similar, suggesting that the chronic effect of diet on postprandial lipoprotein levels are largely determined by fasting triglyceride levels. Fasting triglyceride levels are determined by the rate of endogenous VLDL production by the liver and the rate of

Table V. Rate of Chylomicron Lipolysis *In Vitro* by Purified LPL from Human Milk

Acute fat load	SFA fat diet	w-6 PUFA fat diet	w-3 PUFA diet
	$\mu\text{m}/\text{h}^*$		
SFA	0.104±0.024	0.109±0.021	0.110±0.032
w-6 PUFA	0.165±0.026†	0.161±0.024†	—
w-3 PUFA	—	—	0.164±0.025†

\* Microns of FFA released from 1 mg of chylomicron TG in 1 ml of buffer (pH 8.6) by 10  $\mu\text{l}$  of a standard preparation of LPL (specific activity 3.6  $\mu\text{m}/\text{ml}$  per h) after a 1-h incubation at 37°C ( $n = 7$ ).

† Significantly different from the SFA load by paired  $t$  test  $P < 0.01$ .

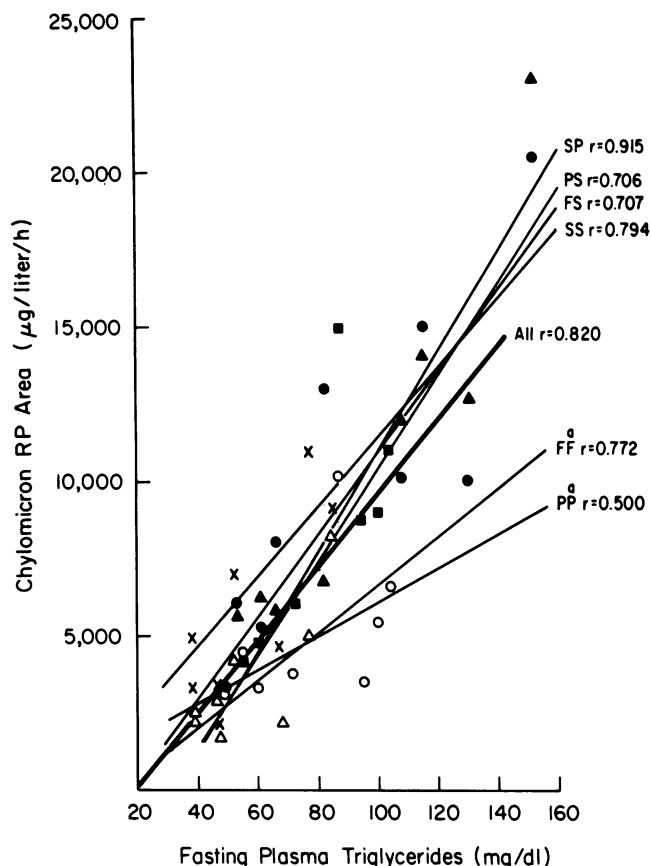


Figure 6. Correlation of postprandial chylomicron and fasting triglyceride levels. Chylomicron RP area is plotted against fasting plasma triglycerides and linear regression lines are constructed for all the fat loads (heavy line), for w-6 PUFA load on w-6 PUFA diet (PP and ○), for w-3 PUFA load on w-3 PUFA diet (FF and △), for SFA load on SFA diet (SS and ●), for SFA load on w-3 PUFA diet (FS and ×), for SFA load on w-6 PUFA diet (PS and ■), and for w-6 PUFA load on SFA diet (SP and ▲). \* $P < 0.05$ , significantly different in slope and intercept from SS, FS, PS, and SP.

catabolism of these particles by lipoprotein lipase. With regard to the latter, there were no differences in post-heparin lipolytic activities on the three diets (Table III). This was also shown by others (12, 19, 67). Another possibility is that the chronic feeding of different types of fat could change the fatty acid composition of VLDL triglycerides and make them more susceptible to lipolysis. It has been shown that w-6 PUFA diets change VLDL fatty acid composition and increase VLDL fluidity (68–70). It has also been reported that lipoprotein lipase is more reactive toward polysaturated triacylglycerol substrates (71). In agreement with this are the observations in this study that show that w-6 and w-3 PUFA containing chylomicrons are more susceptible than SFA containing chylomicrons to lipoprotein lipase-mediated lipolysis in vitro (Table V). In our assay, the w-6 and w-3 PUFA chylomicrons showed similar behavior. Thus, the lower fasting triglycerides seen on the w-6 compared with the SFA diet could be explained on the basis of enhanced susceptibility to lipolysis of triglyceride-rich particles. However, the further decrease in fasting triglyceride levels seen on the w-3 compared with the w-6 PUFA diets may be explained by a decrease in VLDL production as shown by other studies of w-3 PUFA containing diets (57, 72).

Examination of Fig. 6 also shows that the absolute level of fasting triglycerides is not the only determinant of postprandial lipoprotein levels. The regression lines obtained when plotting chylomicron RP area versus fasting triglyceride levels indicates a significantly lower slope for the w-6 PUFA acute fat load on the w-6 PUFA diet (PP) and the w-3 PUFA acute fat load on the w-3 PUFA diet (FF) compared with all the other types of studies ( $P < 0.05$ ). This suggests that on the PUFA diets there was a significant difference in the handling of acute SFA and PUFA loads (compare PS with PP and PS with FF). This was not seen on the SFA diet (compare SS with SP). The difference in metabolizing the different acute fat loads on the PUFA diets were probably due to the different lipolysis susceptibilities of SFA and PUFA containing chylomicrons, as shown in Table V. The lack of a difference in metabolizing acute SFA and PUFA loads on the SFA diet is contrary to the in vitro studies of chylomicron lipolysis. This may occur because increased fasting VLDL levels on the SFA diet may block in vivo chylomicron lipolysis to an extent that prevents seeing a difference between the acute fat loads.

Two other observations relating to dietary fish oil deserve comment. In this study, the w-3 PUFA diet significantly lowered both triglyceride and HDL cholesterol levels. Most perturbations, such as changes in weight, glucose tolerance, physical activity, or fibric acid therapy result in opposite effects on triglyceride and HDL cholesterol levels (73, 74). This suggests that the mechanism of the w-3 PUFA diet effect is fundamentally different. w-3 PUFA diets decrease VLDL production, whereas the other perturbations have their major effect on VLDL catabolism. Decreased VLDL production may provide less surface material for transfer to HDL and result in lower levels. The clinical significance of this is uncertain. The second observation is the significant reduction of the platelet count on the w-3 PUFA diet. This was also shown in previous studies (75, 76) and may contribute to the antiatherogenic effect associated with fish oil-enriched diets.

Our study shows that there are both chronic and acute effects of fat saturation on postprandial lipoprotein levels. This appears to be due to the interaction of chronic diet effects on the catabolic system and acute diet effects on the susceptibility of chylomicrons to lipolysis (Table VI). In addition to their effects on fasting plasma lipoproteins, the substantial reduction of postprandial lipoprotein levels caused by dietary w-6 and, even more so, w-3 PUFA may be a significant contributor to the reduced risk of coronary heart disease observed in individuals consuming these diets.

Table VI. Interactions between Chronic and Acute Effects of Fat Saturation Level on Postprandial Lipoprotein Clearance

Chronic diet	Catabolic machinery	+	Acute meal	Chylomicron lipolysis susceptibility	=	Postprandial lipoprotein catabolic rate
SFA	Low	+	SFA	Low	=	Slow
			W6FA	High	=	Slow
W6FA	Medium	+	SFA	Low	=	Medium
			W6FA	High	=	Medium-fast
W3FA	High	+	SFA	Low	=	Medium-fast
			W3FA	High	=	Fast



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