Effects of Omega-3 Fatty Acids on Intravascular Lipolysis of Very–Low-Density Lipoproteins in Humans

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Very–low-density lipoproteins (VLDLs) are the major carriers of fasting plasma triglyceride (TG). TG-enriched VLDLs become cholesterol (C)-enriched low-density lipoproteins (LDLs) through hydrolysis facilitated by lipoprotein lipase (LPL). Omega-3 fatty acid (n-3 FA) supplementation may increase LDL-C while decreasing plasma TG in hypertriglyceridemic patients. It has been proposed that n-3 FAs increase LDL-C by promoting production of TG-poor VLDL and accelerating conversion of VLDL to LDL. To study the effects of n-3 FA supplementation on in vivo lipolysis of VLDL directly, we treated 11 hypertriglyceridemic subjects with n-3 FA (3.3 g/d). Each participant was studied three times: at baseline, after a 1-month period of run-in olive oil placebo, and after 1 more month of n-3 FA supplementation. Lipolysis was induced by intravenous infusion of heparin for 2 hours. Plasma samples were obtained every 30 minutes for determination of lipids and apoproteins (apos), separation of individual lipoproteins by fast protein liquid chromatography (FPLC), and measurement of LPL and hepatic TG lipase (HTGL) levels. n-3 FA supplementation decreased fasting plasma TG (2.51 ± 0.23 *v* 3.97 ± 0.46 mmol/L), VLDL-TG (1.08 ± 0.18 *v* 2.35 ± 0.35 mmol/L), and VLDL-C (0.39 ± 0.05 *v* 0.72 ± 0.13 mmol/L) while increasing LDL-C (3.59 ± 0.21 *v* 3.00 ± 0.23 mmol/L) and plasma apo B (3.31 ± 0.19 *v* 2.90 ± 0.17 mmol/L). The absolute rate of TG lipolysis correlated with fasting TG ($r = .74$, $P < .005$) and was lower after n-3 FA supplementation (0.11 ± 0.01 mmol/mL/min) as compared with placebo (0.19 ± 0.01, $P < .01$), whereas percent decreases from baseline TG levels were similar at entry onto the study (57.4% ± 2.5%), after placebo (58.8% ± 2.7%), and after n-3 FA (52% ± 3.6%). During lipolysis, LDL-C increased in correlation with the decrease in VLDL-C ($r = -.41$, $P < .03$), consistent with the product-precursor relationship. Postheparin LPL activity at 2 hours correlated inversely with fasting TG ($r = -.53$, $P < .05$), but not with either the decreases in VLDL-TG and VLDL-C or the increase in LDL-C. These data demonstrate that n-3 FA supplementation does not accelerate lipolysis of VLDL and even decreases the VLDL-C pool, which is the precursor of LDL-C. Therefore, the increase seen in fasting LDL-C is probably mediated through mechanisms other than acceleration of VLDL lipolysis.

VERY–LOW-DENSITY lipoproteins (VLDLs) are the major carriers of triglyceride (TG) in fasting plasma.[1] Low-density lipoproteins (LDLs), on the other hand, carry the majority of cholesterol (C) and are atherogenic.[1] VLDLs are converted to LDLs by hydrolysis of VLDL-TG. The lipolysis is initiated by lipoprotein lipase (LPL), an enzyme that resides on endothelial surfaces of many tissues.[2] After interaction with LPL, VLDLs become smaller remnant particles. These are then either directly taken up by the liver or hydrolyzed further into LDLs, possibly by a second lipolytic enzyme, hepatic TG lipase (HTGL).[3] Metabolic fate of the remnants depends on their size and lipid composition; large, TG-rich remnants are preferentially removed by direct hepatic uptake, and smaller, TG-poor particles are further converted to LDLs.[4-6]

Omega-3 fatty acid (n-3 FA) supplementation consistently decreases plasma TG by decreasing hepatic synthesis and secretion of TG.[7-11] Cell culture studies also confirm the inhibitory effect of n-3 FA on TG synthesis.[12-15] Effects of n-3 FA on plasma LDL-C and apoprotein (apo) B can vary depending on the underlying lipoprotein disorder; several studies demonstrated increases in LDL-C and apo B.[16-18] The finding of an increase in LDL-C during the decrease of plasma TG is not unique to n-3 FA supplementation: treatment with gemfibrozil can also cause an increase in LDL-C.[19] We and several other researchers proposed that the common mechanism underlying the increase in LDL-C may be an increase in conversion of VLDL to LDL. Treatment modalities that decrease plasma TG may lead to production of TG-poor VLDL particles, which are preferentially hydrolyzed into LDL instead of being cleared by direct hepatic uptake.

Conversion of VLDL to LDL has been extensively studied with in vitro systems by incubating VLDL with purified LPL.[20,21] These in vitro experiments differ from in vivo conditions in several aspects: they usually lack the second lipolytic enzyme HTGL, and the role of the liver, the major regulator of lipoprotein metabolism, is not accounted for. Hydrolysis of VLDL-TG can also be achieved in vivo by intravenous injection of heparin, which releases tissue-bound LPL and HTGL into the circulation. This approach has been used to study the transfer of various apoproteins from VLDL to high-density lipoprotein (HDL) and production of intermediate-density lipoprotein (IDL),[22-24] but not in the investigation of conversion of VLDL to LDL. One technical limitation has been the need for isolation of individual lipoproteins several times during the lipolytic process. In the present study, we investigated the effects of n-3 FA supplementation on lipolysis of VLDL and production of LDL in vivo by taking advantage of a relatively recent method for separation of lipoproteins, fast protein liquid chromatography (FPLC).[25]

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SUBJECTS AND METHODS

Subjects

Fourteen hypertriglyceridemic subjects (11 men and three women aged [mean ± SD] 51 ± 9 years) were recruited from the Cardiovascular Risk Reduction Clinic of the University of California at Davis Medical Center and the Lipid Clinic of the Veterans Administration Outpatient Facility in Sacramento after provision of informed consent. All patients were being treated by the principal investigator (S.E.K.). At the time of study, patients were following their usual diet. Their weights had been stable for at least 3 months before study, and they had not been on any lipid-lowering medication for at least 2 months before study. The study protocol was approved by the Human Investigation Committee of our institution, and the investigation was conducted in accordance with principles of the Helsinki II Declaration. Exclusion criteria consisted of clinical disorders that may predispose to bleeding (ie, active peptic ulcer disease, recent surgery, treatment with anticoagulants, or blood pressure > 165/95 mm Hg), weight change of more than 5% in the previous 3 months, insulin treatment, untreated hypothyroidism, renal or liver disease, and alcohol intake of greater than 1 oz/d. Diabetic patients who were managed with diet alone or with diet and oral agents were accepted onto the study if their fasting blood glucose level was less than 7.8 mmol/L or hemoglobin A_{1c} was less than 10% (normal, 6% to 8.5%). Two patients had diabetes, one managed with diet alone and the other with oral hypoglycemic agents. Three patients had to leave the study: one for noncompliance with the supplementation protocol, one for excessive alcohol intake, and one after an acute hypertensive crisis.

Study Protocol

Each patient was studied three times: at the start of study (baseline) and after placebo and n-3 FA supplementation periods. Patients first received 5 capsules of placebo containing ethyl esters of 2.6 g oleic acid (18:1n-9) and 0.77 g linoleic acid (18:2n-6), 5.5 mg α- and 5 mg γ-tocopherol, and less than 1 mg per day for 1 month. This was followed by supplementation with 5 capsules of fish oil containing ethyl esters of 2.1 g eicosapentaenoic acid (20:5n-3), 1.2 g docosahexaenoic acid (22:6n-3), 7.5 mg α- and 10.5 mg γ-tocopherol, and 15 mg C per day for another month. Both n-3 FA and identical-appearing placebo capsules were provided by The Fish Oil Test Material Program of the National Institutes of Health (Bethesda, MD). Lipolysis studies were performed after a 14-hour overnight fast, as previously described.[26] After recording weight, height, blood pressure, and 24-hour dietary recall, a 19-gauge butterfly needle was inserted into a forearm vein. After obtaining baseline blood samples, lipolysis was induced by heparin (60 U/kg intravenous push, followed by 60-U/kg/h intravenous infusion for 2 hours). Blood samples were collected in EDTA-containing tubes, first at minute 15 and then every 30 minutes, and kept on ice for separation of lipoproteins and determination of lipolytic activities. Preliminary studies showed that samples kept on ice did not have significant lipolysis, and therefore diethylparanitrophenyl phosphate (paraoxon) was not included in the tubes.[27] Fatty acid profiles of fasting plasma TG and phospholipid fractions were determined by gas chromatography to monitor compliance.

Chemical Assays

Plasma TG and C levels were measured enzymatically. All assays were performed in duplicate and included the required standards and calibrators. Interassay coefficients of variation were 3.6% for TG and 1.9% for C. HDL-C level was measured using the method reported by Warnick et al.[28] The coefficient of variation was 1.8% for this assay. Nonesterified fatty acids (NEFA) were determined by a kit method (WAKO, Richmond, VA). Serum apo B and apo A-I were determined by nephelometry; coefficients of variation were 1.7% for apo B and 1.1% for apo A-I assay. All samples from an individual patient were analyzed in a single assay. Insulin level was measured by a kit method (ICN Biochemicals, Costa Mesa, CA), with a coefficient of variation of 4.9%.

Determination of Fatty Acid Composition

Fatty acid composition of plasma was determined at the Clinical Nutrition Research Unit–Metabolism Core Laboratory at the University of California at Davis. Initially, various lipid fractions (C ester, phospholipid, TG, and fatty acids) were isolated by silicic acid thin-layer chromatography. Then, the specific lipid fraction to be analyzed was scraped from the plate and esterified with 5% HCl in methanol. Methyl esters of fatty acids were extracted in petroleum ether (boiling point, 30° to 60°C), dried with nitrogen, and submitted to analysis by capillary gas chromatography. A Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 50 m × 0.25 mm bonded 007 FFAP fused silica capillary column (Quadrex, New Haven, CT) was used to separate methyl esters of fatty acids. The column temperature was programed to increase from 190° to 220°C at 2°C/min with a final hold, separating 12:0 to 22:6n-3 fatty acids. The detector temperature was 270°C, and the injector temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.4 mL/min and a split ratio of 1:65. Fatty acid methyl esters were identified by comparison with authentic standards, and peak areas are integrated as relative weight (weight%) using a microprocessor.

Separation of Lipoproteins by FPLC

Plasma samples obtained at 0, 30, 60, and 120 minutes of heparin infusion were first submitted to ultracentrifugation for separation of the total lipoprotein fraction (d < 1.21). Then, a 200-uL aliquot of this preparation was applied to a Superose 6 HR column (Pharmacia, Piscataway, NJ).[25,26] The lipoproteins were eluted with 0.9% NaCl containing 0.01% EDTA and 0.1% azide, pH 7.4, at a flow rate of 0.3 mL/min. Material eluted from the column was monitored at 280 nm and collected in 0.6-mL fractions. This method resulted in reproducible separation of VLDL, LDL, and HDL; VLDL was recovered between the elution volumes of 7.2 and 8.4 mL, LDL between 10.2 and 12.6, and HDL between 14.4 and 15.6. However, IDL did not show a distinct peak, and fractions between VLDL and LDL were arbitrarily defined as IDL. TG and C levels were measured in each fraction, followed by calculation of concentrations of TG and C in each lipoprotein peak. Recoveries of lipoprotein lipids from the column were 72% ± 2% for TG and 78% ± 4% for C. These recoveries represent the materials present within the peaks.

Determination of LPL and HTGL Activities

Plasma LPL and HTGL activities were measured as described by Nilsson and Ekman[29] using substrates prepared by sonicating [^{3}H]trioleoylglyceride with lysophosphatidylcholine. Postheparin plasma was diluted either 10 times in 0.9 mol/L NaCl for measurement of LPL level or 20 times in 2 mol/L NaCl for measurement of HTGL level. Diluted plasma was incubated in duplicate with the specific substrate in a total volume of 0.2 mL at 37°C for 30 minutes. At the end of incubation, free fatty acids released were extracted with a mixture of chloroform-methanol-heptane (1.3:1.4:1) followed by potassium carbonate buffer, pH 10.5, and 1 mL of the upper (organic) phase was counted in a scintillation counter. One milliunit of activity was defined as release of 1 μmol free fatty acids/min. Each assay included a high

Table 1. Changes in Fatty Acid Composition of Plasma TGs and Phospholipids at Entry onto the Study (baseline) and After Placebo and n-3 FA Supplementation Periods (n [6ewd] = 6, mean ± SEM)

	TGs			Phospholipids		
Fatty Acid	Baseline	Placebo	Fish Oil	Baseline	Placebo	Fish Oil
PA (16:0)	27.89 ± 2.39	25.55 ± 1.37	24.40 ± 1.96	26.80 ± 1.09	27.02 ± 0.76	26.54 ± 0.78
SA (18:0)	3.89 ± 0.39	3.07 ± 0.16	3.44 ± 0.32	13.44 ± 0.37	13.00 ± 0.38	12.78 ± 0.19
OA (18:1n-9)	33.14 ± 1.83	35.15 ± 0.92	32.26 ± 1.52	9.53 ± 0.61	9.29 ± 0.49	7.65 ± 0.50
LA (18:2n-6)	15.06 ± 2.19	16.87 ± 2.13	18.85 ± 2.59	20.21 ± 0.98	19.16 ± 1.21	16.94 ± 1.64
AA (20:4n-6)	1.03 ± 0.10	1.02 ± 0.10	1.19 ± 0.01	10.60 ± 0.64	11.19 ± 0.76	9.92 ± 0.89
EPA (20:5n-3)	0.15 ± 0.02	0.13 ± 0.01	1.08 ± 0.22*†	0.61 ± 0.06	0.61 ± 0.07	4.68 ± 0.91*†
DHA (22:6n-3)	0.25 ± 0.04	0.26 ± 0.04	1.48 ± 0.30*†	2.33 ± 0.36	2.61 ± 0.38	5.44 ± 0.74*†

Abbreviations: PA, palmitic acid; SA, stearic acid; AA, archidonic acid; OA, oleic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

*$P < .05$ *v* baseline.

†$P < .05$ *v* placebo.

and low enzyme-activity reference standard. All postheparin plasma samples from an individual patient were assayed at the same time. Intraassay variations for both LPL and HTGL assays were less than 2%.

Statistical Analysis

Results are presented as the mean ± SE unless otherwise noted. Data were analyzed by ANOVA with repeated measures. If a significant result was obtained ($P < .05$), Tukey's significant-difference test was performed to evaluate differences between the various data points. Correlations among plasma lipids, apoproteins, and lipolytic activities were calculated using Pearson's product-moment correlations.

RESULTS

Clinical Findings

Both placebo and n-3 FA supplements were well tolerated. Patients' daily energy intakes and weights remained stable throughout the study ([mean ± SEM] daily energy, 1,746 ± 220 kcal at baseline, 1,674 ± 246 during placebo, and 1,809 ± 212 during n-3 FA; weight, 81.3 ± 3.2 kg at baseline, 80.9 ± 3.1 during placebo, and 81.9 ± 3.5 during n-3 FA). Fasting plasma glucose, insulin, and NEFA levels were not affected by n-3 FA (glucose, baseline 8.10 ± 1.05, placebo 7.94 ± 1.05, n-3 FA 7.99 ± 1.08 mmol/L; insulin, baseline 270 ± 48, placebo 258 ± 54, n-3 FA 270 ± 60 pmol/L; NEFA, baseline 0.86 ± 0.07, placebo 1.00 ± 0.10, n-3 FA 0.77 ± 0.09 mEq/L). Fatty acid compositions of plasma TG and phospholipids were altered significantly by n-3 FA supplementation (Table 1).

Effects of n-3 FA Supplementation on Plasma Lipids and Apoproteins in the Fasting State and During Lipolysis

TG. n-3 FA supplementation decreased fasting plasma TG as compared with placebo (2.51 ± 0.23 *v* 3.97 ± 0.46 mmol/L). Heparin-induced lipolysis caused a steady decrease in plasma TG during the first 90 minutes, followed by only a minimal decline. The absolute decrease in TG correlated with fasting TG ($r = .73$, $P < .005$) and was therefore less after n-3 FA supplementation versus placebo (1.10 ± 0.01 *v* 1.87 ± 0.01 mmol/mL/min, $P < .01$). However, percent decreases from baseline in TG were similar during all three periods (57.4% ± 2.5% at entry, 58.8% ± 2.7% after placebo, and 52% ± 3.6% after n-3 FA supplementation) (Fig 1).

Total C and HDL-C. n-3 FA supplementation did not change fasting plasma total C. During lipolysis, total C decreased rapidly during the first 15 minutes and then remained stable (0 minutes 6.15 ± 0.26, 15 minutes 5.77 ± 0.23, and 120 minutes 5.75 ± 0.26 mmol/L). Similar changes were observed during baseline and placebo periods. n-3 FA did not affect fasting plasma HDL-C (Tables 2 and 3).

Apos B and A-I. During n-3 FA supplementation, fasting apo B concentration increased (3.31 ± 0.19 mmol/L) as compared both with baseline (2.90 ± 0.17) and with placebo (2.83 ± 0.17) periods. Lipolysis caused a early and sustained decrease in apo B (0 minutes 3.31 ± 0.19, 15 minutes 3.11 ± 0.20, and 120 minutes 3.16 ± 0.19 mmol/L, $P < .01$), similar to that seen in total C. Fasting apo A-I level was not significantly affected by n-3 FA supplementation. However, apo A-I also decreased early during lipolysis

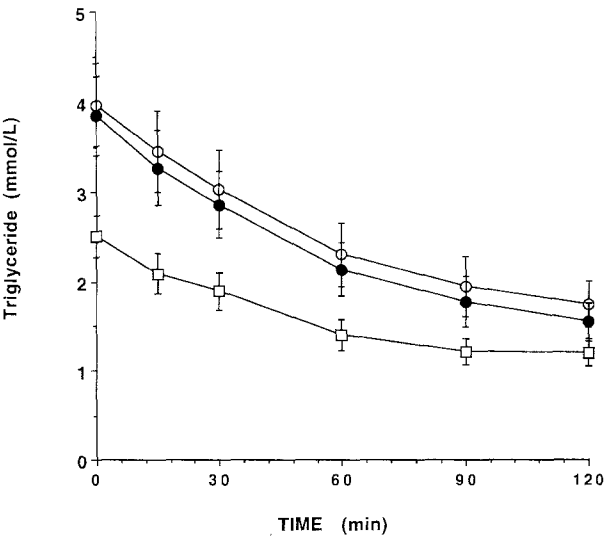

Fig 1. Changes in plasma TG during heparin-induced lipolysis at (○) entry onto the study and after (●) placebo and (□) n-3 FA supplementations (n = 11, mean ± SEM).

Table 2. Changes in Plasma Total C, Apos B and A-I, LPL, and HTGL During Intravascular Lipolysis at Baseline and After Placebo and n-3 FA Periods (mean ± SEM)

Parameter	0 Minutes	15 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	*P* (repeated-measures ANOVA)
Total C (mmol/L)							
BL	6.00 ± 0.18	5.69 ± 0.18	5.77 ± 0.21	5.72 ± 0.21	5.69 ± 0.18	5.74 ± 0.21	<.0005
PL	5.95 ± 0.23	5.61 ± 0.23	5.61 ± 0.23	5.59 ± 0.21	5.56 ± 0.23	5.56 ± 0.23	<.0001
n-3 FA	6.15 ± 0.26	5.77 ± 0.23	5.82 ± 0.23	5.79 ± 0.26	5.79 ± 0.26	5.84 ± 0.26	<.001
Apo B (mmol/L)							
BL	2.90 ± 0.17	2.83 ± 0.17	2.85 ± 0.17	2.90 ± 0.17	2.85 ± 0.17	2.92 ± 0.17	NS
PL	2.83 ± 0.17	2.78 ± 0.17	2.79 ± 0.15	2.83 ± 0.13	2.83 ± 0.15	2.85 ± 0.13	NS
n-3 FA	3.31 ± 0.19*†	3.11 ± 0.20*†	3.15 ± 0.19*†	3.18 ± 0.19*†	3.11 ± 0.19*†	3.16 ± 0.19*†	<.05
Apo A-I (mmol/L)							
BL	4.46 ± 0.36	4.25 ± 0.29	4.21 ± 0.29	4.21 ± 0.29	4.14 ± 0.29	4.25 ± 0.29	<.01
PL	4.56 ± 0.39	4.18 ± 0.36	4.18 ± 0.36	4.18 ± 0.36	4.14 ± 0.32	4.21 ± 0.32	<.01
n-3 FA	4.25 ± 0.36	3.89 ± 0.32	3.96 ± 0.32	3.96 ± 0.32	3.89 ± 0.32	3.96 ± 0.32	<.001
LPL (μU/mL)							
BL	—	74 ± 6	96 ± 11	96 ± 15	79 ± 9	83 ± 12	<.05
PL	—	88 ± 14	110 ± 16	101 ± 14	92 ± 11	92 ± 12	NS
n-3 FA	—	115 ± 18*	127 ± 22	122 ± 19	106 ± 17*	103 ± 12*	NS
HTGL (μU/mL)							
BL	—	649 ± 60	690 ± 69	667 ± 61	690 ± 59	674 ± 78	NS
PL	—	632 ± 75	673 ± 78	654 ± 67	654 ± 71	662 ± 67	NS
n-3 FA	—	611 ± 67	651 ± 70	652 ± 72	652 ± 72	616 ± 68	NS

NOTE. To convert to mg/dL, multiply C by 38.7, apo B by 54, and apo A-I by 28.
Abbreviations: PL, placebo; BL, baseline.
*$P < .05$ *v* BL.
†$P < .05$ *v* PL.

(0 minutes 4.25 ± 0.36, 15 minutes 3.89 ± 0.32, and 120 minutes 3.96 ± 0.32 mmol/L, $P < .001$) (Table 2).

Effects of n-3 FA on Individual Lipoproteins in the Fasting State and During Lipolysis

Changes in individual lipoprotein fractions were determined after isolation by FPLC. Figure 2 depicts typical changes in plasma lipoproteins during lipolysis.

VLDL. n-3 FA supplementation, as compared with placebo, decreased both VLDL-TG (1.08 ± 0.18 *v* 2.17 ± 0.30 mmol/L) and VLDL-C (0.39 ± .05 *v* 0.72 ± 0.13 mmol/L) without altering the ratio of TG to C within the particle (VLDL-TG/VLDL-C ratios are 2.71:1 during placebo and 2.77:1 during n-3 FA periods). During the placebo period, significantly more TG disappeared from the VLDL fraction during lipolysis (decrease in VLDL-TG, 1.84 ± 0.20

Table 3. Changes in C Content of VLDL, IDL, LDL, and HDL During Intravascular Lipolysis at Baseline and After Placebo and n-3 FA Periods (mean ± SEM, n = 11)

Parameter	0 Minutes	30 Minutes	60 Minutes	120 Minutes	*P* (repeated-measures ANOVA)
VLDL-C (mmol/L)					
BL	0.72 ± 0.13	0.54 ± 0.13	0.36 ± 0.08	0.21 ± 0.05	<.0001
PL	0.80 ± 0.16	0.49 ± 0.10	0.28 ± 0.05	0.16 ± 0.03	<.0001
n-3 FA	0.39 ± 0.05*†	0.23 ± 0.05*†	0.13 ± 0.03*†	0.08 ± 0.03*†	<.0001
IDL-C (mmol/L)					
BL	0.49 ± 0.08	0.59 ± 0.08	0.59 ± 0.08	0.47 ± 0.08	<.003
PL	0.49 ± 0.05	0.54 ± 0.08	0.47 ± 0.08	0.41 ± 0.08	NS
n-3 FA	0.36 ± 0.05	0.36 ± 0.08	0.34 ± 0.08	0.26 ± 0.05	<.005
LDL-C (mmol/L)					
BL	2.90 ± 0.23	2.95 ± 0.21	2.95 ± 0.23	3.03 ± 0.26	NS
PL	2.90 ± 0.26	2.90 ± 0.21	3.00 ± 0.18	3.18 ± 0.23	<.04
n-3 FA	3.59 ± 0.21*†	3.24 ± 0.21*†	3.54 ± 0.21*†	3.65 ± 0.23*†	<.05
HDL-C (mmol/L)					
BL	0.85 ± 0.08	0.85 ± 0.05	0.83 ± 0.05	0.96 ± 0.10	NS
PL	0.96 ± 0.10	0.93 ± 0.10	0.98 ± 0.10	1.01 ± 0.10	NS
n-3 FA	0.85 ± 0.10	0.83 ± 0.08	0.83 ± 0.10	0.83 ± 0.08	NS

NOTE. To convert to mg/dL, multiply by 38.7.
*$P < .05$ *v* BL.
†$P < .05$ *v* PL.

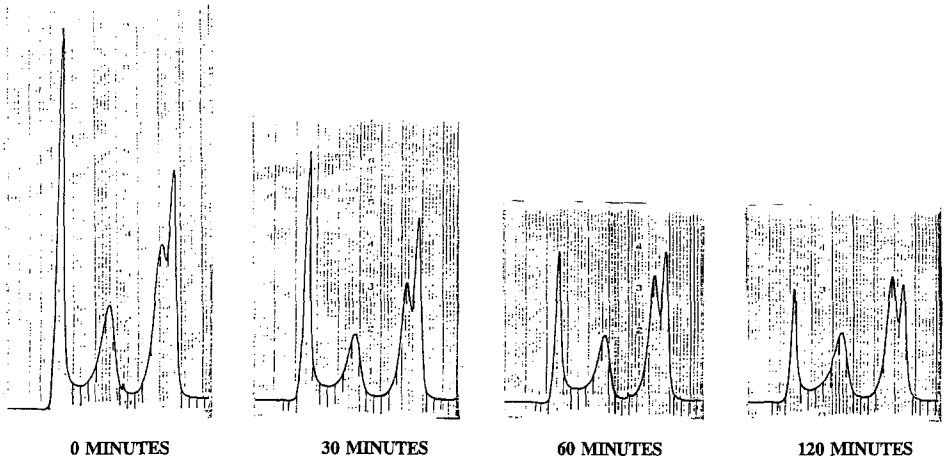

Fig 2. Changes in FPLC patterns of plasma lipoproteins during lipolysis, isolated by FPLC and monitored at 280 nm.

mmol/L/2 h during placebo and 0.97 ± 0.15 during n-3 FA). Similarly, the decrease in VLDL-C was greater during placebo (0.52 ± 0.08 mmol/L/2 h) as compared with n-3 FA supplementation (0.31 ± .03). Decreases in VLDL-TG and VLDL-C correlated with their respective fasting values (Tables 3 and 4).

IDL. n-3 FA caused small decreases in fasting concentrations of IDL-TG (placebo 0.40 ± 0.07 and n-3 FA 0.32 ± 0.05 mmol/L) and IDL-C (placebo 0.49 ± 0.05 and n-3 FA 0.36 ± 0.05 mmol/L). Lipolysis decreased IDL-TG and IDL-C significantly (Tables 3 and 4).

LDL. Fasting LDL-C levels were significantly higher during n-3 FA supplementation versus placebo (3.59 ± 0.21 *v* 2.90 ± 0.23 mmol/L). During lipolysis, changes in LDL-C followed different patterns after n-3 FA supplementation and placebo periods. During placebo, accelerated lipolysis caused a significant increase in LDL-C within 2 hours (0 minutes 2.90 ± 0.26, 30 minutes 2.90 ± 0.21, and 120 minutes 3.18 ± 0.23 mmol/L, $P < .04$). During n-3 FA supplementation, LDL-C first decreased at 30 minutes and returned to fasting values after 2 hours (fasting 3.59 ± 0.21, 30 minutes 3.24 ± 0.21, and 120 minutes 3.65 ± 0.23 mmol/L, $P < .05$). Changes in LDL-C correlated inversely with changes in VLDL-C ($r = -.41$, $P < .03$), in concordance with the precursor-product relationship (Table 3).

HDL. n-3 FA supplementation did not change fasting HDL-TG or HDL-C levels. The majority of patients displayed two separate HDL peaks on FPLC (Fig 2). During intravascular lipolysis, the size of the first HDL peak increased (Tables 3 and 4).

Effects of n-3 FA on Lipases

LPL. LPL activity was determined at five different time points during heparin infusion. Overall responses of LPL, considering all five points of determination, showed an increase in LPL activity with n-3 FA supplementation (86 ± 4 mU/mL at entry, 96 ± 4 after placebo, and 115 ± 4 after n-3 FA; ANOVA repeated measures, $P < .002$). The highest levels of LPL activity were detected at 30 and 60 minutes. Individual LPL activities measured at 15, 90, and

Table 4. Changes in TG Content of VLDL, IDL, LDL, and HDL During Intravascular Lipolysis at the Baseline and After Placebo and n-3 FA Periods (mean ± SEM, n = 11)

Parameter	0 Minutes	30 Minutes	60 Minutes	120 Minutes	*P* (repeated-measures ANOVA)
VLDL-TG (mmol/L)					
BL	2.35 ± 0.35	1.30 ± 0.27	0.82 ± 0.19	0.46 ± 0.15	<.0001
PL	2.17 ± 0.30	1.20 ± 0.27	0.58 ± 0.14	0.33 ± 0.11	<.0001
n-3 FA	1.08 ± 0.18*†	0.44 ± 0.10*†	0.18 ± 0.05*†	0.11 ± 0.03*†	<.0001
IDL-TG (mmol/L)					
BL	0.60 ± 0.16	0.51 ± 0.08	0.38 ± 0.08	0.26 ± 0.08	<.005
PL	0.40 ± 0.07	0.38 ± 0.08	0.32 ± 0.08	0.23 ± 0.07	<.0005
n-3 FA	0.32 ± 0.05	0.26 ± 0.06	0.17 ± 0.05	0.10 ± 0.02	<.005
LDL-TG (mmol/L)					
BL	0.43 ± 0.05	0.51 ± 0.06	0.56 ± 0.05	0.59 ± 0.07	<.005
PL	0.44 ± 0.05	0.51 ± 0.06	0.51 ± 0.06	0.50 ± 0.06	NS
n-3 FA	0.43 ± 0.03	0.53 ± 0.05	0.50 ± 0.05	0.51 ± 0.07	NS
HDL-TG (mmol/L)					
BL	0.15 ± 0.01	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	NS
PL	0.14 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	NS
n-3 FA	0.14 ± 0.01	0.10 ± 0.02	0.10 ± 0.02	0.12 ± 0.02	NS

NOTE. To convert to mg/dL, multiply by 88.6.

*$P < .05$ *v* BL.

†$P < .05$ *v* PL.

120 minutes were higher during n-3 supplementation than those measured at entry onto the study. Activity of LPL at 2 hours correlated inversely with fasting plasma TG ($r = -.53$, $P < .05$). However, changes in VLDL-TG, VLDL-C, or LDL-C were not related to LPL activity (Table 2).

HTGL. Overall responses of HTGL showed a small but significant decrease during n-3 FA supplementation (674 ± 28 mU/mL at entry, 655 ± 31 after placebo, and 635 ± 30 after n-3 FA; ANOVA repeated measures, $P < .03$).

DISCUSSION

This study was performed to investigate whether n-3 FA supplementation promotes production of TG-poor VLDL particles and accelerates lipolysis of VLDL into LDL. The current findings did not support this hypothesis, and demonstrated that supplementation of n-3 FA does not accelerate in vivo lipolysis of VLDL-TG. The absolute rate of TG lipolysis primarily depended on the fasting concentration of VLDL-TG, and was therefore lower during n-3 FA supplementation. The relative rate of lipolysis, adjusted for baseline TG, was not altered by n-3 FAs. n-3 FAs decreased the fasting pools of VLDL-TG and VLDL-C without changing the ratio of C to TG within the particle. During lipolysis, the amount of C that disappeared from the VLDL and appeared in the LDL fraction correlated with the size of the precursor VLDL-C pool. n-3 FA supplementation decreased the precursor VLDL-C pool and therefore reduced the amount of C that appeared in LDL during lipolysis. A similar positive correlation between the size of the VLDL pool and recovery of C and phospholipids in the LDL fraction was previously reported during in vitro lipolysis.[30] To our knowledge, the present study is the first to demonstrate such a relationship in vivo in humans.

Changes in catabolism of VLDL have been investigated primarily by kinetic studies radiolabeling VLDL apo B and/or VLDL-TG,[7-9] which also showed decreases in fasting VLDL-TG and VLDL-C pools. Studies that endogenously labeled TG with radioactive glycerol showed that the decrease in VLDL pool size is primarily due to a lower synthetic rate of TG.[9,31] Effects of n-3 FA on catabolism of VLDL were found to be variable in humans: no change, small change, and major increases in the fractional catabolic rate of VLDL-TG have been reported.[9,31] A study in miniature pigs showed an increase in conversion of VLDL apo B to LDL apo B with n-3 FA supplementation.[32] The variability seen in kinetic studies may be due to the following reasons: These studies require either elimination or severe restriction of dietary fat intake beforehand. It has been shown that restriction of n-3 FAs, even for a short period, may result in a rebound in hepatic production of VLDL-TG and may prevent achievement of a steady state.[9] In addition, studies labeling VLDL apo B may produce different results than those labeling VLDL-TG, since TG and apo B components of VLDL can be catabolized differently.[5] To our knowledge, metabolic response of VLDL-C to n-3 FAs has not been studied by kinetic methods, since VLDL-C has not yet been successfully radiolabeled.

Our study provided information about both TG and C moieties of VLDL during lipolysis. Unfortunately, we were unable to determine changes in VLDL apo B, since the eluate obtained from FPLC was too dilute for accurate measurement of VLDL apo B levels. The findings of this report and results of previously published kinetic studies, taken together, suggest that an increase in lipolysis of VLDL into LDL may initially play a role in increasing LDL-C and apo B levels. However, after a new steady state is established, increases sustained in LDL-C and apo B are probably maintained through other mechanisms. It was previously reported that n-3 FA decreases apo B–mediated binding of LDL to receptors in Hep G2 cell cultures[33] and apo E–mediated binding to fibroblasts,[34] suggesting an inhibition of LDL clearance.

The primary action of n-3 FA is believed to be inhibition of hepatic TG synthesis, by suppressing activities of diacylglycerol acyltransferase,[35] phosphatidate phosphohydrolase,[36] malic enzyme, and glucose-6-phosphate dehydrogenase.[37] In this study, we also found an increase in the activity of LPL, which correlated inversely with fasting plasma TG, suggesting a possible increase in clearance of TG. We have previously reported a transient increase in postheparin LPL activity during supplementation of n-3 FA in subjects with type II diabetes mellitus.[16] In the present study, we also noted a small but significant decrease in the activity of HTGL during n-3 FA supplementation. On the other hand, Harris et al[38] and Nozaki et al[39] did not find any change in postheparin LPL or HTGL during n-3 FA supplementation in healthy control subjects or hypertriglyceridemic patients, respectively. Discrepancies between our findings and those reported by Harris et al and Nozaki et al may be due to differences in study populations, diets, and methods of heparin administration. Harris et al[38] studied younger healthy male and female subjects, whereas Nozaki et al[39] exclusively studied older men. Both of these investigations used well-defined study diets, whereas we kept patients on their usual diets. The previous studies used a single bolus of heparin injection to measure LPL and HTGL, whereas we administered heparin by continuous infusion. In all these studies, including ours, only enzyme activity was measured; it is not clear whether n-3 FA alters LPL enzyme mass.

An important observation was that despite the finding of an inverse correlation between baseline plasma TG level and LPL activity, there was no correlation between the magnitude of decrease in plasma TG or VLDL-TG and LPL at any time point during lipolysis. The amount of decrease in plasma TG and VLDL-TG was directly related to the baseline levels. One possible explanation for this finding may be that when LPL is attached to endothelial surfaces the relative abundance of LPL influences the degree of lipolysis. However, once large amounts of LPL are suddenly released into the circulation, the rate-limiting factor becomes the relative abundance of the substrate.

We noted that there was minimal lipolysis after 90

minutes of heparin infusion, despite the fact that both plasma TG levels and LPL activity were still high. This finding suggested that factors other than VLDL-TG concentration and LPL activity were limiting the rate of lipolysis. It is known that the lipolytic process depends not only on availability of LPL but also on adequate amounts of fatty acid acceptors in plasma.[40] Furthermore, excess amounts of free fatty acids can stop and even reverse lipolysis.[40] Therefore, the decrease in the rate of lipolysis with time may have been due to saturation of fatty acid acceptors. Another possibility is that TG values measured in plasma may have underestimated the actual magnitude of lipolysis, since glycerol released into plasma interferes with the enzymatic assay for TG, producing higher values. To overcome this problem, TG values were also measured in individual lipoproteins separated by FPLC. Changes in VLDL-TG mirrored changes in plasma TG and therefore excluded the possibility of underestimation of TG lipolysis.

n-3 FA supplementation increased fasting apo B concentration, suggesting an increase in the particle number of LDL, as previously reported.[16] During lipolysis, apo B decreased during the first 15 minutes, without any further decrease. Disappearance of apo B from the vascular space may be due to several reasons: It has been reported that in vitro lipolysis exposes biologically unreactive apo E and apo B on VLDL and promotes their binding to the LDL receptor.[41] In vivo heparin injection causes uptake of chylomicron remnants and IDL by the liver,[24] which may be mediated by LDL-receptor–related protein.[42,43] Heparin enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix.[44] Fatty acids and lipases that are released to the circulation after heparin surround lipoproteins, masking the epitopes of apo B and producing artificially low readings. There was also an early and sustained decrease in plasma apo A-I during lipolysis, without any significant change in either HDL-C or HDL-TG; the etiology of the decrease in apo A-I is not clear.

We also monitored changes in plasma glucose, insulin, and NEFA, since n-3 FA supplementation can increase plasma glucose, decrease insulin secretion, and improve insulin sensitivity.[16,45] In our study population, there was no change in these parameters.

In summary, this study confirmed that n-3 FA supplementation increased fasting LDL-C and apo B concentrations while decreasing plasma TG. This increase in LDL-C did not appear to be due to an acceleration in catabolism of VLDL into LDL, since there was no increase in lipolysis of VLDL-TG during n-3 supplementation, and furthermore, VLDL-C, the precursor pool for LDL-C, was decreased. Therefore, we propose that the increase in fasting LDL-C seen during n-3 FA supplementation is mediated by other mechanisms, such as a decrease in clearance of LDL.

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