



## Correlation of serum triglyceride and its reduction by $\omega$ -3 fatty acids with lipid transfer activity and the neutral lipid compositions of high-density and low-density lipoproteins

Henry J. Pownall<sup>a,\*</sup>, Danièle Brauchi<sup>a</sup>, Cumhuri Kiliç<sup>a</sup>, Karin Osmundsen<sup>b</sup>, Quein Pao<sup>a</sup>, Charlotte Payton-Ross<sup>a</sup>, Antonio M. Gotto Jr.<sup>a</sup>, Christie M. Ballantyne<sup>a</sup>

<sup>a</sup> Department of Medicine, MS A-601, Baylor College of Medicine and The Methodist Hospital, 6565 Fannin St., Houston TX 77030, USA

<sup>b</sup> Pronova Biocare a.s., Lysaker, Norway

Received 9 February 1998; received in revised form 8 September 1998; accepted 6 November 1998

### Abstract

Serum triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) concentrations are inversely correlated and mechanistically linked by means of lipid transfer activities. Phospholipid transfer activity (PLTA) moves phospholipids among serum lipoproteins; cholesteryl ester transfer activity (CETA), which exchanges cholesteryl esters (CE) and TG among lipoproteins, is stimulated by nonesterified fatty acids (NEFA). The aims of this study were (a) to develop a quantitative model that correlates the neutral lipid (NL = CE + TG) compositions of HDL and LDL with serum TG concentration; (b) identify the serum lipid determinants of CETA and PLTA, and; (c) identify the effects of serum TG reductions on the neutral lipid compositions of HDL and LDL, serum NEFA concentrations, and on PLTA and CETA. These aims were addressed in 40 hypertriglyceridemic subjects before and after treatment with an 85% concentrate of  $\omega$ -3 fatty acids (Omacor<sup>TM</sup>) and in 16 untreated normolipidemic subjects. In vivo, the NL compositions of LDL and HDL were described by a mathematical model having the form of adsorption isotherms: HDL – (TG/NL) =  $(0.90 \pm 0.07)$  serum TG /  $(7.0 \pm 1.2 \text{ mmol/l} + \text{serum TG})$  and LDL – (TG/NL) =  $(0.65 \pm 0.08)$  serum TG /  $(4.9 \pm 1.5 \text{ mmol/l} + \text{serum TG})$ . Reduction of serum TG was associated with reductions in HDL – (TG/NL), serum NEFA concentration, and serum CETA but not PLTA. These data suggest that both hypertriglyceridemia and the attendant elevated serum CETA but not PLTA are determinants of HDL and LDL composition and structure and that serum TG concentrations are good predictors of the NL compositions of HDL and LDL. © 1999 Published by Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:**  $\omega$ -3 Fatty acids; High-density lipoprotein structure; Lipid metabolism; Lipid transfer; Low-density lipoprotein structure; Serum triglyceride

**Abbreviations:** Apo, apolipoprotein; CE, cholesteryl esters; CETA, CE transfer activity; CETP, CE transfer protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFA, nonesterified fatty acids; NL, neutral lipid (triglyceride and cholesteryl esters); PC, phosphatidylcholine; PLTA, phospholipid transfer activity; PLTP, phospholipid transfer protein; SOPC, *sn*-1-stearoyl-*sn*-2-oleoyl PC; TG, triglyceride; VLDL, very low density lipoprotein.

\* Corresponding author. Tel.: +1-713-798-4160; fax: +1-713-798-4121.

E-mail address: hpownall@bcm.tmc.edu (H.J. Pownall)

### 1. Introduction

Hypertriglyceridemia is associated with derangements in other serum lipid and lipoprotein analytes, including decreased concentrations of high-density lipoprotein cholesterol (HDL-C) [1] and the occurrence of small, dense low-density lipoproteins (LDL) [2,3]. The serum activities of cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are important qualitative determinants of the composition of

serum lipoproteins [4,5]. CETP mediates the exchange of HDL cholesteryl esters (HDL-CE) and LDL cholesteryl esters (LDL-CE) for very low density lipoprotein triglyceride (VLDL-TG); CETP also mediates the transfer of phospholipids among lipoproteins [6]. In hypertriglyceridemic subjects, many of the LDL-CE and HDL-CE are displaced by TG derived from the large pool of VLDL-TG through a reaction that is mediated by CETP [7,8]. The role of PLTP, which transfers phospholipids among lipoprotein subclasses, is less clear. PLTP stimulates cholesteryl ester transfer activity (CETA) in vitro [9,10] and appears to be important in HDL remodelling [11–13].

Hypotriglyceridemic interventions lead to profound changes in serum lipoprotein metabolism and the serum lipid profile. In addition to lowering serum TG concentrations, fibric-acid derivatives increase serum HDL-C and CETA [14] and reduce the magnitude of postprandial lipemia [15]. However, some of these effects are a function of the plasma lipid levels. In normolipidemic subjects but not in hypertriglyceridemia, CETA correlated with VLDL-TG. In contrast, in hypertriglyceridemia, CETA correlated with endogenous CETA activity [16]. In hypercholesterolemic patients,  $\omega$ -3 fatty acids reduce serum TG concentrations with little or no attendant effect on serum HDL-C concentrations; at the same time, serum CETA [17,18] and postprandial lipemia decrease [19]. Thus, changes in serum CETA are a function of the type of hyperlipidemia and the intervention used to manage plasma lipid levels.

Key questions about the remodelling of lipoproteins in hypertriglyceridemic subjects lead to several hypotheses. First, although there is a positive relation between serum TG concentration and the TG content of HDL and of LDL, can this relation be described by a quantitative model based on the molecular mechanism? Moreover, does TG reduction lead to predictable changes in the TG content of HDL and LDL? We hypothesize that the neutral lipid (NL) content of HDL and LDL as defined by TG/NL, where  $NL = TG + CE$ , increases according to a saturation model that has the form of an adsorption isotherm, and that this model is supported by a mechanism in which CETP mediates the exchange of VLDL-TG for HDL-CE and LDL-CE. Furthermore, we hypothesize that the changes in the TG content of HDL and LDL following a hypolipidemic intervention can be predicted by a saturation model. Second, is the exchange of VLDL-TG for HDL-CE a major determinant of serum HDL-C? We hypothesize that the exchange of VLDL-TG for HDL-CE decreases the amount of cholesterol carried by HDL, thereby lowering serum HDL-C concentrations. Third, is serum phospholipid transfer activity (PLTA) elevated in hypertriglyceridemic subjects, and if so, is it lowered by a hypolipidemic intervention? We hypothesize, given the structural and functional homology between PLTP and

CETP [20], that PLTA like CETA will be higher in hypertriglyceridemic subjects than in normolipidemics, and that reduction of serum TG will lower serum CETA and PLTA. Finally, serum nonesterified fatty acids (NEFA) are a possible source of fatty acids for hepatic TG synthesis. Studies in animal models have shown that serum NEFA concentrations are lowered by dietary consumption of  $\omega$ -3 fatty acids [21,22]. We hypothesize that reduction of serum TG by a hypolipidemic intervention will be associated with a reduction in serum NEFA concentrations. These hypotheses were addressed by (a) a study of 40 hypertriglyceridemic subjects who were given a concentrate of  $\omega$ -3 fatty acids or a corn oil placebo for 6 weeks and of 16 untreated normolipidemic subjects, and (b) in vitro studies of CETP-mediated transfer of VLDL-TG for LDL-CE and for HDL-CE. The primary clinical objective of the study was to determine the effects of treatment with an 85% concentrate of  $\omega$ -3 fatty acids on serum TG concentration and on the serum concentration and/or composition of other lipid variables that we hypothesized to be mechanistically linked to hypertriglyceridemia and serum TG reduction.

## 2. Methods

### 2.1. Design and patients

Sixteen untreated normolipidemic subjects were enrolled as controls (TG range: 0.75–3.0 mmol/l, or 66–262 mg/dl) from the laboratory staff and 40 patients with severe type IV hypertriglyceridemia were investigated in these studies. The latter group entered a randomized, double-blind, parallel group study comparing 6 weeks of treatment with  $\omega$ -3 fatty acids with a placebo. The  $\omega$ -3 fatty acids were in the form of an 85% concentrate of their ethyl esters (Omacor<sup>TM</sup>; Pronova Biocare, Lysaker, Norway), which has been shown to reduce serum TG concentrations [23] and the placebo used was corn oil.

Subjects eligible for randomization were men and women aged 18–70 years with mean fasting serum TG  $\geq 5.6$  mmol/l (500 mg/dl) but  $\leq 22.6$  mmol/l (2000 mg/dl) despite dietary counseling (see below). Exclusion criteria were treatment with a fibrate  $< 3$  months before entering the dietary phase; treatment with another  $\omega$ -3 fatty acid product, cod-liver oil, or a dietary fiber with lipid-lowering effects  $< 4$  weeks before entering the dietary phase; consumption of cold-water fish more than once a week; myocardial infarction or another serious disease  $< 6$  months before baseline; serum alanine transaminase  $> 3$  times the upper limit of normal; fasting serum glucose  $> 16.7$  mmol/l (300 mg/dl); serum creatinine  $> 176.8$   $\mu$ mol/l; platelets  $< 60 \times 10^9/l$ ; hemoglobin  $< 100$  g/l; pregnancy or breast-feeding;

Table 1  
Study schedule<sup>a</sup>

	Screening	Dietary lead-in				Randomization	Double-blind intervention ( $\omega$ -3 fatty acids or placebo)					
		-6	-4	-2	-1	0	1	2	3	4	5	6
Week												
Visit	1	2	3	4	5	6	7	8	9	10	11	12
Dietary instruction		x										
Dietary assessment				x				x				x
Serum TG	x	x		x	x	x		x		x	x	x
Serum lipid profile						x						x
Capsule count								x		x		x
Safety assessment			x	x	x	x		x	x	x	x	x

<sup>a</sup> Note: All assessments were at clinic visits except for questionnaire safety assessments conducted by telephone during weeks -4, 1 and 3.

alcohol or drug abuse; and type 1 diabetes mellitus. The protocol was reviewed and approved by the Baylor College of Medicine and The Methodist Hospital Institutional Review Board. Participants gave written informed consent and were monetarily compensated.

The clinical study comprised 6 weeks of dietary lead-in (preceded by a screening visit and followed by randomization) and 6 weeks of double-blind active intervention ( $\omega$ -3 fatty acids or placebo) (Table 1). Clinic visits were scheduled for mornings after minimum 12 h fasting and 24 h abstention from alcohol. Volunteers confirmed to have fasting serum TG of 5.6–22.6 mmol/l (500–2000 mg/dl) at a screening visit received oral and written instruction on the American Heart Association Step I Diet [24] from a registered dietician. Alcohol use was discouraged; however, a maximum of 2 drinks per day (1 drink = 44 ml liquor, 148 ml wine, or 355 ml beer) was permitted. Patients were encouraged, but not required, to exercise moderately. After 6 weeks of dietary therapy, eligibility for the active intervention phase was determined by the mean of the fasting serum TG concentrations from weeks 2 and 1. Patients whose mean TG value was not reduced sufficiently (< 5.6 mmol/l) by diet were randomized to receive  $\omega$ -3 fatty acids or placebo beginning at week 0. Patients were told to continue the diet (and physical activity, if any) throughout the trial during which dietary assessments were performed by a registered dietician.

Corn oil or Omacor was given at 4 g/day, a dosage shown to be effective for TG lowering by previous studies of other  $\omega$ -3 fatty acid formulations [25]. Corn oil has previously been shown to be neutral with respect to alterations in blood lipid concentrations [26,27]. The compositions of Omacor and corn oil are shown in Table 2. Approximately 90% of the fatty acids in Omacor are  $\omega$ -3 isomers, and about 84% are eicosapentaenoic and docosahexaenoic acids, the essential hypotriglyceridemic components (Table 2). Each capsule contained approximately 1 g of fat (9 kcal). Patients were instructed to take capsules with a meal and were

free to take either four capsules once daily or two capsules twice daily. Apart from the study medication, all lipid-lowering drugs, as well as cod-liver oil and other  $\omega$ -3 products, were proscribed for the duration of the study. All other medications were allowed if deemed necessary for the welfare of the patient by a physician. Patients taking concomitant medication were stabilized on their dosage before entry into the study and instructed not to vary the dosage for the duration of the study.

The baseline serum TG value for the assessment of efficacy was defined as the median of fasting values from weeks -2, -1, and 0. The serum TG value with treatment was defined as the median of fasting values from weeks 4, 5, and 6. Multiple TG measurements were averaged because TG concentrations fluctuate considerably, particularly in severe hypertriglyceridemia

Table 2  
Composition of  $\omega$ -3 fatty acids and corn oil placebo

Component	$\omega$ -3 fatty acids	Corn oil
<i>Fatty acid (weight %)</i> <sup>a</sup>		
16:0		10.0
18:0		2.3
18:1 $\omega$ -9		26.8
18:2 $\omega$ -6		56.3
18:3 $\omega$ -6		1.2
18:4 $\omega$ -3	2.2	
20:4 $\omega$ -3	2.2	
20:4 $\omega$ -6	2.2	
20:5 $\omega$ -3	44.4	
21:5 $\omega$ -3	1.8	
22:5 $\omega$ -3	2.4	
22:5 $\omega$ -6	1.0	
22:6 $\omega$ -3	36.2	
20:5 + 22:6 $\omega$ -3	80.6	
Total $\omega$ -3	89.2	
Cholesterol (mg/g)	0.5	6.8
Vitamin E (IU/g)	4.8	4.8

<sup>a</sup> Fatty acids accounting for < 1.0% of weight are not shown.

[28,29]. Other lipid values were determined at weeks 0 and 6 only.

Compliance as assessed by the mean of capsule counts of unused medication at weeks 2, 4 and 6 was 95% (range 85–100%) for the treated group and 96% (range 83–100%) for the placebo group. Safety was monitored through a weekly questionnaire administered at the clinic or by telephone. Perceived side effects were reviewed by the physician to assess seriousness and relation to trial medication. Alanine transaminase and aspartate transaminase, blood pressure, and glucose concentration were determined at weeks 0 and 6.

### 3. Materials

Egg phosphatidylcholine (PC), cholesteryl oleate and cholesterol were obtained from Sigma Chemical (St. Louis, MO). [ $^3\text{H}$ ]cholesteryl oleate (47 mCi/mmol) was purchased from Amersham Life Science (Arlington Heights, IL); [ $^3\text{H}$ ]*sn*-1-stearoyl-*sn*-2-oleoyl PC (SOPC) was prepared by the methylation of *sn*-1-stearoyl-*sn*-2-oleoyl phosphatidylethanolamine with [ $^3\text{H}$ ]CH<sub>3</sub>I (Amersham) by using the method of Patel et al. [30] and purified by normal-phase high-performance liquid chromatography [31]. Apolipoprotein A-I (apo A-I) was isolated and purified as previously described [32].

### 4. Analytical methods

After an overnight fast and a 15 min period of complete inactivity, venous blood samples were collected in serum separator tubes, and transferred to ice. Serum was separated by low speed centrifugation at 4°C. Aprotinin and phenyl methyl sulfonyl fluoride were added to each sample at a final concentration of 10 kallikrein units/ml and 0.1 mM, respectively. Major serum lipoprotein classes were separated by KBr density gradient ultracentrifugation using a Beckman SW-40 swinging bucket rotor run for 22 h at 40 000 rpm at 20°C in a Beckman L5-65 ultracentrifuge [33,34]. Lipoprotein bands were aspirated sequentially from the tops of the centrifuge tubes by Auto-Densi IIC (Haakebuchler Instrument, Saddlebrook, NJ), and 0.3 ml fractions were collected. VLDL, LDL and HDL were isolated and stored at –70°C and later analyzed.

Total serum TG, haematological analytes, and other routine nonlipid laboratory values were determined by National Health Laboratories (Houston, TX), a certified laboratory using established methods. The TG, cholesterol, and CE components of lipoproteins were measured by using enzymatic kits from Boehringer–Mannheim Diagnostics (Indianapolis, IN). NEFA were assayed on the day of collection or after thawing of quick-frozen serum (stored at –20°C), using an enzymatic kit from Boehringer–Mannheim.

#### 4.1. Serum cholesteryl ester transfer activity

Lipid transfer assays were performed with 39 mM phosphate buffer (pH 7.4) containing 30 mM Na<sub>2</sub>HPO<sub>4</sub>, 8 mM KH<sub>2</sub>PO<sub>4</sub>, and 0.025% EDTA unless otherwise indicated. CETA was measured by a previously described method [35,36] using reassembled HDL (R HDL) as the CE donor and LDL as the acceptor with some minor modifications. R HDL were prepared from a mixture of 22.5 μmol of egg PC, 7.5 μmol of cholesterol, and 0.3 μmol cholesteryl oleate including 2.5 μCi [ $^3\text{H}$ ]cholesteryl oleate. This mixture was dried under nitrogen, lyophilized for 1 h, and dissolved in 1 ml of absolute ethanol, which was rapidly injected into 20 ml phosphate buffer at room temperature through a syringe equipped with a 26 gauge needle. After stirring for 15 min, 1.9 ml of 200 mM sodium cholate and 9 mg of apo A-I in phosphate buffer were added to the lipid mixture while stirring. The mixture was incubated for 30 min at room temperature and dialyzed at 4°C against phosphate buffer. The resulting R HDL was diluted to a final volume of 27.5 ml with phosphate buffer.

LDL was isolated from the serum of normolipidemic donors by sequential ultracentrifugation with KBr between  $d = 1.006$  and  $1.063$  g/ml. The LDL was further purified by heparin–Sepharose CL 4B chromatography, and the low salt fraction discarded. The LDL was dialyzed against phosphate buffer at 4°C and passed through a 0.45 μm filter.

The assay mixture consisted of 110 μl R HDL containing 90 nmol of egg PC, 30 nmol cholesterol, 1.2 nmol of [ $^3\text{H}$ ]cholesteryl oleate (8.33 μCi/μmol) and 36 μg apo A-I as the CE donor, 27 μl LDL containing 107 μg LDL protein as the acceptor, and 1–3 μl serum, which contains endogenous CETA, in a final volume of 300 μl phosphate buffer containing 60 mmol NaCl and 270 μmol/ml 5,5'-dithiobis(2-nitrobenzoic acid), which suppresses the activity of lecithin:cholesterol acyltransferase. The CETP assay was linear up to 10 μl; all activities were normalized to 1 μl. The samples were incubated at 37°C for 3 h, after which the tubes were placed in an ice bath and the LDL was precipitated with 30 μl of 60 mmol MgCl<sub>2</sub> and 30 μl of 0.1% dextran sulfate in phosphate buffer containing 60 mmol NaCl. After 20 min in an ice bath, the assay mixtures were centrifuged at 14 000 rpm for 10 min at 4°C. The supernatant containing the R HDL was collected. The precipitated LDL was dissolved in 100 μl of 0.1 M NaOH. Radioactivity in the supernatant and in the precipitate was quantified by liquid scintillation counting. The CE transfer was calculated according to:

$$\text{CETA (\%)} = \left( \frac{\text{cpm}_{\text{precipitate}}}{\text{cpm}_{\text{precipitate}} + \text{cpm}_{\text{supernatant}}} \right) \times 100\% \quad (1)$$

Nonspecific CETA was calculated by applying Eq. (1) to an assay in which serum was replaced by buffer. CETA was calculated as the difference between total CE transfer and nonspecific CE transfer. CETA was expressed as percent transfer per  $\mu\text{l}$  serum during a 3 h incubation.

#### 4.2. Serum phospholipid transfer activity

With minor modifications, a previously described method [37] was used to determine PLTA. PC vesicles were used as a phospholipid donor, and HDL<sub>3</sub> was used as acceptor. The donor was prepared from 12.5  $\mu\text{mol}$  egg PC and 3.7  $\mu\text{Ci}$  [<sup>3</sup>H]SOPC, which were dried under nitrogen and lyophilized for 1 h. The dried lipid residue was dissolved in 0.3 ml absolute ethanol and rapidly injected through a 26 gauge needle into 4.7 ml of phosphate buffer under continuous stirring. After incubation of the mixture for 30 min at room temperature, the ethanol was removed by extensive dialysis at 4°C against phosphate buffer. Acceptors of HDL<sub>3</sub> were isolated by sequential flotation of serum adjusted to a density of  $d=1.12$  g/ml, and then  $d=1.21$  g/ml with KBr. The HDL<sub>3</sub> was dialyzed extensively against phosphate buffer at 4°C and passed through a 0.45  $\mu\text{m}$  filter.

The assay mixture consisted of PC vesicles (91 nmol of PC) as the donor of PC, and HDL<sub>3</sub> (96 nmol of PC) as the acceptor of PC, plus 2–3  $\mu\text{l}$  serum, which contains the endogenous PLTA, from each subject and phosphate buffer containing 60 mmol NaCl to give a final volume of 280  $\mu\text{l}$ . The assay incubation was carried out at 37°C for 45 min. After incubation, the assay tubes were placed in an ice bath, and PC vesicles present in the assay mixture were precipitated by adding 40  $\mu\text{l}$  of 200 mM MgCl<sub>2</sub> and 80  $\mu\text{l}$  of 0.0085% dextran sulfate in phosphate buffer containing 60 mmol NaCl. After standing for 20 min in an ice bath, the assay mixtures were centrifuged at 14 000 rpm for 10 min at 4°C. The supernatant solution including HDL<sub>3</sub> was removed and the precipitated LDL was dissolved in 200  $\mu\text{l}$  0.1 M NaOH. The radioactivity in the supernatant and in the precipitate was quantified by liquid scintillation counting. Nonspecific transfer was determined as described for CETA, and protein-mediated transfer was calculated using Eq. (1).

#### 5. Effects of very low density triglyceride on triglyceride/neutral lipids in vitro

Two experiments examined the relation of CETA to LDL-(TG/NL) and HDL-(TG/NL). In the first, VLDL ( $d < 1.006$  g/ml) were removed by ultracentrifugation of fresh normolipidemic human serum obtained from The Methodist Hospital Blood Donor Center (Houston, TX). VLDL in various concentrations were then re-

turned to the VLDL-depleted serum along with a sufficient volume of Tris-buffered saline solution to bring the sample to the original serum volume. The samples were incubated at 37°C for 8 h, after which the VLDL, LDL and HDL were isolated by sequential flotation at  $d=1.006$ , 1.063 and 1.21 g/ml, respectively.

In the second experiment, VLDL was removed from the normolipidemic serum by flotation at  $d=1.006$  g/ml, and LDL and HDL were floated together at  $d=1.21$  g/ml. The mixture of LDL and HDL was then combined with various amounts of VLDL-TG, recombinant CETP (rCETP; final concentration 5  $\mu\text{g}/\text{ml}$ ), and sufficient buffer to bring the final volume of the mixture to the original serum volume. The rCETP was isolated from Chinese hamster ovary cells (Columbia Innovations, NY) according to the method of Weinberg et al. [38]. The samples were incubated at 37°C for 8 h, after which the VLDL, LDL and HDL were isolated by sequential flotation at  $d=1.006$  (VLDL),  $d=1.063$  g/ml (LDL) and  $d=1.21$  g/ml (HDL). After each of the two experiments was completed, the CE and TG concentrations of the isolated lipoproteins were determined, and the ratio TG/NL was calculated.

#### 6. Statistical methods

Analyses of the effects of  $\omega$ -3 fatty acids and placebo were intent-to-treat, using the statistical package Minitab for Windows, version 10.1. To establish the appropriate statistical method for further analysis, the Anderson–Darling normality test was used to determine the frequency distribution of each variable. The Wilcoxon's signed rank test or the Student's *t*-test was used to compare groups, and the Mann–Whitney test or the two-sample *t*-test was used to compare differences within a group. All significance levels are treated as two-sided. No statistical adjustment for multiplicity was performed. A two-tailed probability of  $P < 0.05$  was considered statistically significant.

#### 7. Results

##### 7.1. Patient characteristics

After screening, 24 men and 17 women were randomized to receive  $\omega$ -3 fatty acids ( $n=20$ ) or placebo ( $n=21$ ). One subject randomized to  $\omega$ -3 fatty acids did not complete the study and was omitted from data analysis. All other subjects were included in all analyses. There were no significant differences between the intervention groups at baseline in mean ( $\pm$  S.E.) age, height or weight ( $\omega$ -3 fatty acids:  $51.4 \pm 1.9$  years;  $168.9 \pm 2.7$  cm;  $85.2 \pm 4.3$  kg. Placebo:  $50.7 \pm 2.1$  years;  $172.8 \pm 2.2$  cm;  $87.3 \pm 3.8$  kg). Mean body weight in

Table 3  
Effects of intervention on median lipid concentrations<sup>a</sup>

Analyte <sup>b</sup>	$\omega$ -3 fatty acids ( $n = 19$ )				Placebo ( $n = 21$ )				Between 1 group $P$ with Rx <sup>d</sup>
	Baseline	With Rx	% Change	$P^c$	Baseline	With Rx	% Change	$P^c$	
TG	9.0 (801)	5.8 (512)	-38.9	0.001	8.8 (786)	7.5 (664)	-7.8	NS	0.001
Total cholesterol	8.4 (326)	7.5 (288)	-9.9	0.004	8.5 (328)	8.5 (328)	-5.6	NS	0.099
HDL-C	0.44 (17.0)	0.47 (18.0)	+5.9	0.057	0.46 (18.0)	0.41 (16.0)	-5.9	NS	0.023
LDL-C	1.1 (43.0)	1.4 (53.0)	+16.7	0.007	1.6 (60.0)	1.5 (57.0)	-4.2	NS	0.013
VLDL-C	4.8 (185)	3.5 (136)	-29.2	0.001	4.6 (179)	4.6 (177)	-7.3	NS	0.002

<sup>a</sup> Note: NS indicates not significant; TG was measured in serum at weeks -2, -1 and 0 for the baseline median and at weeks 4, 5 and 6 for the treatment median. Other lipids were determined in serum at weeks 0 and 6. The difference between the sum of lipoprotein-cholesterol and total cholesterol is due to VLDL-cholesterol and albumin-cholesterol, which was not collected from the density gradients that were used to isolate the major lipoprotein fractions.

<sup>b</sup> Analytes reported as mmol/l (mg/dl).

<sup>c</sup> Wilcoxon signed rank test was used to compare medians.

<sup>d</sup> Mann-Whitney analysis was used to compare medians.

both groups remained stable between baseline and the end of treatment ( $\omega$ -3 fatty acids +0.4%; placebo -0.3%). Eight patients had a prior diagnosis of type 2 diabetes mellitus with impaired glucose tolerance. Four of the eight were treated with insulin, two were treated with oral agents, and in two diabetes was controlled by diet and other lifestyle measures. Fasting serum glucose <16.7 mmol/l was maintained for the duration of the study in all patients. No serious side effects were reported by patients; alanine or aspartate transaminase values, blood pressure, and glucose concentrations (week 0 compared with week 6) remained within enrolment criteria.

## 7.2. Effects on lipid concentrations

There were no significant differences between the two intervention groups in median baseline lipid concentrations (Table 3). A substantial reduction in TG with  $\omega$ -3 fatty acids was seen after 2 weeks of treatment and persisted throughout the remainder of the trial (Fig. 1). With active intervention, median TG was reduced 38.9% from baseline in the  $\omega$ -3 fatty acid group ( $P = 0.001$ ) compared with a 7.8% reduction (not significant) in the placebo group; the difference between the changes in the two groups was significant ( $P = 0.001$ ). There were no significant differences between men and women in changes in TG concentration (comparison not shown).  $\omega$ -3 Fatty acids also significantly reduced median serum total cholesterol and VLDL-C (-9.9 and -29.2%, respectively) and significantly increased LDL-C (+16.7%) from baseline. The increase in median serum HDL-C (+5.9%) neared significance ( $P = 0.057$ ). However, the changes in each of these four cholesterol measurements were significant compared with the placebo (Table 3). Treatment with  $\omega$ -3 fatty

acids reduced median NEFA concentration from 0.86 to 0.66 mmol/l ( $P < 0.05$ ). Within the placebo group the changes were from 0.89 to 0.85 mmol/l ( $P > 0.05$ ); the difference between the  $\omega$ -3FA and placebo groups was not significant.

## 7.3. Effects of triglyceride lowering on high and low-density lipoprotein compositions

At baseline, median HDL-(TG/NL) and LDL-(TG/NL) in the intervention groups were not significantly different (Table 4). With TG-lowering intervention, median HDL-(TG/NL) and LDL-(TG/NL) decreased by 26.7% ( $P = 0.003$ ) and 21.3% ( $P = 0.009$ ), respectively

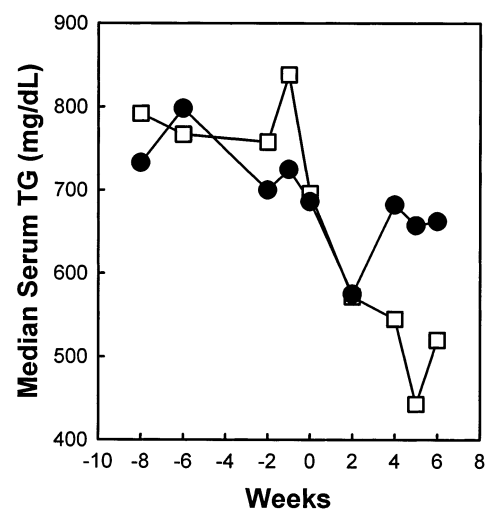


Fig. 1. Effects of  $\omega$ -3 fatty acids on mean ( $\pm$  S.E.M.) fasting serum TG concentration.  $\omega$ -3 Fatty acids subjects ( $n = 19$ ) =  $\square$ ; placebo subjects ( $n = 21$ ) =  $\bullet$ . Serum TG was not measured at weeks -5, -4, -3, or 1. Randomization to  $\omega$ -3 fatty acids or placebo was at week 0.

Table 4  
High and low-density lipoprotein weight ratios before and after intervention and in normolipidemic subjects<sup>a</sup>

Analyte	$\omega$ -3 Fatty acids ( <i>n</i> = 19)				Placebo ( <i>n</i> = 21)				+Rx, placebo vs. $\omega$ -3 fatty acids <sup>b</sup>	Control ( <i>n</i> = 16)
	Baseline	+Rx	% Change	<i>P</i> <sup>c</sup>	Baseline	+Rx	% Change	<i>P</i> <sup>c</sup>		
HDL-(TG/NL)	0.51	0.38	−26.7	0.003	0.49	0.53	+4.0	NS	0.006	0.18
LDL-(TG/NL)	0.45	0.29	−21.3	0.009	0.38	0.43	+4.8	NS	NS	0.17

<sup>a</sup> Note: NS indicates not significant. All values are medians. (TG/NL) determinations were made in serum at weeks 0 and 6 in the  $\omega$ -3 fatty acids and placebo groups. (TG/NL) determinations were made in serum in the normolipidemic (control) group.

<sup>b</sup> Two-sample *t*-test. There were no significant differences between the intervention groups at baseline.

<sup>c</sup> Student's *t*-test.

(Table 4). There were no significant changes in HDL-(TG/NL) and LDL-(TG/NL) in the placebo group with intervention.

At baseline, HDL-(TG/NL) was higher than LDL-(TG/NL) for both hypertriglyceridemic groups ( $\omega$ -3 fatty acids: 0.51 versus 0.45; placebo: 0.49 versus 0.38). In contrast, median HDL-(TG/NL) and LDL-(TG/NL) were similar for the 16 normolipidemic subjects (0.18 and 0.17, respectively) and lower than baseline and post-treatment values for the hypertriglyceridemic subjects (Table 4).

The correlation of HDL-(TG/NL) and LDL-(TG/NL) with serum TG was fitted to a model in which VLDL-TG exchanges for HDL-CE and LDL-CE on a time scale that is fast compared to HDL and LDL turnover. According to this model, an increase in VLDL-TG will increase the TG content of HDL and LDL while decreasing their CE content. Mathematically, this model is described by a hyperbolic function having the form of an adsorption isotherm (Fig. 2). The isotherms are described by:

$$\begin{aligned} \text{HDL-(TG/NL)} \\ = (0.90 \pm 0.07) \\ \text{serum TG} / (620 \pm 107 \text{ mg/dl} + \text{serum TG}) \end{aligned} \quad (2a)$$

$$\begin{aligned} \text{HDL-(TG/NL)} \\ = (0.90 \pm 0.07) \\ \text{serum TG} / (7.0 \pm 1.2 \text{ mmol/l} + \text{serum TG}) \end{aligned} \quad (2b)$$

$$\begin{aligned} \text{LDL-(TG/NL)} \\ = (0.65 \pm 0.08) \\ \text{serum TG} / (436 \pm 134 \text{ mg/dl} + \text{serum TG}) \end{aligned} \quad (3a)$$

$$\begin{aligned} \text{LDL-(TG/NL)} \\ = (0.65 \pm 0.08) \\ \text{serum TG} / (4.9 \pm 1.5 \text{ mmol/l} + \text{serum TG}) \end{aligned} \quad (3b)$$

where values are  $\pm$  S.E. The mathematical description of the relation between LDL-(TG/NL) and serum TG is similar to that previously described for a group of

untreated hypertriglyceridemic subjects [7]. The serum TG value ( $4.9 \pm 1.5$  mmol/l) at which LDL-(TG/NL) is half of LDL-(TG/NL)<sub>max</sub> is lower than the corresponding value for HDL-(TG/NL) ( $7.0 \pm 1.2$  mmol/l), which indicates that HDL has a lower affinity for TG than

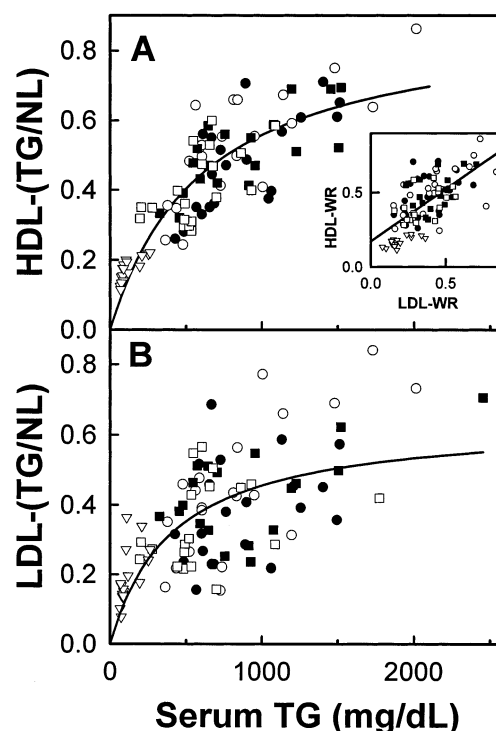


Fig. 2. (A) HDL-(TG/NL) and LDL-(TG/NL) as a function of fasting TG concentration.  $\omega$ -3 fatty acids subjects (*n* = 19) at baseline =  $\blacksquare$  and after intervention =  $\square$ ; placebo subjects (*n* = 21) at baseline =  $\bullet$  and after intervention =  $\circ$ ; normolipidemic subjects (*n* = 16) =  $\nabla$ . Measurements in active intervention groups were made in serum at weeks 0 and 6. Measurements in normolipidemic subjects were made in serum. For both HDL-(TG/NL) and LDL-(TG/NL), data fit a hyperbolic equation having the form of an isotherm. A: HDL-(TG/NL) as a function of serum TG concentrations. (B) LDL-(TG/NL) as a function of serum TG concentrations. Data were fitted to a hyperbolic function given in Eqs. (2a), (2b), (3a) and (3b) (See Text). Insert shows the line of regression of HDL-(TG/NL) vs. LDL-(TG/NL) ( $r = 0.68$ ). HDL-(TG/NL) for all groups ( $n = 96$ ) is significantly different ( $P < 0.05$ ) from LDL-(TG/NL) for all groups ( $n = 96$ ). Values are  $\pm$  S.E.

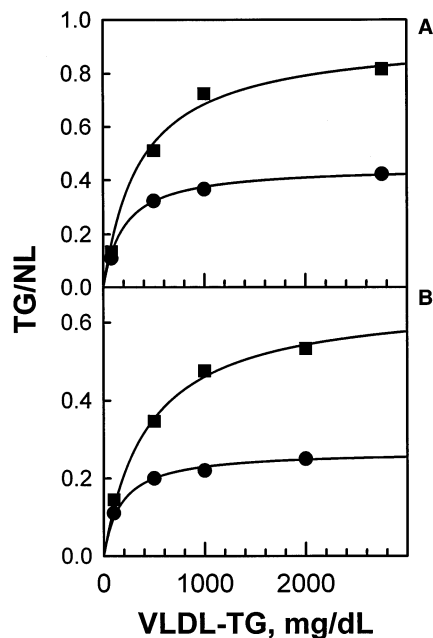


Fig. 3. In vitro studies: effects of lipoprotein-deficient serum and CETP on HDL-(TG/NL) and LDL-(TG/NL). HDL-(TG/NL) = ■; LDL-(TG/NL) = ●. Panel A: lipoprotein-deficient serum. VLDL in four concentrations was combined with VLDL-free serum. The samples were incubated at 37°C for 8 h, after which the VLDL, LDL and HDL were isolated by sequential flotation at  $d = 1.006$  g/ml (VLDL),  $d = 1.063$  g/ml (LDL) and  $d = 1.21$  g/ml (HDL). The CE and TG contents of the HDL and LDL were measured to enable calculation of HDL-(TG/NL) and LDL-(TG/NL). The hyperbolic functions are described by HDL-(TG/NL) =  $(0.97 \pm 0.06)$  serum TG /  $(4.7 \pm 1.0$  mmol/l + serum TG) and LDL-(TG/NL) =  $(0.46 \pm 0.01)$  serum TG /  $(2.6 \pm 0.3$  mmol/l + serum TG). HDL-(TG/NL) is significantly different from LDL-(TG/NL) ( $P < 0.01$ ). Panel B: a mixture of LDL and HDL was combined with rCETP and four concentrations of VLDL. Incubation and analysis were the same as in A. The hyperbolic functions are described by HDL-(TG/NL) =  $(0.64 \pm 0.03)$  serum TG /  $(4.2 \pm 0.7$  mmol/l + serum TG) and LDL-(TG/NL) =  $(0.26 \pm 0.01)$  serum TG /  $(1.6 \pm 0.2$  mmol/l + serum TG). HDL-(TG/NL) is significantly different from LDL-(TG/NL) ( $P < 0.01$ ). HDL-(TG/NL) and LDL-(TG/NL) in lipoprotein-deficient serum are significantly different from HDL-(TG/NL) and LDL-(TG/NL), respectively, in the presence of CETP (both  $P < 0.01$ ). Values are  $\pm$  S.E.

LDL. However, the asymptote for HDL-(TG/NL) ( $0.90 \pm 0.07$ , Eq. (1)) is greater than that for LDL-(TG/NL) ( $0.65 \pm 0.08$ , Eqs. (2a) and (2b)), suggesting that with elevated TG, the NL core of HDL has a greater capacity than that of LDL for TG.

This model was further tested in vitro. Incubation of increasing concentrations of VLDL-TG in VLDL-depleted serum increased both HDL-(TG/NL) and LDL-(TG/NL) (Fig. 3A). For both, the data have the form of adsorption isotherms for which the asymptotes for HDL-(TG/NL) and LDL-(TG/NL) were  $0.97 \pm 0.06$  and  $0.46 \pm 0.01$ , respectively. These data were similar to observed in vivo. The asymptote for HDL-(TG/NL) was greater than that of LDL-(TG/NL). Moreover, the values of the asymptotes for HDL-

(TG/NL) ( $0.97 \pm 0.06$ ) and LDL-(TG/NL) ( $0.46 \pm 0.01$ ) were similar to their respective values ( $0.90$  and  $0.65$ ) found in vivo. Thus, the in vivo associations of increased HDL-(TG/NL) and LDL-(TG/NL) with increased serum TG can be replicated in vitro. Upon in vitro incubation of physiologic concentrations of HDL and LDL with various concentrations of VLDL-TG in the presence of rCETP, HDL-(TG/NL) and LDL-(TG/NL) increased as VLDL-TG increased (Fig. 3B); however, the HDL-(TG/NL) and LDL-(TG/NL) asymptotes were lower than those found both in vivo (Fig. 2) or in VLDL-depleted serum (Fig. 3A).

If one of the determinants of HDL-C is the fraction of NL in HDL that is CE, one would expect HDL-C to vary inversely with HDL-(TG/NL). To test this, we compared serum HDL-C concentrations with HDL-(TG/NL). Although the data of Table 4 would seem to support this hypothesis, the relation is not simple. According to Fig. 4, the data for the normal subjects are confined to a region of the plot corresponding to a high serum HDL-C ( $0.98$ – $1.68$  mmol/l;  $38$ – $65$  mg/dl) and a low value for HDL-(TG/NL) ( $< 0.3$ ). In contrast, most of the data for the hypertriglyceridemic subjects are distributed over a wide range of HDL-(TG/NL) extending from  $0.3$  to  $0.9$ , and a narrow range of HDL-C that is typically low ( $0.48 \pm 0.03$ ,  $18.7 \pm 1.3$ ,  $0.53 \pm 0.04$  mmol/l, and  $20.5 \pm 1.7$  mg/dl, respectively, for the  $\omega$ -3 fatty acid and placebo groups before treatment). Treatment with  $\omega$ -3 fatty acid decreased HDL-(TG/NL) by 25% (Table 4). However, there was only a modest increase in HDL-C (median = +5.9%, Table 3

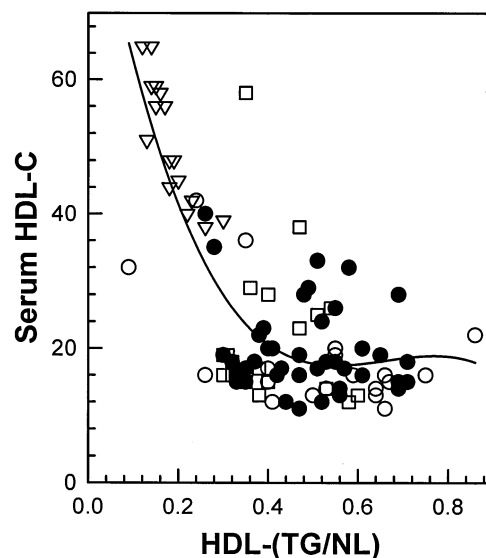


Fig. 4. HDL-C concentration in relation to HDL-(TG/NL).  $\omega$ -3 Fatty acids subjects ( $n = 19$ ) at baseline = ■ and after intervention = □; placebo subjects ( $n = 21$ ) at baseline = ● and after intervention = ○; normolipidemic subjects ( $n = 16$ ) = ▽. Measurements were as described in Fig. 2. The line is a third-order regression of HDL-C compared with HDL-(TG/NL).

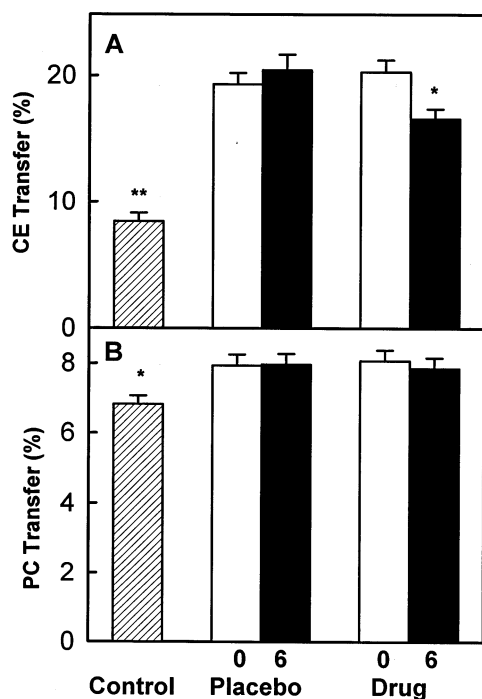


Fig. 5. Serum lipid transfer activities in treated and untreated hypertriglyceridemic subjects and in normolipidemic control subjects. A: serum CETP activities \*pre- and post drug values are statistically significant different,  $P < 0.05$ ; \*\*values for normal subjects are different from hypertriglyceridemic subjects before taking drug or placebo,  $P < 0.001$ . B: serum PC transfer activities; \*values for normal subjects are different from hypertriglyceridemic subjects before taking drug or placebo,  $P < 0.05$ . Error bars represent mean  $\pm$  S.E.

and Fig. 4). Thus, in hypertriglyceridemia a wide range of HDL-(TG/NL) that is  $> 0.3$  co-exists with a relatively narrow range of HDL-C that is typically low. If this model is correct, it suggests that hypolipidemic interventions must bring HDL-(TG/NL) below 0.3 in order to effect an increase in HDL-C. Alternatively, underlying metabolic differences between normolipidemic and hypertriglyceridemic subjects may require different models for the relation of HDL-C with HDL-(TG/NL).

#### 7.4. Effects of serum triglyceride lowering on cholesteryl ester and phospholipid transfer activities

A comparison of serum CETA and PLTA for control, placebo-treated and drug-treated subjects is given in Fig. 5. Mean serum CETA in both the placebo- and drug-treated hypertriglyceridemic patients was more than double that of normolipidemic controls (Fig. 5A). Among the patients receiving placebo there was no significant reduction in CETA after 6 weeks. On the other hand, there was a statistically significant 20% reduction in CETA among the patients receiving the drug. CETA observed after drug treatment was similar to that of untreated patients with similar TG values.

Before drug treatment, the mean PLTA among hypertriglyceridemic patients was 13% higher than that of normolipidemic controls. At the end of the 6 weeks of treatment there was no significant change in the serum PLTA among patients receiving placebo or drug (Fig. 5B).

In Fig. 6, CETA is compared with several serum lipid analytes. As previously reported in hypercholesterolemic patients [39], there was a positive correlation ( $r^2 = 0.38$ ) between serum CETA and serum total cholesterol levels. However, CETA also correlated positively with VLDL-TG ( $r^2 = 0.41$ ), serum TG ( $r^2 = 0.47$ ) and HDL-(TG/NL) ( $r^2 = 0.55$ ). On the other hand, serum PLTA showed no significant correlation with serum TG, total cholesterol or CETA (data not shown).

## 8. Discussion

Intake of  $\omega$ -3 fatty acids improves platelet function, blood viscosity, blood flow and blood pressure, as well as serum HDL-C and TG concentrations, all of which are thought to be related to risk for coronary heart

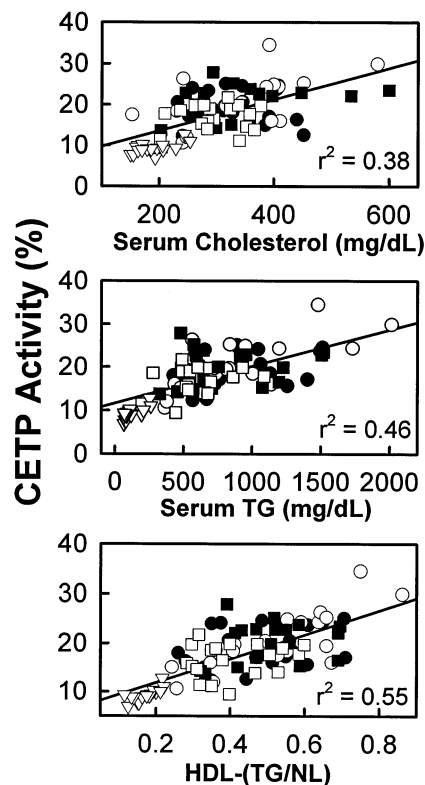


Fig. 6. Serum CETA as a function of (A), serum cholesterol ( $r^2 = 0.38$ ); (B), serum TG ( $r^2 = 0.47$ ) and (C), TG/NL in isolated HDL ( $r^2 = 0.55$ ). CETA was measured in 16 non treated controls, 19 drug treated patients and 21 placebo controls at the beginning and the end of the trial. The data represent the percent [ $^3$ H]CE transfer from donors of R HDL to acceptors of LDL when incubated with serum from each subject. Assignments symbols are the same as those in Fig. 2.

disease events [40–43]. The 85% concentrate of  $\omega$ -3 fatty acids found in Omacor is more concentrated than the formulations used in many previous studies (average composition  $\approx$  22%  $\omega$ -3 fatty acids) and may offer similar benefits with better compliance at a lower caloric intake [24].

Six weeks of  $\omega$ -3 fatty acid treatment in men and women with hypertriglyceridemia (TG at least 5.6 mmol/l, or 500 mg/dl) reduced median serum fasting TG by a statistically significant 38.9% from baseline (from 9.0 to 5.8 mmol/l, or from 801 to 512 mg/dl) (Table 3, Fig. 1). Although median TG was not normalized by this intervention, current U.S. guidelines note that for most patients with very high TG, therapy can be considered successful if it reduces serum TG to  $<$  5.6 mmol/l (500 mg/dl) [23]. Reduction of very high TG is important for control of risk for acute pancreatitis [23,44–46] and may be helpful to reduce risk for coronary heart disease [23,44,45,47]. However, the relation between hypertriglyceridemia and coronary heart disease risk may be indirect, reflecting risk factors that are associated with elevated TG, including decreased HDL-C concentration, small, dense LDL and increased postprandial lipemia [2,3,48–50].

Several secondary endpoints, including HDL-(TG/NL), LDL-(TG/NL) and serum levels of total cholesterol, HDL-C, LDL-C, VLDL-C and NEFA concentrations, were selected for assessment because we hypothesized that they are mechanistically linked to the metabolism of TG-rich lipoproteins. As observed with other hypotriglyceridemic interventions, such as nicotinic acid, and fibric-acid derivatives [51–54], and other fish oil products [24],  $\omega$ -3 fatty acids increased not only HDL-C but also LDL-C (Table 3). Because the drug contains minimal cholesterol ( $\approx$  0.5 mg per 1 g capsule), the increase in LDL-C can be attributed to the  $\omega$ -3 fatty acid content of the formulation. The increases in HDL-C and LDL-C were accompanied by a large decrease in VLDL-C ( $-$  29.2%) such that total cholesterol was significantly reduced ( $-$  9.9%). Although the difference in the change in NEFA concentration in the  $\omega$ -3 fatty acids and placebo groups did not reach significance, the reduction within the  $\omega$ -3 fatty acid group was significant.

Our data support a model of NL trafficking in which CETP mediates the partitioning of TG and CE between VLDL and HDL and LDL. Both HDL-(TG/NL) and LDL-(TG/NL) increased as a function of serum TG concentration, and both decreased as serum TG was lowered with  $\omega$ -3 fatty acids (Table 4, Fig. 2). These results suggest that the TG contents of HDL and LDL are determined by total serum TG and that the same mechanism mediates the transfer of VLDL-TG to both HDL and LDL. These conclusions are supported by our *in vitro* data showing that incubation of HDL and LDL with increasing concentrations of VLDL-TG in

the presence of VLDL-deficient serum is accompanied by increases in HDL-(TG/NL) and LDL-(TG/NL) that simulate the *in vivo* observations (Fig. 4A). Moreover, the observation of a similar effect when lipoprotein-deficient serum was replaced by buffer containing rCETP (Fig. 4B) suggests that CETP has the essential activity that mediates the changes in HDL-(TG/NL) and LDL-(TG/NL) in hypertriglyceridemic serum. For both HDL-(TG/NL) and LDL-(TG/NL), the maximum value observed in the presence of rCETP was lower than that found *in vitro* in the presence of lipoprotein-deficient serum (Fig. 4A) or *in vivo* in the hypertriglyceridemic and normolipidemic subjects (Fig. 2). This finding suggests that whole serum contains other factors that potentiate CETA. HDL-(TG/NL) was higher than LDL-(TG/NL) both *in vivo* (Fig. 2, Table 4, Eqs. (1), (2a) and (2b)) and *in vitro* (Fig. 4). It is somewhat surprising that HDL-(TG/NL) was consistently higher than LDL-(TG/NL), since *in vivo* the former would be expected to lose some of its TG through the action of hepatic lipase. However, since a similar difference was found *in vitro*, HDL may have a greater intrinsic capacity for TG than LDL.

As previously reported, serum CETA correlated with serum cholesterol. McPherson et al. [39] reported a correlation of CETP mass with total cholesterol but not with TG. However, the number of hypertriglyceridemic subjects was small and the severity of the hypertriglyceridemia was much lower. In our study, which included a wider range of serum TG levels, a good correlation was found. Thus, it would appear that serum CETA can be elevated in the presence of both hypercholesterolemia and hypertriglyceridemia, and that a determinant of CETA is the presence of elevated NL levels due to hypercholesterolemia or hypertriglyceridemia. Both of these conditions reflect an increase in the number of lipoprotein particles and one mechanism by which elevations of CETP might occur is through its association with lipoproteins. This would reduce the fraction of CETP available for removal as the free protein. On the other hand, we observed the highest correlation of CETA with HDL-(TG/NL) ratio. Particles with a high TG/NL ratio may bind more strongly to CETP. The high correlation may simply reflect the relation of TG/NL to underlying TG and CE levels. Irrespective of the mechanism, in hypertriglyceridemic subjects greater CETA is available for transfer TG into HDL and LDL.

In our study, serum PLTA was not a sensitive function of serum TG concentrations. Although the difference in mean serum PLTA among normolipidemic and hypertriglyceridemic subjects was statistically significant, the difference was small. In addition, reduction of serum TG did not change PLTA significantly. Thus, differences in the remodelling of lipoproteins are likely to be connected to serum lipid levels and CETA but not PLTA.

According to the reverse cholesterol transport hypothesis [55], HDL is the initial acceptor of peripheral tissue cholesterol, which is esterified by lecithin: cholesterol acyltransferase and transferred to LDL by CETP. Hypertriglyceridemia may interfere with this process by lowering the amount of CE carried by HDL and LDL. Reduction of serum TG in the  $\omega$ -3 fatty acid group was accompanied by a significant reduction of both HDL-(TG/NL) and LDL-(TG/NL) (Fig. 2) and an obligatory increase in the fraction of NL in HDL and LDL occurring as CE. TG/NL reflects the relative amount of NL that is TG; therefore, a low TG/NL corresponds to a high fraction of NL that is CE. Hypolipidemic treatments such as  $\omega$ -3 fatty acids may therefore be of value both to reduce serum TG and to increase HDL-C by decreasing HDL-TG. However, increased HDL-C was found only when HDL-(TG/NL) was  $< 0.3$  (Fig. 3). As shown in Fig. 2, HDL-(TG/NL)  $< 0.3$  corresponded to serum TG  $< 3.4$  mmol/l (300 mg/dl). Thus, we suspect that those individuals in whom serum TG can be brought below 3.4 mmol/l (300 mg/dl) are the most likely to experience a significant increase in HDL-C with  $\omega$ -3 fatty acids.

There are currently two possible models to explain the alterations in the serum lipid profile that occur with  $\omega$ -3 fatty acid treatment. The first model suggests that  $\omega$ -3 fatty acids, like other formulations containing  $\omega$ -3 fatty acids, may divert hepatic fatty acids to  $\beta$ -oxidation [21,22,56,57]. As a consequence, there may be less TG synthesis, an increase in apo B-100 degradation [58] and a reduction in the size and number of secreted VLDL particles. With the reduction in VLDL, less TG may be available for the CETP-mediated transfer to HDL and to LDL. An alternative model suggests that the decreases in serum TG may reduce the amount of substrate available for hydrolysis by lipoprotein lipase. As a consequence of the reduced substrate concentration, the amount of lipolysis product, NEFA, is also reduced. Our data do not provide definitive support for either model; however, other *in vitro* data have suggested that  $\omega$ -3 fatty acids increase hepatic fatty acid oxidation [56,57,59,60], a finding that provides strong support for the first model.

Consideration of our *in vivo* and *in vitro* data together suggests that elevated serum TG concentration and CETA are the two major determinants of the altered HDL and LDL structure found in hypertriglyceridemia. Treatment with  $\omega$ -3 fatty acids appears to change the lipid profile of individuals with elevated TG to one that may be less atherogenic by changing LDL structure; lowering serum CETA, serum TG and VLDL-C; and increasing serum HDL-C. Decreasing the TG content of LDL alters its structure [7,61,62], enhances its binding to the LDL receptors on fibroblasts [7,39] and reduces its intracellular degradation [63–65].  $\omega$ -3 Fatty acids increases the CE content of

HDL in a predictable way and may make the HDL more resistant to the hepatic lipase-mediated conversion to smaller, more dense HDL. Thus, in hypertriglyceridemic patients,  $\omega$ -3 fatty acids reduce serum TG concentrations and improve other mechanistically related lipid risk factors.

## Acknowledgements

This work was supported by Pronova Biocare, Lysaker, Norway, the manufacturer of Omacor, and grants from the National Institutes of Health (HL-56865, HL-30914). The authors thank Shi-Jing Qu, who provided recombinant CETP, and Brook Watts, who provided editorial assistance.

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