Postprandial chylomicrons and VLDLs in severe hypertriacylglycerolemia are lowered more effectively than are chylomicron remnants after treatment with n−3 fatty acids[1,2]

Sabine Westphal, Matthias Orth, Andreas Ambrosch, Karen Osmundsen, and Claus Luley

ABSTRACT

Background: n−3 Fatty acids lower plasma triacylglycerols not only in the fasting state but also in the postprandial state. However, it is not known whether chylomicrons, chylomicron remnants, and VLDLs are all affected equally or whether some lipoprotein species are lowered preferentially.

Objective: Lipoproteins, including large and small chylomicron remnants, were determined specifically with the aid of a newly developed method involving a combination of size-exclusion chromatography and fluorometric determination of retinyl palmitate, which served as a marker for exogenous fat.

Design: Twelve hypertriacylglycerolemic men were treated for 6 wk with 4 capsules containing 85% fish-oil concentrate/d; each capsule contained 850 mg n−3 fatty acid ethyl esters (49.1% eicosapentaenoic acid by wt and 32.2% docosahexaenoic acid by wt). Oral-fat-tolerance tests were performed before and after the treatment. Blood samples were drawn in the fasting state and until 8 h postprandially.

Results: Treatment with n−3 fatty acids reduced the fasting VLDL-triacylglycerol concentration by 44% ($P < 0.05$) and postprandial chylomicrons and VLDLs at 4, 6, and 8 h ($P < 0.05$) by 49–64% and 36–43%, respectively. Chylomicron remnants were reduced only in the late postprandial phase: large chylomicron remnants by 19% at 6 h and by 43% at 8 h ($P < 0.05$) and small chylomicron remnants by 31% at 8 h ($P < 0.05$).

Conclusion: n−3 Fatty acids effectively lower chylomicrons and VLDLs, but their effect on chylomicron remnants was observed only in the late postprandial phase. *Am J Clin Nutr* 2000;71:914–20.

KEY WORDS n−3 Fatty acids, fish oil, hypertriacylglycerolemia, postprandial effects, lipoproteins, chylomicron remnants, chylomicrons, VLDL, men

INTRODUCTION

Regular intake of n−3 fatty acids lowers plasma triacylglycerol concentrations in both normal and hypertriacylglycerolemic individuals (1–3). This has been observed not only in the fasting but also in the postprandial state (4–7). In the postprandial state, 3 lipoprotein species can be affected: chylomicrons, chylomicron remnants, and VLDL. Knowledge of the specific effects on these lipoprotein species is important for assessing the antiatherogenic potential of this treatment. Although chylomicrons do not appear to have any atherogenic potential, chylomicron remnants are thought to be atherogenic on the basis of experiments in cell cultures (8), in animals (9), and in humans with type III hyperlipoproteinemia (10, 11). The importance of VLDL in atherogenesis is still under discussion (12).

Determinations of chylomicrons, chylomicron remnants, and VLDL can be methodologically complicated because many of their physical and chemical properties are identical. To overcome the methodologic problems associated with the separation of VLDL, chylomicrons, and chylomicron remnants, we used a novel approach—a combination of size-exclusion chromatography and fluorometric determination of retinyl palmitate. This allowed improved separation and quantification of chylomicrons and their remnants of different sizes (13).

SUBJECTS AND METHODS

Subjects

Twelve men from the lipid clinic of the Magdeburg University Hospital with fasting triacylglycerol concentrations >3.4 mmol/L (>300 mg/dL) were included in the study. The men were aged 46 ± 6 y ($\bar{x}$ ± SEM) and had a body mass index of 27.7 ± 3.6 (in kg/m^2). The exclusion criteria were type III hyperlipoproteinemia, thyroid dysfunction, a fasting blood glucose concentration >7.8 mmol/L (140 mg/dL), alcohol abuse (>2 drinks/d), a serum creatinine concentration >176.8 μmol/L (2.0 mg/dL), a serum alanine aminotransferase concentration >3 times the upper limit of the normal range, consumption of fish meals with a high fat content >1 time/wk, treatment with lipid reduction agents or n−3 fatty acid supplements within 2 mo of enrollment, and age >65 y. An ethics committee approved the protocol and all patients gave their written informed consent.

[1] From the Institute of Clinical Chemistry, Magdeburg University Hospital, Magdeburg, Germany and Pronova Biocare, Lysaker, Norway.

[2] Address reprint requests to S Westphal, Institute of Clinical Chemistry, Leipziger Strasse 44, D-39120 Magdeburg, Germany.

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TABLE 1
Patient characteristics and the effects of n−3 fatty acids on fasting lipids[1]

	Before treatment	After treatment
Total cholesterol (mmol/L)	9.4 ± 0.9	8.0 ± 0.5
Total triacylglycerol (mmol/L)	13.6 ± 2.6	8.2 ± 1.6[2]
VLDL triacylglycerol (mmol/L)	13.5 ± 2.8	7.6 ± 1.9[2]
VLDL cholesterol (mmol/L)	5.9 ± 1.0	3.3 ± 0.7[2]
LDL cholesterol (mmol/L)	2.6 ± 0.7	3.8 ± 0.7[2]
HDL cholesterol (mmol/L)	0.88 ± 0.06	0.92 ± 0.07
Lipoprotein lipase (μg/L)	488 ± 31	544 ± 45

[1] $\bar{x}$ ± SEM; n = 12. Patients were treated with n−3 fatty acids for 6 wk and blood samples were taken in the fasting state.
[2] Significantly different from before treatment, $P < 0.05$.

The fasting lipid concentrations of the patients are listed in **Table 1**. Most of the patients suffered from severe hypertriacylglycerolemia and 10 of the 12 had type V hyperlipoproteinemia. LDL- and HDL-cholesterol concentrations were particularly low. The distribution of apolipoprotein (apo) E genotypes was as follows: *E3/E3* (n = 6), *E3/E4* (n = 5), and *E2/E3* (n = 1).

n−3 Fatty acid treatment

The patients were treated with 4 lipid-containing capsules (Omacor; Pronova Biocare, Lysaker, Norway) per day for 6 wk. Each capsule contained 850 mg n−3 fatty acid ethyl esters (49.1% eicosapentaenoic acid (EPA) by wt and 32.2% docosahexaenoic acid (DHA) by wt (**Table 2**). Patients were advised to swallow the capsules before consuming their main meals to minimize gastrointestinal discomfort. Compliance was assessed by capsule counting and by analyzing the fatty acid profile in the phospholipid fraction of serum collected at baseline and after 6 wk of treatment. Concentrations of n−3 fatty acids were determined by gas-liquid chromatography after chloroform-methanol extraction and transmethylation (14).

Oral-fat-tolerance test

Oral-fat-tolerance tests were performed before and after treatment. The first blood sample was drawn after a 12-h fast. The fatty meal consisted of 30% whipping cream (3 mL/kg body wt) mixed with 60 000 IU (32.8 mg) retinyl palmitate/m^2 body surface (Arovit; Hoffmann-La Roche, Basel, Switzerland). The fatty meal contained 30 g fat (18.2 g saturated and 9.04 g monounsaturated fatty acids), 3.5 g carbohydrates, and 2.5 mg protein/g whipping cream and was ingested within 15 min between 0730 and 0800. Blood samples were taken immediately before and 4, 6, and 8 h after the oral-fat-tolerance test. No other source of energy was provided but drinking water was allowed ad libitum. The participants did not engage in physical activity during the test and exercise was avoided during the 24 h before the tests. Venous blood samples were drawn under standardized conditions and serum was separated from the blood cells by centrifugation for 10 min at 3000 × g and 10 °C. Lipoproteins were analyzed within 24 h.

Determination of lipids and conventional lipoproteins

The method of the Lipid Research Clinics was used to determine lipids in VLDL, LDL, and HDL, with the modification that LDL was precipitated with phosphotungstic acid/Mg^{2+}. The concentrations of triacylglycerol and cholesterol were determined by commercial enzymatic methods in a random-access analyzer (Hitachi 911; Boehringer Mannheim, Mannheim, Germany). All reagents and calibrators were from Boehringer Mannheim. Apo E phenotypes were determined by immunofixation after isoelectric focusing in agarose, as described previously (15).

Characterization of triacylglycerol-rich lipoproteins

The separation and determination of triacylglycerol-rich lipoproteins according to size and retinyl palmitate content were conducted as described recently (13, 16). Briefly, the fluorescence of triacylglycerol and retinyl palmitate was measured in serum eluate fractions obtained by size-exclusion chromatography. The elution profiles of triacylglycerol and retinyl palmitate fluorescence of 1 fasting and 3 postprandial samples collected from a representative individual before and 4, 6, and 8 h after oral fat loading are shown in **Figure 1**A. The figure illustrates the postprandial appearance and disappearance of chylomicrons (first triacylglycerol and fluorescence peaks from the left) and also those of chylomicron remnants (second fluorescence peaks from the left). The kinetics of these peaks are depicted in two-dimensional graphs that are derived from Figure 1A. Triacylglycerols of chylomicrons (a) are shown in Figure 1B and the fluorescence of chylomicrons (b), large chylomicron remnants (c), and small chylomicron remnants (d) are shown in Figure 1C. Although the size of the chylomicrons and the large chylomicron remnants was defined by the respective peak maximum, the size of the small chylomicron remnants was arbitrarily defined by the size of VLDLs taken from the VLDL-triacylglycerol peak from the fasting sample (Figure 1A). The reproducibility of the determinations was tested with one postprandial serum sample that was analyzed 7 times. The CVs for chylomicrons, large chylomicron remnants, and small chylomicron remnants were 9.2%, 10.9%, and 11.5%, respectively.

Lipoprotein lipase

Lipoprotein lipase was measured in EDTA-treated plasma taken 20 min after an intravenous injection of 100 IU heparin/kg body wt. The test was carried out directly after the last blood sample was taken for each oral-fat-load test (8 h). The MARKIT-

TABLE 2
Composition of the lipid-containing capsules[1]

Component	Capsule
Fatty acid *(% by wt)*	
16:3n−4	1.9
16:4n−1	2.8
18:4n−3	4.3
20:4n−6	3.4
20:5n−3	49.1
21:5n−3	1.1
22:5n−3	1.6
22:6n−3	32.2
Cholesterol (mg/g)	0.9
Retinol (mg/kg)	8.0
Vitamin D (mg/kg)	0.8
Vitamin E (mg DL-α-tocopherol)	3.3

[1] Omacor (Pronova Biocare, Lysaker, Norway). The analyses were conducted by Pronova Biocare.

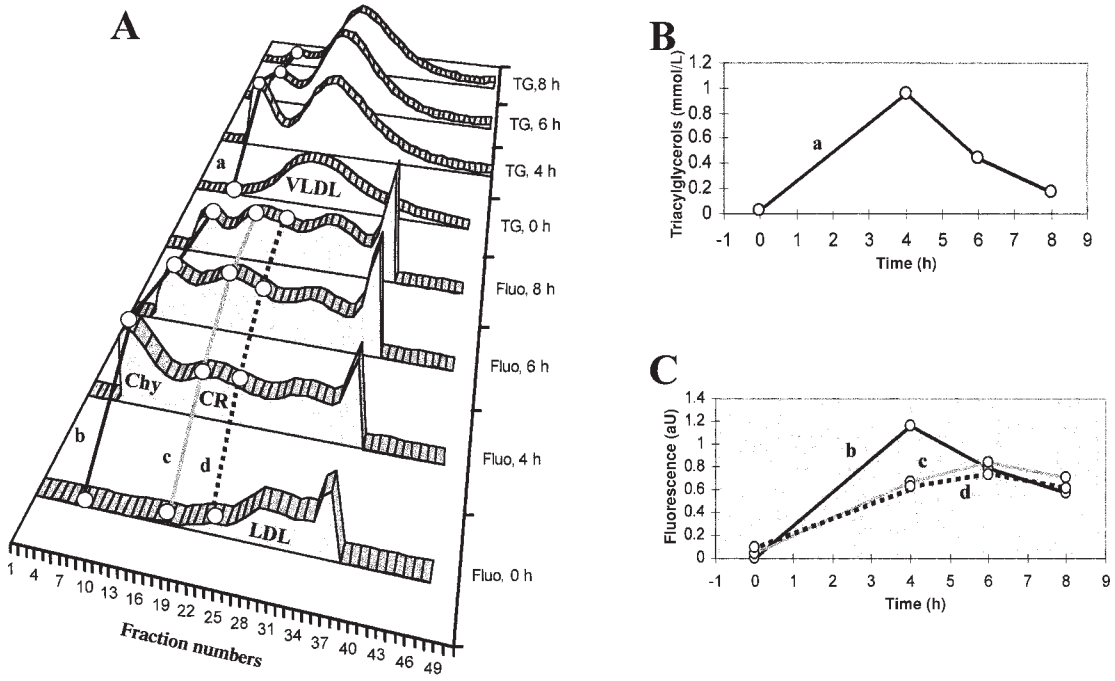

FIGURE 1. Elution profiles of triacylglycerol and retinyl palmitate fluorescence (Fluo) in 1 fasting and 3 postprandial blood samples from a representative individual before (0 h) and 4, 6, and 8 h after oral fat loading (A). For quantification, the peak heights of the profiles of triacylglycerol and retinyl palmitate fluorescence were transferred to corresponding two-dimensional panels. Shown in panel B is triacylglycerols of chylomicrons (a) and shown in panel C is fluorescence of chylomicrons (b), large chylomicron remnants (c), and small chylomicron remnants (d). Although the size of the chylomicrons and the large chylomicron remnants is defined by the respective peak, the size of the small chylomicron remnants was arbitrarily defined by the size of VLDLs taken from the VLDL triacylglycerol peak from the fasting sample (*see* "VLDL" in panel A). aU, arbitrary units; Chy, chylomicrons; CR, chylomicron remnants.

F lipoprotein lipase one-step sandwich immunoassay kit (Dainippon, Osaka, Japan) was used for quantification. This kit determines the mass of lipoprotein lipase and has been shown to correlate with enzyme activity (17).

Statistical evaluation

Results are presented as means ± SEMs. Differences between baseline and treatment values were tested for significance with the Wilcoxon's signed-rank test for paired samples, with *P* values of 0.05 considered significant. To estimate the overall response of triacylglycerol during the 8-h postprandial period, the area under the postprandial curve (AUC) was calculated by the trapezoid rule. SPSS for WINDOWS (version 7.5; SPSS Inc, Chicago) was used for the analyses.

RESULTS

Patient compliance

All patients followed the treatment protocol as required, as confirmed by capsule counting and by EPA and DHA concentrations in the phospholipid fraction of serum, which increased significantly after treatment by 156% (EPA: from 34.03 ± 18.58 to 87.08 ± 33.54 μmol/L; $P < 0.0029$) and 40% (DHA: from 118.8 ± 33.37 to 165.98 ± 61.13 μmol/L; $P < 0.015$). No adverse events were reported.

Effects of the treatment on fasting lipids and lipoprotein lipase

In the fasting state, the most pronounced effect of the treatment was on VLDL-triacylglycerol concentrations, which decreased by 44% (Table 1). Total cholesterol decreased at the same time by 14%. This net effect on total cholesterol was the result of a pronounced 40% decrease in VLDL cholesterol and a 45% increase in LDL cholesterol from 2.6 to 3.8 mmol/L. No significant differences were found for HDL cholesterol or for lipoprotein lipase.

Effects of the treatment on postprandial lipids

The kinetics of triacylglycerol in serum, chylomicrons, and VLDL as separated by size-exclusion chromatography are shown in **Figure 2**. Serum triacylglycerol concentrations decreased with treatment at all time points (0, 4, 6, and 8 h) in relatively equal proportions (32–39%). This was also the case for VLDL triacylglycerol (36–43%). The greatest reductions were observed for chylomicron triacylglycerol, which decreased by 49%, 58%, and 64% at 4, 6, and 8 h, respectively. The AUCs for serum triacylglycerol, VLDL, and chylomicrons decreased significantly by 34%, 38%, and 54%, respectively.

Effects on postprandial retinyl palmitate fluorescence

The kinetics of retinyl palmitate fluorescence in chylomicrons and in small and large chylomicron remnants are presented in

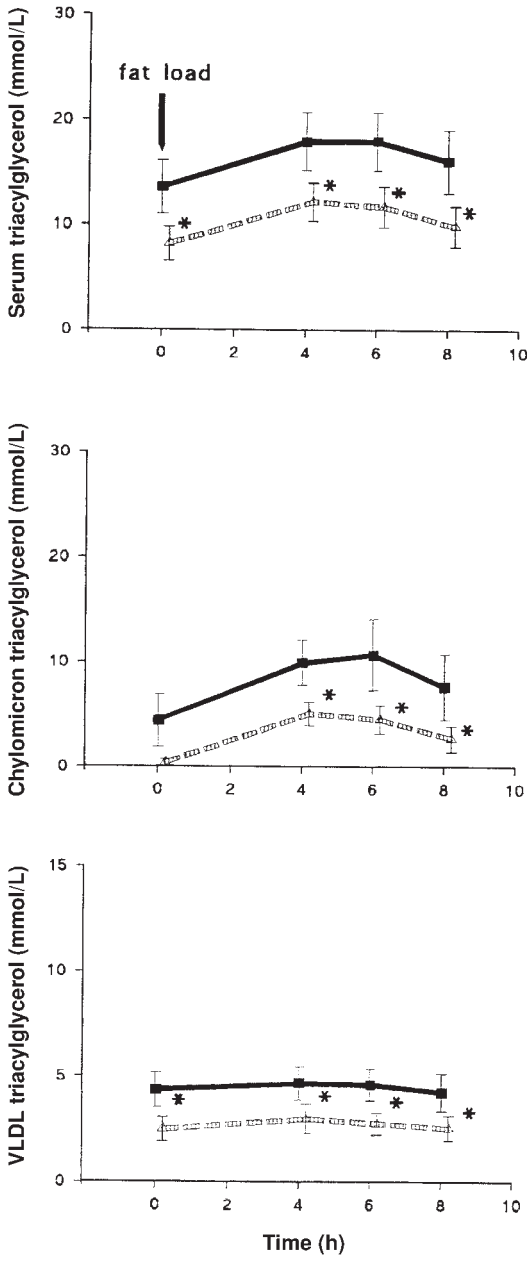

FIGURE 2. Mean (±SEM) concentrations of postprandial triacylglycerol in serum, chylomicrons, and VLDL as separated by size-exclusion chromatography and fluorometry before (▬) and after (▭) treatment with n−3 fatty acids. n = 12. *Significantly different from before treatment, $P < 0.05$.

Figure 3. An obvious difference between fluorescence and triacylglycerol is naturally observed in the fasting state, when no retinyl palmitate has yet entered the bloodstream. During the postprandial phase, the kinetics of fluorescence in chylomicrons paralleled those of chylomicron triacylglycerol, peaking after 6 h. A comparison of the kinetics of chylomicron fluorescence with that of large and small chylomicron remnants showed clearly that the curves became flatter and the peaks appeared later as lipoprotein size decreased. Chylomicron fluorescence decreased by 29% at 4 h, by 28% at 6 h, and by 33% at 8 h. The fluorescence of large chylomicron remnants decreased by 27% at 6 h and by 43% at 8 h. The fluorescence of small chylomicron remnants decreased significantly (by 31%) 8 h after the oral fat load. The decreases in the AUCs of large and small chylomicron remnants were less pronounced and not significantly different.

DISCUSSION

We used a new method, a combination of size-exclusion chromatography and fluorometry, to examine whether decreases in triacylglycerols after treatment with n−3 fatty acids are relatively equal for all triacylglycerol-rich lipoproteins postprandially or whether certain lipoprotein species decrease significantly more so than others. Although we confirmed the lowering effect of n−3 fatty acids on postprandial triacylglycerols, we also extended the current understanding by showing that this lowering affects large lipoproteins earlier and to a greater extent than it does the smaller species, which decrease later and to a smaller degree.

Effects of n−3 fatty acid treatment on chylomicrons, VLDL, and LDL

A pronounced lowering of chylomicrons after n−3 fatty acid treatment was described by many other authors (6, 18, 19). In a recent elegant study in rats, Harris et al (20) reported that this reduction is not caused by effects on chylomicron production as had been suggested previously (21, 22). Rather, their findings suggest that n−3 fatty acids accelerate chylomicron lipid clearance by facilitating lipoprotein lipase–mediated lipolysis. This involvement of lipoprotein lipase was not confirmed by our data nor by the data of Weintraub et al (23) or of Desager et al (24), but the apparent absence of involvement may have been due to the different methods used to measure lipoprotein lipase activity by the different investigators. Specifically, Weintraub et al, Desager et al, and we used heparin stimulation to release the endothelium-bound enzyme before lipoprotein lipase measurement. Harris et al (25), on the other hand, determined lipoprotein lipase activity without heparin stimulation, which may reflect the in vivo lipolytic potential better than the unphysiologic use of heparin.

Various additional mechanisms for improved chylomicron clearance have been proposed: *1*) chemical surface alteration to improve the action of lipoprotein lipase (26), *2*) differences in chylomicron size because larger particles are hydrolyzed more easily (27), *3*) increased lipoprotein lipase activity during increased margination (binding to the endothelium) (20), and *4*) reduced competition between chylomicrons and VLDL concentrations for LPL because of reduced VLDL concentrations (28, 29).

However, our data also confirm the well-known and pronounced decrease in VLDLs after n−3 fatty acid treatment, which is known to be due to an inhibition of 1,2-diacylglycerol–sterol *O*-acyltransferase (30) or phosphatidate phosphatase (31), resulting in a >50% reduction in the synthesis rate of VLDL (32). Thus, despite the fact that chylomicrons are a preferred substrate for lipoprotein lipase (33), we do not exclude the possibility that a decrease in VLDL triacylglycerol of 44% makes more lipoprotein lipase available, allowing accelerated hydrolysis of incoming and competing chylomicrons. A common and saturable lipolytic removal mechanism of this kind was postulated by Brunzell et al (34) after stimulation of VLDL production in normo- and hypertriacylglycerolemic humans.

An increase in LDL cholesterol—a frequent finding in hypertriacylglycerolemic patients (1)—reflects the accelerated

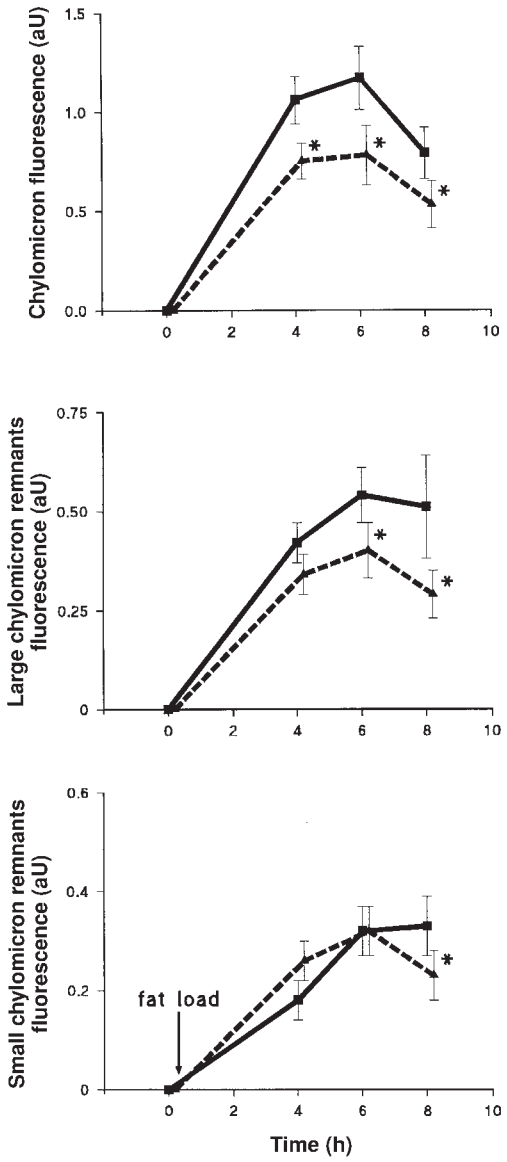

FIGURE 3. Mean (±SEM) postprandial retinyl palmitate fluorescence in chylomicrons, large chylomicron remnants, and small chylomicron remnants as separated by size-exclusion chromatography and fluorometry before (▬▬) and after (▬ ▬ ▬) treatment with n−3 fatty acids. $n = 12$. *Significantly different from before treatment, $P < 0.05$.

degradation of triacylglycerol-rich lipoproteins to denser lipoproteins. The extent of this increase depends on baseline VLDL concentrations. If a substantial proportion of total cholesterol is in the VLDL fraction (as was the case in our patients), a marked lowering of VLDL can outweigh the increase in LDL cholesterol and decrease total cholesterol. A marked relative increase in LDL cholesterol is not necessarily a cause for concern because LDL cholesterol is often low in pronounced hypertriacylglycerolemia and its final concentration may still be in the normal range (29). There is no such increase in LDL cholesterol in patients with moderate hypertriacylglycerolemia or in healthy individuals. This was shown recently by Tinker et al (19), who also observed a postprandial redistribution of cholesterol toward triacylglycerol-rich lipoproteins and proposed a redirection of cholesterol to the liver, which would therefore be beneficial.

Effects of n−3 fatty acid treatment on chylomicron remnants

Fewer data are available on the kinetics of chylomicron remnants after n−3 fatty acid treatment. Three studies carried out in humans generally agree that chylomicron remnants can also be lowered by n−3 fatty acids (23, 35, 36). However, the 3 studies differed considerably, eg, in the selection of patients and in the method used to measure chylomicron remnants. Pschierer et al (35) studied patients with severe hypertriacylglycerolemia (triacylglycerol concentration: 15.0 mmol/L, or 1293 mg/dL), but only in the fasting state. They found an 80% decrease in apo B48 (density < 1.006 kg/L) in the supernate after n−3 fatty acid treatment. However, because the supernate contained both chylomicrons and chylomicron remnants, a specific effect on each class of lipoproteins could not be derived from the data. Dallongeville et al (36) determined fasting β-VLDL concentrations in patients with type III and type IV hypertriacylglycerolemia before and after n−3 fatty acid treatment. Although these authors described a lowering of β-VLDL in these patients, differentiation between VLDL and chylomicron remnants was not possible. The only study of postprandial chylomicron remnants was carried out by Weintraub et al (23) in normolipidemic subjects. They determined retinyl palmitate concentrations in chylomicrons [Svedberg flotation unit (S_f) >1000] and in chylomicron remnants (S_f < 1000). Treatment with n−3 fatty acids lowered both these indexes considerably throughout the postprandial phase, but a delay in the lowering of chylomicron remnants was not observed. The discrepancy between this finding and our data cannot be explained by methodologic differences. As discussed elsewhere (13), ultracentrifugation tends to overestimate the concentration of chylomicrons in the S_f >1000 fraction and to underestimate chylomicron remnants in the S_f < 1000 fraction. It may therefore be assumed that the latter contains preferably higher-density chylomicron remnants. If this assumption is correct, the method used by Weintraub et al (23) should yield results similar to those of ours for small chylomicron remnants, ie, a treatment effect only in the late postprandial phase. A potential explanation for this discrepancy might be that Weintraub et al (23) studied healthy individuals (fasting triacylglycerol concentration: <1.2 mmol/L, or 100 mg/dL), whereas we studied patients with marked hypertriacylglycerolemia.

Although retinyl ester labeling has been used in many studies to assess chylomicron remnants (37–39), leading to important findings about chylomicron remnant metabolism, the validity of this label has been questioned. A major criticism concerns the potential exchange of retinyl esters between exogenous and endogenous lipoproteins (*see* reference 38 for a review). This point of view, however, is controversial. The degree of exchange appears to be small [7% into LDL (39)] or nonexistent, as in the study by Sprecher et al (40), who investigated chylomicron retinyl palmitate clearance in severely hyperchylomicronemic patients. These authors did not find any significant retinyl ester transfer from chylomicrons to apo B-100–containing particles. However, if such an effect were to occur, the transfer of retinyl esters to VLDL would underestimate rather than overestimate the late lowering of small chylomicron remnants and the "true" effect might be even more pronounced than described here.

In any discussion of remnant elimination it must be remembered that this process occurs mainly via hepatic receptors and

requires apo E as a key prerequisite. Apo E can be found in all lipoproteins, with a major proportion in the HDL of fasting normolipidemic individuals. With increasing triacylglycerol-rich lipoprotein concentrations, however, apo is redistributed into larger lipoproteins, where it may eventually facilitate their hepatic removal (41). Thus, we assume that if the concentrations of incoming chylomicrons are very high, they might absorb most of the available apo E, removing it from the other lipoproteins. According to this hypothesis, chylomicron remnants may not acquire sufficient apo E as long as the chylomicron concentrations remain elevated. Only when chylomicron concentrations decrease, in the late postprandial phase, can sufficient apo E become available for chylomicron remnants, which in turn accelerates their removal. This hypothesis could explain the discrepancy between our data and those of Weintraub et al (23) because in their study the subjects were exclusively normolipidemic; thus, the postprandial triacylglycerol elevation was moderate (maximum triacylglycerol concentration: 3.0 mmol/L, or 260 mg/dL). In our severely hypertriacylglycerolemic patients, in contrast, the mean postprandial triacylglycerol concentration was ≈4 times higher (12.18 mmol/L, or 1078 mg/dL). Thus, the proposed competition of triacylglycerol-rich lipoproteins for a limited quantity of apo E may be effective predominantly in patients with pronounced hypertriacylglycerolemia. In conclusion, n−3 fatty acids effectively lower chylomicrons and VLDL, but their effect on chylomicron remnants only becomes evident in the late postprandial phase.

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