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Is hypertriglyceridemic very low density lipoprotein a precursor of normal low density lipoprotein?

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Abstract The precursor-product relationship of very low density (VLDL) and low density lipoproteins (LDL) was studied. VLDL obtained from normal (NTG) and hypertriglyceridemic (HTG) subjects was fractionated by zonal ultracentrifugation and subjected to in vitro lipolysis. The individual subfractions and their isolated lipolysis products, as well as IDL and LDL, were rigorously characterized. A striking difference in the contribution of cholesteryl ester to VLDL is noted. In NTG subfractions, the cholesteryl ester to protein ratio increases with decreasing density (VLDL-I → VLDL-III). This is the expected result of triglyceride loss through lipolysis and cholesteryl ester gain through core-lipid transfer protein action. In HTG subfractions there is an abnormal enrichment of cholesteryl esters that is most marked in VLDL-I and nearly absent in VLDL-III. Thus, the trend of the cholesteryl ester to protein ratios is reversed, being highest in HTG-VLDL-I and lowest in VLDL-III. This is incompatible with the precursor-product relationship described by the VLDL → IDL → LDL cascade. In vitro lipolysis studies support the conclusion that not all HTG-VLDL can be metabolized to LDL. While all NTG subfractions yield products that are LDL-like in size, density, and composition, only HTG-VLDL-III, whose composition is most similar to normal, does so. HTG VLDL-I and VLDL-II products are large and light populations that are highly enriched in cholesteryl ester. We suggest that this abnormal enrichment of HTG-VLDL with cholesteryl ester results from the prolonged action of core-lipid transfer protein on the slowly metabolized VLDL mass. This excess cholesteryl ester load, unaffected by the process of VLDL catabolism, remains entrapped within the abnormal particle. Therefore, lipolysis yields an abnormal, cholesteryl ester-rich product that can never become LDL.—**Oschry, Y., T. Olivecrona, R. J. Deckelbaum, and S. Eisenberg.** Is hypertriglyceridemic very low density lipoprotein a precursor of normal low density lipoprotein? *J. Lipid Res.* 1985. **26:** 158–167.

Supplementary key words VLDL remnants • lipoprotein lipase • triglycerides • cholesteryl esters • lipid transfer reaction

The current view on the metabolism of the apoB-containing lipoproteins VLDL, IDL, and LDL predicts a precursor-product relationship between the larger particles and the smaller ones (1). Accordingly, smaller VLDLs are produced from larger particles as a result of lipolysis, and IDL and LDL represent products of continuous triglyceride hydrolysis. This view is supported mainly by apoB turnover studies in human subjects (2–6) and by studies of VLDL catabolism in vitro (7, 8). However, when the VLDL → IDL → LDL cascade was examined in hypertriglyceridemic subjects, incomplete conversion of VLDL-apoB precursors to LDL was consistently observed (6, 9–12). Moreover, the in vitro studies demonstrated that at least some of the LDL-like particles produced by lipolysis are larger than normal LDL and contain surplus cholesteryl ester molecules (7). These observations suggest that hypertriglyceridemic VLDL (HTG-VLDL) may differ from normotriglyceridemic VLDL (N-VLDL) and that such differences may cause abnormal metabolism of VLDL in hypertriglyceridemia.

VLDL is present in plasma as a multidisperse population. Particles vary in size between 300 and 800 Å and in molecular mass between 5 and 100 million daltons (13, 14). Whenever studied, abnormalities of VLDL metabolism in HTG are predominantly apparent in the larger sized and lighter fractions (11, 12, 15). Therefore it is possible that differences between N- and HTG-VLDL exist mainly in light density fractions. The present investigation was undertaken to evaluate the nature of differences between N- and HTG-VLDL that may be responsible for the altered metabolic behavior of the latter. To this end, we have determined the potentialities of forming LDL particles from VLDL density subfractions present in the circulating plasma of normo- and hypertriglyceridemic human subjects. The study indeed demonstrated that the in vitro lipolytic products of large sized HTG-VLDL cannot possibly form LDL and must be excluded from the VLDL → LDL cascade.

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; CE, cholesteryl ester; HTG, hypertriglyceridemic; N, normal; PAGE, polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Subjects and preparation of lipoproteins

Human subjects with hypertriglyceridemia were identified in the Hadassah Hospital Lipid Clinic. All subjects were followed in the clinic for at least 6 months (and as long as 5 years) and were treated with the clinic's "type IV" isocaloric diet (40% of calories from fat, 40% complex carbohydrates, 20% protein, and less than 300 mg of cholesterol per day). Ten male patients (28–50 years old) with plasma triglyceride levels between 400 and 1200 mg/dl agreed to participate in the study, and each donated one unit of blood for isolation of lipoproteins. The ten patients had high VLDL and normal or even low LDL-cholesterol levels. Family history of hypertriglyceridemia was found in six patients. Lack of enough first degree relatives prevented extensive family investigations in the other four patients, and of more exact familial diagnosis in all patients. All patients were at stable weight during the 4 weeks prior to phlebotomy, and none was on lipid-lowering or cardiac drugs. Two patients that had had previous myocardial infarction were free of cardiac symptoms. None of the patients suffered from diabetes mellitus and all had normal renal, hepatic and thyroid function. One unit of blood was obtained at morning hours (8–10 AM) after a 14–16-hr fast. The blood was collected in plastic bags containing citrate–phosphate–dextrose (CPD) solution and plasma was separated promptly by low speed centrifugation (5000 rpm, 30 min) at 4°C. VLDL was separated at plasma density of 1.006 g/ml after 16 hr centrifugation in a 60 Ti rotor at 50,000 rpm. The VLDL was washed and concentrated by one additional spin at density of 1.006 g/ml. IDL (d 1.006–1.019 g/ml) and LDL (d 1.019–1.063 g/ml) were separated likewise at their respective density intervals. VLDL subfractions, IDL, and LDL were then prepared by zonal ultracentrifugation as described below.

Six normotriglyceridemic male subjects (24–46 years old) volunteered to donate one unit of plasma to serve as control samples. The procedures for blood drawing and separation of plasma lipoproteins were identical to those described above.

Zonal ultracentrifugation

Native and post-lipolysis lipoproteins were subjected to zonal ultracentrifugation as described by Patsch et al. (16). All runs were carried out in a Beckman Spinco L5-50 ultracentrifuge equipped to accept the Ti 14 zonal rotor. Gradients and solutions were introduced into the rotor by a Beckman model 141 piston gradient pump. Samples were separated in nonequilibrium rate runs at 15°C on 665 ml NaBr gradients (pH 7.4) spanning d 1.00–1.15 g/ml and containing 350 μM disodium EDTA. The gradients were pumped in from the periphery at the rate of 50 ml/min with the rotor spinning at 3000 rpm. The density of each sample was adjusted to that of the heaviest part of the gradient with solid NaBr, brought to 30 ml with heavy stock solution, and injected to the rotor periphery. This was followed by a 20-ml cushion of heavy solution to ensure that the entire sample was inside the rotor. The rotor was then sealed, accelerated, and spun at 42,000 rpm for 45 min. At the end of the run the rotor was decelerated to 3000 rpm and its contents were displaced by pumping heavy solution into the rotor from the periphery at the rate of 50 ml/min. The rotor effluent was monitored by continuous measurement of absorbance at 280 nm by an ISCO model UA-5 Absorbance Monitor equipped with a quartz flow-through cell. Fractions of 25 ml were collected. Individual lipoproteins were pooled and dialyzed at 4°C against normal saline (0.15 M NaCl, 0.01% EDTA, and 0.01% NaN_3; pH 7.4) with at least three changes of dialyzate. Samples were then concentrated to a volume of 3–6 ml by vacuum dialysis (17) and dialyzed again as above.

Whole VLDL distributed along the zonal effluent as seen in **Fig. 1**. The first 25 ml of effluent, containing aggregates and very large particles, was discarded. Particles eluting from 26–100 ml were collected and designated as VLDL-I. The 101–200-ml fraction was designated as VLDL-II, and the 201–350-ml fraction as VLDL-III. Each subfraction was pooled, dialyzed, and concentrated as described above. Recentrifugation of isolated samples showed them to be distinct density subfractions (Fig. 1). The elution profiles of IDL and LDL are also indicated in the figure.

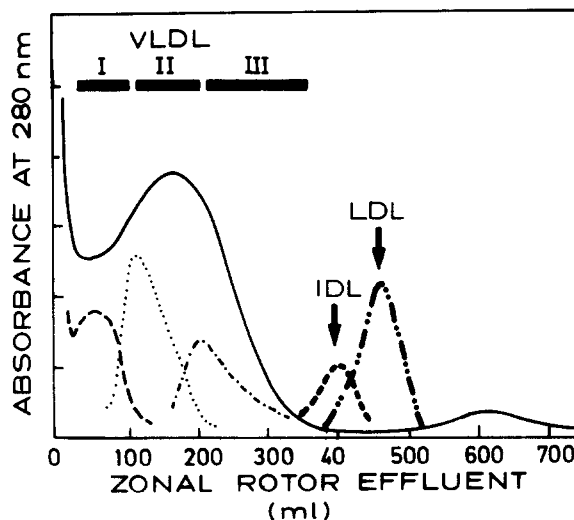

Fig. 1 Zonal elution profiles of total VLDL, VLDL subfractions, IDL, and LDL. VLDL from a hypertriglyceridemic subject was fractionated by rate zonal ultracentrifugation. The eluate was divided into three subfractions as indicated by the solid bars (upper left). These fractions were pooled and concentrated. IDL (d 1.006–1.019 g/ml), LDL (d < 1.019–1.063 g/ml) and subfraction aliquots were re-centrifuged under identical conditions. The elution profiles of total VLDL (—), VLDL-I (- - - -), VLDL-II (· · · ·), VLDL-III (- · - · -), IDL (- - -), and LDL (- · · · · · -) are superimposed on the figure.

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Lipolysis of VLDL subfractions

Lipolysis of the three very low density lipoprotein subfractions was performed in vitro by incubation with lipoprotein lipase purified from bovine milk (7). The enzyme was prepared in Umea, Sweden (18) and sent to Jerusalem, Israel, in dry ice. The incubation mixture included the VLDL subfraction (2-5 mg of protein), lipoprotein lipase, and bovine serum albumin (essentially fatty acid-free fraction V powder, Sigma Laboratories). The amount of albumin was adjusted to that of subfraction triglycerides at a ratio of 3-5 moles TG fatty acids per mole albumin. Triglyceride hydrolysis was initiated with 25-50 μl of lipoprotein lipase [7-15 μg protein, 25-50 mU activity (18)] and incubation at 37°C was continued for 1-2 hr when a complete clearing of the incubation system occurred. The lipolytic product was obtained by subjecting the whole incubation mixture to zonal gradient centrifugation, as described above. In some experiments a duplicate incubation system containing human HDL_3 (5-10 mg of protein) was also examined.

Analytical procedures

Lipoprotein-protein was determined by the method of Lowry et al. (19) using bovine serum albumin as a reference. Phospholipids were determined by the Bartlett procedure (20) and triglycerides in the AutoAnalyzer II following the Lipid Research Clinics protocol (21). Unesterified and total cholesterol were determined by the cholesterol oxidase-cholesterol esterase method using a commercial kit (Boehringer-Mannheim, Germany). Cholesteryl ester was calculated by the difference. SDS polyacrylamide gel electrophoresis (SDS-PAGE) of lipoprotein apoproteins in 10% acrylamide was performed according to established procedures (22). SDS-PAGE in 4% gels was performed according to Kane, Hardman, and Paulus (23). Phospholipids were separated by thin-layer chromatography using a solvent system of chloroform-methanol-water-acetic acid 70:25:4:1 (v/v). The lecithin and sphingomyelin spots were identified and scraped off the plate and their phosphorus content was determined as above.

Electron microscopy

Lipoproteins were negatively stained with 2% sodium phosphotungstate, pH 7.4, on collodion-carbon-coated grids. Electron micrographs were obtained with a Phillips 300 electron microscope at instrument magnification of 90,000. For size determination, at least 100 particles were measured for each fraction.

RESULTS

Five patients were classified as moderately hypertriglyceridemic (plasma TG levels 245 to 600 mg/dl, mean = 452 ± 81) and five as severely hypertriglyceridemic (plasma TG levels 800 to 1200 mg/dl, mean = 1047 ± 74). Plasma TG levels of the normotriglyceridemics ranged between 46 to 187 mg/dl (mean = 105 ± 28).

The chemical composition of the VLDL density subfractions, of IDL, and of LDL is shown in **Table 1**. When the data from normotriglyceridemic (NTG) subfractions are compared with those from moderately and severely hypertriglyceridemic subjects, the following differences are found. The contributions of protein and TG to total VLDL mass decrease while those of cholesteryl ester and free cholesterol increase. No consistent changes are found in total VLDL phospholipids, but the lecithin to sphingomyelin molar ratio decreases ($P < 0.05$). Hence, the weight ratio of CE to protein increases considerably in HTG ($P < 0.005$) while that of CE to phospholipids does so to a lesser extent ($P < 0.005$). These differences are most marked in the VLDL-I subfraction and tend to disappear in VLDL-III; VLDL-II values are intermediate. A reverse change in compositional variation is observed in LDL: protein and TG contributions to total mass increase with hypertriglyceridemia while those of CE and free cholesterol decrease. Thus, the mass ratio of CE to protein in LDL from subjects with severe hypertriglyceridemia is about 50% that of normotriglyceridemic LDL ($P < 0.001$) and the CE to phospholipid ratio compares as 75-80% ($P < 0.01$). The CE to TG mass ratios decrease markedly in the three groups of subjects [7.75 ± 0.80, 3.34 ± 0.23, and 2.05 ± 0.34 (mean ± SE, $P < 0.001$), respectively]. Interestingly, there is also a slight decrease in the ratio of total core (cholesteryl ester and triglyceride) to total surface components (protein, phospholipid, and free cholesterol): 0.484 ± 0.08, 0.463 ± 0.08, and 0.444 ± 0.03, respectively. This difference, however, was not significant. The composition of IDL was intermediate between VLDL-III and LDL.

The contribution of intestinal lipoproteins to total VLDL was assessed by SDS-PAGE using 4% acrylamide gels and visual inspection of the gels. Three examples are shown in **Fig. 2**. Small amounts (per total protein) of low molecular weight apoB (B-48) were found in most preparations, and they were more abundant in HTG. In VLDL from each individual donor there were no consistent differences among density subfractions. ApoE was also identified in these gels, and appeared to be more abundant in HTG preparations (especially VLDL-I) than in normals.

Zonal effluent profiles of post-lipolysis VLDL preparations are shown in **Fig. 3**. In VLDL fractions from NTG subjects, post-lipolysis particles eluted at or close to the elution volume of LDL, 375-500 ml. Post-lipolysis VLDL-I usually eluted somewhat later than either post-lipolysis VLDL-II or VLDL-III. A completely different picture was observed in moderate and severe HTG. Post-lipolysis VLDL-I eluted as a symmetrical peak at a considerably

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TABLE 1. Chemical composition and composition ratios of normo- and hypertriglyceridemic VLDL density subfractions, IDL and LDL

	Composition					Weight Ratios		
	Protein	TG	CE	FC	PL	LE/SP	CE/PR	CE/PL
	mg/100 mg lipoprotein							
VLDL-I								
N (6)	8.5 ± 1.1	68.9 ± 1.7	5.0 ± 0.3	3.3 ± 0.5	14.3 ± 0.4	5.22 ± 0.2	0.66 ± 0.07	0.36 ± 0.02
M (5)	7.3 ± 0.8	64.8 ± 1.8	8.8 ± 0.9	5.6 ± 0.3	13.8 ± 0.5	4.66 ± 0.2	1.22 ± 0.08	0.64 ± 0.06
S (5)	6.7 ± 0.8	63.4 ± 1.3*	9.6 ± 0.6**	5.6 ± 0.4**	14.7 ± 0.5	4.27 ± 0.6*	1.50 ± 0.16**	0.65 ± 0.04**
VLDL-II								
N (6)	11.9 ± 0.6	56.1 ± 2.1	9.0 ± 0.8	4.6 ± 0.2	18.4 ± 0.9	5.21 ± 0.1	0.76 ± 0.06	0.49 ± 0.02
M (5)	10.1 ± 0.9	57.2 ± 2.5	9.3 ± 0.8	5.9 ± 0.3	17.4 ± 1.1	4.25 ± 0.5	0.93 ± 0.08	0.54 ± 0.03
S (5)	9.6 ± 0.8*	54.3 ± 2.9	11.9 ± 1.2*	6.3 ± 0.2**	17.9 ± 1.2	4.16 ± 0.8*	1.24 ± 0.09**	0.66 ± 0.03**
VLDL-III								
N (6)	12.9 ± 0.8	52.1 ± 2.9	12.2 ± 1.1	5.0 ± 0.3	17.8 ± 1.2	4.25 ± 0.2	0.95 ± 0.08	0.69 ± 0.04
M (5)	13.1 ± 0.6	48.6 ± 2.9	13.6 ± 1.9	6.5 ± 0.2	18.2 ± 1.1	3.65 ± 0.5	1.08 ± 0.13	0.75 ± 0.10
S (5)	12.4 ± 0.8	46.4 ± 4.4	14.4 ± 2.0	6.9 ± 0.4**	19.8 ± 1.6	3.31 ± 0.4*	1.15 ± 0.29	0.71 ± 0.06
IDL								
N (4)	17.2 ± 1.4	21.5 ± 3.6	30.5 ± 1.3	8.6 ± 0.3	22.3 ± 1.0	2.31 ± 0.4	1.80 ± 0.10	1.37 ± 0.03
M (4)	19.2 ± 1.4	23.0 ± 3.9	28.9 ± 2.1	8.8 ± 0.6	20.0 ± 0.5	2.17 ± 0.2	1.52 ± 0.11	1.44 ± 0.10
S (3)	17.5 ± 1.2	25.2 ± 1.1	28.7 ± 3.4	10.2 ± 1.3	18.4 ± 2.7	2.21 ± 0.1	1.36 ± 0.21*	1.15 ± 0.07*
LDL								
N (6)	21.5 ± 0.6	5.8 ± 0.7	42.4 ± 0.6	9.1 ± 0.4	21.2 ± 0.6	2.08 ± 0.1	1.99 ± 0.08	2.01 ± 0.08
M (5)	24.9 ± 0.8	12.3 ± 1.6	34.0 ± 1.8	8.5 ± 0.3	20.3 ± 0.7	2.46 ± 0.3	1.37 ± 0.09	1.69 ± 0.14
S (5)	30.5 ± 0.7**	15.2 ± 1.6**	29.1 ± 1.4**	6.4 ± 0.5**	18.9 ± 0.9*	2.38 ± 0.3	0.96 ± 0.05**	1.55 ± 0.12**

Data are means ± SEM for six normotriglyceridemic (N), five moderately severe hypertriglyceridemic (M), and five severely hypertriglyceridemic (S) subjects. Differences between normal and severely hypertriglyceridemic samples were tested for significance by Student's *t*-test. Significantly different data are shown by * for $P < 0.05$ and ** for $P < 0.01$ or less. TG, triglyceride; CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid; LE, lecithin; SP, sphingomyelin; PR, protein.

lower volume (150–300 ml) than the analogous NTG preparation. Post-lipolysis VLDL-II also eluted early and only post-lipolysis VLDL-III eluted at volumes comparable to the NTG product. As is evident from a comparison of Figs. 1 and 3, post-lipolysis HTG-VLDL-I eluted at volumes similar to VLDL-III and post-lipolysis HTG-VLDL-II eluted between VLDL-III and IDL.

The chemical composition of post-lipolysis VLDL preparations and the degrees of lipolysis are shown in **Table 2** and **Table 3**. For all preparations a mean degree of more than 93% triglyceride hydrolysis was achieved. The following differences between groups of subjects (NTG and moderate or severe HTG) and VLDL density subfractions (VLDL-I, -II, or -III) are noteworthy. All post-lipolysis VLDL-I preparations are different from the corresponding LDL (see Table 1) and all are enriched with free cholesterol and, to a lesser extent, phospholipids (especially sphingomyelins). Post-lipolysis VLDL-I from NTG is relatively enriched with protein and poor in CE, whereas the opposite findings are observed in HTG, especially when severe. This changed composition is best reflected by the CE to protein weight ratio which is 1.27 in NTG but 3.05 and 4.10 for moderate and severe HTG, respectively. The corresponding value for NTG-LDL is 2.01. Post-lipolysis VLDL-II exhibits the same trend, but to a lesser degree. The composition of post-lipolysis VLDL-III, in contrast, is similar among the subjects and, with the exception of some free cholesterol and phospholipid enrichment, also similar to the composition of NTG-LDL. Thus, the CE to protein ratio of post-lipolysis VLDL-III from any source was similar to that of NTG-LDL and ranged between 1.79 and 1.90. SDS-PAGE revealed that, during the course of lipolysis, apoprotein C was deleted from the VLDL and was almost undetectable

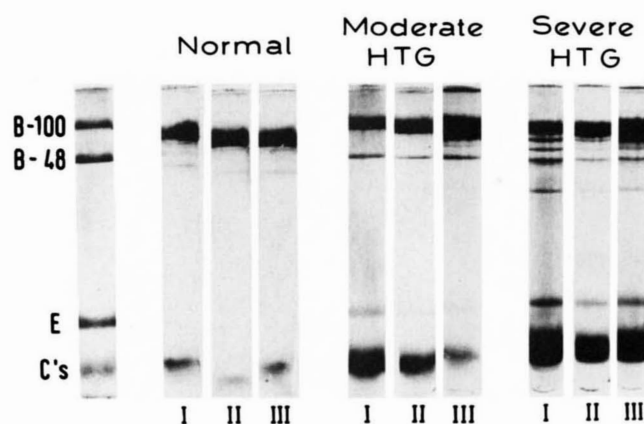

Fig. 2 SDS-PAGE of apoproteins of VLDL subfractions. Separation of VLDL-I, -II, and -III apoproteins on 4% SDS was performed as described in Methods. The figure shows a control gel (left) containing equal amounts of B-100 and B-48 (rat VLDL) and apoproteins present in VLDL fractions from normo-, moderately, and severely hypertriglyceridemic patients.

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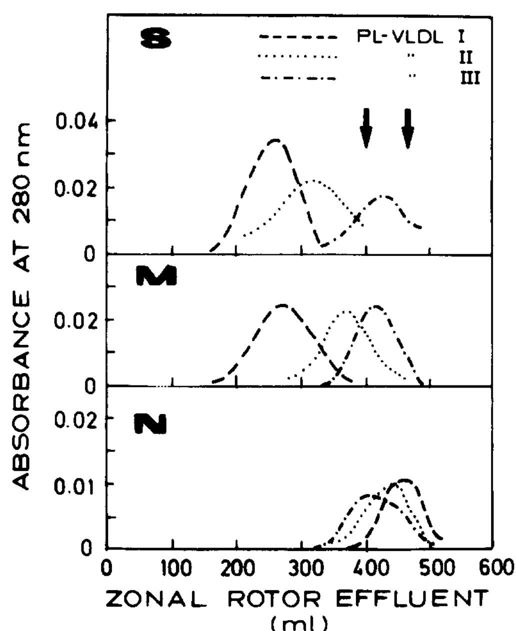

Fig. 3 Elution profiles of post-lipolysis VLDL subfractions (PL-VLDL-I, -II, and -III). VLDL subfractions isolated from normal (N), moderately (M), and severely hypertriglyceridemic (S) subjects were lipolyzed with lipoprotein lipase and each whole incubation mixture was separated by zonal ultracentrifugation. Elution profiles from each class are superimposed and the relative positions of IDL (left) and LDL (right) are indicated by heavy arrows in the upper frame.

in the post-lipolysis preparations. ApoB was retained as the major apoprotein of the post-lipolysis particle. Small amounts of albumin were frequently found in the isolated post-lipolysis VLDL, more so in samples prepared from HTG subjects.

Negatively stained electron micrographs of all VLDL and post-lipolysis VLDL preparations were obtained (**Fig. 4**). No striking differences were found among VLDL isolated from the various subjects. The density subfractions contained populations of spherical, quite homogenous particles with the diameters of VLDL-I, VLDL-II, and VLDL-III ranging between 417 and 570 Å, 348 and 417 Å, and 290 and 331 Å, respectively. Post-lipolysis VLDL preparations also contained predominantly spherical particles with occasional "vesicular-empty" structures. However, marked differences were found among post-lipolysis particles obtained from different subjects, mainly in VLDL-I and VLDL-II. In severe or moderate HTG, post-lipolysis VLDL-I constituted populations of particles that were 50–100% larger than the corresponding plasma LDL and ranged in diameter between 300 and 400 Å. Post-lipolysis VLDL-II from these subjects was also considerably larger than LDL and only post-lipolysis VLDL-III was of diameter comparable to LDL. In contrast, the post-lipolysis product of all three subfractions from NTG subjects contained particles of diameter similar to LDL.

To determine whether some of the abnormalities found in HTG-VLDL subfraction products were due to the absence of HDL from the lipolysis system, experiments in three HTG subjects (two with severe and one with moderate hypertriglyceridemia) were carried out in parallel with and without HDL_3 in the incubation mixture. There was no effect of the presence of HDL on the zonal elution profile or electron micrograph appearance of post-lipolysis particles (data not shown). The chemical composition of the particles was also very similar other than a small, insignificant but consistent decrease in free cholesterol content (**Table 4**).

The experimental design also enabled us to estimate the degree of elimination of components from VLDL

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TABLE 2. Chemical composition and composition ratios of post-lipolysis VLDL subfractions

	Composition					Weight Ratios		
	Protein	TG	CE	FC	PL	LE/SP	CE/PR	CE/PL
			mg/100 mg lipoprotein					
Post-lipolysis VLDL-I								
N (3)	23.7 ± 2.6	7.2 ± 0.2	29.7 ± 3.6	15.7 ± 3.4	23.8 ± 2.9	2.01 ± 0.3	1.27 ± 0.15	1.33 ± 0.33
M (3)	13.1 ± 0.6	5.0 ± 0.6	39.7 ± 0.2	18.7 ± 1.1	23.4 ± 1.7	1.46 ± 0.2	3.05 ± 0.12	1.88 ± 0.19
S (5)	11.8 ± 1.1**	4.1 ± 0.9*	46.4 ± 1.1**	17.6 ± 0.6	20.2 ± 1.1	1.20 ± 0.1*	4.10 ± 0.45**	2.32 ± 0.16*
Post-lipolysis VLDL-II								
N (4)	22.2 ± 1.8	7.5 ± 1.6	30.6 ± 2.4	14.7 ± 0.8	24.9 ± 2.0	2.23 ± 0.2	1.41 ± 0.17	1.27 ± 0.18
M (5)	16.3 ± 1.3	3.7 ± 1.9	38.2 ± 3.5	19.6 ± 2.2	24.1 ± 1.6	1.68 ± 0.2	2.67 ± 0.40	1.62 ± 0.21
S (4)	15.3 ± 1.3**	6.2 ± 1.9	39.1 ± 3.6*	16.5 ± 0.7	23.0 ± 1.9	2.08 ± 0.1	2.64 ± 0.43*	1.83 ± 0.41
Post-lipolysis VLDL-III								
N (5)	20.7 ± 1.3	7.3 ± 1.0	36.4 ± 2.0	12.4 ± 0.5	23.2 ± 0.9	1.98 ± 0.2	1.80 ± 0.17	1.58 ± 0.13
M (4)	20.7 ± 1.4	6.7 ± 1.4	36.2 ± 2.7	13.0 ± 0.7	23.6 ± 1.3	2.09 ± 0.2	1.79 ± 0.20	1.63 ± 0.27
S (5)	18.7 ± 1.2	8.4 ± 1.3	37.0 ± 1.3	13.1 ± 0.4	22.9 ± 0.8	1.71 ± 0.2	1.90 ± 0.14	1.64 ± 0.11

Data are means ± SEM. The number of normal (N), moderately (M), and severely (S) hypertriglyceridemic samples are given in parentheses. Differences between normal and severely hypertriglyceridemic samples were tested for significance by Student's *t*-test. Significantly different data are shown by * for $P < 0.05$ and ** for $P < 0.01$ or less. TG, triglyceride; CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid; LE, lecithin; SP, sphingomyelin; PR, protein.

TABLE 3. The extent of triglyceride hydrolysis in VLDL subfractions

	S-HTG	M-HTG	NTG
	% of triglyceride hydrolyzed		
VLDL-I	98.9 ± 0.3	98.2 ± 0.3	98.1 ± 0.1
VLDL-II	95.6 ± 1.3	98.8 ± 0.9	96.9 ± 0.6
VLDL-III	93.2 ± 3.0	94.0 ± 1.0	94.1 ± 2.0

Values are means ± SEM for the samples presented in Table 2. S-HTG, severely hypertriglyceridemic; M-HTG, moderately hypertriglyceridemic; NTG, normotriglyceridemic.

(**Fig. 5**). This was calculated from the change in the weight ratio of the content of each component compared to that of cholesteryl ester for each subfraction as a result of lipolysis. Assuming that cholesteryl ester molecules remain with the lipolyzed particle, any change in such ratios indicates deletion or addition of components during lipolysis. As expected, we found deletion of 40–60% of the VLDL protein and 51–72% of the VLDL-phospholipid. While differences among subjects were relatively small, variations among VLDL-I, -II, and -III were as expected from their different diameters, i.e, relatively more protein and phospholipid disappeared from larger particles as compared to smaller particles. The behavior of free cholesterol was markedly different from other components in that considerably fewer molecules disappeared from the subfraction VLDL during the course of lipolysis.

DISCUSSION

The present investigation was undertaken to determine critically the nature of VLDL particles in subjects with hypertriglyceridemia and the potentialities of forming LDL from VLDL precursors of different density, size, and composition. Ultracentrifugation in a zonal rotor, which had previously been employed to prepare VLDL density subfractions (16), seemed especially suitable for the present experimental design. With this method it was possible to prepare very reproducible density fractions from whole VLDL and to separate post-lipolysis VLDL populations by a single 45-min spin immediately after the incubation period. The method also allows relative esti-

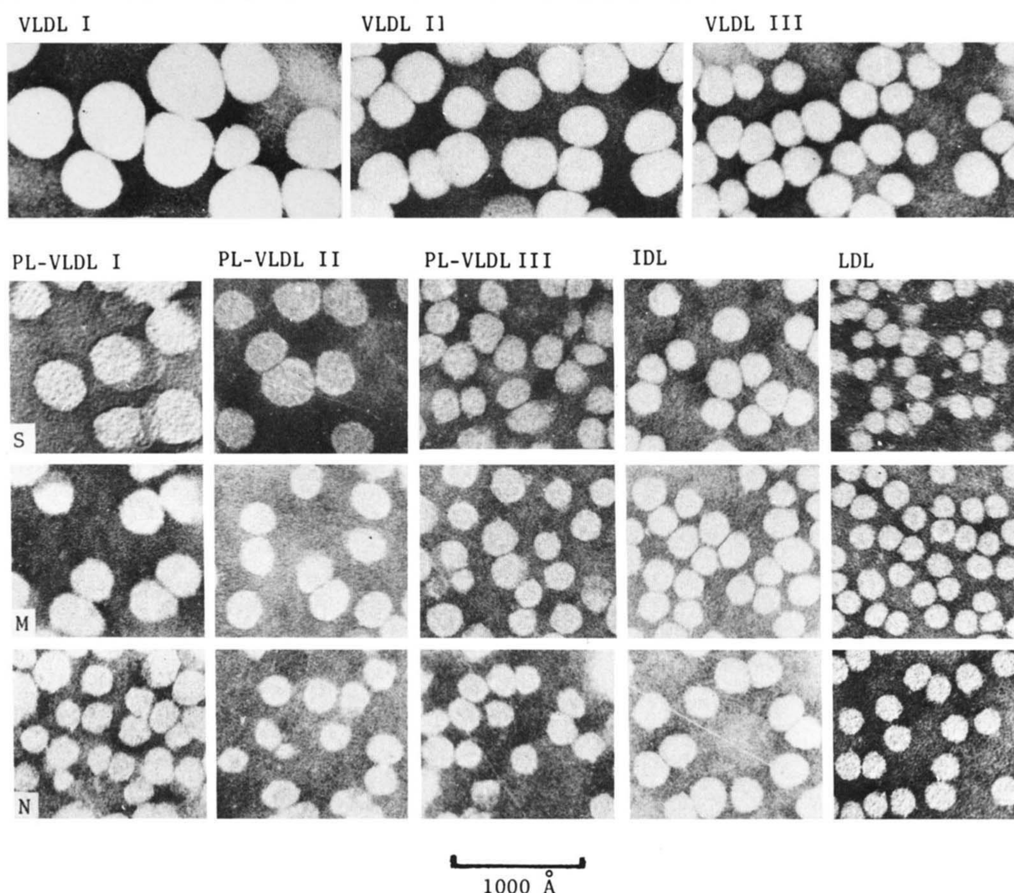

Fig. 4 Negatively stained electron micrographs of VLDL subfractions, post-lipolysis (PL) products, IDL, and LDL. Top row shows representative subfraction preparations. Lower rows show (from left to right), lipolysis products of VLDL-I, VLDL-II, and VLDL-III, and of IDL and LDL from the same subject. Results obtained from a severely hypertriglyceridemic subject (S) are shown in the second row, from a moderately HTG subject (M) in the third row, and from a normolipemic subject (N) in the bottom row.

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TABLE 4. Chemical composition of the lipolytic products of VLDL subfractions produced in the presence (+) and absence (−) of HDL_3

Post-lipolysis VLDL	HDL_3	Lipoprotein Composition				
		Protein	TG	CE	FC	PL
		mg/100 mg lipoprotein				
I (2)	−	11.6	3.2	46.4	18.0	20.8
I (2)	+	11.9	4.9	47.7	15.8	19.7
II (3)	−	16.2 ± 1.4	8.7 ± 2.1	33.3 ± 3.0	17.3 ± 2.1	24.6 ± 1.1
II (3)	+	14.8 ± 2.2	11.6 ± 4.2	35.1 ± 7.8	16.7 ± 2.4	21.7 ± 2.7
III (3)	−	19.9 ± 4.7	8.7 ± 1.7	34.7 ± 8.1	15.2 ± 1.7	21.6 ± 2.7
III (3)	+	18.8 ± 1.7	11.8 ± 0.7	30.4 ± 6.0	14.0 ± 1.8	24.9 ± 2.3

Data are means ± SEM. The number of samples determined is given in parentheses. TG, triglyceride; CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid.

mates of particle density (according to the elution volume) and direct sampling of the lipoprotein fractions.

The chemical composition of VLDL density subfractions prepared by a variety of methods (ultracentrifugation in swinging bucket rotors, zonal ultracentrifugation, and gel filtration) has been reported by several investigators (4, 13, 14, 16, 24–27). In general, denser and smaller particles are relatively enriched with surface components (proteins, phospholipids, and free cholesterol) and depleted of core triglycerides as compared to lighter and larger populations. This general rule was also observed in the present study. Nonetheless, significant differences between HTG patients and normal subjects were observed, especially in the lighter subfractions, VLDL-I and VLDL-II. In HTG, these fractions were enriched in CE and free cholesterol and contained less protein and TG.

The significance of these differences is elusive when values are expressed as percent of total weight. However, when they are expressed as CE to protein weight ratios, a distinctive difference among subjects becomes apparent. In NTG samples, this ratio increases from VLDL-I to VLDL-II, VLDL-III, and LDL (0.66, 0.76, 0.95, and 1.99, respectively). In HTG subjects, this trend is reversed. For samples from severe HTG subjects the CE to protein ratio is highest in VLDL-I and decreases in VLDL-II, VLDL-III, and LDL (1.50, 1.24, 1.15, and 0.96, respectively). A few studies examining VLDL subfractions in normal and HTG subjects simultaneously are available. In two of these studies, VLDL was fractionated by gel filtration. Analysis of the data of Quarfordt et al. (25) shows that, for particles eluted early from the column (large sized), the total cholesterol to protein ratio in normals ranges between 0.70 and 1.29 (CE/free cholesterol ratios 0.65–1.0), while in HTG the ratio is 1.80–1.90 (CE/free cholesterol ratios 2.1–3.3). In the study reported by Sata, Havel, and Jones (14), CE/protein ratios in the three lighter fractions prepared from type IV patients were 1.70–1.92-times higher than in normals, while in the heavier fractions the ratios were alike. Similar observations, albeit less striking quantitatively, were reported by Packard et al. (26). We must, therefore, conclude that in HTG the lighter large-sized VLDL particles contain many more CE molecules per protein than in normals and that these differences tend to disappear in the denser small-sized fractions.

The trend of increasing CE to protein ratios from VLDL-I to VLDL-III observed in NTG subjects is consistent with the accepted metabolic pathway wherein larger and lighter VLDL molecules are reduced to smaller and denser VLDLs and finally to LDL (1–6). This is directed by two concomitant processes: lipolysis by lipoprotein lipase and the core-lipid (cholesteryl ester and triglyceride) transfer reaction. Lipolysis, besides removing

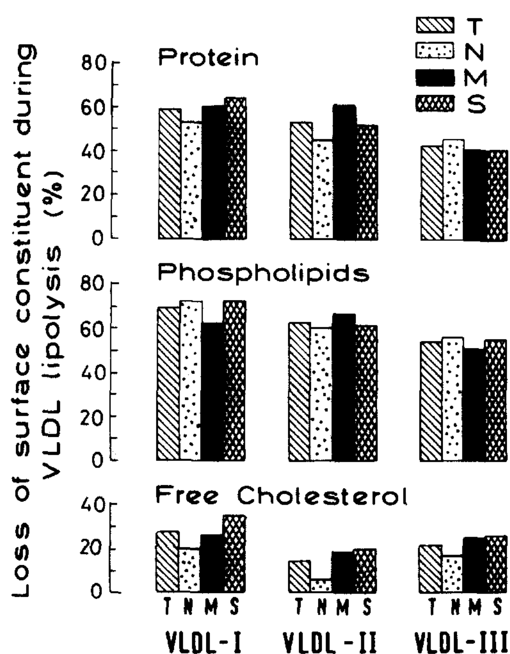

Fig. 5 Loss of surface constituents from VLDL subfractions during lipolysis. Losses are calculated relative to cholesteryl ester content as described in the text. N, normotriglyceridemia; M, moderate hypertriglyceridemia; S, severe hypertriglyceridemia; T, average loss for all three groups.

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triglyceride, is also associated with exclusion of apoprotein C species from VLDL (1, 3, 4). The core-lipid transfer process shifts CE molecules to VLDL (see below). Hence, both processes would result in an increase in the relative, and even the total CE content in the diminishing particle. The higher CE to protein ratio observed in VLDL-I and VLDL-II from severe HTG and, to a lesser extent, in moderate HTG, is not compatible with this description. An explanation lies in the extended circulation time of HTG-VLDL. This allows for prolonged action of the core-lipid transfer protein(s) on VLDL resulting in an enrichment with CE. VLDL has recently been shown to be a preferred acceptor of transferred CE molecules (28) and, of the different VLDLs, the lighter and larger particles are most avid in this respect (S. Eisenberg, unpublished results). Thus, the abnormal enrichment of HTG-VLDL with CE would be most marked in VLDL-I, least marked in VLDL-III, and the decreasing CE/protein ratios observed here would result.

This process should also generate abnormal LDL. The core-lipid transfer reaction accomplishes the enrichment of VLDL by sequestering CE from lipoproteins rich in these molecules and replacing them with TG (29–36), part of which could subsequently be hydrolyzed (36). This prediction is amply verified. We find HTG-LDL to be smaller, poorer in CE, and enriched in TG as compared to NTG-LDL. In another report (37), we also demonstrate that the degree of this abnormality correlates with increasing plasma TG levels. Similar observations are also presented for HDL, the other CE-rich lipoprotein (37).

The CE/protein ratio of HTG-IDL does not comply with the decreasing ratio observed in HTG lipoproteins. This noteworthy observation is also consistent with our hypothesis. We can expect VLDL-III, as an almost normal precursor of LDL, to first diminish to an intermediate relatively richer in CE. This IDL, being of relatively short half-life (6), would hardly be modified by the core-lipid transfer protein(s). However, the LDL produced from this particle, having a much longer circulation time, would be subject to core lipid exchange and the CE/protein ratio would drop.

Several reports have been made indicating that not all HTG-VLDL is metabolized to LDL. Apoprotein B turnover studies show variously that 40–67% of VLDL apoB is not recovered in LDL apoB and an alternative removal pathway was suggested (6, 9–12). Turnover studies of VLDL subfraction apoB by Nestel and his colleagues (11, 12) indicate that most of the HTG apoB diverted from the LDL pathway originates in the lighter density subfractions. In contrast, most NTG-VLDL apoB is recovered in the LDL fraction. Our results may clarify this important yet unresolved metabolic abnormality. As discussed above, the lipolytic reduction of large VLDL particles to smaller ones is accompanied by an increase in the CE/protein ratio. How, then, could HTG-VLDL-I and VLDL-II be precursors of VLDL-III and finally LDL when the measured CE/protein values demonstrate the opposite trend? The abnormal CE enrichment of these subfractions might, therefore, preclude their being precursors of LDL.

On the basis of the considerations discussed above, we suggest that VLDL metabolism in hypertriglyceridemia is abnormal. The main abnormality is inability of part or all of the large-sized and less dense particles (VLDL-I and VLDL-II) to serve as precursors of smaller particles and to complete the VLDL → IDL → LDL cascade. That inability is due to abnormal enrichment of VLDL-I and, to a lesser extent, VLDL-II with cholesteryl esters. This precludes their conversion to smaller-sized denser particles. We can supply further support for this hypothesis. Zonal elution profiles and electron micrographs clearly indicate that the immediate in vitro post-lipolysis products of HTG-VLDL-I and VLDL-II are not LDL nor are they "LDL-like." Analysis of these products indicates that while nearly all the apoC has been deleted, the CE to protein ratios are much higher than that of LDL (4.1 and 2.64 for severe HTG-VLDL-I and VLDL-II, respectively, as opposed to 1.99 for normal LDL). In contrast, the products of NTG-VLDL-I and VLDL-II are "LDL-like" in size and density and have CE to protein ratios lower than that of LDL (1.27 and 1.41, respectively). This lack of CE could readily be filled by the core-lipid exchange reaction in vivo. Similarly, the lipolytic products of VLDL-III from all sources have an LDL-like size, density, and chemical composition. The CE to protein ratios for post-lipolysis VLDL-III as compared to the corresponding LDL are somewhat low for NTG subjects and high for HTG subjects, but these disparities are small and could also be accounted for by the cholesteryl ester-triglyceride exchange reaction. These observations might reflect contamination of HTG-VLDL with chylomicron remnants, but we do not believe so for the following considerations. First, the amount of intestinal apoB, as judged by SDS-PAGE in our study and as recently reported (38), is relatively small. Therefore, if contamination did distort our results it would have to be due to a small number of particles of either very large diameter or of very high cholesteryl ester content. We, however, were not able to detect very large particles in any of the many electron micrographs inspected throughout the study. Furthermore, large and light particles elute in the first 25 ml of the zonal rotor volume and this was discarded in every preparation. Neither did we find any evidence for an extra population especially rich in cholesteryl ester in any of the post-lipolysis preparations. Such a population would, of necessity, yield a distant lipolytic product but, as can be seen in Fig. 3, post-lipolysis VLDL-I and VLDL-II eluted as single, rather symmetrical peaks. Because of these considerations we suggest that the higher amounts of apoB-48 in HTG-VLDL reflect the presence of intestinal VLDL that accompanies the increased VLDL levels found in HTG and that

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all VLDL particles are enriched with cholesteryl esters and free cholesterol.

We therefore conclude that while all VLDLs in NTG subjects are potential LDL precursors, only the VLDL-III subfraction of the HTG patient can so qualify. HTG-VLDL-I and VLDL-II, due to their excess CE content, must have a different destiny. The following differences in the metabolism of N- and HTG-VLDL are suggested. In normotriglyceridemic subjects, VLDL is released into the plasma compartment with an amount of cholesteryl ester. The nascent VLDL may comprise particles in a range of sizes and densities or it may be composed only of a very large and light TG-rich population. In either case, once in circulation, they undergo concomitant lipolysis and CE uptake. Thus, the nascent particle (or range of particles) diminishes to smaller and denser VLDLs and the VLDL-I → VLDL-II → VLDL-III → IDL → LDL cascade is achieved. In hypertriglyceridemia, VLDL is produced in excess and cleared slowly from the plasma (39, 40). During their prolonged circulation, VLDL-I and VLDL-II become abnormally enriched in CE at the expense of LDL and HDL. As a result, the larger HTG-VLDLs cannot be lipolyzed to VLDL-III and LDL, and large CE-rich products, which are subsequently removed from the plasma compartment, are produced. HTG-VLDL-III, having no precursor in the plasma compartment, must therefore be secreted as a discrete population and serve as the only precursor of LDL. The metabolic fate of the lipolysis products of large HTG-VLDL remains to be elucidated. ■

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