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Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoC-III in hypercholesterolemia and hypertriglyceridemia

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Abstract Apolipoprotein (apo) E and apoC-III concentrations in VLDL and LDL are associated with coronary heart disease. We studied the relationship between apoE and apoC-III and the abnormal concentrations and distribution of apoB lipoproteins in 10 hypercholesterolemic and 13 hypertriglyceridemic patients compared with 12 normolipidemic subjects (mean age, 45 years). Sixteen distinct types of apoB lipoprotein particles were separated by first using anti-apoE and anti-apoC-III immunoaffinity chromatography in sequence and then ultracentrifugation [light VLDL, dense VLDL, IDL, and LDL, with apoE with or without apoC-III ($E^+C\text{-III}^+$, $E^+C\text{-III}^-$) or without apoE with or without apoC-III ($E^-C\text{-III}^+$, $E^-C\text{-III}^-$)]. The concentrations of VLDL particles with apoC-III ($E^+C\text{-III}^+$, $E^-C\text{-III}^+$) were increased in the hypertriglyceridemic group compared with the hypercholesterolemic and normolipidemic groups. These particles were the most triglyceride rich of the particle types, and their triglyceride content was twice as high in hypertriglyceridemics compared with the other two groups. Hypertriglyceridemics had a similar concentration of total $E^-C\text{-III}^-$ particles compared with normolipidemics, but the $E^-C\text{-III}^-$ particles were distributed more to VLDL and IDL than to LDL. Hypercholesterolemic, in contrast, were distinguished from the normolipidemic group by 2-fold higher concentrations of apoB lipoproteins without apoE or apoC-III ($E^-C\text{-III}^-$), mainly LDL, which had high cholesterol content. Nonetheless, both normolipidemics and hypercholesterolemic had apoC-III-containing VLDL, which comprised 68% and 43% of their total VLDL particles. $E^+C\text{-III}^-$ particles were a minor type, comprising <10% of particles in all lipoproteins and patient groups. Therefore, VLDL particles with apoC-III may play a central role in identifying the high risk of coronary heart disease in hypertriglyceridemia, but their substantial prevalence in normolipidemics may be of clinical significance as well.—Campos, H., D. Perlov, C. Khoo, and F. M. Sacks. Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoC-III in hypercholesterolemia and hypertriglyceridemia. *J. Lipid Res.* 2001. 42: 1239–1249.

Supplementary key words metabolism • cholesterol • triglyceride

Apolipoprotein C-III (apoC-III) is an important constituent of triglyceride-rich lipoproteins (1–10). ApoC-III modulates the metabolism of VLDL by inhibiting lipoprotein lipase activation by apoC-II (1–6), and the binding of apoE to hepatic lipoprotein receptors (7–10). ApoC-III

can also inhibit hepatic lipase (11), which plays an important role in the conversion of dense VLDL to IDL and LDL (12, 13). Hypertriglyceridemic patients have increased plasma apoC-III concentrations and production rates compared with normolipidemics (14). In contrast, apoC-III-deficient patients have reduced VLDL triglyceride with rapid conversion of VLDL to LDL (5). These abnormalities associated with high and low apoC-III concentrations have been reproduced in mouse models by altering apoC-III expression (6, 10, 15, 16).

High plasma triglyceride concentration is an independent risk factor for coronary disease (17, 18). However, the specific role of triglyceride-rich lipoproteins in atherosclerosis is poorly understood, in part because triglyceride-rich lipoproteins are heterogeneous in size, density, and composition (19–30) as well as metabolic characteristics (23, 24, 26–28, 31–35). It has been suggested that apoB lipoproteins (VLDL, IDL, LDL) that have apoC-III are atherogenic in humans (36–42). ApoC-III concentrations in plasma and in apoB lipoproteins are higher among patients with coronary disease than among controls (36, 37). A high concentration of apoC-III in apoB lipoproteins is an independent predictor of coronary events (41), and is associated with increased progression of coronary atherosclerotic lesions (38–40).

The interpretation of the role of apoC-III in these studies (36–41) is complicated by the presence of apoE in VLDL in addition to apoC-III (42–44). ApoE can be recognized by several cell surface receptors (27, 45–48), facilitating the clearance of VLDL. Paradoxically, apoE concentrations are high in hypertriglyceridemic patients and are associated with coronary heart disease (36, 41, 49, 50). ApoC-III interferes with the function of apoE to bind cell surface LDL and LDL receptor-related protein receptors (43, 51). We have found that apoE exists mainly together

Abbreviations: apo, apolipoprotein; IAC, immunoaffinity chromatography.

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with apoC-III in the same apoB lipoprotein particles of young normolipidemic women (52). These data suggested that the presence of apoE in apoB lipoproteins is probably a marker of particles with apoC-III as well, and that apoE content, per se, may not be proatherogenic.

The purpose of this study is to evaluate the relationship of apoE and apoC-III to the abnormal apoB lipoprotein particle concentrations in dyslipidemia. We hypothesized that apoC-III-containing lipoproteins play a central role in determining the lipoprotein risk profile of hypertriglyceridemia.

MATERIALS AND METHODS

Study population

The study population consisted of 35 middle-aged men ($n = 28$) and women ($n = 7$), who were free of diabetes, stroke, clinical cardiovascular disease, and thyroid disease. Hyperlipidemic patients were withdrawn from their antilipidemic medications for 3 weeks before sample collection. The following criteria were used for assigning subjects into three groups. The hypertriglyceridemic group was defined as having fasting plasma triglyceride concentrations ≥ 250 mg/dl and LDL cholesterol concentrations ≤ 160 mg/dl; the hypercholesterolemic group as having fasting plasma total cholesterol ≥ 240 mg/dl and triglyceride concentrations ≤ 250 mg/dl; and the normolipidemic group as having fasting plasma cholesterol ≤ 220 mg/dl and triglyceride concentrations ≤ 150 mg/dl. The criteria were designed to produce a hypertriglyceridemic group that did not have combined hyperlipidemia (high triglycerides and high LDL cholesterol), and therefore were completely distinguished from the hypercholesterolemic group; and a hypercholesterolemic group that was completely distinguished from the other two groups. The normolipidemic group was closely matched by age to the hypertriglyceridemic and hypercholesterolemic groups. The final sample size was 13 hypertriglyceridemic, 10 hypercholesterolemic, and 12 normolipidemic patients. All study participants gave informed consent, and the Human Subjects Committees of the Harvard School of Public Health and Brigham and Women's Hospital approved the study.

Immunoaffinity chromatography (IAC) separation

Blood was drawn after a 12-h fast and immediately centrifuged at 4°C to isolate plasma. A mixture containing 2 μ M benzamidine, aprotinin (0.01 mg/ml), PMSF (17.5 μ g/ml), and gentamicin (0.05 mg/ml) was added to the plasma and the samples were immediately sealed under N₂ and frozen at 80°C until they were analyzed. We found that freezing does not materially affect the results. For example, the plasma concentrations of apoB in fresh and frozen samples ($n = 3$), respectively, were for E⁺C-III⁺ particles 0.26 versus 0.31 mg/dl; for E⁻C-III⁺ 2.12 versus 2.14 mg/dl; for E⁺C-III⁻ 0.19 versus 0.27 mg/dl; and for E⁻C-III⁻ 41 versus 44 mg/dl (all NS). Separation of lipoproteins by apoE and apoC-III content was carried out with affinity-purified polyclonal antibodies anti-apoE (kindly provided by Genzyme, Cambridge, MA) and anti-apoC-III (DMA, Arlington, TX), coupled to cyanogen bromide-activated Sephacryl S-1000 resins as previously described (52). For each subject 3 ml of plasma was first incubated with 1 ml of anti-apoE resin for 1 h at room temperature with constant mixing. All incubations and rinses were carried out with disposable Econopac columns (Bio-Rad, Hercules, CA). The unbound fractions (E⁻) were collected by gravity flow from the Econopac columns and the resin was washed with PBS. The bound fraction (E⁺) was eluted by incubation with 3 M NaSCN,

passed through a gel-filtration column using PBS, and then immediately dialyzed against PBS in microconcentrators (Amicon, Beverly, MA). The E⁻ fractions and the dialyzed E⁺ fractions were further incubated with 1.0 ml of anti-apoC-III resin for 4 h at 4°C. The same elution protocol used for the anti-apoE resin was carried out to yield four immunofractions, two with apoE (E⁺C-III⁺, E⁺C-III⁻) and two without apoE (E⁻C-III⁺, and E⁻C-III⁻). ApoE and apoC-III were undetected in the filtrate by silver-stained 10–20% SDS-PAGE (Owl Separation Systems, Portsmouth, NH) and Western blotting. A control pool sample was included in every run. On average 11% of the apoE and 20% of the apoC-III in the control pool were not associated with lipoprotein particles that bound to the respective anti-apoE and anti-apoC-III columns. To estimate the binding efficiency of the columns in the three patient groups, we pooled equal volumes of plasma from the participants. Consistent with the laboratory controls, the efficiency of the apoE column was 95% in normolipidemics, and 96% in hypercholesterolemics and hypertriglyceridemics. The efficiency for the apoC-III column was 78% in normolipidemics, 88% in hypercholesterolemics, and 65% in hypertriglyceridemics. It is possible that in the hypertriglyceridemic group, the immunoreactivity of apoC-III was hindered by increased triglyceride in the particles, or that a higher resin-to-plasma ratio is needed with hypertriglyceridemic subjects. The column efficiency for the apoC-III columns was not improved with an increased resin-to-plasma ratio, although the apoC-III column efficiency in hypertriglyceridemic plasma was 9% better during method development.

Each of the four immunofractions was then separated into four density fractions by using a modification of the Lindgren, Jensen, and Hatch method (20). The IAC separation procedure was carried out before ultracentrifugation, so that any loss of apoC-III and apoE during ultracentrifugation could not affect the separation of particle types by IAC (Fig. 1). To separate VLDL subfractions with Svedberg units of flotation (S_f) 60–400 (light VLDL) and S_f 20–60 (dense VLDL), samples were spun in the outer row of a Beckman (Palo Alto, CA) type 25 rotor at 25,000 rpm in an L8-70M instrument (Beckman) for 1 h at 10°C to collect light VLDL and for 6 h to collect dense VLDL. To separate IDL ($d = 1.006$ – 1.025 g/ml) and LDL ($d = 1.025$ – 1.050 g/ml), the density was raised with KBr, and centrifugation was carried out at 25,000 rpm for 24 h each at 10°C. Silver-stained 10–20% SDS-PAGE of pooled light VLDL from the normolipidemic subjects prepared by IAC and ultracentrifugation showed proportionally less apoE or apoC-III in the unretained compared with the retained fractions (Fig. 2). Lipoprotein concentrations were corrected for losses during IAC and ultracentrifugation. For each subject, a sample was ultracentrifuged to separate light and dense VLDL, IDL, and LDL at the same time that another sample from the same subject was ultracentrifuged after IAC. Cholesterol, triglyceride, and apoB were measured in both the ultracentrifuged-only samples and in those ultracentrifuged after IAC. The concentrations of the lipoprotein fractions separated by ultracentrifugation only, and corrected for plasma concentrations, were used as the standard for the lipoprotein fractions separated by IAC and ultracentrifugation. The overall recoveries for apoB in the lipoprotein immunofractions after IAC and ultracentrifugation were 60% for VLDL, 66% for IDL, and 80% for LDL. The major IAC fractions after ultracentrifugation had similar recoveries when they were compared with their corresponding fraction before ultracentrifugation: 85% for E⁺C-III⁺ lipoproteins, 83% for E⁻C-III⁺, and 86% for E⁻C-III⁻. A lower recovery, 69%, was found for E⁺C-III⁻ lipoproteins, but this particle type represented 1.4% of the total apoB lipoproteins. Thus among the major particles types there does not appear to be selective loss.

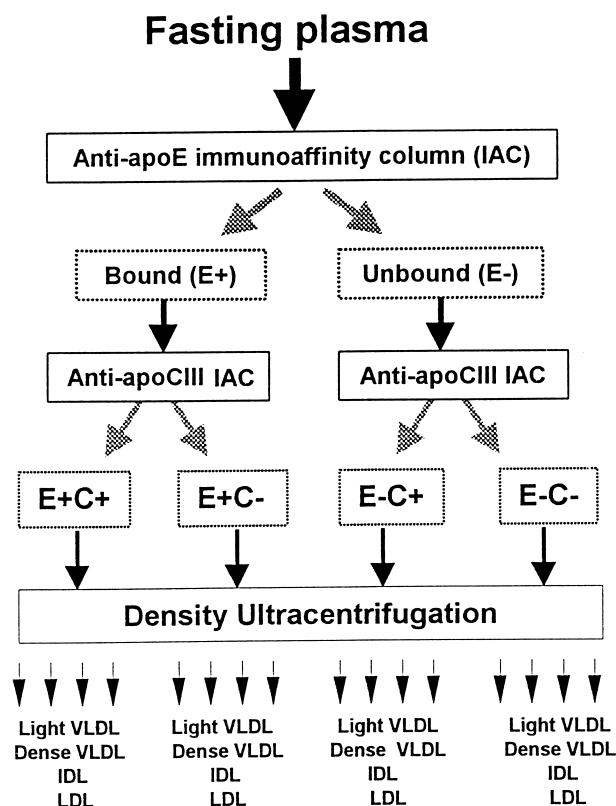


Fig. 1. Separation of lipoproteins with apoB defined by their apoE and apoC-III content. First, lipoproteins with apoE were isolated with affinity-purified polyclonal antibody anti-apoE (kindly provided by Genzyme) coupled to cyanogen bromide-activated Sepharyl S-1000 resins. Bound (E⁺) and unbound (E⁻) lipoproteins were then separated by their content of apoC-III, using the resin coupled to anti-apoC-III (DMA, Arlington, TX). The bound fractions were eluted by incubation with 3 M NaSCN, passed through a gel-filtration column using PBS, and then immediately dialyzed against PBS in microconcentrators (Amicon). Separation by IAC of the four immunofractions (E⁺C-III⁺, E⁺C-III⁻, E⁻C-III⁺, and E⁻C-III⁻) was followed by ultracentrifugation to further isolate four density fractions. Sixteen distinct types of apoB lipoprotein particles were obtained [light VLDL, dense VLDL, IDL, and LDL, with apoE with or without apoC-III (E⁺C-III⁺, E⁺C-III⁻) or without apoE with or without apoC-III (E⁻C-III⁺, E⁻C-III⁻)].

Lipid, apolipoprotein, and APOE polymorphism determination

Whole plasma and lipoprotein immunofractions were assayed for total cholesterol and triglyceride, using enzymatic reagents (Boehringer Mannheim, Indianapolis, IN). Cholesterol determinations in our laboratory are standardized according to the program for research laboratories specified by the Centers for Disease Control and Prevention (Atlanta, GA), and by the National Heart, Lung, and Blood Institute (National Institutes of Health, Bethesda, MD). ApoB was measured with an ELISA (detection limit, 0.1 mg/dl), using a polyclonal antibody (AlerCHECK, Portland, ME). The standards used for measuring apoB in our current study were calibrated to the improved standards of the International Federation of Clinical Chemistry. APOE genotyping was carried out with the polymerase chain reaction followed by restriction endonuclease digestion. APOE gene variants were identified by cleavage with *HhaI* as described by Hixson and Vernier (53).

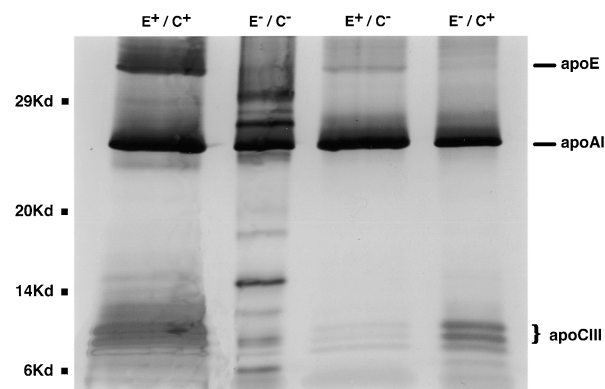


Fig. 2. Silver-stained 10–20% SDS-PAGE of pooled light VLDL from the normolipidemic subjects prepared by IAC and ultracentrifugation.

Statistical analysis

Statistical analyses were performed with Statistical Analysis Systems software (SAS, Cary, NC). ANOVA was used to compare immunofraction cholesterol, triglyceride and apoB concentrations among the groups, and each hyperlipidemic group was compared with the normal group, using Student-Newman-Kuels adjustment for multiple comparisons. All the parameters were log transformed before analysis and data are presented as geometric means $\pm$ SD. Frequencies were compared with the χ^2 statistic. Multiple regression analysis tested the effect of patient group, APOE genotype (33 vs. 34), and gender (male vs. female). Adjustments for gender and APOE genotype did not change the original results and the data are presented without these adjustments. Smoking status had no significant effect on any of the studied parameters. Among the members of the hypertriglyceridemic group there were two subjects who were carriers of the APOE2 allele. When these subjects were excluded the results were basically unchanged and the data are presented including these two subjects.

RESULTS

Subject characteristics

The characteristics and plasma lipid parameters for normolipidemic, hypercholesterolemic, and hypertriglyceridemic patients are shown in **Table 1**. The groups were similar in age, as intended by matching. The hypercholesterolemic and hypertriglyceridemic patients had higher body mass indexes than normolipidemics. Hypercholesteroleemics had more family history of heart and/or vascular disease than the other two groups. As intended, the hypercholesterolemic patients had higher cholesterol concentrations in apoB lipoproteins, particularly LDL, and higher apoB concentrations than normolipidemics. The hypertriglyceridemic patients had much higher triglyceride concentrations than hypercholesteroleemics and normolipidemics, as intended, but their plasma total apoB concentrations were similar to those of the normolipidemics. Hypertriglyceridemic patients also had higher VLDL cholesterol, VLDL triglycerides, and VLDL apoB than the other two groups. HDL cholesterol concentrations were lower in hypercholesterolemic and hypertri-

TABLE 1. Subject characteristics and plasma lipoproteins

Parameter	Normolipidemic (n = 12)	HCHOL (n = 10)	HTG (n = 13)	ANOVA <i>P</i>
Age (years)	46 ± 9	42.0 ± 9	46 ± 7	0.5
Body mass index (kg/cm ²)	23.2 ± 2.5	28.3 ± 3.5 ^a	27.7 ± 3.6 ^a	0.0008
Gender (male/female) ^b	8/4	7/3	13/0	0.07
Current smokers (%) ^b	8	20	0	0.3
Family history of heart and/or vascular disease (%) ^b	60	100	40	0.01
Family history of hyperlipidemia (%) ^b	50	90	44	0.02
ApoB				
Total plasma				
μM	1.62 ± 0.62	3.03 ± 0.82 ^a	1.86 ± 0.58 ^c	0.0002
mg/dl	90 ± 34	169 ± 46	103 ± 32	
Light VLDL (μM)	0.03 ± 0.03	0.04 ± 0.04	0.17 ± 0.11 ^{a,c}	0.0003
Dense VLDL (μM)	0.04 ± 0.03	0.08 ± 0.04 ^a	0.13 ± 0.05 ^{a,c}	0.0001
IDL (μM)	0.09 ± 0.03	0.18 ± 0.08 ^a	0.12 ± 0.04 ^c	0.002
LDL (μM)	1.22 ± 0.54	2.39 ± 0.66 ^a	0.99 ± 0.37 ^c	0.0001
Total d < 1.050 g/ml (μM)	1.42 ± 57	2.72 ± 0.73 ^a	1.48 ± 0.42 ^c	0.0001
Cholesterol				
Plasma total				
μM	4,817 ± 645	8,519 ± 1,567 ^a	5,432 ± 1,493 ^c	0.0001
mg/dl	187 ± 25	330 ± 61	211 ± 58	
Light VLDL (μM)	137 ± 110	206 ± 191	1,130 ± 986 ^{a,c}	0.0001
Dense VLDL (μM)	119 ± 72	268 ± 137 ^a	561 ± 277 ^{a,c}	0.0001
IDL (μM)	287 ± 82	821 ± 324 ^a	437 ± 206 ^{a,c}	0.0001
LDL (μM)	2,018 ± 708	5,541 ± 1,366 ^a	1,408 ± 626 ^{a,c}	0.0001
Total d < 1.050 g/ml (μM)	2,670 ± 760	7,044 ± 1,399 ^a	3,904 ± 1,461 ^{a,c}	0.0001
Triglyceride				
Plasma total				
μM	1,043 ± 317	1,755 ± 720 ^a	4,316 ± 3,160 ^{a,c}	0.0001
mg/dl	91 ± 28	154 ± 63	379 ± 277	
Light VLDL (μM)	237 ± 154	478 ± 391 ^a	2,566 ± 2,292 ^{a,c}	0.0001
Dense VLDL (μM)	215 ± 102	354 ± 188 ^a	863 ± 578 ^{a,c}	0.0004
IDL (μM)	136 ± 29	204 ± 50 ^a	228 ± 194 ^a	0.009
LDL (μM)	187 ± 62	369 ± 108 ^a	206 ± 53	0.0001
Total d < 1.050 g/ml (μM)	812 ± 294	1,556 ± 681 ^a	4,024 ± 3,039 ^{a,c}	0.0001
HDL cholesterol				
μM	1,577 ± 578	1,009 ± 259 ^a	816 ± 194 ^a	0.0001
mg/dl	61 ± 22	39 ± 10	32 ± 8	

Means ± SD. ANOVA Student-Newman-Keuls correction for multiple comparisons. To convert apoB, cholesterol, and triglyceride concentrations to mg/dl divide by 0.018, 25.8, and 11.4, respectively. HTG, hypertriglyceridemic; HCHOL, hypercholesterolemic.

^a *P* < 0.05 compared with normolipidemic subjects.

^b Analysis used the χ^2 statistic.

^c *P* < 0.05 compared with hypercholesterolemic subjects.

glyceridemic patients than in the normolipidemics. There were nine subjects with the *APOE3/4* genotype (one normolipidemic, five hypercholesterolemic, and three hypertriglyceridemic), and two with *APOE3/2* (both hypertriglyceridemic).

Predominant apoB lipoprotein particle types in plasma

Particles without apoE or apoC-III (E⁻C-III⁻). ApoB particles without apoC-III or apoE (E⁻C-III⁻) were the most prevalent of all the particles studied, and represented 89% of all apoB in the total d < 1.050 g/ml lipoprotein fraction in normolipidemic patients, 93% in hypercholesterolemic patients, and 82% in hypertriglyceridemic patients (Table 2). Hypercholesterolemic patients had 2-fold higher concentrations of E⁻C-III⁻ particles than did normolipidemic or hypertriglyceridemic patients. E⁻C-III⁻ carried more of the cholesterol in normolipidemic patients (83%) and hypercholesterolemic patients (88%) than in hypertriglyceridemic

patients (64%) (Table 3). E⁻C-III⁻ particles also carried most of the triglyceride in hypercholesterolemic patients (56%), but only 38–39% in normolipidemic and hypertriglyceridemic patients (Table 4). Thus, in every respect, E⁻C-III⁻ particles are the dominant apoB lipoproteins in the hypercholesterolemic group.

Particles with apoC-III with and without apoE (E⁺C-III⁺ and E⁻C-III⁺). The plasma concentration of apoC-III-containing lipoprotein B particles was 2-fold higher in the hypertriglyceridemic patients compared with normolipidemic and hypercholesterolemic patients (Table 2). ApoE was present in approximately 50% of the apoC-III-containing particles in each patient group. In hypertriglyceridemic patients, 35% of plasma cholesterol was associated with apoC-III-containing particles, compared with 17% in normolipidemic patients and 22% in hypercholesterolemic patients (Table 3). Most of the plasma triglyceride (62%) was found in apoC-III-containing particles in normolipidemic

TABLE 2. ApoB concentrations in light VLDL, dense VLDL, IDL, and LDL separated by immunoaffinity chromatography and ultracentrifugation

ApoB	Normolipidemic (n = 12)	HCHOL (n = 10)	HTG (n = 13)	ANOVA <i>P</i>
	μM	μM	μM	
E⁻C-III⁻				
Light VLDL	0.005 ± 0.006	0.010 ± 0.01 ^a	0.062 ± 0.053 ^{a,b}	0.0001
Dense VLDL	0.010 ± 0.005	0.046 ± 0.03 ^a	0.076 ± 0.030 ^{a,b}	0.0001
IDL	0.062 ± 0.016	0.140 ± 0.06 ^a	0.090 ± 0.030 ^{a,b}	0.0005
LDL	1.17 ± 0.52	2.32 ± 0.64 ^a	0.94 ± 0.38 ^b	0.0001
Total	1.26 ± 0.52	2.53 ± 0.72 ^a	1.21 ± 0.40 ^b	0.0001
E⁺C-III⁺				
Light VLDL	0.013 ± 0.014	0.016 ± 0.014	0.048 ± 0.035 ^{a,b}	0.01
Dense VLDL	0.018 ± 0.013	0.017 ± 0.011	0.021 ± 0.016	0.8
IDL	0.011 ± 0.010	0.020 ± 0.009	0.016 ± 0.008	0.08
LDL	0.012 ± 0.011	0.020 ± 0.008	0.007 ± 0.005	0.03
Total	0.055 ± 0.042	0.078 ± 0.034	0.094 ± 0.056	0.2
E⁻C-III⁺				
Light VLDL	0.008 ± 0.005	0.010 ± 0.012	0.038 ± 0.018 ^{a,b}	0.0004
Dense VLDL	0.007 ± 0.008	0.007 ± 0.006	0.023 ± 0.014 ^{a,b}	0.001
IDL	0.008 ± 0.005	0.008 ± 0.003	0.011 ± 0.007	0.6
LDL	0.023 ± 0.009	0.031 ± 0.014	0.021 ± 0.014	0.4
Total	0.049 ± 0.023	0.059 ± 0.031	0.095 ± 0.046 ^a	0.04
E⁺C-III⁻				
Light VLDL	0.002 ± 0.003	0.003 ± 0.003	0.008 ± 0.008 ^a	0.06
Dense VLDL	0.005 ± 0.005	0.006 ± 0.006	0.006 ± 0.007	0.9
IDL	0.006 ± 0.006	0.011 ± 0.008 ^a	0.003 ± 0.005 ^b	0.02
LDL	0.006 ± 0.005	0.009 ± 0.006	0.002 ± 0.003 ^{a,b}	0.008
Total	0.021 ± 0.016	0.030 ± 0.019	0.019 ± 0.023	0.3

Means ± SD. ANOVA Student-Newman-Keuls correction for multiple comparisons. To convert apoB concentrations to mg/dl divide by 0.018. HTG, hypertriglyceridemic; HCHOL, hypercholesterolemic.

^a *P* < 0.05 compared with normolipidemic subjects.

^b *P* < 0.05 compared with hypercholesterolemic subjects.

and hypertriglyceridemic patients, compared with 44% in hypercholesterolemic patients (Table 4). Thus, apoC-III-containing apoB lipoproteins are more prominent in hypertriglyceridemic patients than in normolipidemic or hypercholesterolemic patients in terms of concentration and the amount of lipid carried, whereas E⁻C-III⁻ particles strongly dominate the apoB lipoproteins of hypercholesterolemic patients.

Particles with apoE but without apoC-III. ApoE-containing particles without apoC-III (E⁺C-III⁻) were the least prevalent and represented <1.5% of all apoB in the d < 1.050 g/ml lipoprotein fraction in all the patient groups. E⁺C-III⁻ particles are more prominent carriers of triglyceride in the hypertriglyceridemics than in the other groups.

Particle types within light VLDL, dense VLDL, IDL, and LDL

Particles with apoC-III, E⁺C-III⁺ and E⁻C-III⁺, predominated in light VLDL of all groups (Table 2 and Fig. 3). As lipoprotein density increased from light VLDL to LDL, E⁻C-III⁻ particles progressively became more prevalent (Fig. 3). The increased light VLDL concentration in hypertriglyceridemic patients compared with normolipidemic and hypercholesterolemic patients was due to increases in all of the particle types, notably 4-fold increases in E⁺C-III⁺ and E⁻C-III⁺ particles and a 10-fold increase in E⁻C-III⁻ particles (Table 2 and Fig. 3). The higher concentration of dense VLDL particles in hypertriglyceridemic patients compared with normolipidemic patients was mostly due

to higher concentrations of E⁻C-III⁻ and E⁻C-III⁺ particles. Hypercholesterolemic patients had higher particle concentrations of all density fractions than normolipidemic patients, due mainly to higher E⁻C-III⁻ particles (Fig. 3). Thus, hypertriglyceridemic and hypercholesterolemic patients have contrasting patterns in their particle types. Hypertriglyceridemics have enrichment of light VLDL particles with apoC-III whereas hypercholesterolemic have more E⁻C-III⁻ particles in denser fractions, particularly IDL and LDL.

Nonetheless, both normolipidemics and hypercholesterolemic had apoC-III-containing VLDL, which comprised 68% and 43% of their total VLDL particles (Table 2 and Fig. 3). Hypercholesterolemic also had higher E⁺C-III⁻ IDL concentrations than normolipidemics (Table 2, Fig. 3).

Cholesterol and triglyceride distribution within particles with and without apoC-III

In hypertriglyceridemic patients, VLDL particles, particularly those containing apoC-III, carried most of the non-HDL cholesterol; only 38% was in LDL E⁻C-III⁻ particles (Table 3, Fig. 4). In contrast, in normolipidemic and hypercholesterolemic patients, LDL E⁻C-III⁻ particles carried 75% and 89% of the non-HDL cholesterol; apoC-III⁻ VLDL particles had less than 5% (Table 3 and Fig. 4). Overall, the non-HDL cholesterol distribution among the particle types and density fractions was similar in the normolipidemic and hypercholesterolemic

TABLE 3. Cholesterol concentrations in light VLDL, dense VLDL, IDL, and LDL separated by immunoaffinity chromatography and ultracentrifugation

Cholesterol	Normolipidemic (n = 12)	HCHOL (n = 10)	HTG (n = 13)	ANOVA <i>P</i>
	μM	μM	μM	
E⁻C-III⁻				
Light VLDL	12 $\pm$ 23	37 $\pm$ 38	278 $\pm$ 277 ^{a,b}	0.0001
Dense VLDL	19 $\pm$ 18	125 $\pm$ 75 ^a	252 $\pm$ 179 ^{a,b}	0.0001
IDL	145 $\pm$ 51	518 $\pm$ 162 ^a	230 $\pm$ 127 ^b	0.0001
LDL	1,882 $\pm$ 678	5,271 $\pm$ 1,307 ^a	1,236 $\pm$ 613 ^{a,b}	0.0001
Total	2,101 $\pm$ 693	6,063 $\pm$ 1,355 ^a	2,186 $\pm$ 1,097 ^b	0.0001
E⁺C-III⁺				
Light VLDL	81 $\pm$ 55	97 $\pm$ 79	499 $\pm$ 399 ^{a,b}	0.0001
Dense VLDL	66 $\pm$ 37	85 $\pm$ 60	145 $\pm$ 112 ^{a,b}	0.03
IDL	94 $\pm$ 39	189 $\pm$ 90 ^a	120 $\pm$ 72 ^b	0.01
LDL	51 $\pm$ 29	93 $\pm$ 33 ^a	45 $\pm$ 21 ^b	0.002
Total	308 $\pm$ 122	503 $\pm$ 219 ^a	842 $\pm$ 537 ^{a,b}	0.0001
E⁻C-III⁺				
Light VLDL	29 $\pm$ 20	47 $\pm$ 63 ^a	201 $\pm$ 120 ^{a,b}	0.0001
Dense VLDL	17 $\pm$ 15	30 $\pm$ 20 ^a	86 $\pm$ 28 ^{a,b}	0.0001
IDL	22 $\pm$ 9	34 $\pm$ 11 ^a	39 $\pm$ 21 ^a	0.01
LDL	59 $\pm$ 30	113 $\pm$ 34 ^a	62 $\pm$ 26 ^b	0.008
Total	136 $\pm$ 58	242 $\pm$ 129 ^a	398 $\pm$ 154 ^{a,b}	0.0001
E⁺C-III⁻				
Light VLDL	11 $\pm$ 7	17 $\pm$ 22	51 $\pm$ 107 ^a	0.02
Dense VLDL	10 $\pm$ 10	18 $\pm$ 24	25 $\pm$ 35	0.3
IDL	18 $\pm$ 15	55 $\pm$ 55 ^a	21 $\pm$ 27	0.09
LDL	18 $\pm$ 16	33 $\pm$ 30	12 $\pm$ 17	0.2
Total	59 $\pm$ 44	133 $\pm$ 127	112 $\pm$ 217	0.2

Means $\pm$ SD. ANOVA Student-Newman-Keuls correction for multiple comparisons. To convert cholesterol concentrations to mg/dl divide by 25.8. HTG, hypertriglyceridemic, HCHOL, hypercholesterolemic.
^a *P* < 0.05 compared with normolipidemic subjects.
^b *P* < 0.05 compared with hypercholesterolemic subjects.

patients (Fig. 4), as was the apoB distribution, with hypercholesteroleemics having higher cholesterol concentrations in the E⁻C-III⁻ particles in each density fraction (Table 3). Among the particle types, those with both apoC-III and apoE had the highest cholesterol content (Fig. 5). The cholesterol content of E⁺C-III⁻ particles was higher in the hypertriglyceridemic group compared with normolipidemic or hypercholesterolemic groups (Fig. 5). In contrast, the hypercholesterolemic group, compared with the other groups, had higher cholesterol contents of the predominant particle type, E⁻C-III⁻ IDL and LDL particles.

In hypertriglyceridemics, nearly half of the total plasma triglyceride was carried in apoC-III⁺ light VLDL; most of the remainder was in other light and dense VLDL particles, and small amounts were in IDL and LDL (Fig. 4 and Table 4). In contrast, normolipidemic and hypercholesterolemic patients had a lower proportion of the triglyceride in apoC-III⁺ VLDL and a higher percentage in apoC-III⁻ IDL and LDL compared with hypertriglyceridemics. Light VLDL E⁺C-III⁺ particles had the highest triglyceride content of the particle types: 19,000 molecules per particle in hypertriglyceridemic patients and 13,000 in normolipidemics and hypercholesteroleemics (Fig. 5).

Effect of APOE genotype and gender on immunoaffinity fractions

We found a significant independent APOE genotype effect (33 vs. 34). APOE3/4 genotype compared with

APOE3/3 was associated with higher E⁻C-III⁻ IDL and LDL cholesterol: 458 versus 290 μM and 3,827 versus 2,824 μM , respectively, *P* < 0.01. Triglyceride in IDL and LDL E⁻C-III⁻ was also higher in association with the APOE3/4 genotype compared with APOE3/3: 148 versus 81 μM , *P* = 0.05 and 293 versus 215 μM , *P* = 0.01. Females had significantly lower LDL apoB E⁻C-III⁺ and E⁺C-III⁺ particles compared with males: 0.012 versus 0.029 μM , *P* = 0.01, and 0.004 versus 0.02 μM , *P* = 0.004.

DISCUSSION

We studied the difference between hypertriglyceridemic and normolipidemic patients, and between hypercholesterolemic and normolipidemic patients, in terms of plasma apoB lipoproteins as defined by their content of apoC-III and apoE, and by their density. This system of particles revealed distinct differentiating characteristics of the hyperlipidemia of hypertriglyceridemic patients, and that of hypercholesteroleemics compared with normolipidemics. The central finding for hypertriglyceridemics was a vastly different particle distribution characterized by increased involvement of apoC-III-containing particles in lighter density lipoproteins. In hypertriglyceridemics, VLDL particles with apoC-III carried most of the apoE as well as disproportionate amounts of the plasma cholesterol and triglyceride. In contrast, the prominent feature of hyper-

TABLE 4. Triglyceride concentrations light VLDL, dense VLDL, IDL, and LDL separated by immunoaffinity chromatography and ultracentrifugation

Triglyceride	Normolipidemic (n = 12)	HCHOL (n = 10)	HTG (n = 13)	ANOVA P
	μM	μM	μM	
E ⁻ C-III ⁻				
Light VLDL	14 ± 13	92 ± 83 ^a	598 ± 612 ^{a,b}	0.0001
Dense VLDL	33 ± 22	152 ± 90 ^a	342 ± 301 ^{a,b}	0.0001
IDL	56 ± 25	134 ± 53 ^a	107 ± 107 ^a	0.004
LDL	131 ± 66	317 ± 102 ^a	145 ± 53 ^b	0.0001
Total	240 ± 110	765 ± 222 ^a	1,224 ± 1,153 ^a	0.0001
E ⁺ C-III ⁺				
Light VLDL	150 ± 103	219 ± 213	1,027 ± 818 ^{a,b}	0.0001
Dense VLDL	108 ± 54	103 ± 80	216 ± 219 ^{a,b}	0.02
IDL	38 ± 10	27 ± 14	52 ± 43	0.08
LDL	16 ± 9	18 ± 7	20 ± 12	0.3
Total	324 ± 163	388 ± 326	1,249 ± 1,354 ^{a,b}	0.0001
E ⁻ C-III ⁺				
Light VLDL	50 ± 34	123 ± 113 ^a	587 ± 312 ^{a,b}	0.0001
Dense VLDL	41 ± 30	59 ± 29	182 ± 61 ^{a,b}	0.0001
IDL	23 ± 10	21 ± 9	42 ± 34 ^{a,b}	0.02
LDL	22 ± 10	22 ± 10	22 ± 11	0.9
Total	143 ± 78	236 ± 156	866 ± 357 ^{a,b}	0.0001
E ⁺ C-III ⁻				
Light VLDL	13 ± 12	22 ± 33	129 ± 293 ^{a,b}	0.0008
Dense VLDL	20 ± 14	24 ± 29	40 ± 67	0.5
IDL	12 ± 9	11 ± 6	14 ± 18	0.8
LDL	9 ± 6	9 ± 5	8 ± 11	0.8
Total	59 ± 40	70 ± 86	196 ± 444 ^{a,b}	0.02

Means ± SD. ANOVA Student-Newman-Keuls correction for multiple comparisons. To convert triglyceride concentrations to mg/dl divide by 11.4. HTG, hypertriglyceridemic, HCHOL, hypercholesterolemic.
^a *P* < 0.05 compared with normolipidemic subjects.
^b *P* < 0.05 compared with hypercholesterolemic subjects.

cholesterolemic patients was increased concentrations of particles without apoC-III and apoE in all densities, but most of all in LDL, and a similar distribution of particles compared with normolipidemics.

The predominant apoB lipoprotein particles in plasma in all of the patient groups were those without apoC-III and apoE. These particles were predominantly found in the LDL density range. Because apoC-III inhibits lipolysis (1–6), particles without apoC-III may be good substrates for lipoprotein and hepatic lipase, which converts them to smaller particles. Studies of apoC-III-deficient patients (5) and mice with deletion of the apoC-III gene (16) support this hypothesis because they show rapid conversion of VLDL to LDL. Thus, triglyceride-rich particles without apoC-III or apoE could be the most important precursor of plasma LDL, and this lipolytic pathway could play an important role in hypercholesterolemic patients, who have high concentrations of these particles.

The apoC-III-containing lipoproteins, the most triglyceride rich of the particle types, were distributed to light VLDL in all patient groups, in distinct contrast to the E⁻C-III⁻ particles, which were in denser lipoproteins. This distribution and composition of apoC-III-containing particles could be due to the direct action of apoC-III as an inhibitor of lipolysis (1–6). The apoC-III-containing particles could be lipolyzed slowly compared with particles without apoC-III, and accumulated in lighter density lipoproteins, VLDL rather than LDL. Hypertriglyceridemic

patients had 4-fold higher apoC-III-containing light VLDL particles, more buoyant and lipid rich, compared with the other patient groups. This exaggerated pattern of apoC-III particles in hypertriglyceridemia could be attributed to decreased lipoprotein lipase activity, often reported in hypertriglyceridemia (54–56), which could compound the intrinsic resistance of apoC-III-containing particles to lipolysis. Low lipoprotein lipase activity could also explain the increased concentration of light and dense VLDL E⁻C-III⁻ and E⁺C-III⁻ particles in hypertriglyceridemics.

Our findings also suggest that apoE may modulate the metabolism of apoC-III⁺ apoB lipoproteins. The plasma total concentrations of E⁻C-III⁺ and E⁺C-III⁺ particles are similar but they are distributed differently across the density classes, with E⁻C-III⁺ being more prevalent in LDL and E⁺C-III⁺ more prevalent in VLDL. In all patient groups, the concentrations of E⁻C-III⁺ LDL were greater than those of E⁺C-III⁺ LDL, a reversal of the relationships in VLDL and IDL, where the concentrations of E⁻C-III⁺ particles were lower than those of E⁺C-III⁺ particles. In normolipidemic and hypercholesterolemic patients, 50–55% of the E⁻C-III⁺ particles were in the LDL density range compared with 20–25% of E⁺C-III⁺ particles. Even in hypertriglyceridemics, who had 70% of the apoC-III-containing particles in VLDL, the concentration of LDL E⁻C-III⁺ particles was 3-fold higher than that of LDL E⁺C-III⁺ particles. This suggests that apoE facilitates the removal of apoC-III-containing particles from plasma and reduces

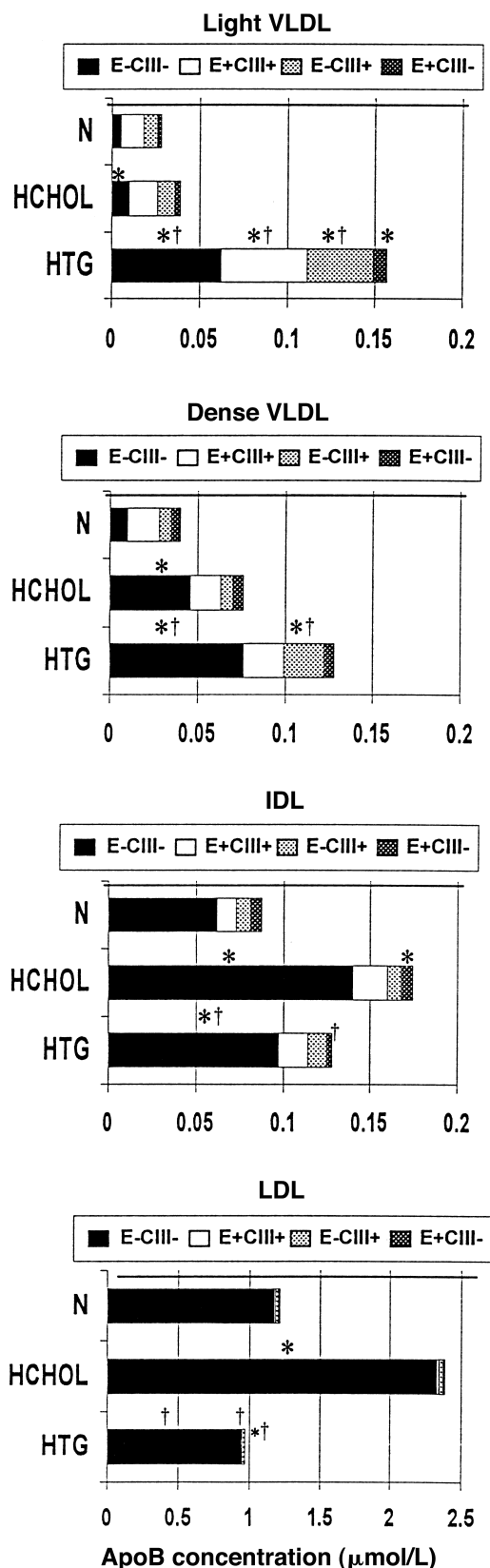


Fig. 3. ApoB concentration of each particle type (E^-C-III^- , E^+C-III^- , E^-C-III^+ , and E^+C-III^+) in each of the lipoprotein density fractions (light VLDL, dense VLDL, IDL, and LDL), in normolipidemic (N), hypercholesterolemic (HCHOL), and hypertriglyceridemic (HTG) patients. * Significantly different ($P < 0.05$) from normolipidemic patients; † significantly different ($P < 0.05$) from hypercholesterolemic patients.

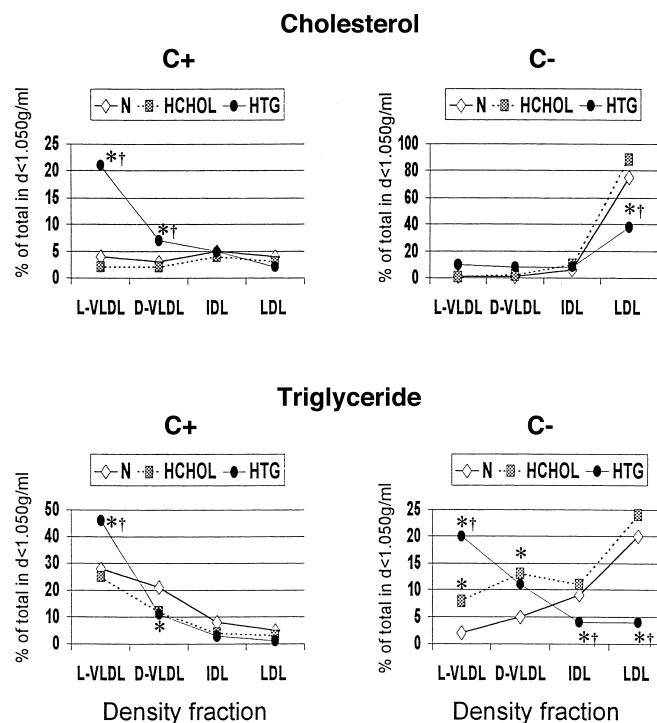


Fig. 4. Lipoprotein density distribution of cholesterol and triglyceride in apoC-III⁺ (E^+C-III^+ and E^-C-III^+) and apoC-III⁻ (E^+C-III^- and E^-C-III^-) particles from normolipidemic (N), hypercholesterolemic (HCHOL), and hypertriglyceridemic (HTG) patients. L-VLDL indicates light VLDL and D-VLDL indicates dense VLDL. * Significantly different ($P < 0.05$) from normolipidemic patients; † significantly different ($P < 0.05$) from hypercholesterolemic patients.

their conversion and accumulation in LDL. The E^-C-III^+ VLDL lacks the apoE ligand for receptor-mediated uptake, and is channeled to the lipolysis pathway, albeit likely slowly, to LDL.

An alternative explanation for reduced lipolysis of E^+C-III^+ VLDL, and a different view of the role of apoE in lipoprotein metabolism, is that apoE itself may inhibit lipolysis (57–59). VLDL from apoE transgenic rabbits and mice had reduced susceptibility to lipolysis in vitro (57, 58), and the addition of human apoE3 decreased the susceptibility of normal mouse chylomicrons and VLDL to lipolysis (58). The apoE-rich lipoproteins had a low apoC-II content, an activator of lipoprotein lipase (58).

The density distribution of E^+C-III^- particles, by far the least prevalent of the apoB lipoproteins, also suggests a role for apoE in enhancing clearance or slowing lipolysis of the particles. Compared with E^-C-III^- particles, the E^+C-III^- particles are mainly in VLDL and IDL rather than LDL. This and their low plasma concentration suggest reduced conversion of E^+C-III^- VLDL and IDL particles to E^+C-III^- LDL, and rapid receptor-mediated clearance of E^+C-III^- particles using apoE as the ligand. In normolipidemic and hypercholesterolemic patients, E^+C-III^- VLDL and IDL particles generally had relatively low triglyceride and cholesterol contents, more like E^-C-III^- than E^+C-III^+ particles. In hypertriglyceridemics, the cholesterol and triglyceride contents of E^+C-III^- VLDL and

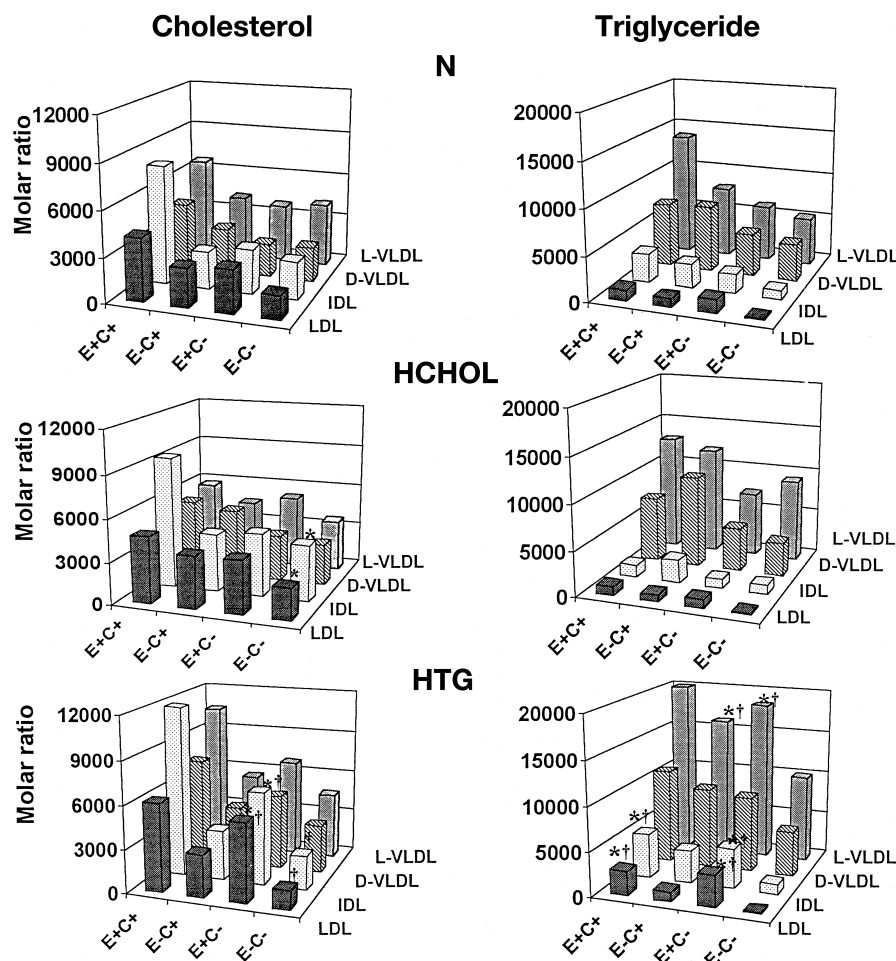


Fig. 5. Cholesterol and triglyceride content of each particle type (E^-C-III^- , E^+C-III^+ , E^-C-III^+ , and E^+C-III^-) in the plasma lipoprotein density fractions (light VLDL, dense VLDL, IDL, and LDL) from normolipidemic (N), hypercholesterolemic (HCHOL), and hypertriglyceridemic (HTG) patients. L-VLDL indicates light VLDL and D-VLDL indicates dense VLDL. * Significantly different ($P < 0.05$) from normolipidemic patients; † significantly different ($P < 0.05$) from hypercholesterolemic patients.

IDL particles were high, more like E^+C-III^+ than E^-C-III^- particles. This suggests that the E^+C-III^- particles are partially lipolyzed E^+C-III^+ particles, and that lipolysis is impaired in hypertriglyceridemics, causing the accumulation of triglyceride-rich E^+C-III^- particles in light VLDL.

Higher triglyceride concentrations in plasma are an independent risk factor for coronary disease (17, 18). However, the plasma concentration of particle types rather than total triglyceride may be more relevant to atherosclerosis. Data from case-control studies show that apoC-III concentrations in apoB relative to non-apoB lipoproteins were increased in survivors of myocardial infarction (37) and in patients before undergoing bypass surgery compared with the control population (36). ApoC-III concentrations in apoB lipoproteins are significant markers of progression of coronary atherosclerosis as determined by coronary angiography in hypercholesterolemic patients (38–40). In normolipidemic patients with myocardial infarction (41), the concentrations of apoC-III and apoE in apoB lipoproteins (LDL and VLDL) predicted new coronary disease events when they were considered indi-

vidually. However, only apoC-III remained a significant predictor when they were included together in multiple regression analysis. This finding is consistent with our data showing that apoE and apoC-III occur mainly together in the particle (52). Furthermore, a high apoC-III content may be responsible for the slower catabolic rates observed in apoB lipoproteins with apoE compared with those without apoE (60). Taken together, the weight of the evidence indicates that triglyceride-rich particles, specifically those with apoC-III, have impaired metabolism and may be atherogenic remnants. Because of the heterogeneity of triglyceride-rich lipoproteins, trials that will evaluate whether triglyceride reduction will reduce coronary risk should measure specific particle types that are potentially atherogenic in order to obtain optimal markers of disease.

In summary, hypertriglyceridemia is distinguished from normolipidemia and hypercholesterolemia by increased apoC-III-containing light VLDL particles (E^+C-III^+ , E^-C-III^+) that are rich in cholesterol and triglyceride. In contrast, hypercholesterolemia is characterized by high concentrations of E^-C-III^- particles distributed to denser lipopro-

teins, particularly LDL. The overall pattern of lipoprotein density and particle type is similar between hypercholesterolemics and normolipidemics. The presence or absence of apoE on apoB lipoproteins does not discriminate among the patient groups as well as apoC-III. Thus, VLDL particles with apoC-III, which have the potential of promoting atherosclerosis, may play a central role in establishing the lipoprotein pattern of hypertriglyceridemia. **BJR**

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