Characterization of remnant-like particles isolated by immunoaffinity gel from the plasma of type III and type IV hyperlipoproteinemic patients

Caroline Marcoux,* Michel Tremblay,* Katsuyuki Nakajima,† Jean Davignon,* and Jeffrey S. Cohn[1,*]

Hyperlipidemia and Atherosclerosis Research Group,* Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7, and Otsuka America Pharmaceutical Inc.,† Rockville, MD 20852

Abstract Previous studies have investigated the potential atherogenicity and thrombogenicity of triglyceride-rich lipoprotein (TRL) remnants by isolating them from plasma within a remnant-like particle (RLP) fraction, using an immunoaffinity gel containing specific anti-apoB-100 and anti-apoA-I antibodies. In order to characterize lipoproteins in this RLP fraction and to determine to what extent their composition varies from one individual to another, we have used automated gel filtration chromatography to determine the size heterogeneity of RLP isolated from normolipidemic control subjects (n = 8), and from type III (n = 6) and type IV (n = 9) hyperlipoproteinemic patients, who by selection had similarly elevated levels of plasma triglyceride (406 ± 43 and 397 ± 35 mg/dl, respectively). Plasma RLP triglyceride, cholesterol, apoB, apoC-III, and apoE concentrations were elevated 2- to 6-fold ($P < 0.05$) in hyperlipoproteinemic patients compared to controls. RLP fractions of type III patients were enriched in cholesterol and apoE compared to those of type IV patients, and RLP of type IV patients were enriched in triglyceride and apoC-III relative to those of normolipidemic subjects. In normolipidemic subjects, the majority of RLP had a size similar to LDL or HDL. The RLP of hyperlipoproteinemic patients were, however, larger and were similar in size to TRL, or were intermediate in size (i.e., ISL) between that of TRL and LDL. Compared to controls, ISL in the RLP fraction of type III patients were enriched in apoE relative to apoC-III, whereas in type IV patients they were enriched in apoC-III relative to apoE. These results demonstrate that: *1*) RLP are heterogeneous in size and composition in both normolipidemic and hypertriglyceridemic subjects, and *2*) the apoE and apoC-III composition of RLP is different in type III compared to type IV hyperlipoproteinemic patients.—Marcoux, C., M. Tremblay, K. Nakajima, J. Davignon, and J. S. Cohn. **Characterization of remnant-like particles isolated by immunoaffinity gel from the plasma of type III and type IV hyperlipoproteinemic patients. *J. Lipid Res.* 1999. 40: 636–647.**

Supplementary key words triglyceride-rich lipoprotein remnants • apoC-III • apoE • LDL • atherosclerosis

Considerable evidence from both clinical and laboratory studies suggests that triglyceride-rich lipoprotein (TRL) remnants play a role in the pathogenesis of atherosclerosis and thrombosis (1–3). TRL remnants are produced in the circulation from apoB-48-containing chylomicrons of intestinal origin or apoB-100-containing very low density lipoproteins (VLDL) of hepatic origin. Triglycerides in the core of these lipoproteins are hydrolyzed by lipoprotein lipase on the surface of vascular endothelial cells resulting in the formation of TRL remnants. Compared to their nascent precursors, these remnants are smaller, more dense, depleted of triglyceride, phospholipid, apolipoprotein (apo) A-I and apos C, and are enriched in cholesteryl esters and apoE (4).

Remnant lipoproteins have been separated from plasma on the basis of their size, density, charge, specific lipid components, or apolipoprotein composition (5, 6). Recently, a more quantitative and clinically applicable assay has been developed, whereby remnant-like particles (RLP) are separated from plasma by immunoaffinity chromatography with a gel containing an anti-apoA-I and a specific anti-apoB-100 monoclonal antibody (JI-H) (7, 8). The former antibody recognizes all high density lipoproteins (HDL) containing apoA-I, while the latter antibody recognizes all apoB-100-containing lipoproteins, except partially lipolyzed TRL remnants. HDL, low density lipoproteins (LDL), and the majority of VLDL are retained by the gel, while the unbound RLP fraction is made up of remnant-like VLDL containing apoB-100, as well as TRL containing apoB-48. The reason why the anti-apoB-100

Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; d, density; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediamine-tetraacetate; FPLC, fast protein liquid chromatography; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; ISL, intermediate-sized lipoprotein; LDL, low-density lipoprotein; RLP, remnant-like particle; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

(JI-H) antibody does not recognize the apoB-100 of apoE-enriched VLDL remnants is not entirely clear, although the amino acid sequence of the apoB-100 epitope region has been found to be homologous to an amphipathic helical region of apoE, suggesting that apoE can compete for binding of the antibody to its epitope on apoB-100 (8).

The plasma concentration of RLP cholesterol is significantly correlated with levels of total triglyceride, VLDL triglyceride, and VLDL cholesterol, and is not strongly related to the plasma concentration of LDL cholesterol or LDL apoB (7, 9, 10). RLP cholesterol concentration is higher in men compared to women (10), older compared to younger subjects (7, 10), postmenopausal compared to premenopausal women (10), the fed versus the fasted state (11), individuals with diabetes (12), patients with familial dysbetalipoproteinemia (13, 14), hemodialysis patients (15, 16), and patients with coronary artery restenosis after angioplasty (17). It has been demonstrated that RLP cholesterol concentration is significantly higher in patients with CAD compared to controls (7, 14, 17–19). The potential atherogenicity of RLP is supported by the observation that RLP can promote lipid accumulation by mouse peritoneal macrophages (20), can stimulate whole blood platelet aggregation (21, 22), and can impair endothelium-dependent vasorelaxation (23).

Although certain physiological and pathophysiological aspects of the RLP fraction have been investigated, relatively little is known about the lipoprotein composition of this fraction, and to what extent this composition varies from one individual to another. Campos et al. (24) have characterized the physical and chemical properties of lipoproteins not recognized by the apoB-100 monoclonal antibody JI-H, which were subsequently isolated by ultracentrifugation at a density less than 1.006 g/ml. These lipoproteins contained more molecules of apoE and cholesteryl esters and fewer molecules of apoC-III per particle than those that were bound. They had slow pre-β-electrophoretic mobility compared to the bound VLDL fraction, consistent with them being remnant-like lipoproteins. Other lipoproteins may however be present when the JI-H monoclonal antibody (together with an anti-apoA-I antibody) is used to isolate RLP by immunaffinity chromatography from total plasma (in the absence of ultracentrifugation). HPLC analysis of RLP fractions isolated in this way from normolipidemic and diabetic subjects has revealed considerable size heterogeneity in RLP, with particles ranging in size from VLDL to HDL (8). Work from our laboratory has shown that the relative amount of lipid and apolipoprotein in RLP can also vary considerably, such that hypertriglyceridemic patients have more triglyceride and apoC-III and less apoE relative to apoB in RLP than normotriglyceridemic individuals (9).

In order to further investigate the apparent heterogeneity of RLP in normolipidemic and hypertriglyceridemic subjects, we have in the present study used automated gel filtration chromatography and measurement of apoB, apoE, and apoC-III to determine the size distribution of lipoproteins in the RLP fraction. We have compared the lipoprotein composition of the RLP fraction of normolipidemic subjects with that of hypertriglyceridemic patients, who did or did not have significant plasma remnant lipoprotein accumulation. We have thus studied a group of type III hyperlipoproteinemic individuals, having an apoE 2/2 phenotype and plasma remnant lipoprotein accumulation (as evidenced by the presence in plasma of β-VLDL), and a group of type IV subjects (without β-VLDL), who were selected to have similar levels of total plasma triglyceride.

METHODS

Subjects

Healthy male normolipidemic subjects (n = 8), who had a plasma triglyceride concentration less than 200 mg/dl (2.3 mmol/l), were recruited from the staff of our research laboratory (mean age (±SE): 41 ± 5 years, BMI: 24 ± 1 kg/m^2). Hyperlipidemic patients were selected from those attending our lipid clinic at the Clinical Research Institute of Montreal. One group of patients (n = 6) was selected who had Fredrickson (25) type III hyperlipoproteinemia (plasma triglyceride >200 mg/dl, an apoE 2/2 phenotype, and a clearly defined β-migrating VLDL band on agarose gel electrophoresis). A second group was selected (n = 9) who had type IV hyperlipoproteinemia (plasma triglyceride >200 mg/dI, LDL cholesterol <130 mg/dl (3.4 mmol/l)). Mean ages were 49 ± 4 years and 45 ± 4 years for the type III and type IV patients, respectively. Both groups had a mean BMI of 29 ± 1 kg/m^2. The normolipidemic and type IV patients had an apoE 3/3 phenotype, except for one normolipidemic and three type IV patients who had an apoE 4/3 phenotype. Neither normolipidemic nor hyperlipidemic subjects were taking medications known to affect plasma lipid levels. Mean plasma lipid and apolipoprotein concentrations for the study groups are shown in Table 1. Hypertriglyceridemic patients were selected so that mean levels of plasma triglyceride for the two hyperlipoproteinemic groups were not significantly different.

Separation of plasma lipoproteins

Blood samples were obtained from subjects who had fasted for 12 h overnight. Blood was drawn under vacuum from an arm vein into tubes containing EDTA (final concentration: 1.5 mg/ml). Plasma was obtained by centrifugation (15 min, 3000 rpm, 4°C) and was stored at 4°C until lipids and lipoproteins were analyzed. RLP and ultracentrifugal fractions were separated within 2 days of plasma isolation. VLDL were separated by ultracentrifugation from 5 ml of plasma, which was spun overnight at d = 1.006 g/ml (50,000 rpm, 10 h, 4°C) in a 50.4 Ti rotor (Beckman Instruments Inc., Palo Alto, CA).

Isolation of RLP from plasma

RLP fractions were isolated from fresh plasma using RLP-Cholesterol Assay Kits (Jimro-II, Japan lmmunoresearch Laboratories, Japan). According to the manufacturer's instructions, 5 μl of plasma was added to 300 μl of gel suspension, consisting of anti-human apoA-I and apoB-100 mouse monoclonal antibodies bound to Sepharose (8). The suspension was gently mixed for 2 h at room temperature with a vertical magnetic-bead oscillator (RLP Mixer J-100A, Photal, Otsuka Electronics, Japan). The mixture was allowed to settle for 15 min. The supernatant (230 μl), containing unbound RLP, was aspirated and stored at 4°C until time of analysis.

Separation of RLP fractions

Analysis of different-sized lipoproteins in the RLP fraction was achieved by separating lipoprotein particles by automated gel fil-

tration chromatography on a fast protein liquid chromatography (FPLC) system. Multiple (n = 95) RLP aliquots, each prepared from 5 μl of plasma, were concentrated (minicon-CS15 concentrators, Amicon, Inc., Beverly, MA) to give a final volume of 1.5–2 ml. They were then manually transferred to a 2-ml sample loop with two washes (250 μl) of 0.15 m NaCI buffer (0.01% EDTA, 0.02% sodium azide, pH 7.2). The system was programmed (Liquid Chromatography Controller LCC-500 Plus) to load and separate samples on a 50-cm column (16 mm internal diameter) packed with cross-linked agarose gel (Superose 6 prep grade, Pharmacia). The column was eluted with saline solution at a rate of 1.0 ml/min, and 25 min after addition of sample, 90 × 1 ml fractions were collected sequentially. Sample elution was monitored spectrophotometrically at optical density 280 nm. TRL-, ISL-, and HDL-sized fractions were pooled according to the optical density elution profile.

Separation of lipoproteins by two-dimensional gel electrophoresis

Lipoproteins in the HDL size range in total plasma and in RLP fractions were separated by two-dimensional non-denaturing gel electrophoresis, as described previously (26). Briefly, plasma samples (30 μl) and RLP fractions (isolated from 30 μl of plasma) were separated in the first dimension (according to their charge) by 0.75% agarose gel electrophoresis (100 V, 8 h, 4°C), and in a second dimension (according to their size) by 3–24% polyacrylamide concave gradient gel electrophoresis (80 V, 20 h, 4°C). A high-molecular weight protein mixture (7.1 nm to 17.0 nm, Pharmacia, Piscataway, NJ) iodinated using IODO-GEN® Iodination Reagent (1,3,4,6-tetrachloro-3α-6α-diphenylglycouril, Pierce Chem. Co., Rockford, IL) was run as a standard on each gel. Electrophoretically separated proteins and lipoproteins were electrotransferred (30 V, 20 h, 4°C) onto nitrocellulose membranes (Hybond ECL, Amersham Life Science, Buckinghamshire, England), and apoE- or apoA-I-containing lipoproteins were detected by incubating membranes with immunopurified polyclonal apoE antibody (Genzyme Corp, Cambridge, MA) or anti-apoA-I antibody (Biodesign, Kennebunk, ME) labeled with ^{125}I. The presence of labeled antibody was detected by autoradiography using XAR-2 Kodak film.

Quantitation of lipids and apolipoproteins

Cholesterol and triglyceride in plasma, RLP fractions from total plasma, and RLP fractions separated by FPLC were determined enzymatically on an autoanalyzer (Cobas Mira, Roche). HDL cholesterol concentration was determined by assaying cholesterol in the supernatant after precipitation of apoB-containing lipoproteins with heparin–manganese (27) from the d > 1.006 g/ml fraction prepared by ultracentrifugation. Plasma apoA-I and apoB concentrations were determined by nephelometry (Behring Nephelometer 100 Analyser). ApoE phenotypes were determined by immunoblotting of plasma separated by IEF minigel electrophoresis (28). ApoE and apoC-III in total plasma, lipoprotein and FPLC fractions were assayed by ELISA (29, 30). ApoB was measured by a non-competitive polyclonal ELISA based on the method of Albers, Lodge, and Curtis (31). Affinity-purified goat polyclonal anti-human apoB antibody (Biodesign, Kennebunk, ME) was used as the capture antibody and monoclonal antibody MB-24, which recognizes both apoB-100 and apoB-48, was used as the detection antibody (kindly provided by Dr. Linda Curtis, Scripps Research Institute, La Jolla, CA). One MB24 epitope exists on every molecule of apoB, irrespective of whether it is apoB-100 or apoB-48. ApoB concentrations were therefore expressed in units of μmol/l, providing a measure of the number of apoB molecules in a given fraction, independent of it being apoB-100 or apoB-48. Cholesterol, apoB, apoC-III, and apoE measured in FPLC elution fractions were corrected to give 100% recovery. Relative amounts of apoB-100 and apoB-48 were determined in plasma RLP fractions by Western blotting of RLP apolipoproteins separated on SDS 3–7% polyacrylamide gradient gels. Twenty RLP fractions isolated from 20 × 5 μl of plasma were pooled for this analysis. ApoB bands were quantitated by densitometry after their chemiluminescent detection with a goat anti-apoB polyclonal antiserum followed by a conjugated rabbit anti-goat IgG antibody. This system had sufficient sensitivity to detect relative amounts of apoB-48 and apoB-100 in whole RLP fractions, but not in RLP after separation by FPLC. RLP apoA-I was assayed by a non-competitive polyclonal ELISA developed in our laboratory according to the method of Bury and Rosseneu (32). Affinity-purified goat polyclonal anti-human apoA-I antibody (Biodesign, Kennebunk, ME) was used as the capture antibody and the detection antibody. Plasma VLDL concentrations were determined by measuring lipids and apolipoproteins in the d < 1.006 g/ml fraction. Plasma LDL cholesterol concentrations were determined by subtracting HDL cholesterol from d > 1.006 g/ml cholesterol. Apolipoprotein molar ratios were determined by dividing uncorrected apolipoprotein concentrations (in μmol/l) obtained by direct measurement of RLP fractions.

Statistical analysis

Statistical analyses were performed with Sigmastat statistical software (Jandel Corporation, San Rafael, CA). Data were expressed as means ± SE. Student's unpaired *t*-test was used for comparisons between groups. Mann-Whitney rank sum tests were performed if data sets were not normally distributed. Kolmogorov-Smirnov tests were used to test normality and equal variance of data sets. Differences with a *P* value less than 0.05 were considered to be statistically significant.

RESULTS

Plasma lipid, apolipoprotein, and RLP levels

Mean plasma lipid and apolipoprotein concentrations for the normolipidemic and hyperlipoproteinemic subjects are shown in **Table 1**. Plasma triglyceride concentrations of type III and type IV hyperlipoproteinemic subjects were (according to patient selection) not significantly different ($P = 0.88$), and were 3- to 4-times higher than that of normolipidemic subjects. Total plasma cholesterol concentrations were also significantly higher in hypertriglyceridemic versus normolipidemic subjects, with type III patients having VLDL cholesterol levels which were on average 2-fold higher than those of type IV patients. Remnant lipoprotein accumulation in type III patients (reflected by the presence in plasma of β-migrating VLDL) was thus, as expected, associated with a significantly ($P < 0.001$) higher VLDL cholesterol to plasma triglyceride mass ratio (0.35 ± 0.03), compared to type IV (0.18 ± 0.01) or control subjects (0.23 ± 0.06). Total plasma apoE concentrations were on average 4.5-times higher in type III and 1.5-times higher in type IV patients than in normolipidemic subjects. Mean plasma apoC-III levels were elevated 2- to 3-fold in hypertriglyceridemic patients versus controls, but as for total plasma triglyceride, type III and type IV patients did not have significantly different apoC-III concentrations.

Mean plasma RLP lipid and apolipoprotein concentrations (in molar units) are shown in **Table 2**. All RLP parameters were significantly elevated in hyperlipoprotein-

TABLE 1. Plasma lipid and apolipoprotein concentrations of normolipidemic and hyperlipoproteinemic subjects

	Normolipidemic (n = 8)	Type III (n = 6)	Type IV (n = 9)	Significance of Mean Differences[a] Type III vs. IV
		mg/dl		
Plasma triglyceride	105.9 ± 10.4	405.9 ± 43.0[c]	397.2 ± 34.6[c]	—
Plasma cholesterol	175.6 ± 9.5	266.6 ± 10.8[c]	209.0 ± 11.8[b]	$P < 0.01$
VLDL cholesterol	22.2 ± 4.5	134.5 ± 9.5[c]	71.2 ± 4.9[c]	$P < 0.01$
LDL cholesterol	105.2 ± 10.1	88.8 ± 7.4	106.4 ± 9.2	—
HDL cholesterol	48.2 ± 5.5	34.1 ± 1.9	31.4 ± 2.3[b]	—
ApoB	105.9 ± 9.4	104.6 ± 7.7	133.9 ± 5.8[b]	$P < 0.01$
ApoC-III	11.7 ± 1.3	28.6 ± 3.5[c]	29.6 ± 2.6[c]	—
ApoE	4.1 ± 0.3	18.1 ± 0.8[c]	6.8 ± 0.5[c]	$P < 0.001$
ApoA-I	122.6 ± 4.1	135.6 ± 3.8	125.9 ± 7.8	—

Values represent means ± SE.

[a] Significantly different by unpaired *t*-test, or by Mann-Whitney rank sum when groups were not normally distributed.

[b] $P < 0.05$; [c] $P < 0.001$; significantly different from normolipidemic control group by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.

emic patients compared to controls. RLP cholesterol levels were on average 5-times higher in type III subjects and 2-times higher in type IV subjects compared to controls (43.6 ± 4.0 vs. 15.5 ± 2.0 vs. 8.3 ± 0.6 mg/dl, respectively). Despite no significant difference between their levels of total plasma triglyceride, type III patients had significantly ($P < 0.001$) higher levels of RLP cholesterol and RLP apoE (2.5- to 3-fold) than type IV patients. The ratio of RLP cholesterol to total plasma triglyceride was therefore significantly elevated in type III versus type IV patients (0.25 ± 0.03 vs. 0.09 ± 0.01 in mol/mol, 0.111 ± 0.012 vs. 0.038 ± 0.002 in mg/mg, $P < 0.001$). RLP triglyceride, apoC-III, and apoB concentrations were not significantly different in type III compared to type IV patients. If it is assumed that each remnant particle contained one molecule of apoB, type III and type IV patients had similar numbers of circulating RLP containing apoB. Western blot analysis revealed that virtually all RLP apoB was apoB-100 in normolipidemic subjects (**Table 3**). In type IV patients, the RLP apoB-48 to apoB-100 ratio was 0.11 ± 0.08 (range: 0 to 0.41). Considerably more apoB-48 was detected in the RLP fraction of type III patients (apoB-48 to apoB-100 ratio: 0.95 ± 0.31, n = 3; range: 0.39 to 1.46), demonstrating that as much as one-half of RLP apoB was apoB-48 in type III patients, although this varied from one patient to another. Less than 1% of total plasma apoA-I was recovered in the RLP fraction. Mean plasma concentrations of RLP apoA-I (determined by ELISA) were 0.4 ± 0.1, 0.6 ± 0.1 and 0.7 ± 0.1 mg/dl in normolipidemic, type III and type IV subjects, representing 0.34 ± 0.07%, 0.48 ± 0.04% and 0.58 ± 0.06% of total plasma apoA-I, respectively.

RLP composition

The lipid and apolipoprotein composition of RLP fractions from normolipidemic and hypertriglyceridemic subjects was assessed by expressing RLP lipids and apolipoproteins as molar ratios (mol/mol) relative to apoB (**Table 4**). For every molecule of apoB in the RLP fraction of normolipidemic subjects (which was >95% apoB-100), there was a similar number of both triglyceride and cholesterol molecules (3000 to 4000), and on average 33 molecules of apoC-III and 7 molecules of apoE. Compared to normolipidemic control subjects, type III patients had twice as many triglyceride and cholesterol molecules in

TABLE 2. Plasma RLP lipid and apolipoprotein concentrations of normolipidemic and hyperlipoproteinemic subjects

	Normolipidemic (n = 8)	Type III (n = 6)	Type IV (n = 9)	Significance of Mean Differences[a] Type III vs. IV
		mmol/l		
RLP triglyceride	0.23 ± 0.04	1.15 ± 0.16[d]	1.03 ± 0.18[d]	—
RLP cholesterol	0.21 ± 0.02	1.13 ± 0.10[d]	0.40 ± 0.05[c]	$P < 0.001$
		μmol/l		
RLP apoB	0.08 ± 0.01	0.17 ± 0.03[b]	0.15 ± 0.02[b]	—
RLP apoC-III	2.25 ± 0.29	6.89 ± 0.86[d]	8.01 ± 1.13[d]	—
RLP apoE	0.41 ± 0.05	1.63 ± 0.07[d]	0.60 ± 0.05[c]	$P < 0.001$

Values represent means ± SE.

[a] Significantly different by unpaired *t*-test, or by Mann-Whitney rank sum when groups were not normally distributed.

[b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$; significantly different from normolipidemic control group by unpaired *t*-test, or by Mann-Whitney rank sum when groups were not normally distributed.

TABLE 3. Ratio of apoB-48 to apoB-100 in the RLP fraction of normolipidemic and hyperlipoproteinemic subjects

	Normolipidemic (n = 4)	Type III (n = 3)	Type IV (n = 5)
ApoB-48/apoB-100	0.02 ± 0.01	0.95 ± 0.31	0.11 ± 0.08

Values represent means ± SE for a subgroup of normolipidemic, type III, and type IV subjects. Amount of apoB-48 relative to apoB-100 was determined by densitometric scanning of films after chemiluminescent immunodetection of electrophoretically separated apolipoproteins (see Methods).

RLP for every molecule of apoB (50% of apoB molecules on average being apoB-48). Their RLP fractions were also enriched in apoC-III and apoE (though this did not reach statistical significance). RLP fractions from type IV patients were enriched in triglyceride and apoC-III compared to controls, but not in cholesterol or apoE. Cholesterol to apoB and apoE to apoB ratios were thus significantly higher ($P < 0.01$) in type III compared to type IV subjects, and the RLP fraction of type IV subjects was significantly enriched (2- to 3-fold) in apoC-III relative to apoE.

RLP subfractions

The lipoprotein composition of RLP fractions from normolipidemic and hypertriglyceridemic subjects was further investigated by separating RLP on the basis of their size by automated gel filtration chromatography. The elution profile of lipoproteins in the RLP fraction, as detected by the presence of cholesterol, apoB, apoE, and apoC-III, is shown for a normolipidemic, type III and type IV subject in **Fig. 1**. The majority of lipoproteins in the RLP fraction of normolipidemic subjects had a size similar to LDL or HDL. Lipoproteins in the RLP fraction of hypertriglyceridemic patients, however, tended on average to be larger, and had a size similar to TRL or were intermediate in size between TRL and LDL. In order to compare the size distribution of lipoproteins in RLP fractions from different subjects in quantitative terms, material in FPLC elution tubes were pooled to give three major fractions: the first containing TRL (tubes 6 to 16), the second containing lipoproteins intermediate in size between TRL and LDL or similar in size to LDL, designated the intermediate-sized lipoprotein (ISL) fraction (tubes 17 to 30), and the third containing lipoproteins similar in size to HDL (tubes 31 to 50) (29). The relative and absolute amounts of cholesterol and apolipoproteins in these three fractions were determined for each subject. Mean results are shown in Table 4. In normolipidemic control subjects, the majority of RLP cholesterol and apoB (80% and 77%, respectively) was found to be associated with LDL-sized lipoproteins in the ISL fraction. Sixteen percent of total RLP cholesterol was on average found in association with HDL-sized particles. The distribution of apoE between different-sized lipoproteins in the RLP fraction was, however, different from that of cholesterol and apoB. Only 21% of total RLP apoE was found in ISL, and the majority of RLP apoE (66%) was associated with HDL-sized lipoproteins, which tended to have a size distribution larger than the cholesterol-containing HDL-sized particles in RLP (top panel, Fig. 1). A significant proportion of RLP apoC-III (33%) was isolated in TRL-sized particles, and 45% was found associated with particles in the HDL fraction. Two-dimensional gel electrophoretic separation of HDL-sized lipoproteins in normolipidemic plasma and in RLP derived from the same sample demonstrated that HDL-sized apoE-containing lipoproteins in the RLP fraction were not associated with apoA-I. As shown in **Fig. 2**, plasma apoE-containing lipoproteins in the HDL size range had a particle diameter ranging from 9 to 18.5 nm (left-hand side of top panel), whereas apoA-I-containing lipoproteins were smaller, the majority having an hydrated density less than 10 nm (left-hand side of bottom panel). Immunoaffinity separation of RLP from normolipidemic plasma resulted in 30–50% of total plasma HDL apoE to be detected in the unbound RLP fraction (as assessed by densitometric scanning). This is consistent with FPLC data showing that the concentration of apoE in the HDL-sized RLP fraction was 0.27 ± 0.04 μmol/l, which is about 40% of the concentration of apoE in plasma HDL of normolipidemic subjects (0.67 ± 0.09 mmol/l, 2.3 ± 0.3 mg/dl, n = 10 (29)). These lipoproteins escaped capture by the immunoaffinity gel probably because they contained no apoA-I or apoB. They have previously been shown by immunoblotting analysis to be devoid of apoB (26). Trace amounts of apoA-I not bound by the RLP affinity gel (shown as a faint region of apoA-I reactivity on the right-hand side of the membrane in the bottom panel of Fig. 2), did not co-migrate (and were thus not associated) with apoE in HDL-sized particles.

TABLE 4. Molar ratio (mol/mol) between lipids and apolipoproteins in the RLP fraction of normolipidemic and hyperlipoproteinemic subjects

	Normolipidemic (n = 8)	Type III (n = 6)	Type IV (n = 9)	Significance of Mean Differences[a] Type III vs. IV
RLP triglyceride/apoB	3429 ± 889	7305 ± 847[b]	7135 ± 722[c]	—
RLP cholesterol/apoB	3306 ± 717	7633 ± 1480[b]	2878 ± 286	$P < 0.01$
RLP apoC-III/apoB	32.7 ± 6.3	45.6 ± 8.6	57.9 ± 6.7[b]	—
RLP apoE/apoB	6.9 ± 2.0	11.4 ± 2.1	4.7 ± 0.7	$P < 0.01$
RLP apoC-III/apoE	5.9 ± 0.9	4.2 ± 0.4	13.2 ± 1.3[c]	$P < 0.01$

Values represent means ± SE.
[a]Significantly different by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.
[b]$P < 0.05$; [c]$P < 0.01$; significantly different from normolipidemic control group by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.

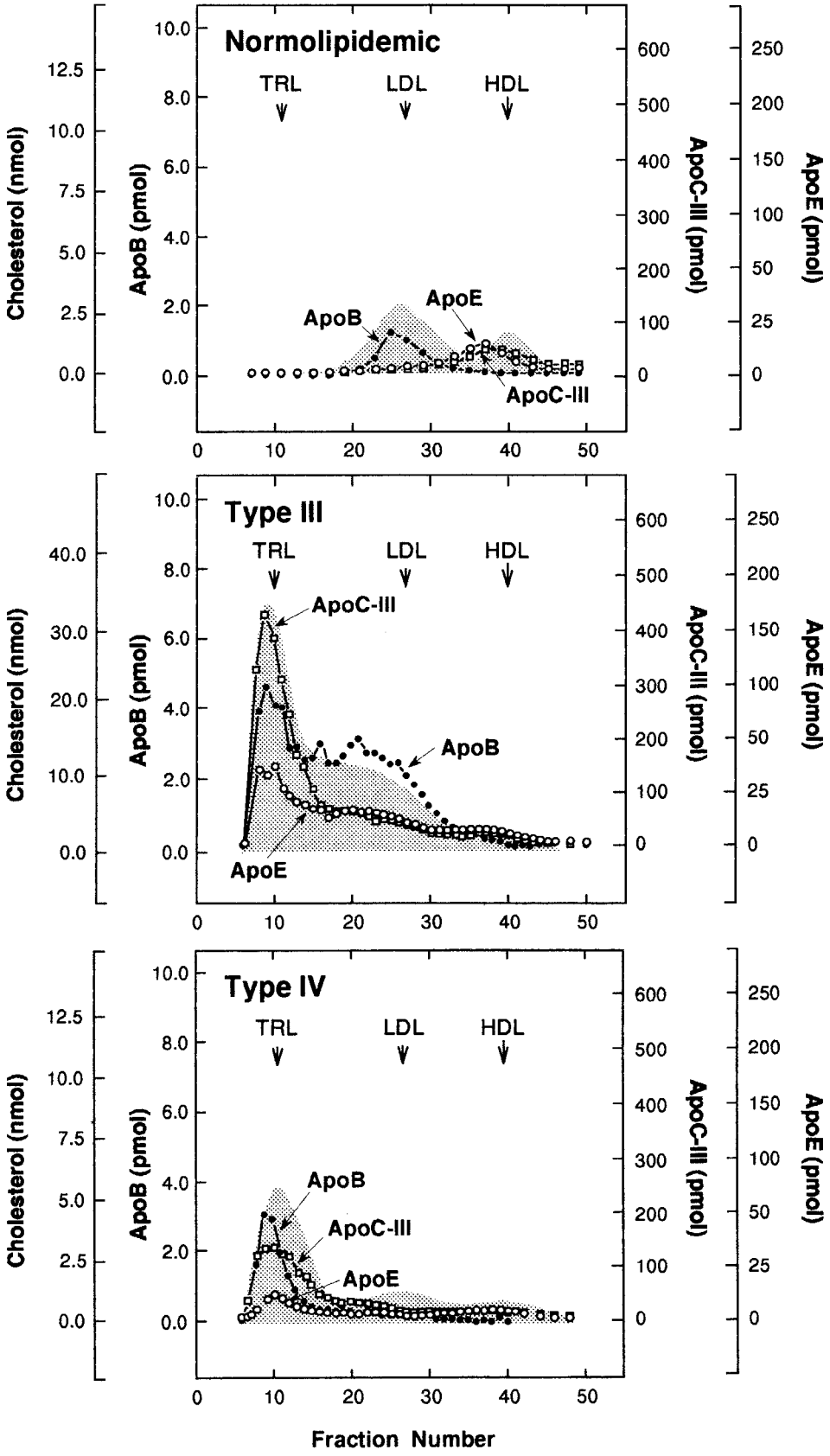

Fig. 1. Separation of lipoproteins in the RLP fraction according to size by automated gel filtration chromatography on an FPLC system. Results are shown for RLP from the plasma of a normolipidemic subject (top panel), a type III (middle panel), and a type IV patient (bottom panel). The elution profile of cholesterol is indicated by the shaded areas, and the elution of lipoproteins corresponding to the size of VLDL, LDL, and HDL is arrowed in each panel. The amount of apoB and apoE in each elution fraction is indicated by the closed and open circles, respectively, while apoC-III is indicated by the open squares. Plasma concentration of RLP cholesterol for the three subjects was 9.1, 56.6, and 13.2 mg/dl, respectively. The scale of the y-axis is the same in all cases, except for cholesterol in the middle panel.

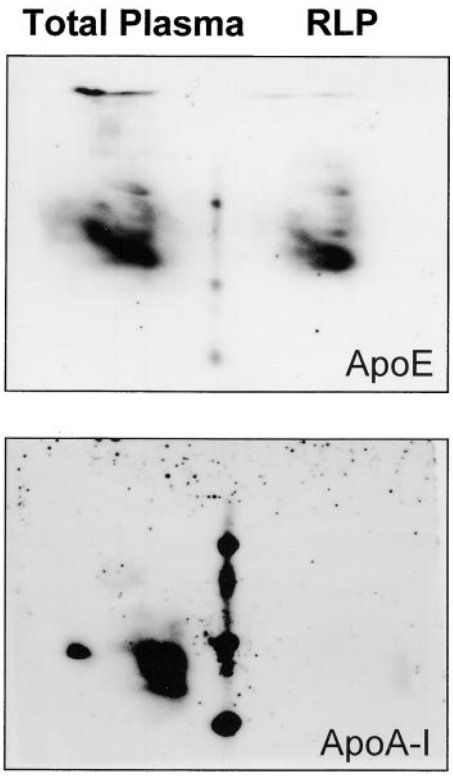

Fig. 2. Two-dimensional gradient gel electrophoretic separation of HDL-sized lipoproteins in total plasma and in the RLP fraction. Total plasma (30 μl) and RLP isolated from 30 μl of the same plasma sample were separated in the first dimension according to charge (from left to right) by agarose gel electrophoresis, and then according to size in the second dimension (top to bottom) by polyacrylamide gradient (3–24%) gel electrophoresis. ApoE and apoA-I were detected with ^{125}I-labeled human anti-apoE and anti-apoA-I antibodies (top and bottom panels, respectively) after transfer of proteins to a nitrocellulose membrane. ^{125}I-labeled molecular weight standards are shown in the middle lane and the three bands in the top panel represent proteins with hydrated diameters corresponding to 17.0, 9.5, and 7.1 nm (from top to bottom); in the bottom panel, five standards are visible corresponding to hydrated diameters of 17.0, 12.2, 9.5, 8.2, and 7.1 nm.

Elevated levels of RLP cholesterol in type III subjects were associated with a significant increase in the presence of RLP having the size of TRL and ISL, as reflected by significantly higher levels of cholesterol, apoB, apoC-III, and apoE in the TRL fraction and of cholesterol, apoC-III, and apoE in the ISL fraction of RLP (**Table 5**). Concentrations of cholesterol, apoC-III, and apoE were not different in HDL-sized RLP of type III patients compared to controls. Similarly, in type IV patients, a significant increase in the presence of TRL- and ISL-sized RLP was reflected by significantly increased levels of TRL cholesterol, apoB, apoC-III, and apoE and of ISL apoE and apoC-III. Concentration of cholesterol in ISL of RLP from type IV patients was not increased, and was in fact 25% lower than that of controls. The concentration of cholesterol, apoC-III, and apoE in HDL-sized lipoproteins of RLP was not different in type IV patients compared to controls. The 3-fold mean elevation in RLP cholesterol concentration in type III compared to type IV patients was due to a 3-fold increase in cholesterol in both TRL- and ISL-sized particles. Similarly, apoE levels in TRL and ISL of RLP were significantly ($P < 0.001$) higher in type III compared to type IV patients. There was no difference, however, in the number of particles in the TRL and ISL fractions of RLP of type III versus type IV patients (as reflected by similar TRL and ISL apoB levels), nor a difference in apoC-III levels in these two fractions. HDL-sized RLP of type III contained similar amounts of cholesterol, but higher amounts of apoE and lower amounts of apoC-III, compared to type IV patients.

RLP subfraction composition

In order to compare the composition of different-sized lipoproteins in the RLP fraction, molar ratios between different parameters in TRL, ISL, and HDL were calculated for both normolipidemic and hypertriglyceridemic subjects. ApoB was taken as the reference protein for TRL and ISL, and apoA-I (total plasma RLP apoA-I) was used as the reference protein for HDL. Mean results are shown in **Table 6**. Significantly increased levels of TRL-sized RLP in type III patients relative to controls (Table 5) were associated with a significant increase in the cholesterol/apoB ratio and a decrease in the apoC-III/apoE ratio in these particles. In comparison, increased levels of TRL-sized RLP in type IV patients were associated with a significant though smaller increase in the cholesterol/apoB ratio and no significant difference in the apoC-III/apoE ratio in these particles relative to controls. The number of ISL-sized particles in the RLP fraction of the three groups (reflected by RLP ISL apoB concentrations, Table 5) was not significantly different; however, there were differences in the lipid and apolipoprotein composition of these particles. ISL-sized RLP of type III patients were enriched in cholesterol and apoE, and they had a decreased apoC-III/apoE ratio compared to type IV patients. ISL-sized RLP of both type III and type IV patients had significantly higher apoC-III/apoB ratios than normolipidemic subjects. Apolipoprotein ratios in HDL-sized fractions are also presented in Table 6. As not all particles in the HDL-sized fraction necessarily contained apoA-I (Fig. 2), these data have been presented for comparative purposes only and they do not reflect the composition of individual particles. Relative to the control group, the HDL-sized RLP fraction of type III patients had less apoE and that of type IV patients had less apoC-III, resulting in type III patients having a significantly reduced RLP-HDL apoC-III/apoE ratio compared to type IV patients ($P < 0.001$).

RLP compared to lipoproteins of similar size in plasma

The question was posed, to what extent did the apolipoprotein composition of different-sized lipoproteins isolated in the RLP fraction differ from similar-sized lipoproteins in total plasma? In the present study, we did not isolate TRL, ISL, and HDL fractions by FPLC from total plasma, so this comparison could not be made directly in the same samples. These parameters have, however, been measured previously in our laboratory in other groups of normolipidemic, type III, and type IV individuals (9, 24).

TABLE 5. Relative and absolute cholesterol and apolipoprotein concentrations of different-sized lipoproteins in the RLP fraction of normolipidemic and hyperlipoproteinemic subjects

	Normolipidemic (n = 8)	Type III (n = 6)	Type IV (n = 9)	Significance of Mean Differences[a] Type III vs. IV
Cholesterol				
TRL	0.01 ± 0.01	0.69 ± 0.08[d]	0.23 ± 0.05[d]	$P < 0.001$
	(4.3 ± 2.6%)	(61.1 ± 3.6%)	(52.4 ± 5.5%)	
ISL	0.17 ± 0.02	0.37 ± 0.06[b]	0.13 ± 0.01[b]	$P < 0.001$
	(80.3 ± 4.2%)	(32.1 ± 3.5%)	(36.0 ± 4.5%)	
HDL	0.03 ± 0.01	0.07 ± 0.02	0.046 ± 0.01	—
	(15.5 ± 4.4%)	(6.8 ± 2.1%)	(11.3 ± 2.6%)	
ApoB				
TRL	0.02 ± 0.01	0.09 ± 0.02[c]	0.08 ± 0.02[c]	—
	(19.6 ± 4.4%)	(49.3 ± 2.2%)	(54.6 ± 5.4%)	
ISL	0.06 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	—
	(76.6 ± 4.4%)	(47.7 ± 2.5%)	(43.0 ± 5.0%)	
HDL	0.003 ± 0.001	0.007 ± 0.005	0.003 ± 0.001	—
	(3.7 ± 1.4%)	(3.0 ± 2.0%)	(2.4 ± 0.6%)	
ApoC-III				
TRL	0.82 ± 0.19	3.76 ± 0.78[d]	5.07 ± 0.93[d]	—
	(32.8 ± 4.9%)	(52.0 ± 5.5%)	(60.9 ± 3.0%)	
ISL	0.58 ± 0.11	2.52 ± 0.23[d]	1.81 ± 0.23[d]	$P < 0.05$
	(24.9 ± 3.1%)	(40.0 ± 6.5%)	(23.1 ± 1.3%)	
HDL	0.88 ± 0.11	0.60 ± 0.16	1.12 ± 0.12	$P < 0.05$
	(44.6 ± 7.8%)	(8.1 ± 1.4%)	(15.8 ± 2.2%)	
ApoE				
TRL	0.05 ± 0.01	0.68 ± 0.07[d]	0.26 ± 0.4[d]	$P < 0.001$
	(13.2 ± 2.1%)	(41.5 ± 3.0%)	(40.9 ± 4.2%)	
ISL	0.08 ± 0.01	0.61 ± 0.03[d]	0.13 ± 0.01[b]	$P < 0.001$
	(20.8 ± 1.6%)	(38.0 ± 3.1%)	(21.9 ± 1.6%)	
HDL	0.27 ± 0.04	0.34 ± 0.06	0.21 ± 0.02	$P < 0.05$
	(65.8 ± 3.2%)	(20.4 ± 3.1%)	(37.2 ± 3.8%)	

Values represent means ± SE. Absolute RLP lipid and apolipoprotein concentrations are expressed in mmol/l and μmol/l, respectively; concentrations are also expressed in percentage term (in parentheses) relative to total.

[a] Significantly different by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.

[b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$; significantly different from normolipidemic control group by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.

These published results are summarized in **Table 7**. Comparing results in Tables 6 and 7, TRL-sized RLP of both normolipidemic and type IV subjects tended to have 2- to 3-fold higher apoC-III/apoB and apoE/apoB ratios than lipoproteins of similar size in total plasma. ISL-sized RLP of both normolipidemic and type IV subjects tended to have 3- to 6- fold higher apoC-III/apoB and apoE/apoB ratios. ApoC-III/apoE ratios of TRL and ISL in RLP were very similar to apoC-III/apoE ratios in TRL and ISL of total plasma, indicating that irrespective of the type of subject, RLP were enriched to the same extent in apoC-III as apoE, compared with lipoproteins of similar size in total plasma.

DISCUSSION

Although the potential atherogenicity and thrombogenicity of RLP have been investigated in a number of studies (10–23), acceptance of this methodology for measuring the plasma concentration of remnant lipoproteins, and for using this measurement to predict the presence of coronary disease (18, 19), depends upon a better understanding of the type of lipoprotein particles isolated in this remnant lipoprotein fraction. The objective of the present study was therefore to characterize the lipoprotein composition of RLP isolated from the plasma of normolipidemic subjects, and to compare these results with those obtained from hypertriglyceridemic subjects, who did or did not have significant plasma remnant lipoprotein accumulation.

Our results demonstrate that RLP are a heterogeneous group of plasma lipoprotein particles, which vary in both size and composition in a given individual, as well as in normolipidemic compared to hypertriglyceridemic subjects. It has previously been documented that patients with type III hyperlipoproteinemia have elevated levels of plasma RLP cholesterol that are significantly higher than those of patients with other forms of hyperlipidemia (12). This is consistent with results of the present study, whereby type III hyperlipoproteinemic patients had plasma RLP cholesterol levels that were 3-fold higher than those of type IV patients (Table 2), despite similar levels of total plasma triglyceride (Table 1). They therefore had significantly elevated RLP cholesterol to total plasma triglyceride ratios. In addition, type III patients had significantly higher levels of RLP apoE, but not apoB or apoC-III, indicating that RLP of type III patients were enriched in cholesterol and apoE compared to those of type IV patients. Enrichment of remnant lipoproteins with apoE in type III patients is consistent with the well-accepted concept that impaired binding of apoE2 to hepatic receptors (33, 34)

TABLE 6. Ratio (mol/mol) between apolipoproteins of different-sized lipoproteins in the RLP fraction of normolipidemic and hyperlipoproteinemic subjects

	Normolipidemic (n = 8)	Type III (n = 6)	Type IV (n = 9)	Significance of Mean Differences[a] Type III vs. IV
TRL				
RLP cholesterol/apoB	418 ± 199	9801 ± 2472[d]	2920 ± 213[d]	$P < 0.01$
RLP apoC-III/apoB	82.2 ± 26.8	45.8 ± 6.3	70.3 ± 10.7	$P < 0.05$
RLP apoE/apoB	6.6 ± 2.7	9.1 ± 1.3	4.3 ± 0.9	$P < 0.05$
RLP apoC-III/apoE	17.4 ± 4.8	5.2 ± 0.6[b]	21.3 ± 3.6	$P < 0.01$
ISL				
RLP cholesterol/apoB	3251 ± 489	5055 ± 869	2674 ± 566	$P < 0.05$
RLP apoC-III/apoB	12.1 ± 3.7	39.6 ± 11.4[d]	35.5 ± 8.6[c]	–
RLP apoE/apoB	1.7 ± 0.4	9.1 ± 1.8[c]	2.5 ± 0.3	$P < 0.01$
RLP apoC-III/apoE	6.9 ± 1.0	4.1 ± 0.3[b]	14.8 ± 2.1[b]	$P < 0.01$
HDL				
RLP apoC-III/apoA-I	7.1 ± 1.9	3.0 ± 0.9	4.8 ± 0.6	—
RLP apoE/apoA-I	2.1 ± 0.2	1.6 ± 0.3	0.9 ± 0.1[d]	$P < 0.05$
RLP apoC-III/apoE	3.9 ± 0.8	2.0 ± 0.6	5.2 ± 0.3[b]	$P < 0.001$

Values represent means ± SE.

[a] Significantly different by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.

[b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$; significantly different from normolipidemic control group by unpaired *t*-test or by Mann-Whitney rank sum when groups were not normally distributed.

and incomplete conversion of apoE2-containing TRL to LDL (35) are primary reasons for reduced plasma clearance (36) and subsequent accumulation of remnant lipoproteins in these individuals.

In contrast, RLP of type IV patients were found to be enriched in triglyceride and apoC-III relative to normolipidemic subjects (Table 4). Smaller RLP particles isolated in the ISL fraction of RLP from type IV patients, rather than larger TRL-sized RLP, were found to be enriched in apoC-III relative to apoB (Table 6). The RLP apoC-III/apoE ratio was subsequently 3-fold higher in type IV compared to type III patients, due to a significantly higher apoC-III/apoE ratio in both TRL- and ISL-sized particles. Fundamental differences therefore exist in the apolipoprotein composition of RLP isolated from patients with different forms of hyperlipoproteinemia. Smaller RLP of type IV patients tend to be enriched in apoC-III relative to apoB, while in type III patients they are enriched in apoE as well as apoC-III (Table 6). These results support recent work from our laboratory, which has shown that remnant lipoproteins isolated by gel filtration chromatography (i.e., as ISL) from total plasma of type IV patients are enriched in apoC-III (i.e., ISL apoC-III to apoE ratios are significantly higher in type IV compared to normolipidemic or type III subjects (30)). Increased amounts of apoC-III on remnant lipoproteins could be a cause or a consequence of plasma remnant lipoprotein accumulation, although results of recent studies with mice overexpressing apoC-III suggest that apoC-III is a causative factor (37–39). This is consistent with the concept that apoC-III can inhibit the recognition and uptake of TRL by the liver (40, 41), and has the potential to inhibit both lipoprotein and hepatic lipase (42–45).

Previous studies have indicated that remnant lipoproteins are enriched in apoE and depleted in C apolipoproteins (4, 46). When remnant-like VLDL were isolated from plasma as lipoproteins that did not bind to the monoclonal antibody JI-H (with a density less than 1.006 g/ml), they were similarly reported to be enriched in apoE and depleted in apoC (24). We have found that both TRL- and ISL-sized RLP of normolipidemic and type IV subjects had significantly higher apoE to apoB ratios than lipoproteins of similar size in total plasma. However, TRL- and ISL-sized RLP also had significantly higher apoC-III to apoB ratios than lipoproteins of similar size in total plasma, indicating that they were enriched and not depleted in apoC-III. RLP were enriched in apoC-III and apoE to a similar extent in different-sized particles and to a similar extent in individuals with different lipid phenotypes (i.e., the apoC-III to apoE ratio was similar in RLP and in lipoproteins of similar size in total plasma, Tables 6 and 7). These data suggest that an increase in the amount of apoC-III relative to apoB, in addition to an increase in apoE relative to apoB, is a characteristic feature of particles that are not recognized by the JI-H monoclonal antibody. This points out the importance of apoC-III in defining the

TABLE 7. Ratio (mol/mol) between apoB, apoE, and apoC-III in different-sized lipoproteins isolated from the plasma of normolipidemic and hyperlipoproteinemic subjects (data derived from refs. 9 and 30)

	Normolipidemic	Type III	Type IV
TRL			
ApoC-III/apoB	38.32 ± 4.63	na	41.56 ± 4.41
ApoE/apoB	2.29 ± 0.22	na	1.94 ± 0.17
ApoC-III/apoE	17.28 ± 2.10	6.21 ± 0.58	21.28 ± 1.11
ISL			
ApoC-III/apoB	3.04 ± 0.55	na	6.44 ± 0.83
ApoE/apoB	0.30 ± 0.03	na	0.44 ± 0.03
ApoC-III/apoE	9.83 ± 0.86	4.78 ± 0.58	14.77 ± 1.87

Values represent means ± SE for normolipidemic (n = 10), type III (n = 8), and type IV (n = 10) subjects; na, data not available.

characteristics of remnant lipoproteins which accumulate in human plasma, and raises the possibility that apoC-III, or other C apolipoprotein, might play a role in the lack of recognition of remnant particles by the JI-H antibody. The exact reason why the JI-H antibody recognizes VLDL and LDL but not partially degraded VLDL remnants has not yet been established, although it has been suggested that apoE can compete for binding of JI-H to its specific epitope on apoB (8).

Analysis of the size distribution of lipoproteins in the RLP fraction revealed that the majority of RLP in normolipidemic subjects had a size similar to LDL or HDL. The majority of RLP in hyperlipoproteinemic patients were however larger and were similar in size to TRL, or were intermediate in size between TRL and LDL (Fig. 1). This result is in agreement with cholesterol elution profiles of lipoproteins in RLP separated by HPLC (7, 8, 12) and supports the concept that the majority of RLP isolated from the plasma of hypertriglyceridemic subjects have a size compatible with them being products of TRL hydrolysis and catabolism. Whether all particles in the RLP fraction of normolipidemic subjects can similarly be considered to be products of TRL hydrolysis must however be carefully assessed. One possibility is that these lipoproteins simply represent trace amounts of normal LDL or HDL that escape capture by antibodies in the immunoaffinity gel. As far as the LDL-sized particles are concerned, there are two pieces of evidence which suggest that this is not the case. First, comparison of data in Tables 6 and 7 shows that RLP isolated in the ISL fraction of normolipidemic subjects were enriched 3- to 6-fold with both apoC-III and apoE relative to lipoproteins in the ISL fraction of total plasma. Thus, intermediate- and LDL-sized RLP contained increased amounts of apoC-III and apoE and did not have the characteristics of apoB-only-containing LDL. Second, in a previous study (9), we found that subjects with normal plasma triglyceride levels (<200 mg/dl), but significantly different LDL cholesterol levels (i.e., normolipidemic and type IIa hyperlipoproteinemic subjects with LDL-cholesterol levels of 115 ± 9 vs. 180 ± 3 mg/dl, respectively), had similar RLP cholesterol (0.22 ± 0.01 vs. 0.21 ± 0.01 mmol/l) and similar RLP apoB levels (0.06 ± 0.01 vs. 0.06 ± 0.01 μmol/l). Subjects with elevated plasma triglyceride levels (>200 mg/dl) but significantly different LDL cholesterol levels (i.e., type IV and type IIb hyperlipoproteinemic subjects; LDL cholesterol: 116 ± 8 vs. 181 ± 8 mg/dl, respectively), also had similar RLP cholesterol (0.58 ± 0.11 vs. 0.50 ± 0.07 mmol/l) and RLP apoB levels (0.15 ± 0.02 vs. 0.15 ± 0.03 μmol/l). Increased levels of plasma LDL therefore do not result in more LDL-sized particles to be recovered in the RLP fraction. These data together support the conclusion that intermediate- or LDL-sized lipoproteins in the RLP fraction of normolipidemic subjects (representing around 80% of total plasma RLP cholesterol) do not simply represent contaminating amounts of LDL.

As far as the HDL-sized RLP are concerned, we have previously proposed that these particles represent apoE-containing HDL-sized lipoproteins that do not contain apoB or apoA-I (9). The presence in plasma of apoE-containing HDL devoid of apoA-I has been described by a number of authors (26, 47–49). Direct evidence for the presence of these lipoproteins in the RLP fraction of normolipidemic subjects has been provided in the present study by results of two-dimensional gel electrophoretic analysis of plasma RLP fractions (Fig. 2), which demonstrated that ⅓ to ½ of total plasma HDL apoE was on average isolated in the RLP fraction of normolipidemic subjects. These particles were responsible for transporting about ⅔ of apoE isolated in the RLP fraction of normolipidemic subjects and about ⅓ in hyperlipoproteinemic subjects (Table 5). They may represent lipoproteins which are formed as excess surface material is released from TRL during lipolysis (50), and may serve as a reservoir of apolipoproteins (apoE, as well as C apolipoproteins), which transfer to TRL during the early stages of lipolysis (51). An additional explanation is that a significant proportion of this material represents "HDL-sized" complexes of apoE bound to particular plasma proteins. For example, we have recently documented the presence in human plasma of apoE bound to α_2-macroglobulin (α_2M) (52), which is a complex having a hydrated density of 18.5 nm. This apoE/α_2M complex is present in the RLP fraction, as evidenced by the dark spot of apoE migrating above the first 17.0 nm molecular standard in Fig. 2. It is inappropriate to describe these latter particles as being "remnant-like," as they are essentially lipid-free protein complexes. They make little contribution, however, to the total amount of cholesterol in RLP. As far as apoA-I-containing HDL are concerned, we have determined that these HDL, which escaped capture by the immunoaffinity gel, represented less than 1% of total plasma apoA-I, contributing less than 1% of plasma HDL cholesterol to the RLP fraction. This would have been equivalent to 12.40, 8.79, and 8.01 μmol/l of cholesterol for the normolipidemic, type III, and type IV subjects, respectively, representing 5.9%, 0.8%, and 2.0% of total RLP cholesterol. Together, HDL-sized particles were found to be responsible for 15%, or in the case of hypertriglyceridemic subjects, 5–10% of total cholesterol in the RLP fraction (Table 5). If from a clinical perspective, it were considered important to quantitate RLP in the absence of these HDL-sized lipoproteins, measurement of RLP apoB rather than RLP cholesterol would be an appropriate alternative.

In conclusion, the present study has demonstrated that the immunoaffinity-isolated RLP fraction is composed of a heterogeneous group of plasma lipoproteins varying in both size and composition. This heterogeneity is apparent in individual subjects, and is particularly evident when comparing normolipidemic and hypertriglyceridemic subjects. Thus, the apolipoprotein composition of similar-sized RLP can vary, depending on a patient's form of hypertriglyceridemia, e.g., compared to normolipidemic subjects, intermediate-sized RLP in type III patients are enriched in apoE relative to apoC-III, whereas in type IV patients they are enriched in apoC-III relative to apoE. It remains to be determined to what extent these size and compositional differences are of relevance to the pathogenesis of atherosclerosis.

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