Sources and properties of triglyceride-rich lipoproteins containing apoB-48 and apoB-100 in postprandial blood plasma of patients with primary combined hyperlipidemia

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Abstract **Although editing of apolipoprotein (apo)B in the small intestine, yielding apoB-48, is thought to be nearly complete in adult humans, small amounts of intestinal apoB-100 may also be produced. We have evaluated the fraction of unedited apoB secreted from the intestine postprandially in subjects with primary combined hyperlipidemia, a disorder in which secretion of apoB-100 into the blood is increased. Three hours after these subjects and healthy controls were fed a fat-rich meal containing retinol, the distribution of retinyl esters (RE) between plasma triglyceride-rich lipoprotein (TRL) fractions containing apoB-100 and apoB-48 was measured under conditions minimizing transfer of RE between lipoprotein particles. The estimated maximal percentage of unedited intestinal apoB-100 (~3%) was not increased in subjects with primary combined hyperlipidemia, suggesting that reduced editing of intestinal mRNA does not contribute to the pathogenesis of this disorder. Postprandially, the triglyceride content of TRL containing apoB-48 more than doubled, leading to a 20% increase in mean diameter, yet the surface concentration of phospholipids and soluble apolipoproteins (apoE and total apoC) was unchanged. Furthermore, the surface concentrations of these components did not differ among TRL containing apoB-48 and two smaller fractions of apoB-100 TRL with distinct immunoreactivities. These findings suggest that available surface area is a major determinant of the particle content of each of these surface components of TRL species of differing size and origin.**—Kovar, J., and R. J. Havel. **Sources and properties of triglyceride-rich lipoproteins containing apoB-48 and apoB-100 in postprandial blood plasma of patients with primary combined hyperlipidemia.** ***J. Lipid Res.*** **2002.** 43: **1026–1034.**

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The alimentary lipemic response in humans involves triglyceride-rich lipoproteins (TRL) containing apolipoprotein (apo)B-100 as well as apoB-48. The alimentary response of TRL-triglyceride (TG) mainly reflects the increased size of apoB-48 containing TRLs, but TRLs containing apoB-100 contribute most of the increase in the number of TRL particles (1). In humans, as in most mammals, apoB-100 is thought to be the sole or predominant form of apoB secreted by the liver, whereas apoB-48 is universally considered the sole or predominant form of apoB secreted by the intestine (2). The editing of apoB mRNA that yields apoB-48 is developmentally regulated in the human intestine, such that the edited fraction increases during gestation (3). Liver transplantation data indicate that little or no apoB-48 is secreted from the human liver and also that large postprandial TRL containing apoB-100 are of hepatogenous, not intestinal, origin (4). Several studies, however, have found editing of intestinal apoB mRNA to be incomplete in adults, in the range of 80–95% (5–7). In one of these (7), the fraction edited was unchanged 3 h after healthy adults ingested a fat-rich meal. Immunocytochemical studies have usually (8–10), but not always (4), found evidence of epitopes specific for apoB-100 in the small intestine. By immunogold cytochemistry at the electron microscopic level, apoB-100-specific epitopes have been localized to Golgi cisternae of intestinal epithelial cells, consistent with local synthesis of apoB-100 (8, 9). ApoB-100 accounted for about 5% of newly synthesized apoB secreted from intestinal organ cultures (6), and a similar percentage of the mass of apoB extracted from freshly isolated small intestinal epithelium (A. Tanaka, personal communication). Quantitative in

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Abbreviations: apo, apolipoprotein; CE, cholesteryl ester(s); FC, free cholesterol; PL, phospholipid(s); RE, retinyl ester(s); TC, total cholesterol; TG, triglyceride(s); TRL, triglyceride rich lipoprotein(s).

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vivo data on the fraction of human TRL particles secreted from the intestine that contain apoB-100 are not available.

We have used dietary retinol as a marker for postprandial intestinal TRL to evaluate the magnitude of apoB-100 secretion from the human intestine. We chose to study patients with primary combined hyperlipidemia because the total rate of secretion of apoB-100 into the blood is thought to be increased in this disorder (2). We tested the hypothesis that reduced editing of intestinal mRNA contributes to this increased rate of apoB-100 secretion by comparing the fraction of retinyl esters (RE) found in TRL containing apoB-100 in patients with combined hyperlipidemia with that found in healthy, normolipidemic subjects. Because our experimental design required separation of TRL containing apoB-48 from those containing apoB-100, we were also able to obtain novel information about the composition of these two components of TRL during postprandial as well as postabsorptive conditions.

METHODS

Subjects

Seven patients with combined hyperlipidemia, five men and two women, from the Lipid Clinic of the University of California, San Francisco were studied. The diagnosis was based on plasma TG and cholesterol concentrations above the 95th percentile of corresponding age- and sex-standardized population values (11). In addition to disorders causing secondary hyperlipoproteinemia, exclusion criteria included an apoE-2/2 phenotype and use of lipid-lowering drugs within 6 weeks.

Two normolipidemic, healthy men were studied, one of them on two occasions. A third normolipidemic man was studied in a separate experiment to evaluate the extent of RE transfer in vitro.

Protocol

On the morning of study, 25 ml of venous blood was drawn from the subjects who had fasted for about 12 h. They then ingested a breakfast consisting of a milkshake containing skimmed milk, whipping cream, sucrose, and cocoa mix powder (1.5 g of fat per kg body weight) (12) and grape juice containing 2,000 IU of vitamin A (Aquasol A, Armour Pharmaceutical, Kanakee IL) per kg body weight. After 3 h, 45 ml of venous blood was drawn. To determine the rate of transfer of RE in vitro, the same breakfast, without vitamin A, was provided.

Blood samples were placed into chilled tubes containing 5 μl of 10% EDTA, pH 7.4, and 1 μl of benzamidine (0.3 g/ml) per ml and kept on ice. Plasma was separated by centrifugation (2,000 *g*, 10 min, 4°C).

Protocols for blood drawing and use were approved by the Committee on Human Research of the University of California, San Francisco.

Preparation of immunoaffinity columns

Two monoclonal antibodies that recognize epitopes specific to apoB-100 were used to separate apoB-48-rich TRL from two immunochemically distinct fractions of apoB-100 TRL. The epitope of monoclonal antibody JI-H is located in the region of residues 2291–2318, just distal to the C-terminus of apoB-48 (13), and that of monoclonal antibody 4G3 is located between residues 2,980 and 3,084 (14). JI-H monoclonal antibody purified from ascites fluid (Japan Immunoresearch Laboratories, Takasaki City, Japan) was coupled to CNBr-activated Sepharose 4B (Pharmacia), 20 mg/g of gel, according to the recommendations of the manufacturer. Before use, the columns were conditioned with normal human plasma. Their capacity, determined by loading increasing amounts of human LDL, was >1.25 mg of apoB-100/ml of gel. 4G3 monoclonal antibody purified from ascites fluid (Ottawa Heart Institute, Ottawa, Canada) was coupled to CNBr-activated Sepharose 4B, 11 mg/g of gel, under the same conditions. The gel was preconditioned in the same way and its capacity determined to be >1.2 mg of apoB-100/ml of gel.

Separation of TRL fractions

Fresh whole plasma was subjected to immunoaffinity chromatography on JI-H columns. The unretained fraction was then separated on columns of 4G3 (**Fig. 1**). For each experiment, 2 ml of plasma were loaded onto each of six JI-H immunoaffinity columns (0.7 × 8 cm). The unbound fraction was eluted with 8 ml of 150 mM NaCl containing 1.3 mM EDTA, pH 7.4, and benzamidine (0.3 g/l) (saline/E/B) at a flow rate of 5 ml/h. The columns were then washed with another 20 ml at a flow rate 12 ml/h, following which the bound fraction was eluted with 20 ml of 3 M NaSCN containing BSA (2 g/l) and benzamidine (0.3 g/l) (NASCN/B). Columns were routinely washed with another 10 ml of NaSCN buffer followed by 50 ml of saline/E/B.

Two approximately 12 ml portions of freshly obtained JI-H-unbound fraction were loaded on two 4G3 columns (6 × 1.5 cm). The columns were then tightly closed and placed on a rocking table (Hema-Tek, Miles, Inc.) for 1 h. The columns were then returned to the vertical position and eluted with saline/E/B at flow rate of 15 ml/h. After approximately 40 ml of unbound fraction had been collected from each column, the columns were washed with 20 ml of saline/E/B. The bound fraction was then eluted with 20 ml NaSCN/B at flow rate 15 ml/h and the columns were washed as above.

The unbound fraction from 4G3 columns was concentrated in a micro protein dialysis/concentrator (Biomolecular Dynamics), equipped with a 50,000 molecular weight cut-off membrane, against saline/E/B overnight. The bound fractions from the JI-H and 4G3 columns were dialyzed thrice against 4 liters of saline/E/B exchanged at 40 min intervals. The bound fractions were then concentrated overnight in the dialysis/concentrator against saline/E/B.

Throughout the entire separation procedure, temperature was maintained at 6–8°C to minimize transfer of core components of lipoproteins mediated by CETP and samples, dialysis flasks, and concentrators were kept on ice. To protect RE from degradation, exposure of samples to light was minimized by covering the tubes, columns, concentrators, and dialysis flasks with aluminum foil.

Separation of lipoproteins

Portions of plasma, dialyzed and concentrated JIH-bound fractions, concentrated 4G3-unbound fraction, and dialyzed and concentrated 4G3-bound fraction were subjected to ultracentrifugation (Beckman 50.3 rotor, 39,000 rpm, 4°C, 20 h). The four floating fractions, obtained after tube slicing, contained total, JI-H-bound, JI-H-unbound/4G3-unbound, and JI-H-unbound/4G3-bound TRL. TRL unbound to JI-H and 4G3, containing predominantly apoB-48, are hereafter called apoB-48-rich TRL; TRL bound to JIH are called bound apoB-100 TRL; and TRL that were unretained by JI-H but were bound to 4G3 are called unbound apoB-100 TRL (Fig. 1).

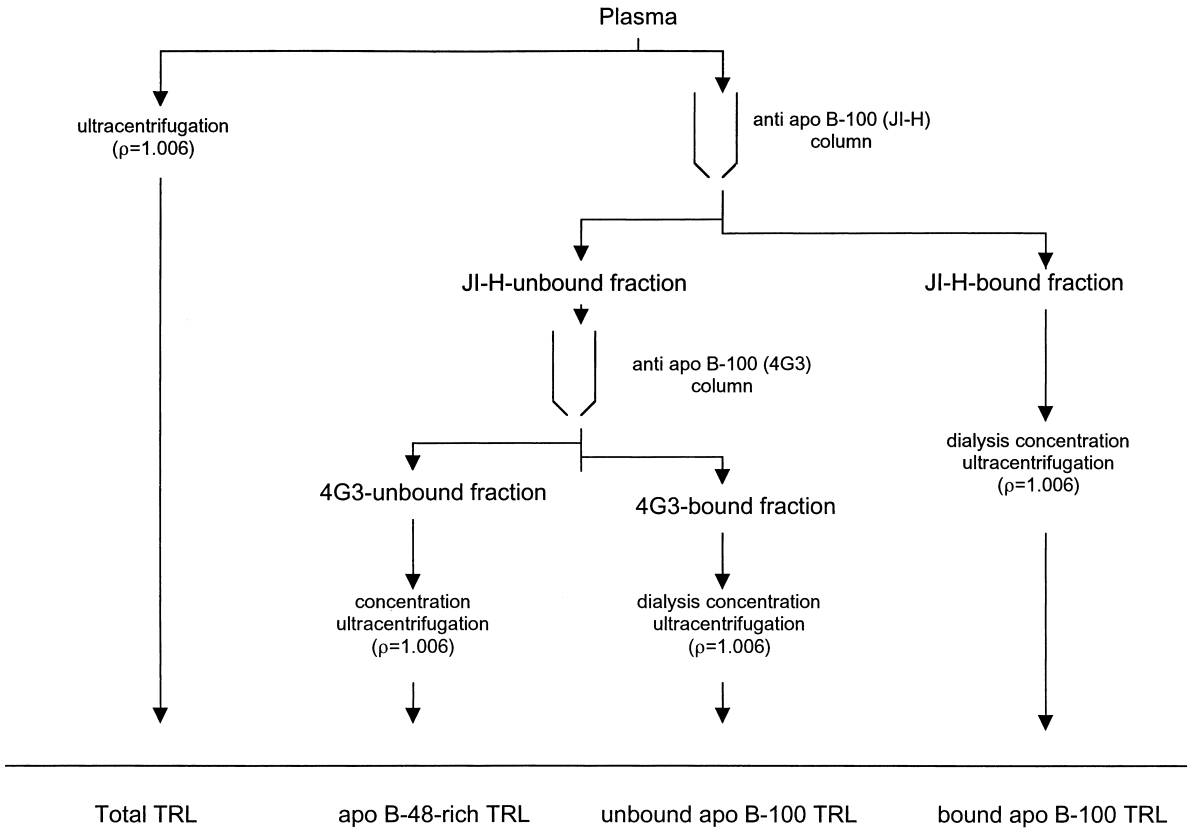

Fig. 1. Procedure for separating lipoprotein fractions containing apolipoprotein (apo) B-48 and apoB-100 on JI-H and 4G3 immunoaffinity columns followed by ultracentrifugation to separate triglyceride rich lipoproteins (TRLs). Terminology used here to designate the three TRL fractions is indicated at the bottom of the figure. In postprandial plasma samples from the seven patients with combined hyperlipidemia, the values (mean and SD) for mass ratios of apoB-48:apoB-100 were 0.052 (0.022) and 1.62 (2.01) for total and apoB-48-rich TRL, respectively; the percentage distribution (mean and SD) of TRL apoB-100 among the three fractions was 7.2 (5.6) in apoB-48-rich TRL, 14.8 (8.2) in unbound apoB-100 TRL, and 78.1 (11.5) in bound apoB-100 TRL.

Total IDL and LDL were separated from the ρ = 1.006 infranate of unfractionated plasma by sequential ultracentrifugation (15). HDL were separated from apoB-containing lipoproteins by the method of Warnick et al. (16).

Analyses

TG, total cholesterol (TC), free cholesterol (FC), phospholipids (PL), and total protein were estimated in plasma and lipoprotein fractions (17–20). RE were assayed by HPLC (21). The sensitivity of this assay was 5 ng.

Portions of all TRL subfractions, IDL, and LDL were delipidated with 10 volumes of ice-cold ethanol-ether (3:1, v/v) for at least 16 h. Protein was pelleted by centrifugation at 1,500 rpm, washed with an equal volume of cold ether, and solubilized in SDS-sample buffer. After residual ether was removed by applying a gentle stream of nitrogen, tubes were heated for 3 min in a boiling water bath, and cooled to room temperature. Apolipoproteins were separated on 3–10% linear polyacrylamide slab gels, stained, and quantified with a laser densitometer, utilizing values for the chromogenicity of the pure apolipoproteins. The chromogenicities of apoB-100, apoB-48, and apoE obtained with this method have been given previously (22). Chromogenicities of apoC-I, apoC-II, and apoC-III-1 are 105, 78, and 87 volume U/μg apolipoprotein, respectively (L. Kotite and R. Havel, unpublished observations). The chromogenicity of apoC-III-1 was used to estimate apoCs, which migrate together on these gels.

Calculations

Lipoprotein composition. The average molecular composition of lipoproteins was calculated relative to apoB (one molecule per particle) (2). Molecular weights (kDa) were taken as 512 for apoB-100, 243 for apoB-48, 34.2 for apoE, 8.8 for apoCs, 0.885 for TG, 0.387 for FC, 0.646 for cholesteryl esters (CEs), and 0.774 for PL. We assumed that the residual apoB-100 in apoB-48-rich TRL has the same composition as that found in unbound apoB-100 TRL. We therefore subtracted the amounts of lipids and apolipoproteins attributable to apoB-100 particles from the total amounts found in apoB-48-rich TRL to estimate the molecular composition of particles containing solely apoB-48 (hereafter called apoB-48 TRL).

Particle volumes and surface densities. The average diameter of particles in each fraction was calculated from their composition (23). Given the skewed distribution of particle sizes, the average diameter of particles in the TRL fractions is likely to be somewhat smaller than the calculated values. To calculate the densities of certain components on the surface of spherical TRL fractions, we first calculated the surface areas corresponding to the respective diameters. The density of surface components was then calculated and expressed as molecules/μm^2.

Transfer of RE among lipoprotein particles during fractionation

To evaluate the extent to which RE found in apoB-100-containing TRL fractions were transferred from apoB-48 TRL dur-

ing the separation procedures, we mixed RE-containing rat lymph chylomicrons with post-prandial plasma and subjected the mixture to the standard procedure used in this study. Chylomicrons were isolated from the intestinal lymph of a rat given Intralipid (24) containing added vitamin A. Chylomicrons were separated from the lymph by ultracentrifugation (Beckman 40.3 rotor, 35,000 rpm, 24°C, 60 min). Chylomicrons containing 82.5 mg TG and 0.119 mg RE in a volume of 0.15 ml were mixed with 22.5 ml of plasma obtained from a healthy subject (C3) after he had consumed the standard breakfast without vitamin A. The complete separation procedure was carried out as described above and the percentage of total TRL-RE in the three TRL fractions was determined.

Statistical analysis

Nonparametric methods were used because of the likely skewed distribution of measured analytes in lipoprotein particles and the small number of subjects. As appropriate, the Wilcoxon signed-rank test or Friedman statistic followed by a variant of Student-Newman-Keuls procedure for nonparametric multiple comparisons was used (25).

RESULTS

Concentration and composition of lipoproteins in postabsorptive and postprandial states

Fasting plasma and lipoprotein lipid values on the day of study in the seven patients with combined hyperlipidemia and three control subjects are shown in **Table 1**. In the hyperlipidemic patients, TRL-TG rose by an average of 55% 3 h after the fat-rich meal (**Table 2**). TRL-cholesterol, PL, apoB-48, and apoE concentrations also increased significantly, but the 8% increment in TRL-apoB-100 concentration was not significant ($P = 0.08$). Approximately 55%, 80%, and 70% of the increment in TRL-TC, TG, and PL was in the apoB-48-rich fraction, all of which increased significantly, as did TG in the bound fraction of apoB-100 TRL. As observed previously in normolipidemic subjects (1), apoB-100 TRL accounted for most of the postprandial increment in TRL particles.

In IDL, the concentrations of apoB-48 (0.17 vs. 0.10 mg/dl, $P = 0.031$) and apoE (0.37 vs. 0.28 mg/dl, $P = 0.031$) also rose postprandially, but that of apoB-100 and lipid components did not. There were no significant changes in components of LDL and HDL (data not shown).

The calculated molecular composition of the apoB-containing lipoprotein fractions in the postabsorptive and postprandial samples is shown in **Table 3**. In postabsorptive samples, the average number of TG molecules was highest in apoB-48 TRL, intermediate in unbound apoB-100 TRL, and lowest in bound apoB-100 TRL. The same pattern was observed for all other measured components. Both apoB-48 TRL and unbound apoB-100 TRL contained more CE per particle than IDL or LDL ($P < 0.01$). Postprandially, the number of TG in apoB-48 TRL more than doubled, whereas that of CE fell by 28%. The average number of all other components also increased, but only that of apoCs was significant. Bound apoB-100 TRL became enriched in TG, FC, and PL. IDL also became enriched in cholesterol, apoB-48, and apoE postprandially, but no changes in the particle composition of LDL were observed.

TABLE 1. Postabsorptive plasma lipid concentrations in study subjects

Subject	Sex	Age	TG	TC	HDL-C
				mg/dl	
Combined hyperlipidemia					
P1	M	43	180	271	44
P2	M	35	283	247	41
P3	M	27	146	270	53
P4	M	55	281	266	40
P5	F	75	167	327	42
P6	F	46	563	233	44
P7	M	46	447	272	57
Mean			295	269	46
(SD)			(157)	(29)	(7)
Normolipidemia					
1a	M	70	119	175	46
1b			108	176	
2	M	35	53	154	39
3	M	59	179	205	44

All apoB-containing lipoproteins were enriched in RE postprandially. Enrichments were greatest in those fractions containing apoB-48 and were by far the lowest in LDL. For those components that are excluded from the core of TRL, it was possible to calculate their average concentration on the surface of the three TRL fractions (**Table 4**). The average diameter of particles in each fraction was first estimated (23) from the data in Table 3 in order to estimate the total average surface area. Then, from the data in Table 3, the surface concentration of apoE, apoCs, and PL was calculated. As expected from the compositional data, apoB-48 TRL were significantly larger than each of the apoB-100 TRL fractions, and unbound apoB-100 TRL were significantly larger than bound apoB-100 TRL in both postabsorptive and postprandial samples. The diameter of apoB-48 TRL and bound apoB-100 TRL increased postprandially, but that of unbound apoB-100 TRL did not. In contrast, the concentration of none of the surface components differed between any of the TRL fractions in either of the samples, nor were significant changes in surface concentration observed in the postprandial samples. Thus, not only the concentration of PL, which occupy most of the surface of these spherical lipoproteins, but also that of exchangeable proteins appeared to be related primarily to the available surface area of the particles.

Similar relationships between particle size and surface concentration were observed in the three postprandial samples from the two control subjects. The range of average particle diameters (nm) in the three samples was 58.7–62.9 for apoB-48 TRL, 37.4–49.6 for unbound apoB-100 TRL, and 35.6–37.0 for bound apoB-100 TRL. The surface concentrations of apoE and apoCs were in the same range observed in the samples from the hyperlipidemic subjects.

Content of RE in lipoproteins

RE were detectable in total TRL of all hyperlipidemic patients in the postabsorptive state, but could be detected

TABLE 2. Concentration of postabsorptive and postprandial TRL subfractions in seven subjects with combined hyperlipidemia

	ApoB-100	ApoB-48	ApoE	ApoCs	TG	TC	FC[f]	EC[f]	PL[f]	RE
					mg/dl					*μg/dl*
Postabsorptive lipoproteins										
total TRL	9.54	0.54	2.07	10.32	214.5	46.3	23.0	27.8	62.4	1.83
	(4.57)	(0.38)	(1.60)	(4.53)	(145.8)	(32.3)	(13.3)	(19.9)	(36.7)	(2.13)
apoB-48-rich TRL	0.72	0.43	0.63	2.26	66.6	14.0	6.7	8.8	15.7	1.13[g]
	(0.93)	(0.37)	(0.83)	(2.02)	(77.5)	(15.1)	(6.6)	(9.8)	(15.7)	(2.06)
unbound apoB-100 TRL	1.16	0.00	0.27	1.19	27.6	5.9	3.5	3.1	7.4	0.14[g]
	(1.15)		(0.23)	(1.03)	(20.0)	(4.4)	(2.5)	(1.9)	(5.3)	(0.24)
bound apoB-100 TRL	6.57	0.00	1.16	5.29	111.2	28.7	15.2	15.7	35.2	0.30[g]
	(2.34)		(0.47)	(2.19)	(55.4)	(13.2)	(5.5)	(7.8)	(15.3)	(0.51)
Postprandial lipoproteins										
total TRL	10.33[e]	0.67[a]	2.52[c]	11.96[e]	333.5[a]	50.6[b]	25.4[d]	29.5	76.2[b]	218.97[a]
	(4.04)	(0.35)	(1.33)	(2.95)	(182.2)	(30.9)	(13.3)	(18.2)	(40.4)	(69.68)
apoB-48-rich TRL	0.90[e]	0.58[a]	0.76	3.74[a]	154.0[a]	16.4[b]	8.7[b]	9.4	28.0[b]	193.38[a]
	(0.82)	(0.33)	(0.44)	(2.41)	(117.1)	(13.4)	(5.9)	(8.2)	(20.1)	(76.39)
unbound apoB-100 TRL	1.24	0.00	0.29	1.40	32.1	5.8	3.2	3.4	9.0	1.49[a]
	(0.83)		(0.19)	(0.78)	(18.9)	(3.7)	(1.8)	(1.7)	(4.9)	(0.54)
bound apoB-100 TRL	6.89	0.00	1.32[e]	5.73	127.9[b]	30.8	16.4	16.2	39.4[d]	3.33[a]
	(2.50)		(0.54)	(2.14)	(58.0)	(13.5)	(6.6)	(7.5)	(16.2)	(1.39)

All values [mean (SD)] have been corrected for the average recoveries of TG and TC in the three TRL fractions as related to total TRL. Correction factors were 1.13 for postabsorptive and 1.19 for postprandial lipoproteins.

[a] $P = 0.015$, [b] $P = 0.031$, [c] $P = 0.047$, [d] $P = 0.063$, [e] $P = 0.078$: postabsorptive versus postprandial lipoproteins (Wilcoxon signed-rank test).

[f] n = 6.

[g] RE in fasting TRL fractions was detectable only in three patients.

in TRL fractions from only three of them (Table 2). No RE were detected in postabsorptive IDL, LDL, or HDL.

In postprandial samples, RE increased more than 100-fold in TRL and also became detectable in IDL and LDL. Although 97% of TRL-RE was in the fraction containing apoB-48, RE could also be quantified in the two TRL fractions containing solely apoB-100 (Table 2). The concentration of RE was higher in bound than in unbound apoB-100 TRL, but the particle enrichment was 4-fold higher in the unbound fraction (Table 3). More substantial amounts of RE were found in postprandial IDL, which also contained apoB-48. RE were also detected in postprandial LDL, but the concentration was much lower than in IDL and the TRL fractions containing solely apoB-100.

To estimate the fraction of TRL bearing apoB-48 and apoB-100 secreted from the intestine, we calculated the percentage of RE associated with each of the TRL fractions from the patients and control subjects. Providing that TRL bearing apoB-48 or apoB-100 that are secreted from the intestine postprandially are of a comparable size, these percentages should be proportional to the number of particles secreted. As shown in **Table 5**, 2.25–5.16% of RE was associated with apoB-100 TRL in the seven samples from the hyperlipidemic patients and 5.18–6.80% in the three samples from the two control subjects. These values overestimate the fraction of TRL secreted from the intestine that contain apoB-100 to the extent that RE are transferred, in vivo or in vitro, to these TRL from TRL bearing apoB-48 (all of which are assumed to be secreted from the intestine).

To estimate the fraction of RE that could be transferred from apoB-48 TRL to apoB-100 TRL during the extensive procedures that we employed after blood was drawn, we added rat chylomicrons enriched in RE to postprandial plasma and carried this sample through the standard separation procedures. As only 0.4% of the RE was recovered in apoB-100 TRL (Table 5), most of the RE found in apoB-100 TRL likely was either secreted directly from the intestine or transferred from secreted apoB-48 TRL to apoB-100 TRL in vivo.

DISCUSSION

Our findings provide in vivo evidence that the human intestine secretes a small but measurable fraction of apoB-100 in TRL, both in healthy adults and in those with primary combined hyperlipidemia. It must be emphasized, however, that our estimates are maximal. We found very little transfer of RE from apoB-48 TRL to apoB-100 TRL during our prolonged separation procedures (Table 5). However, RE could transfer from nascent chylomicrons or chylomicron remnants to other lipoproteins in the blood during the 3 h after the fat-rich meal was ingested. The magnitude of such transfer has been evaluated by others who have incubated chylomicrons with human blood plasma. Wilson and Chan (26) found little transfer of RE from rat chylomicrons to lipoproteins in human blood plasma during incubation at 37°C. Even with incubation at body temperature for 5 h, Berr and Kern (27) found very limited transfer of RE from a human plasma chylomicron fraction to lipoproteins of higher density. Although transfer activity increases in concert with TRL-TG levels postprandially (28), the fact that the concentration of RE was much lower in our postprandial LDL particles than in the two apoB-100 TRL particles suggests that most RE in the latter reflects intestinal secretion of TRL containing

TABLE 3. Composition of postabsorptive and postprandial TRL subfractions, IDL, and LDL in seven subjects with combined hyperlipidemia

	ApoB-48 Particles	ApoE/ Particle	ApoC/ Particle	TG/ Particle	TC/ Particle	FC/ Particle[k]	CE/ Particle[k]	PL/ Particle[k]	RE/ Particle
	% of all apoB particles	*molecules*							
Postabsorptive lipoproteins									
apoB-48 TRL	100.0	6.35[g] (2.93)	110.10[e,g] (39.61)	27200[g] (13030)	14390[f,g] (4730)	5430[f,h] (1210)	8970[e,h] (2600)	7140[f,h] (2410)	0.49[l] (0.89)
unbound apoB-100 TRL	ND	3.96[i] (1.28)	63.81[i] (16.84)	17370[i] (5670)	8960[j] (4020)	4640 (2260)	4330 (1850)	4200 (1630)	0.05[l] (0.08)
bound apoB-100 TRL	ND	2.66 (0.59)	46.33 (6.59)	9410 (1900)	5440 (970)	2720 (330)	2720 (730)	3310 (800)	0.04[l] (0.05)
IDL[k]	6.8 (4.1)	1.33 (0.32)	13.17 (4.09)	1890 (400)	3420 (1120)	1180 (380)	2240 (750)	1360 (190)	ND
LDL[k]	ND	0.14 (0.12)	2.40 (1.09)	230 (70)	2570 (970)	710 (250)	1860 (730)	750 (120)	ND
Postprandial lipoproteins									
apoB-48-TRL	100.0 (0.0)	7.68[e,g] (4.20)	131.55[e,g] (38.97)	63090[b,e,g] (33210)	12540[e,g] (3590)	6100[e,h] (980)	6440[c,e,g] (2680)	10600[f,g] (2460)	175.11[a,e,g] (57.66)
unbound apoB-100 TRL	ND	3.62[i] (0.80)	73.48[i] (17.02)	15910[i] (2400)	6490 (1360)	3160 (880)	3330 (630)	3820 (260)	2.19[a,i] (2.22)
bound apoB-100 TRL	ND	2.70 (0.44)	48.42 (2.27)	10630[b] (1840)	5860 (940)	2960[b] (470)	2900 (520)	3640[b] (760)	0.54[a] (0.41)
IDL[k]	11.9[b] (4.9)	1.76[b] (0.27)	14.42 (3.12)	2800 (700)	3720[d] (700)	1350 (290)	2370 (460)	1930 (900)	6.27 (7.56)
LDL[k]	ND	0.14 (0.12)	1.95 (1.16)	240 (60)	2780 (710)	760 (160)	2030 (560)	780 (130)	0.06 (0.05)

Values are means (SD). ND, not detectable.
[a] $P = 0.015$, [b] $P = 0.031$, [c] $P = 0.047$, [d] $P = 0.063$; postabsorptive versus postprandial lipoproteins (Wilcoxon signed-rank test).
Student-Newman-Keuls test if significant differences between groups were revealed with Friedman test (e–j):
[e] $P < 0.01$, [f] $P < 0.05$ (apoB-48-TRL vs. unbound apoB-100-TRL).
[g] $P < 0.01$, [h] $P < 0.05$ (apoB-48-TRL vs. bound apoB-100-TRL).
[i] $P < 0.01$, [j] $P < 0.05$ (unbound apoB-100-TRL vs. bound apoB-100-TRL).
[k] n = 6.
[l] RE in fasting TRL fractions was detectable only in three patients.

apoB-100. Specifically, postprandial LDL contained only 0.06 molecules of RE per particle (some of which could be in apoB-48 particles) (29), whereas bound apoB-100 TRL particles contained 9-fold and unbound apoB-100 particles contained 37-fold more RE than LDL (Table 3). Based upon the estimated diameters of bound and unbound apoB-100 TRL, their core volumes are approximately 7- and 9-fold larger than that of LDL (diameter taken as 22 nm). Even if in vivo rates of transfer of RE from chylomicrons to lipoproteins containing apoB-100 are proportional to core volume, it is thus likely that RE in unbound apoB-100 TRL are predominantly secreted with these particles from the intestine. By this reasoning, RE in bound apoB-100 TRL may be derived to a larger extent from post-secretory transfer of RE from chylomicrons.

Given the 80-fold lower number of RE molecules per particle in unbound apoB-100 TRL than in apoB-48 TRL (Table 3), it is evident that the former particles are overwhelmingly of hepatic origin. This is consistent with the conclusion of Linton et al. (4) with respect to larger TRL containing apoB-100 that accumulate in postprandial plasma. Of note, unbound apoB-100 TRL comprise about 75% of the TRL included in a clinical test for "remnant-like particles" in postabsorptive human blood plasma (29).

The extent to which retinol ingested with a fat-rich meal appears in postprandial lipoproteins containing apoB-100 is controversial. Consistent with our data that show only 1–2% of RE in LDL 3 h after ingestion of retinol, several other investigators have found little RE in LDL after ingestion of vitamin A with a fat-rich meal (27, 30, 31). Krasinski et al., however, found 9% of RE in LDL 3 h postprandially, increasing progressively at later times (32). Furthermore, in additional studies, Cohn et al. have, like us, used monoclonal antibodies specific for apoB-100 to determine the distribution of RE in TRL containing apoB-48 and apoB-100 postprandially (33). Over a period of 12 h, they found 75% of the increment in RE to be in apoB-48 TRL and 25% to be in apoB-100 TRL. As early as 4 h after the meal, the percentage in apoB-100 TRL was appreciable. In considering the basis for the apparent difference between our observations and those of Krasinski et al. and Cohn et al., it is notable that they fed retinyl palmitate with the fat-rich meal whereas we, like other investigators who found little RE in LDL (27, 30, 31), fed an aqueous dispersion of retinol (Aquasol A). Like Karpe et al. (34), who also fed retinyl palmitate with a fat-rich meal, Krasinski et al. (32) and Cohn et al. (33) found that the peak concentration of RE in plasma and TRL was delayed as compared with peak concentrations of TG, apoB-48, and apoB-100 in TRL. In contrast, some other investigators who fed retinol found considerably less delay in the attainment of peak RE as compared with peak TG. Notably, the amplitude and duration of excursions of RE and

TABLE 4. Diameter of lipoprotein particles and surface concentration of apolipoproteins and phospholipids in postabsorptive and postprandial TRL subfractions from seven subjects with combined hyperlipidemia

	Diameter	Surface Concentration		
		ApoE	ApoC	PL[f]
	nm	*molecules/μm² × 10⁻³*		
Postabsorptive lipoproteins				
apoB-48-TRL	52.2[c,d]	0.757	13.6	811
	(5.4)	(0.208)	(5.10)	(113)
unbound apoB-100 TRL	42.8[e]	0.644	11.1	720
	(5.1)	(0.219)	(2.84)	(177)
bound apoB-100 TRL	36.5	0.643	11.4	782
	(2.0)	(0.201)	(2.12)	(117)
Postprandial lipoproteins				
apoB-48 TRL	63.8[a,c,d]	0.561	10.9	791
	(7.2)	(0.191)	(1.77)	(142)
unbound apoB-100 TRL	41.7[e]	0.660	12.7	726
	(2.3)	(0.193)	(2.99)	(77)
bound apoB-100 TRL	38.2[b]	0.576	10.7	796
	(1.2)	(0.116)	(1.05)	(128)

Values are means (SD).

[a] $P = 0.031$, [b] $P = 0.063$; postabsorptive versus postprandial lipoproteins (Wilcoxon signed-rank test).

Student-Newman-Keuls test if significant differences between groups were revealed with Friedman test (c–e):

[c] $P < 0.05$ (apoB-48-TRL vs. unbound apoB-100-TRL).

[d] $P < 0.01$ (apoB-48-TRL vs. bound apoB-100-TRL).

[e] $P < 0.05$ (unbound apoB-100-TRL vs. bound apoB-100-TRL).

[f] n = 6.

TG in a chylomicron fraction of plasma were found to be superimposable in a study comparing subjects with homozygous or heterozygous lipoprotein lipase deficiency with normolipidemic control subjects (31). Thus, in these subjects who were fed cream fat together with aqueous retinol as Aquasol A, retinol and TG fatty acids evidently were absorbed at comparable rates. Karpe et al. (34) have speculated that the use of soybean oil as compared with dairy cream may account for delayed absorption in their study and in the studies of Krasinski et al. Cohn et al. (33), who fed retinyl palmitate together with dairy cream, still observed a considerable delay in the attainment of peak concentrations of RE in apoB-48 TRL and even greater delay in apoB-100 TRL. These data lead us to suggest that ingested retinyl palmitate may be absorbed more slowly relative to dietary fat than retinol, with the consequence that more vitamin A is absorbed in smaller TRL secreted several hours after fat absorption is largely complete. The appearance of RE in apoB-100 TRL and in LDL under these circumstances could reflect substantial transfer (via CETP) from smaller apoB-48 TRL to lipoproteins containing apoB-100. In contrast, we suggest that transfer of RE from large chylomicrons that contain most absorbed TG and RE in the first few hours after fat is fed is much more limited. Although experiments to test this hypothesis are needed, we suggest that dietary retinol may be a more suitable tracer for intestinal lipoproteins than retinyl palmitate in feeding studies.

Previously, Milne, Marcel, and their associates have used a set of two monoclonal antibodies to separate apoB-48 TRL and apoB-100 TRL from total TRL of fasting subjects with familial dysbetalipoproteinemia, familial hypertriglyceridemia, as well as normolipidemia (35, 36). They have shown that apoB-48 TRL are relatively protein-poor and that both fractions contain apoE and the three major apoCs. In subjects with familial hypertriglyceridemia and a single normolipidemic subject, apoB-48 TRL were enriched in TG and depleted in CE as compared with apoB-100 TRL, whereas the reverse was the case for those with dysbetalipoproteinemia. Bjorkegren et al. have used the same method to study postprandial changes in the concentration of apoB-48-rich TRL and apoB-100 TRL fractions (Sf 60–400 and 20–60) in healthy, normolipidemic subjects (37). As they excluded large particles of Sf >400 from their analyses, it is difficult to compare their data with ours. Our data extend the observations of these investigators to subjects with primary combined hyperlipidemia and provide the first data on the surface density of intestinal and hepatic TRL in postprandial as well as postabsorptive blood plasma.

TABLE 5. Percentage of retinyl esters associated with TRL subfractions 3 h after feeding a fat-rich meal containing added retinol

	ApoB-48 TRL	Unbound ApoB-100 TRL	Bound ApoB-100 TRL	Total ApoB-100 TRL
			%	
Combined hyperlipidemia				
1	94.99	1.86	3.15	5.01
2	97.75	1.04	1.21	2.25
3	94.84	1.67	3.49	5.16
4	97.24	1.68	1.09	2.76
5	97.11	1.21	1.69	2.89
6	97.65	1.19	1.16	2.35
7	98.62	0.68	0.70	1.38
Mean	96.89	1.33	1.78	3.11
(SD)	(1.43)	(0.42)	(1.09)	(1.43)
Controls				
1	94.82	2.09	3.09	5.18
1b	93.20	2.92	3.88	6.80
2	93.75	3.11	3.14	6.25
3 (in vitro)	99.58	0.17	0.25	0.42

Values for each fraction have been corrected for apoB-48 recovery as related to total TRL, assuming equal recovery of apoB-48 and apoB-100 from JI-H and 4G3 columns.

Although the TG content of apoB-48 TRL more than doubled postprandially, their average diameter increased by only about 20%, consistent with other data showing that most apoB-48 particles in postprandial TRL have Sf rates below 400 (34). This small increment of size may reflect in part the rapid hydrolysis of TG in large chylomicrons, yielding appreciably smaller chylomicron remnants (38). However, estimates of the RE content of apoB-48 TRL of varying flotation rates suggest that most intestinal TRL secreted 3–6 h after a fat-rich meal remain well within the size range of hepatogenous TRL (34). Our data suggest that some intestinal TRL in postprandial plasma may be as small as IDL (Table 3).

PL occupy most of the surface membrane of TRL and it would therefore be predicted that their surface density is a constant feature of TRL of differing size and origin. Our data show this to be the case for apoB-48 TRL and two fractions of apoB-100 TRL in which apoB-100 exhibits distinctive immunoreactivity. Less predictable was our observation that the average surface density of apoE and the combined apoCs is also a constant feature of these three TRL fractions and that their surface density as well as that of PL is unchanged postprandially. In fact, this observation is rather unexpected considering that TRL species may differ in content of apoE (39, 40) as well as apoC-III (the most prevalent apoC) (40), and that the relative amounts of apoE and apoCs change during lipolytic degradation of TRL (41). In other studies, we have found the surface density of apoC-II in bound apoB-100 TRL from fasting normolipidemic subjects to be significantly lower, and that of apoE to be significantly higher than in a combined fraction containing both apoB-48 TRL and unbound apoB-100 TRL (29). No differences were observed for apoC-I, apoC-III, and PL, however. Taken together, these combined data suggest that available surface area is a major determinant of the average content of soluble apolipoproteins in TRL species of differing size and origin.

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