Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants

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Abstract The size of cholesterol-rich lipoprotein particles is a strong determinant of whether they may be deposited in the arterial wall and by this become potentially atherogenic. This study deals with the in vivo transformation of larger-sized chylomicrons and chylomicron remnants to smaller-sized remnants. Twelve healthy men aged 22 to 45 years were given a fatty meal to which retinyl palmitate (RP) had been added. Plasmapheresis was performed 4½ h after meal intake to isolate approximately 400 ml plasma. The RP-rich plasma was re-injected to the subject 24 h later. The RP content was determined in whole plasma and in Svedberg flotation rate fractions (S_f) >400, S_f 60–400 and S_f 20–60. A compartmental model was developed for the kinetic analysis. Lipoprotein fractions showed minimal signs of aggregation, thus arguing for well-preserved postprandial lipoproteins. Approximately a fourth [23% (4–68%)] of the RP-containing lipoproteins in the S_f >400 pool was converted to smaller species (S_f 60–400). Conversion of material from the S_f 60–400 to the S_f 20–60 fraction could not be detected. In a second study a large bolus dose of a triglyceride emulsion (Intralipid) was injected to subjects shortly after the RP-labeled plasma to investigate the endothelial binding of the chylomicron/chylomicron remnants. RP material in the S_f >400 fraction rapidly returned to plasma, arguing for margination of chylomicrons, whereas the corresponding effect was minimal in the S_f 60–400 and S_f 20–60 fractions. The formation of small chylomicron remnants from the larger chylomicron/chylomicron remnant species is limited and large chylomicron/chylomicron remnants are not evenly distributed in plasma, rather they show signs of being marginated to the vascular endothelium.—**Karpe, F., T. Olivecrona, A. Hamsten, and M. Hultin.** Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. *J. Lipid Res.* 1997. **38:** 949–961.

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Dietary fat is efficiently absorbed by the intestine, and a majority of the lipid content of the food is incorporated into chylomicrons and secreted by enterocytes into the thoracic duct. A tissue-specific variant of apoB, apoB-48, is the structural protein of the chylomicron particle. After acquiring an appropriate composition of exchangeable apolipoproteins (apoC and E), a substantial mass of the triglyceride content is rapidly hydrolyzed by lipoprotein lipase (LPL). Essentially, the non-triglyceride core components and apoB-48 stay with the lipoprotein particle until final whole-particle uptake ensues, presumably by specific receptor-mediated processes.

Studies describing the rapid metabolic events of the in vivo handling of chylomicrons in humans are sparse. The decay of the triglyceride component was first studied by Nestel (1). He obtained human triglyceride-labeled lymph chylomicrons and re-injected this lipoprotein fraction into healthy subjects and patients with coronary heart disease (CHD). The triglyceride component disappeared from blood with a half-life ranging between 5 and 8 min in healthy subjects, whereas the rate of disappearance was prolonged in the mildly to moderately hypertriglyceridemic CHD patients. There was a strong linear relationship between the fasting triglyceride level and the half-life of chylomicron triglycerides. Subsequently, Grundy and Mok (2) used a completely different approach to quantify the chylomicron triglyceride clearance rate. They used a long-term, presumably steady-state, intra-duodenal triglyceride infusion and calculated the triglyceride clearance capacity

Abbreviations: CHD, coronary heart disease; FCR, fractional catabolic rate; IDL, intermediate density lipoprotein; LRC, Lipid Research Clinic; RP, retinyl palmitate; RT, residence time; S_f, Svedberg flotation rate.

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with a result very similar to the one found by Nestel; the chylomicron triglyceride clearance rate had a half-life of 4.5 ± 2.9 min in healthy males. A first attempt to quantify the chylomicron particle clearance in humans was done by Schaefer, Jenkins, and Brewer (3). They labeled human chylomicrons with ^{125}I and traced the disappearance of apoB after re-injection of the chylomicrons. Kinetic information was not provided but it can be read from the graphs that approximately 80% of the chylomicron-bound apoB had disappeared after 1 h with the generation of approximately 20% of the initial chylomicron apoB radioactivity in the VLDL fraction in the one subject studied.

Intravenous injection of well-defined lipid emulsion particles has been used to simulate chylomicron turnover in humans (4, 5). If the emulsion particle composition and size are similar to chylomicrons, the metabolic fate of emulsion particles also seems to agree well with that of chylomicrons. With the use of this technique, patients with documented coronary heart disease have a slower removal of emulsion particles compared to healthy subjects (5).

Labeling of the chylomicron core components with vitamin A has been used in a few studies involving chylomicron turnover in humans. Cortner and coworkers (6) administered an intraduodenal infusion of a triglyceride emulsion together with vitamin A to healthy subjects, subjects with hypertriglyceridemia, and subjects carrying the apoE2/2 phenotype. It was assumed that absorption of lipids and the vitamin was rapid and uniform. The decay of retinyl palmitate (RP) from lipoprotein fractions was analyzed in a multicompartmental model, separately analyzing Svedberg flotation rate (S_f) >1000 lipoproteins and lipoproteins in the S_f range 20–1000. The calculated half-life of the latter fraction was grossly prolonged in hypertriglyceridemic subjects and in particular in type III patients. The authors concluded that the delayed chylomicron remnant clearance in subjects with endogeneous hypertriglyceridemia may be largely secondary to overproduction of VLDL particles, whose remnants compete with chylomicron remnants for removal by the liver via apoE receptor-mediated endocytosis. Berr and Kern (7) used an alternative approach to study the turnover of RP-labeled postprandial lipoproteins; RP-labeled plasma was obtained by plasmapheresis and re-injected to subjects 2 days later. Blood samples were then taken during 3 h and 30 min, and in most subjects the disappearance of RP from plasma obeyed first-order kinetics, but in some subjects RP was cleared bi-exponentially. The fractional removal rate of RP was estimated to 0.037 ± 0.037 min^{-1}. In a subsequent study a greater heterogeneity of the clearance pattern of RP was detected (8). Berr (8) also concluded that there was no substrate–product relationship between the disappearance of RP from a d < 1.006 kg/l to a d > 1.006 kg/l fraction. This finding fits well with an old investigation of apoB-48 turnover in the rat, which showed that <1% of ^{125}I-labeled apoB-48-containing lymph chylomicrons was found in the IDL/LDL fraction after injection into rats (9). However, recent evidence for the existence of minute amounts of apoB-48 in the human LDL fraction has been obtained using refined immunological methods (10).

With the development of appropriate methods to accurately quantify the plasma concentration of apoB-48 (10–13) it was possible to investigate the relationship between the "true" marker for chylomicrons and their remnants (i.e., apoB-48) and RP which has been extensively used as a surrogate marker for intestinal lipoproteins (14). It was quite obvious that the very large postprandial triglyceride-rich lipoproteins (S_f >400) had more RP molecules per apoB-48 molecule than the corresponding remnant lipoprotein fractions (S_f 60–400 and S_f 20–60). One explanation for this apparent paradox was that the RP content in the remnant fractions (S_f 60–400 and S_f 20–60) did not originate to any major extent from the S_f >400 fraction. Accordingly, there are a number of observations pointing in the direction of a lack of a substrate–product relationship between larger-sized chylomicrons and apoB-48-containing lipoproteins in fractions that are normally regarded as remnant lipoprotein fractions.

A critical issue of the present work is to investigate to what extent there is in vivo transformation of larger-sized chylomicron or chylomicron remnant species to smaller-sized remnants, i.e., lipoproteins that might be implicated in the development of atherosclerosis due to their ability to penetrate the vascular endothelium. In order to answer these questions in vivo, turnover studies were performed in humans and a compartmental model for the kinetic analysis was developed.

MATERIAL AND METHODS

Subjects

A total of 16 healthy men aged 22 to 45 years were investigated. The data from 12 subjects (experiments) are shown as four experiments could not be evaluated due to extremely low levels of RP in the postprandial plasma. Mean fasting plasma cholesterol and triglyceride levels were 4.87 ± 0.83 mmol/l (mean ± SD, range 3.87–6.83) and 1.66 ± 0.83 mmol/l (0.79–3.75), respectively. HDL and LDL cholesterol levels were deter-

mined after an overnight ultracentrifugation (removal of VLDL) and precipitation of the bottom fraction with heparin/$MgCl_2$, essentially according to the LRC protocol (15). HDL was 1.07 mmol/l (0.78–1.99) and LDL was 3.18 mmol/l (2.41–4.74). None of the subjects had an apoE2 allele; the distribution of apoE genotypes was E3/E3 (n = 7), E3/E4 (n = 4), and E4/E4 (n = 1). The body mass index varied from 20.4 to 31.0 kg/m^2 with a mean of 24.2 ± 3.2 kg/m^2. The plasma volume of the subjects was calculated by a nomogram with age and body weight as input variables (16).

Experimental procedures

Participants were admitted early in the morning to the Clinical Research Unit for a mixed meal-type of oral fat load. They had been fasting for 12 h and asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 3 days. An emulsion consisting of soybean oil [Karlshamns Oils & Fats AB, Karlshamn, Sweden, 50 g/m^2 body surface area (16)], glucose (50 g/m^2), egg-white protein (Sigma 0500, 25 g/m^2), dried egg yolk (Sigma 0625, 6.3 g/m^2), and 200 ml water prepared with some lemon flavor (60.2% fat, 13.3% protein, and 26.5% carbohydrate by energy) was ingested within 10 min between 7:00 and 7:30 AM. Five tablets containing the equivalent of 250,000 IU of vitamin A as RP (Arovit, Hoffman-La Roche, Basle, Switzerland) were added to the meal. The subjects were instructed to chew the tablets carefully. The test meal was well tolerated by all subjects. Four hours and 30 min after meal intake, plasmapheresis was performed to isolate approximately 400 ml of postprandial plasma containing RP-labeled triglyceride-rich lipoproteins. The plasma was recovered in two sterile plastic bags (approximately 200–220 ml plasma in each) prefilled with 70 ml of a CPD (citrate/phosphate/dextrose) buffer, pH 7.0, as anticoagulant, and stored protected from light overnight at 4°C on a slowly shaking tray. Twenty-four hours later the plasma was brought to room temperature just before it was re-injected intravenously as a bolus to the subject. A procedure similar to this has been described by Berr (8). Subjects were instructed to ingest a light and essentially fat-free breakfast at 07:00 AM and the infusion took place at 11:00–11:30 AM that day. The infusion was completed within 4–5.5 min. Blood samples were taken from the other arm before (three base-line samples during 7.5 min) and 1, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 120 180, 240, and 360 min after the infusion had been completed.

A second study was undertaken to investigate the effect of a triglyceride emulsion on the metabolism of the RP-labeled lipoproteins. The emulsion is to some extent chylomicron-like as its lipid droplets have a size and metabolic fate not too dissimilar to chylomicrons (17, 18), but they do not contain cholesterol and the phospholipid content is not chylomicron-like. The cholesterol content of the lipid emulsion has been argued to be of importance for a chylomicron-like metabolic fate of the emulsion particles (19). Three subjects were asked to return for this study. The experimental set-up was identical until blood sampling after the re-injection of plasma. In this study a primed infusion of Intralipid® 100 mg/ml (Pharmacia-Upjohn, Sweden) was given, initiated with a bolus dose (0.15 g/kg body weight) given 10 min after re-injection of plasma followed by a continuous infusion (0.15 g/kg per hour). Blood samples were taken prior to re-injection and at 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 15, 17, 20, 40, and 60 min. The injection of Intralipid® was made at 10 min and the 10-min blood sample was taken after the Intralipid® injection had been completed, which typically took 75 s.

Analytical procedures

Venous blood samples were drawn into pre-cooled sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na_2EDTA (final concentration 4 mM), which were instantly put into icewater and protected from light. Plasma was recovered by low-speed centrifugation (1,750 *g*, 20 min) at 1°C and kept at this temperature throughout the preparation procedure. PMSF (10 mmol/l, dissolved in isopropanol) and aprotinin (1,400 μg/ml) (Trasylol, Bayer, Leverkusen, Germany) were immediately added to the isolated plasma before fractionation of triglyceride-rich lipoproteins to final concentrations of 10 μmol/l and 28 μg/ml, respectively.

Triglyceride-rich lipoproteins were subfractionated by cumulative density gradient ultracentrifugation (20). Plasma was adjusted to d 1.10 kg/l with solid NaCl. A density gradient consisting of 4 ml of d 1.10 kg/l plasma and 3 ml each of d 1.065, d 1.020, and d 1.006 kg/l NaCl solutions was then formed in Beckman Ultraclear tubes (Beckman, Palo Alto, CA). Ultracentrifugation was performed in a Beckman SW40 Ti swinging bucket rotor at 40,000 rpm and 15°C. Consecutive runs calculated to float S_f >400 (32 min), S_f 60–400 (3 h 28 min) and S_f 20–60 (16 h) lipoprotein fractions were made. After each ultracentrifugation, the top 0.5 ml of the gradient containing the respective lipoprotein subclass was aspirated, and a d 1.006 kg/l NaCl solution was used to refill the tubes before the next run. Plasma and fractions were protected from day light and always kept at 4°C.

Whole plasma and isolated lipoprotein fractions were assayed for their RP content as described previously (14). Fasting and peak postprandial samples were also assayed for their apoB-48 and apoB-100 content (10).

Statistical analysis

Conventional methods were used to calculate means, medians, and standard deviations (SD). SD or the full range is given in the text. For skewed variables, as for example plasma triglycerides and some kinetic variables, the median and range are given. Associations between lipoprotein parameters were determined by calculation of Pearson correlation coefficients after skewed variables had been log-normalized.

Compartmental modeling

SAAM II version 1.0 (SAAM Institute, Redmond, WA) was used for compartmental modeling and for fitting data to exponential functions. The data was weighted using the POIS (0.1) weighting scheme, which assigns Poisson statistics, such that SD = $\sqrt{0.1 * |data_{ij}|}$ (21). The a priori identifiability analysis of the model was done by Dr. Maria-Pia Saccomani, University of Padua, Italy, using the GLOBI software (22). When constraining k(0,3) = k(0,1) and k(0,4) = k(0,2), all rate constants and distribution volumes were uniquely identifiable.

Ethical considerations

The study protocol was approved by the local ethics committee at the Karolinska Hospital. All subjects gave informed consent.

RESULTS

Triglycerides, apoB-48, and RP in plasma

The fat-rich meal increased plasma triglycerides from 1.66 mmol/l (0.79–3.75) to 4.11 mmol/l (1.34–10.39). The latter sample was taken 4.5 h after meal intake, i.e., just before the plasmapheresis started. In the S_f >400 fraction apoB-48 rose from not detectable (nd, less than 0.02 mg/l) to 0.41 mg/l (nd-3.0). The apoB-48 content in the S_f 60–400 fraction increased from 0.81 mg/l (nd-1.84) to 2.42 mg/l (0.05–4.9) whereas there was no increase of apoB-48 in the S_f 20–60 fraction [1.06 mg/l (nd-2.11) to 0.95 mg/l (0.05–1.86)].

The plasma levels and the distribution of RP between fractions deserves special attention as it forms the basis for the present work (**Table 1**). Although all subjects received the same dose of RP there was a more than 10-fold difference among the plasma RP levels at the time of plasmapheresis. Some subjects had a dominance of RP in the S_f >400 fraction whereas others had more material in the S_f 60–400 fraction. Only a minor proportion of RP was present in the S_f 20–60 fraction.

Storing plasma overnight did not affect the total plasma RP level (Table 1), nor did it induce any major changes in the distribution of RP among the different subfractions of triglyceride-rich lipoproteins. The amount of RP in the S_f >400 fraction tended to increase (+14% compared to the sample isolated just before plasmapheresis, $P < 0.05$) but the source for this increase was not obvious as the concentration of RP in the S_f 60–400 fraction was not changed. The RP concentration in the S_f 20–60 fraction was generally low and decreased with storage, but this decrease could not account for the increase in the S_f >400 fraction. In summary, samples taken from the subject before initiation of the plasmapheresis and from the bag after storage were almost similar, showing minimal signs of lipoprotein aggregation and thus arguing for the presence of well-preserved large postprandial triglyceride-rich lipoproteins in the infusate.

If it is assumed that all RP molecules are carried by apoB-48-containing lipoproteins, the average number of RP molecules for each apoB-48 can be calculated. Assuming that the molecular weight of apoB-48 is 260 kD, the S_f >400 particles contained 10,150 (1,350–23,780) RP molecules per apoB-48, while the S_f 60–400 particles contained only 540 (270–2,880).

Injection of RP-labeled plasma

A total of 536 ± 32 ml anticoagulated plasma (corresponding to 395 ml whole plasma) was injected within 4.5 ± 0.4 min. The total amount of RP infused was 5.25 μmol (0.86–15.53), with 2.19 μmol (0.48–8.73) contained in the S_f >400 fraction, 1.39 μmol (0.31–3.96) in the S_f 60–400 fraction, and 0.16 μmol (0.08–0.65) in the S_f 20–60 fraction.

The instantaneous increase and slower decay of RP in subfractions of triglyceride-rich lipoproteins from one subject is shown in **Fig. 1.** The first phase of decay of the S_f >400 fraction was rapid with a $t_{1/2}$ of 23 min and both the S_f >400 and the S_f 60–400 fractions exhibited at least one slower component with a $t_{1/2}$ of approximately 6 h (370 min). The decay of RP from the S_f 20–60 fraction exhibited either first-order kinetics or no detectable clearance at all (**Table 2**). In the S_f 60–400 fraction some cases showed an increase or a shoulder-like appearance of the RP curve during the first half hour after injection, but this was never seen in the S_f 20–60 fraction. This increase, or shoulder, in the RP-curve argues for transformation of S_f >400 particles to S_f 60–400 particles, while the complete lack of a shoulder in the RP concentration curve of the S_f 20–60 fraction, showing a very slow turnover, argues against a substrate/product relationship with the S_f 60–400 fraction. Based on these preliminary findings a kinetic model was formulated (**Fig. 2**).

TABLE 1. Retinyl palmitate in plasma and subfractions of triglyceride-rich lipoproteins at 4.5 h after meal intake (before plasmapheresis) and after storing the plasma (just before re-injection)

Subject	Plasma RP before Plasmapheresis	Plasma RP after Storage	%[a]	RP in S_f >400 after Storage	%[a]	RP in S_f 60–400 after Storage	%[a]	RP in S_f 20–60 after Storage	%[a]
	μM	μM		μM		μM		μM	
1	12.2	13.0	106	2.8	97	5.1	126	1.43	92
2	13.7	13.9	101	6.4	90	4.4	105	1.10	88
3	30.8	28.2	92	14.9	102	4.4	53	0.46	67
4	7.4	6.8	94	2.2	116	2.8	91	0.57	42
5	1.7	1.5	88	0.8	115	0.5	102	0.18	83
6	12.5	10.1	81	4.4	141	3.3	84	0.31	64
7	10.4	10.9	104	2.1	111	1.7	123	0.33	79
8	23.9	22.8	93	6.2	112	2.9	88	0.26	88
9	9.4	9.8	93	3.3	138	3.4	85	0.32	69
10	22.0	22.6	104	9.7	112	5.7	130	0.89	76
11	4.9	4.5	102	2.6	102	1.5	71	0.33	51
12	4.4	5.9	92	2.3	142	2.1	129	0.20	80
Mean			99 ± 12		114 ± 17[b]		99 ± 25		73 ± 15[c]

Confidence limits were calculated for the difference between the RP content before and after storage. RP, retinyl palmitate; S_f, Svedberg flotation rate.

[a]Percent column shows the percentage of RP material compared with a sample analyzed before storage.

[b]Denotes a significant difference from 100% with a 95% confidence limit but not with a 99% confidence limit.

[c]Denotes a significant difference from 100% with a 99% confidence limit.

Kinetic model

The clearance of RP from the S_f >400 and S_f 60–400 fractions is biphasic, accounting for the need of at least two compartments within each of these fractions, one with a rapid and one with a slow turnover. As we have shown that the injected material was well preserved, it was assumed that injection was made into the rapidly turning over compartments (1 and 3). This assumption may be marred due to the obvious fact that the injected material was already partly metabolized. The RP material still present in blood from the day before was modeled as slowly turning over material and thus confined to compartments 2 and 4. As there was evidence for a shoulder in the curve for RP decay in the S_f 60–400 fraction in most experiments, a transfer from the rapid compartment in S_f >400 to S_f 60–400 was added [k(3,1)]. Material was also allowed to leave compartment 1 [k(0,1)] directly or to feed into compartment 2 [k(2,1)], the slowly turning over compartment in S_f >400. The S_f 60–400 level of the model is similar; material is allowed to leave compartment 3 directly [k(0,3)] or to transfer to the slow compartment 4 [k(4,3)]. In the preliminary data analysis it became evident that the disappearance rate from the slowly turning over compartments, k(0,2) and k(0,4), were poorly defined by the data (data were only collected during approximately one half-life). These rates rather seemed to be fairly similar and k(0,2) and k(0,4) could therefore be assigned equal constants without compromising the data fits. Furthermore, only a small proportion of the RP material left plasma by this route. The rate constants out from the rapidly turning over compartments, k(0,1) and k(0,3), could also be set equal without compromising the data fits. As the turnover of RP in the S_f 20–60 fraction could be described as a monoexponential decay, only one compartment was needed to model these data. The distribution volumes for RP in the different fractions were set equal within each fraction but were allowed to adjust freely (**Table 3**).

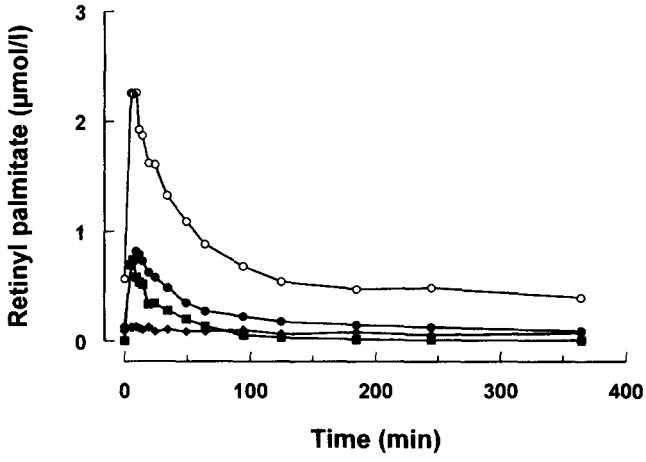

Fig. 1. Infusion of 395 ml RP-labeled plasma to subject #10. The infusion lasted for 4 min and each plasma sample (○) was separated into S_f >400 (■), S_f 60–400 (●), and S_f 20–60 (◆) fractions by density gradient ultracentrifugation.

TABLE 2. Calculated rate constants for RP in the S_f >400 fraction (compartments 1 and 2), the S_f 60–400 fraction (compartments 3 and 4), and the S_f 20–60 fraction (compartment 5)

Subject	k(0,1) and k(0,3)	k(0,2) and k(0,4)	k(2,1)	k(3,1)	k(4,3)	k(0,5)
	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}
1	0.0201	0.0035	0.0224	0.0900	0.0045	0.0027
2	0.0041	0.0130	0.0071	0.0005	0.0071	0.0024
3	0.0316	0.0064	0.0276	0.0063	0.0205	0.0008
4	0.0480	0.0034	0.0212	0.0381	0.0312	0.0009
5	0.0430	0.0023	0.0147	0.0379	0.0260	<0.0001
6	0.0150	0.0062	0.0144	0.0078	0.0350	0.0015
7	0.0563	0.0068	0.0250	0.0193	0.0373	0.0030
8	0.0149	0.0018	0.0008	0.0051	0.0043	<0.0001
9	0.0045	0.0085	0.0082	0.0022	0.0052	0.0003
10	0.0263	0.0018	0.0012	0.0055	0.0056	0.0002
11	0.0643	0.0051	0.0414	0.0560	0.0386	<0.0001
12	0.0209	0.0039	0.0054	0.0350	0.0021	0.0040
Median (Range)	0.0236 (0.0041–0.0643)	0.0045 (0.0018–0.0130)	0.0146 (0.0008–0.0414)	0.0135 (0.0005–0.0900)	0.0138 (0.0021–0.0386)	0.00085 (<0.0001–0.0040)

To analyze the a priori identifiability of this model we used the newly developed software GLOBI (17). Identifiability testing verifies whether the proposed model in conjunction with the present experiment is appropriate for estimating the unknown parameters (23, 24). With the above-mentioned constraints [k(0,1) = k(0,3) and k(0,2) = k(0,4)] all parameters in the model were uniquely identifiable.

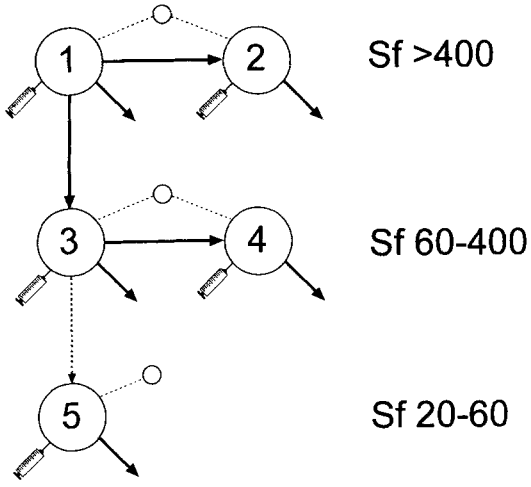

Fig. 2. Compartmental model for the clearance of the infused RP-labeled plasma. Material is entering at S_f >400, S_f 60–400, and S_f 20–60 levels. Compartments 1 and 3 are rapidly turning over, whereas compartments 2 and 4 are slow. Material entering compartments 1 and 3 can undergo direct removal or be fed into the slow compartments. Material from compartment 1 can also be transferred to compartment 3. Entry of material from compartment 3 into compartment 5 could not be detected in any subject, and these compartments are therefore connected with a dashed arrow. The rationale behind the compartmental model is described in the text.

Kinetics of chylomicrons/chylomicron remnants

Using the developed model, the full length of the experiments was modeled, including the infusion of the labeled plasma (4–5.5 min). The fits are shown in **Fig. 3** and the rate constants are given in Table 2, while the model-derived kinetic parameters are shown in **Table 4.** A substantial proportion of the material initially confined to the S_f >400 fraction was removed from plasma without being converted to smaller species or being transferred to a slow compartment, i.e., removed via k(0,1) (45% of the pool, ranging from 15 to 80%). Approximately one-fourth of the pool was converted to smaller species [via k(3,1), 23% (4–68)]. Another

TABLE 3. Distribution volumes (V_d) for RP in the S_f >400 fraction (compartments 1 and 2) and the S_f 60–400 fraction (compartments 3 and 4) obtained after fitting the data in the model

Subject	Plasma Volume	V_d S_f >400	V_d S_f 60–400
#	*liter*	*liter*	*liter*
1	2.81	5.24	5.19
2	3.60	7.59	3.47
3	3.50	5.49	1.33
4	3.22	3.56	3.36
5	3.40	6.35	6.56
6	3.36	5.24	3.52
7	3.30	3.91	3.07
8	3.70	6.03	3.10
9	3.10	5.29	2.33
10	3.31	7.78	4.77
11	3.26	5.70	2.61
12	3.35	5.72	4.10
Median (Range)	3.33 (2.81–3.70)	5.59 (3.56–7.78)	3.41 (1.33–6.56)

Plasma volume was calculated for each subject (14).

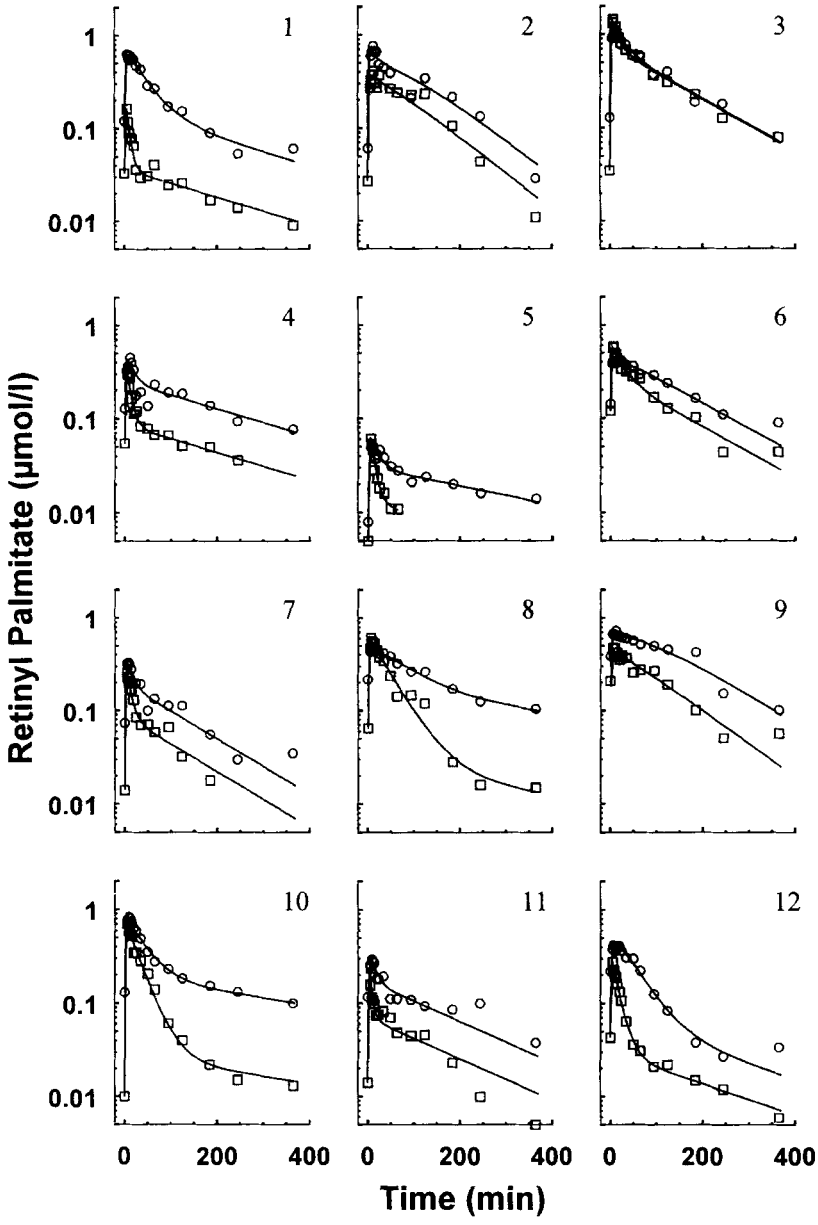

Fig. 3. The plasma clearance of RP from the S_f >400 and S_f 60–400 fractions in the twelve healthy male subjects were fitted to the model shown in Fig. 2. Datapoints are S_f >400 (□) and S_f 60–400 (○) RP levels. The solid lines show the fits obtained using the model, and the kinetic data are shown in Tables 2, 3, and 4.

fourth of the pool was converted to lipoproteins with the same density (flotation rate), but with a slower removal rate [via k(2,1), 22% (4–61)]. The residence time (RT) for chylomicrons/chylomicron remnants in the rapidly turning over compartment of S_f >400 fraction (compartment 1) was 15.8 min (6.2–86 min), which agrees well with other estimations of the turnover of the very large postprandial triglyceride-rich-lipoprotein particles. When the data were fitted to the model-derived equations, the distribution volumes for the different lipoprotein classes were freely adjustable. In all subjects the distribution volume of the S_f >400 fraction was larger than the estimated plasma volume with a median of 171% (110–235) of the plasma volume (Table 3).

The origin of RP-labeled material in the S_f 60–400 fraction was dual. First, some material was transferred from the S_f >400 fraction via k(3,1) and second, material was also injected directly into this subfraction. A majority of the material was rapidly removed [the RT in the rapid compartment of the S_f 60–400 fraction, compartment 3, was 25.7 min (9.7–103)] and a minor proportion was transferred to lipoprotein particles with a slow turnover, i.e., compartment 4. As previously shown, there was no further transfer of material downwards the lipolytic cascade, i.e., no material entered the S_f 20–60

TABLE 4. Model-derived kinetic parameters of chylomicron and chylomicron remnant metabolism

Subject #	S_f >400				S_f60–400			
	Fraction of Pool Transferred from			RT	Fraction of Pool Transferred from		RT	RT
	1 to out	1 to 2	1 to 3	Comp. 1	3 to out	3 to 4	Comp. 3	Comps 2 and 4
				min			*min*	*min*
1	0.15	0.17	0.68	7.5	0.82	0.18	41.0	285
2	0.35	0.61	0.04	85.7	0.37	0.63	89.5	77
3	0.48	0.42	0.10	15.3	0.61	0.39	19.2	157
4	0.45	0.20	0.36	9.3	0.61	0.39	12.6	295
5	0.45	0.15	0.40	10.5	0.62	0.38	14.5	429
6	0.40	0.39	0.21	26.9	0.30	0.70	20.0	161
7	0.56	0.25	0.19	9.9	0.60	0.40	10.7	147
8	0.72	0.04	0.25	48.2	0.78	0.22	52.3	545
9	0.30	0.55	0.15	67.3	0.46	0.54	103	118
10	0.80	0.04	0.17	30.4	0.83	0.17	31.4	542
11	0.40	0.26	0.34	6.2	0.62	0.37	9.7	197
12	0.34	0.09	0.57	16.3	0.91	0.09	43.4	256
Median (Range)	0.43 (0.15–0.80)	0.22 (0.04–0.61)	0.23 (0.04–0.68)	15.8 (6.2–85.7)	0.61 (0.30–0.91)	0.38 (0.09–0.54)	25.7 (9.7–103)	226 (99–545)

The S_f >400 and S_f 60–400 fractions contain one rapid (1 and 3) and one slow (2 and 4) compartment each. Direct removal from the fraction is designated 1 to out and 3 to out; S_f, Svedberg flotation rate; RT, residence time.

fraction from the S_f 60–400 fraction. The model-derived calculation of the distribution volume for the S_f 60–400 fraction was similar to the estimated plasma volume [110% (40–190%)].

Correlations between kinetic variables and plasma lipid and lipoprotein levels

Correlations between fasting plasma triglycerides, LDL and HDL cholesterol levels, and the rate constants were sought. The fasting plasma triglyceride level was negatively correlated (borderline significance) with the rapid removal out of compartments 1 and 3 (k(0,1) and k(0,3), $r = -0.55$, $P = 0.07$). There was no correlation between the triglyceride level and the rate of entry of material from the S_f >400 into the S_f 60–400 fraction [k(3,1)], ($r = -0.39$, $P = 0.19$). Using the peak postprandial triglyceride level gave essentially the same correlations. The rate of entry of material from the rapid compartment in S_f 60–400 to the slow compartment [k(4,3)] was negatively related (borderline significance) to the fasting triglyceride level ($r = -0.56$, $P = 0.06$), in contrast to the rate from compartment 1 into 2 [k(2,1)], ($r = 0.05$, $P = 0.86$). The LDL cholesterol level was significantly related to the transfer of material from S_f > 400 to S_f 60–400 [k(3,1)], $r = -0.60$, $P = 0.04$, but not related to any other kinetic parameter (**Fig. 4**). The HDL cholesterol level, on the other hand, did not correlate significantly with any kinetic parameter. The relative pool size of RP in S_f 60–400 (RP in the S_f 60–400 fraction/RP in whole plasma) seemed to be linked to the conversion rate of material from compartment 1 to 3 [k(3,1)] ($r = 0.58$, $P < 0.05$). This indicates that the conversion rate of material from the S_f >400 to the S_f 60–400 fraction is a major determinant of the pool size of S_f 60–400. It also indicates that the injected material is a mixture of already metabolized and de novo synthesized chylomicron/chylomicron remnant particles.

Primed infusion of a chylomicron-like triglyceride emulsion after injecting the chylomicron/chylomicron remnant-rich plasma

The modeling of turnover of chylomicrons/chylomicron remnants predicted a distribution volume for lipoprotein particles contained in the S_f >400 fraction that

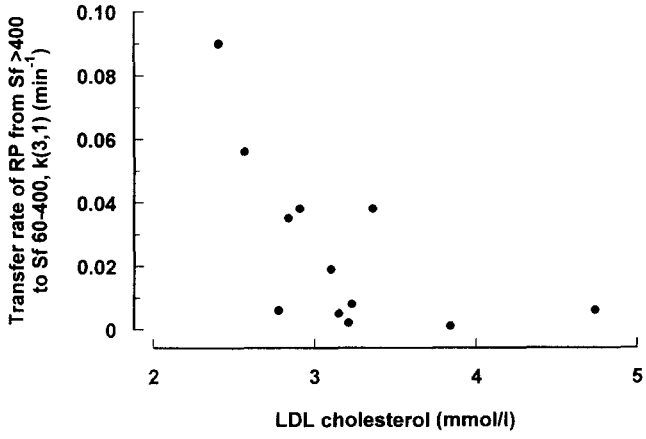

Fig. 4. Relationship between the transfer rate of RP from the S_f >400 fraction to the S_f 60–400 fraction and the LDL cholesterol concentration.

was 1.5–3 times larger than the plasma volume. This could hardly represent a miscalculation of the plasma volume. Instead, immediate uptake or loss of material or the presence of a reversible endothelial binding site for triglyceride-rich lipoproteins are reasonable causes for such an effect. In the latter case it should be possible to compete out the binding of endothelially attached chylomicrons with a chylomicron-like triglyceride emulsion. Accordingly, RP-labeled plasma was first injected, followed after 10 min by a primed infusion of Intralipid®. Three subjects (#5, #8, and #12) were asked to return for this study 3–6 months after the first study. During the first 10 min, RP in whole plasma, S_f >400, S_f 60–400, and S_f 20–60 fractions was cleared as in the first experiment (**Fig. 5**). Upon the immediate elevation of plasma triglycerides from 1.97, 0.87, and 0.82 mmol/l to 5.35, 4.04, and 3.03 mmol/l, respectively, with the primed infusion of Intralipid®, the total plasma RP level was hardly affected. In contrast, the emulsion induced a rapid increase of RP in the S_f >400 fraction. In fact, the height of this second peak exceeded the highest initial level of RP in the S_f >400 fraction in all subjects. In the S_f 60–400 fraction the RP increased somewhat less after injection of the emulsion while there were no major effects of the emulsion on the RP level in the S_f 20–60 fraction. These findings argue for a reversible, most likely endothelial, margination of large chylomicron/chylomicron remnants.

Two important conclusions might be drawn from these results. First, it is justifiable to use a theoretical distribution volume which is considerably larger than the estimated plasma volume in the kinetic calculations. Second, margination is a physiological entity and appears to be more pronounced for the very large chylomicrons/chylomicron remnants (S_f >400) compared to smaller species (S_f >400) compared to smaller species (S_f 60–400 and S_f 20–60).

DISCUSSION

The present study dealt with the intravascular metabolism of chylomicrons/chylomicron remnants in humans. Chylomicrons and their remnants were traced with RP. We show that the formation of smaller chylomicron remnants from larger species is limited, but not insignificant. Furthermore, the largest chylomicrons/chylomicron remnant species are not evenly distributed within the vascular system, rather they show signs of being marginated to the vascular wall.

The classic concept of how chylomicrons are metabolized indicates that extremely triglyceride-loaded chylomicron particles are secreted from the intestine. After they have acquired an appropriate composition of regulatory apolipoproteins (for example apoC-II), LPL-mediated lipolysis of the triglyceride core content takes place with the sequential formation of smaller triglyceride-depleted chylomicron remnants. These remnant particles have a more or less well-founded reputation of being vicious atherogenic lipoproteins. A major issue of the present investigation was to investigate the efficiency of this system, i.e., whether and, in that case, how the largest chylomicron/chylomicron remnants are sequentially delipidated forming smaller remnants. In a previous study on the postprandial lipoprotein metabolism in normal healthy men, we were surprised to see a delayed postprandial rise as well as a low absolute level of RP in the S_f 20–60 fraction after intake of RP together with a mixed meal (12). Furthermore, the apoB-48 level in that subfraction hardly increased in response to fat intake. This led us to hypothesize that very few of the largest chylomicron/chylomicron remnants eventually formed smaller chylomicron remnants, rather they were removed from plasma after transient lipolysis as large remnants (remnants formed already in the S_f >400 fraction or in the S_f 60–400 fraction). This concept is in accord with two recent studies performed in rats (25, 26). First, compartmental analysis of the disappearance of triglycerides and retinyl esters from labeled chylomicrons indicated that rat chylomicron remnants left blood with approximately half the original triglyceride content retained within the particle (25). Second, Windler and coworkers (26) have demonstrated that very large chylomicron remnants are rapidly removed via a non-LDL-receptor pathway, in contrast to small chylomicron remnants, which were slowly removed through the LDL receptor.

Berr and coworkers (8) concluded that there was a complete dissociation between the disappearance of RP in d < 1.006 kg/l and appearance of RP in d > 1.006 kg/l, indicating removal of RP material from d < 1.006 kg/l without conversion to d > 1.006 kg/l, i.e., loss of substrate/product relationship between the fractions. In retrospect this is not very surprising as d 1.006 kg/l indicates the border between VLDL and IDL/LDL. From other studies it is known that there is almost no apoB-48 in the IDL/LDL fractions (12,14) and the appearance of RP in this fraction is more likely to depend on RP being transfered by cholesteryl ester transfer reactions (27, 28).

A comparison with VLDL (the apoB-100 system) is justified. It has recently been shown that the smallest VLDL particles (S_f 20–60 fraction) are direct precursors of LDL (29). These particles have approximately 5–10,000 triglyceride molecules per particle and roughly 90–95% of these have to be lipolyzed in order for the particle to attain the density and size of LDL. The liver

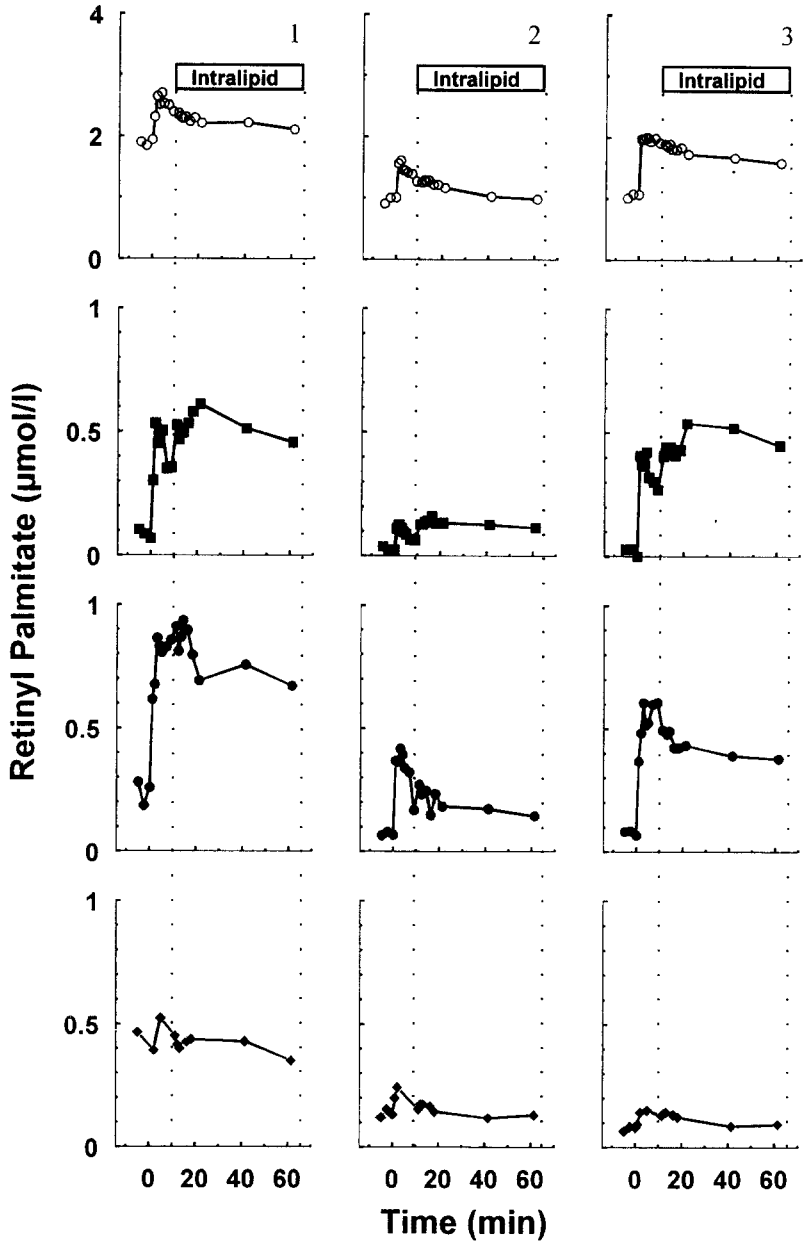

Fig. 5. RP-labeled plasma was returned after a second oral fat load and plasmapheresis on a separate occasion to subjects #5, #8 and #12. At time +10 min a bolus dose of Intralipid was administered intravenously (0.15 g/kg body weight), immediately followed by a constant infusion of 0.15 g/kg per h. The RP contents in plasma (○) and $S_f > 400$ (■), S_f 60–400 (●), and S_f 20–60 (◆) fractions are shown in the figure.

also secretes large VLDL (S_f 60–400) particles, which typically have 15–20,000 triglyceride molecules per particle, but these very triglyceride-rich lipoprotein particles seem to have difficulty in efficiently forming LDL (30), in particular in hypertriglyceridemic subjects. Rather, they are, to a major extent, removed from plasma as VLDL remnants together with a considerable amount of core triglycerides. In this context it is not surprising that chylomicrons with an estimated triglyceride content of 200,000–1,000,000 triglyceride molecules per particle (S_f >400 particles) have difficulty in forming S_f 60–400 or S_f 20–60 particles.

We detected substantial amounts of RP in the S_f 60–400 fraction, and the ratio between RP and apoB-48 implies that these RP-labeled particles have not been formed from the S_f >400 fraction (substantially fewer number of RP molecules per apoB-48, a constant amount of the core label would indicate a substrate/product relationship between S_f >400 and S_f 60–400 fraction). How is that? The rate of apoB-48 synthesis from enterocytes is essentially not influenced by the lipid flux over the cell (31, 32). Instead, when there is an increased demand for lipid transport, each apoB-48-containing lipoprotein carries more lipid. Thus, there is a transition from secretion of small apoB-48-containing lipoproteins in the fasting state to secretion of very large

particles in the fed state, albeit some degree of secretion of small apoB-48-containing particles seems to persist in the fed state (33). Similarly, lymph chylomicrons in the early postprandial phase are smaller than those in the late state (34). The proximal intestine seems to be more capable of transporting lipids compared to the distal part, in which intracellular lipids accumulate after a long-term intraluminal triglyceride infusion (35). In summary, there are certainly a number of indications in the literature pointing in the direction of considerable heterogeneity in size of the apoB-48-containing lipoproteins secreted from the intestine. Accordingly, we assume that the intestine is secreting both very large chylomicrons (S_f >400) and smaller apoB-48-containing lipoprotein particles (S_f 60–400) in the fed state and that the latter particles carry fewer RP molecules per particle compared to the larger ones (most likely the same ratio between triglycerides and RP as in large chylomicrons).

In order to be hydrolyzed by LPL, the chylomicron has to attach to the vascular endothelium. This study shows that the largest chylomicron/chylomicron remnants (S_f >400) have a greater tendency to attach to the endothelium than the RP-containing particles in the S_f 60–400 fraction. Furthermore, S_f >400 chylomicron/chylomicron remnants have a shorter RT in plasma. Thus, it appears that the increased contact with LPL also decreases the time that the larger chylomicron/chylomicron remnants are present in plasma. The action of LPL on the S_f >400 particles is the prerequisite for the formation of smaller chylomicron remnant particles, i.e., the appearance of material in the S_f 60–400 fraction. Theoretically, there are a number of possible determinants for the relative efficiency of the lipolytic pathway. Choi and coworkers (36) have shown that apoB and LPL have a protein–protein interaction, which may indicate that the LDL level is of importance in determining the relative occupancy of possible LPL binding sites. We were therefore intrigued by the negative relation between the LDL cholesterol level and the rate of transfer of RP from the S_f >400 to the S_f 60–400 fractions. Subjects with a high LDL cholesterol level had slower lipolytic conversion of the largest chylomicrons/chylomicron remnants (S_f >400 fraction) to the smaller chylomicron remnant species (S_f 60–400 fraction). This supports the notion that the total apoB level in plasma is an important determinant for the efficiency of the lipolytic system. This finding may have substantial implications for subjects with excessively elevated LDL apoB levels as, for example, patients with familial hypercholesterolemia or hyperapobetalipoproteinemia.

Use of RP as surrogate marker for chylomicrons/chylomicron remnants have potential pitfalls. Indeed, RP is an indirect measure of chylomicron/chylomicron remnant particles. RP is known to exchange between lipoprotein particles in humans, a sign of which is the appearance of RP in apoB-100 particles (28). However, we do not think that this is a problem in the present study as the metabolic fate of the majority of infused material is rapid, while the exchange of RP between lipoprotein particles is a slow process reaching significance only in the late postprandial phase (6–8 after fat intake) (28). During storage, RP was probably exchanged to some extent but, as shown in Table 1, this did not seriously affect the distribution of RP between the different fractions. Most likely the collected plasma was already in equilibrium; thus, the storage did not affect the net distribution of RP between the different fractions. RP has been argued to be sensitive to LPL-mediated hydrolysis (37). It was, however, shown in vitro by Hultin, Savonen, and Olivecrona (25) that the LPL-mediated consumption of RP starts only at the point when triglyceride stores have been depleted. This situation is not likely to occur under the present experimental conditions.

The use of RP as a marker of chylomicron/chylomicron remnant particles, as in the present study, has one inherent problem. If it is assumed that an S_f >400 particle is converted to an S_f 60–400 particle, this is the effect of LPL, i.e., loss of a number of triglyceride molecules but retaining almost all RP molecules. As an average S_f >400 particle has considerably more RP molecules per particle compared with a S_f 60–400 particle, the transferred particles should have more RP molecules per particle compared with the native S_f 60–400 particles. In the next step, the S_f 60–400 particles can either be converted to a slow compartment or be removed from plasma without being converted to S_f 20–60 particles, but theoretically this pool is now heterogeneous. Accordingly, the relative pool mass transfer from the S_f > 400 to the S_f 60–400 fraction, which was 23% (4–68) of all RP in the S_f >400 fraction, may be grossly overestimated in terms of number of transferred lipoprotein particles.

Knowing the relationship between RP and apoB-48 in the fractions made it possible to calculate the transport rates of apoB-48 in the S_f >400 and S_f 60–400 fractions from the present data. When the FCR of the S_f >400 fraction (compartments 1 and 2) was multiplied by the apoB-48 content of the fraction, it was calculated that 1.03(0.20–20.3) μg apoB-48 was catabolized per min from the S_f >400 fraction. The corresponding value for S_f 60–400 fraction was 5.7(0.16–20.9) μg/min. Less than a quarter of the RP material in the S_f >400 fraction was transferred to the S_f 60–400 fraction, making the apoB-48 mass contribution of the larger to the smaller chylomicron species very marginal. A previous

study dealt with the transport rate of apoB-48 using endogenous labeling of apolipoproteins with stable isotopes (38). The described turnover rates of apoB-48 were approximately 4 pools/day, which seems to be a very slow course for a metabolic event with a known $t_{1/2}$ of minutes. One explanation for this is that the stable isotope technique is not suitable for tracing very rapid metabolic processes. However, it is interesting to note that the RT values for the slow compartments in the present study agree well with findings of Lichtenstein and coworkers (38). This may depend on the fact that the technique used by Lichtenstein and coworkers is only accurate for tracing the slow components of the chylomicron turnover system.

There are two potentially important implications of the present study. First, a majority of the chylomicrons are removed from plasma as very large chylomicrons/chylomicron remnants, long before they have reached a size that could implicate them in atherogenesis, i.e., the S_f 60–400 or the S_f 20–60 fraction (39). In fact, data arguing for this phenomenon have also been derived from studies on chylomicron turnover in rabbits (40). Second, margination of chylomicron/chylomicron remnants seems to be a physiological phenomenon, which in itself is not surprising as chylomicrons must have physical contact with the endothelial-bound LPL. Margination needs to be taken into account in future kinetic studies of triglyceride-rich lipoproteins. It is quite clear that for these rapidly metabolized lipoprotein particles it is inaccurate to assume that the plasma volume is equal to the distribution volume.

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