Characterization of chylomicron remnant clearance by retinyl palmitate label in normal humans[1]

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Abstract To characterize chylomicron remnant clearance by the liver, plasma elimination of retinyl palmitate-labeled chylomicron remnants was studied in 18 healthy subjects, ages 21–42 years. Autologous plasma containing retinyl palmitate-labeled chylomicrons and their remnants was injected intravenously, and retinyl palmitate disappearance was measured in serial plasma samples in all subjects and in lipoprotein fractions in 11 subjects. The injected doses (n = 18) ranged from 0.34 to 7.11 μmol retinyl palmitate in d ≤ 1.006 g/ml particles with an average molar ratio of 330/1 of retinyl palmitate/apoB-48 (n = 8). The label distributed in the intravascular space and exhibited apparent first order elimination, monoexponential in 6 and biexponential in 12 subjects. The first rapid component k_1 ($t_{½}$ 18.8 ± 11.4 min, n = 18) was shown to represent retinyl palmitate in particles of d ≤ 1.006 g/ml, i.e., chylomicron remnants, and the second slow component k_2 ($t_{½}$ 123 ± 62 min, n = 12) small amounts of retinyl palmitate (11 ± 7%) injected in d > 1.006 g/ml particles (therefore excluded from analysis). Assuming a single-compartment model, initial rates of elimination (= dose × k_1) of labeled chylomicron remnants obeyed (P = 0.06) Michaelis-Menten saturation kinetics: K_m was 921 ± 305 nmol retinyl palmitate label and V_{max} 124 ± 14 nmol/min corresponding to 0.88 nM apoB-48 for K_m and 0.25×10^{-3} nmol apoB-48 · min^{-1} · g^{-1} liver for V_{max}. Their elimination was limited neither by the injected triglyceride dose nor theoretically by the liver blood flow. After the intake of 70 g of fat (cream) containing retinyl palmitate, the plasma retinyl palmitate concentration exceeded the estimated saturation concentration for 7 h. In conclusion, physiological chylomicron remnant catabolism by the liver appears to be saturable by ordinary lipid intake in healthy humans.—**Berr, F.** Characterization of chylomicron remnant clearance by retinyl palmitate label in normal humans. *J. Lipid Res.* 1992. **33:** 915–930.

Supplementary key words apoB-48 • apoE isotypes • alimentary lipemia

Dietary and biliary cholesterol are absorbed into the intestinal mucosa, esterified, and incorporated into chylomicrons which are transported to plasma in lymph. Apolipoprotein C-II is added to the chylomicrons and core triglyceride is hydrolyzed by lipoprotein lipase, leaving remnants with cholesteryl ester in their core (1–4). Chylomicron remnants are removed from plasma mainly by the hepatocytes by a receptor-mediated process (4–7).

The rate and regulation of plasma clearance of chylomicron remnants have not yet been defined in the intact organism. Studies of the isolated perfused rat liver showed saturation kinetics for hepatic uptake of chylomicron remnants (5, 8). Elimination half-times for small doses of remnants were 2–10 min, probably close to maximum velocity (9–11). In intact animals (12, 13) and humans (14–21), by contrast, the reported half-times of plasma removal of chylomicron remnants range from 10 to 53 min. But the critical issue has not been addressed: whether in the intact organism this clearance process has limited capacity saturable by a physiologic load of chylomicron remnants.

The kinetics of plasma clearance of chylomicron remnants in humans has not been systematically investigated for dose-dependence, because a suitable labeling technique has not been available. Exogenous labeling of apoB-48 in chylomicrons with radioiodine is unsatisfactory; less than 10% of the radioiodine is incorporated specifically into apoB-48 and more than 90% into other, exchangeable apolipoproteins and lipid components (14, 16). Vitamin A esters, especially retinyl palmitate, have been used in rabbits (22) and later in humans (17, 21, 23–28) as endogenous label of the core of chylomicron remnants. After a vitamin A test meal (23–28), the course of the retinyl palmitate plasma concentration reflects the balance between input of retinyl palmitate-labeled chylomicrons into the plasma and formation and elimination of their remnants. Specifically the elimination of the retinyl palmitate label was studied with the intravenous clearance test after injection of labeled chylomicrons in a bolus of autologous plasma that had been harvested 2 days earlier

Abbreviations: RP, retinyl palmitate; VLDL, very low density lipoproteins (d < 1.006 g/ml): LDL, low density lipoproteins (d 1.019–1.063 g/ml): HDL, high density lipoproteins (d 1.063–1.21 g/ml); apo, apolipoprotein; k_e, first order decay constant.

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by plasmapheresis after a vitamin A meal (17–19). The label apparently distributed in the intravascular space and disappeared by a mono- or biexponential first order process (17, 18). The half-time of the major, rapid plasma disappearance increased with the administered dose (17, 18) suggesting saturable elimination. However, chylomicron remnant elimination rates could not be calculated, since it was uncertain whether the second, slow plasma disappearance, mainly of label in d > 1.006 g/ml lipoproteins (18, 19), also reflected chylomicron remnant removal. Which kinetic process represents the initial rate of plasma removal of chylomicron remnants? Do the two kinetic components reflect a single elimination process with lipolysis of d ≤ 1.006 to d > 1.006 g/ml particles prior to removal? Or do they represent two labeled particle classes of d ≤ 1.006 and d > 1.006 g/ml cleared at different rates?

To characterize the elimination process, the distribution of retinyl palmitate in lipoproteins of injected plasma, its relation to the chylomicron remnant marker apoB-48 (29–32) and the hepatic lipoprotein marker apoB-100 (32, 33), and the clearance of retinyl palmitate from d ≤ 1.006 and d > 1.006 g/ml lipoproteins were investigated. Based on these findings a kinetic model was postulated to calculate the rate of plasma elimination of chylomicron remnants and analyze its dose dependence for all published studies (17–19) and three additional studies of high doses of label.

METHODS

Subjects

The protocol was approved by the Human Subjects Committee of the University of Colorado School of Medicine and written informed consent was obtained prior to study. All participants were healthy, paid volunteers, ages 21 to 41 years (**Table 1**). In women, studies were done during the first 7 days of the menstrual cycle and a rapid HCG test was performed to exclude pregnancy. The participation in previous protocols is listed in Table 1; only control studies were evaluated for saturation kinetics.

Data from 18 healthy subjects are reported. They include 15 subjects previously reported (17–19) (#3 and #6, whose data (17) were not suitable for computer curve fitting, were excluded) and three additional subjects. Data from all 18 subjects were used for the kinetic analysis of plasma elimination of chylomicron remnants. Plasma retinyl palmitate disappearance in individual lipoprotein fractions was analyzed in 11 subjects.

Study design

All subjects underwent the study according to the same uniform protocol, which has been described in detail (17). After an overnight fast, a large dose of retinyl palmitate (60 mg/m² body surface area as retinol equivalent, i.e., 200,000 I.U. vitamin A /m²) was given orally with cream (39% fat) (100 ml/m² body surface area). At 5–6 h after intake, i.e., at peak serum concentration of retinyl palmitate, 2 units of lipemic plasma were obtained by plasmapheresis (17). The plasma was stored in ACD buffer, pH 7.0, light-shielded, at room temperature for 42 h. At that time all retinyl palmitate given on day 1 had been cleared from the subject's circulation. Concentrations of retinyl palmitate, cholesterol, and triglycerides and the distribution of retinyl palmitate (n = 18) and apolipoproteins B-48 and B-100 (n = 8) in lipoprotein fractions were measured in the stored plasma. The autologous plasma (range 180–643 ml) was pulse-injected into an antecubital vein within less than 3.3 min at a rate of 142 ± 36 ml/min. Except for transient symptoms (<5 min) of hypocalcemia (hot flushes, paresthesias) no adverse reactions occurred. Frequent blood samples were obtained during the next 4 h for measurement of retinyl palmitate concentration in plasma and, in four studies (#s 15, 18, 19, 20), in d ≤ 1.006 g/ml lipoprotein precipitates as well as in d > 1.006 g/ml infranatants. In 11 studies (Table 1, #10–#20), additional blood samples were drawn for separation of lipoproteins by ultracentrifugation and measurement of retinyl palmitate in each. ApoB-48 and apoB-100 were determined in samples from five of these subjects (12, 15, 16, 17, 19). All subjects fasted during the procedure.

The retinyl palmitate concentration of labeled plasma was 5866 ± 3989 nM (n = 18) and the amount of retinyl palmitate given was 0.36 to 7.33 μmol, as calculated from the volume of plasma injected multiplied by its retinyl palmitate concentration. The injection of 387 ± 170 ml plasma (equivalent to 8.6 ± 4.3% of the estimated blood volume) lowered the hematocrit from 44.2 ± 4.5% to 39.3 ± 5.0% at 10 min after injection (n = 18); it returned 33% toward baseline during the remaining 3.5 h of study.

A single subject (#20) was studied three times the same day, with increasing doses of retinyl palmitate, which had been injected with increasing bolus volumes of the same, pooled autologous plasma (stored for 42, 43.5, and 46 h, respectively). After 46 h storage, 97% of the injected retinyl palmitate had been in d ≤ 1.006 g/ml lipoproteins.

Two additional healthy subjects took the standard amount of retinyl palmitate in cream (70 g fat), as used with the test meal for endogenous labeling, and plasma retinyl palmitate levels were measured for 24 h.

Analyses

All plasma samples were stored in the dark at −20°C prior to analysis. Retinyl palmitate concentration in plasma and in individual lipoprotein fractions was measured by reverse-phase high performance liquid chromatography as previously described (17, 18). Lipoproteins of

TABLE 1. Characteristics of study volunteers and their participation in study protocols

Subject No.	Protocol Participation[a]	Age	Height	Body Weight	Ideal Weight[b]	Serum Lipid Levels			Apoe Isotype
						TG	Chol	HDL Chol	
		yr	*cm*	*kg*	*%*		*mg/dl*		
1 m	1,5	23	163	59	105	75	223	63	
2 f	1,5	25	163	57	97	135	204	65	4/3
3 f	1	24	182	68	100	44	179	54	
4 f	1,5	32	162	67	118	78	236	65	3/3
5 m	1,5	30	178	66	94	36	156	63	
6 m	1	25	183	75	101	57	208	42	
7 m	1,5	21	172	65	100	115	200	52	4/3
8 m	1,5	23	180	72	101	130	216	52	3/3
9 f	2,5	24	159	66	119	158	162	44	2/3
10 m	2,5	26	190	75	97	75	125	35	3/3
11 f	2-5	28	155	54.5	109	70	108	62	
12 f	2-5	25	156	69.5	124	66	175	49	3/3
13 m	2,5	35	186	68	94	66	120	38	
14 f	3-5	26	170	55	96	58	173	57	
15 f	3-5	41	163	50	93	58	159	46	3/3
16 f	3-5	29	164	61	111	83	186	41	4/2
17 f	3-5	40	164	51	97	66	179	57	4/3
18 m	4,5	24	173	74	108	85	173	43	3/3
19 f	4,5	25	161	53	100	55	135		3/3
20 m	4,5	25	182	69	98	102	146	52	3/3
Median	n = 18	26	164/170	66	101	75	173	52	
Range	10 f,8 m	21-41	155-190	50-75	94-125	36-158	108-236	35-65	

Abbreviations: TG, triglycerides; Chol, cholesterol; m, male; f, female.

[a]Study protocol numbers: 1, first study protocol (17); 2, heparin study protocol (18); 3, contraceptive steroid study (19); 4, study of kinetic heterogeneity in lipoprotein fractions (this paper only); 5, dose-dependency study (this paper only).

[b]Statistical Bulletin #40; Metropolitan Life Insurance Co., Nov.-Dec., 1959.

d ≤ 1.006 g/ml were precipitated using a modification (34) of the method of Burstein, Scholnick, and Morfin (35). For 1.0-ml plasma samples (containing 1.5% EDTA), 50 μl of 5% sodium heparin and 60 μl of 2 M $MgCl_2$ were used. The samples were vortexed 2 min, allowed to precipitate 30 min, and centrifuged 10 min at 11,000 *g* in a Microfuge (Beckman Instruments, Palo Alto, CA). The infranatant solution was carefully aspirated and checked for lack of precipitate. The precipitates were resuspended in 800 μl 0.02 M Tris-HCl buffer (pH 7.7) containing 0.6% NaCl and 0.5% sodium citrate and the flotation step was repeated 3 times. Intraassay coefficient of variation for retinyl palmitate measurement was 8.4% in precipitates and 13.2% in infranatants (n = 10). Recovery was 94.5 ± 9.1%. The following lipoprotein classes were isolated by sequential ultracentrifugation (36) from the top of a discontinuous salt gradient (13.5 ml, d 1.006-1.10 g/ml; rotor SW 40.1, Beckman Instruments) (17): chylomicrons (4.5×10^6 *g*-min; d < 0.90 g/ml); VLDL fraction A (17.5×10^6 *g*-min; ca. d 0.933 g/ml); VLDL fraction B (31.2×10^6 *g*-min; ca. d 0.957 g/ml); VLDL fraction C (152×10^6 *g*-min; ca. d 0.967-1.006 g/ml). After the last centrifugation step, the gradient was fractionated (2 ml VLDL fraction C; 3 ml approximate density 1.010-1.020 g/ml; 2.5 ml visible LDL band; 2 ml d 1.06-1.09 g/ml; 4 ml plasma infranatant d 1.09-1.10 g/ml). Recovery of retinyl palmitate from ultracentrifugation ranged from 88% to 105%.

ApoB-48 and apoB-100 were separated by SDS polyacrylamide gel electrophoresis. Lipoprotein fractions were dialyzed against 5 mM ammonium bicarbonate containing 0.02% EDTA and 0.02% sodium azide at 4°C and delipidated 3-5 times with three fold excess of diethyl ether. Protein concentration was quantitated (37) using a colorimetric kit with bovine serum albumin as standard (Protein Microassay[R], Bio-Rad Laboratories GmbH, Munich, Germany). Protein content of lipoprotein fractions was corrected for volume changes (predialysis to postdelipidation), and by the factor 1.67 for the fraction of apoB (vide infra), to account for the diminished color response of human apoB standard (Sigma Chemical Co., St. Louis, MO) compared to bovine serum albumin. Protein concentrations (μg/ml) in lipoprotein fractions of postprandial plasma injected for clearance studies (n = 8) were as follows: chylomicrons 18 ± 9, VLDL A 14 ± 6.5, VLDL B and C 125 ± 10, LDL fraction (d 1.02-1.06 g/ml) 421 ± 56. Lipoprotein aliquots (5-15 μg) were lyophilized, heated for 10 min at 95°C in buffer (0.5 mM EDTA, 1% SDS, 5% glycerol, 5% mercaptoethanol; pH 8.3), applied to 0.75 mm wide, 1.0-20% polyacrylamide gradient gels using a Hoefer SE-500 slab gel apparatus (Hoefer, San Francisco, CA), and electrophoresed (38). Gels were stained with Coomassie blue (29), destained in 35% methanol with 10% acetic acid, and restained with silver nitrate (39) (staining kit, Bio-Rad Laboratories GmbH, Munich, Germany) (**Fig. 1**).

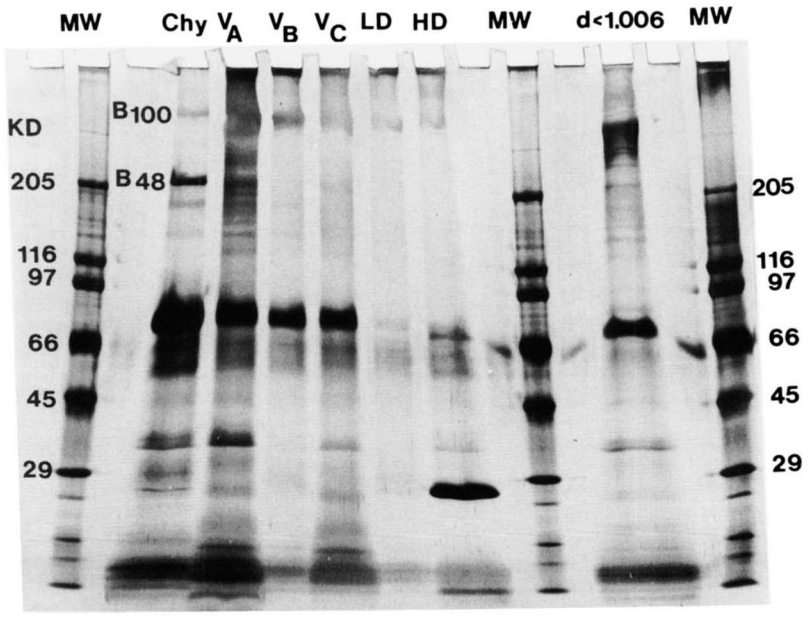

Fig. 1. Electrophoretic separation of apolipoproteins B-48 and B-100 in lipoproteins obtained from stored plasma (subject #12). The plasma had been harvested by plasmapheresis 4 h after intake of vitamin A in cream and stored for 46 h; prior to reinjection into the donor, an aliquot was taken for ultracentrifugation of lipoprotein fractions and analysis by SDS-PAGE (1–20% gradient gel; silver stain) as described in Methods. Abbreviations: MW, standard of molecular weight markers (29,000–205,000); d $\leq$ 1.006, standard of d $\leq$ 1.006 g/ml lipoproteins from postprandial plasma; lipoprotein fractions (10–12 μg protein/sample): Chy, chylomicron; V_A, VLDL A; V_B, VLDL B; V_C, VLDL C; LD, LDL (3 μg protein); HD, d 1.06–1.09 g/ml.

ApoB-100 and apoB-48 bands were identified with reference to a molecular weight marker protein standard (Sigma Chemical Co., St. Louis, MO) and a lipoprotein standard obtained from the d < 1.006 g/ml fraction of postprandial plasma. As the densitometry values (ELSCRIPT densitometer; Fa. Hirschmann, D-8010 Unterhaching, Germany) of the bands of apoB-48 and apoB-100 were in agreement between the Coomassie stain and the silver stain of the lipoprotein standard on the gels, the apoB fractions of the more sensitive silver stains were multiplied by the total protein concentration (μg/ml) for each lipoprotein sample to obtain the concentrations of apoB-100 and apoB-48, which then were converted to the nanomolar concentrations. The lower limit of detection was 1 nM apoB-48. The interassay (n = 8) coefficient of variation was 8.5% for total protein and 14.5% for apoB-48 in the d < 1.006 g/ml lipoprotein standard. Apolipoprotein E (apoE) phenotyping was performed on the serum of 13 subjects (40) by Dr. H. J. Menzel, Innsbruck. Plasma triglyceride and cholesterol concentrations were measured by standard techniques (41).

Pharmacokinetic analysis of retinyl palmitate plasma disappearance curves

After subtraction of the baseline level, the retinyl palmitate concentrations in plasma or in the d $\leq$ 1.006 g/ml lipoprotein fractions were tested for fit of the entire curve to a mono-, bi-, or triexponential function using an exponential stripping computer program (42), and the best fit was chosen (43). Final fit of the resulting mono- or biexponential function to the data was computed with a nonlinear least squares program (44). The apparent volume of distribution, V_d, was calculated as the dose divided by the y-intercept of the total plasma retinyl palmitate disappearance curve, and it was compared to the plasma volume estimated from age, sex, and body weight (45). When the analysis indicated biexponential disappearance, the contribution of each slope to total plasma retinyl palmitate clearance was calculated from the respective first order rate constant, k_e, divided by the sum of both k_es; this formula (18, 19) is derived from the formula of the plasma clearance of retinyl palmitate ($= V_d \times k_e$) on the assumption (46, 47) of a common V_d for all labeled lipoproteins of d > 1.063 g/ml. Bivariate relationships were tested by linear regression analysis (48).

MODEL DEVELOPMENT

General assumptions

Chylomicron remnants were defined functionally as the rapidly cleared remnants of chylomicrons that had been endogenously labeled in the core with retinyl palmitate. The use of retinyl palmitate as endogenous label of

chylomicrons and their remnants in humans is based on the following assumptions (supported by the references): incorporation of retinyl palmitate selectively into the core of chylomicrons prior to secretion by the enterocytes (49); negligible intravascular hydrolysis of retinyl palmitate (17), or extrahepatic removal of labeled chylomicron remnants (4, 49, 50); low transfer to other lipoproteins (17); complete plasma clearance of chylomicron remnants by hepatocytes (49–51); no resecretion of retinyl palmitate by the liver (17, 49, 52) except in the dog (13); and virtually no background level of plasma retinyl palmitate in fasting humans (17). These assumptions are widely accepted (17, 21, 23–26). The disappearance of retinyl palmitate after intravenous injection of labeled chylomicrons in autologous plasma complies with two assumptions: *a)* the retinyl palmitate-labeled lipoproteins rapidly (within 5 min) distribute virtually in a single compartment, the intravascular space (17–19); *b)* the elimination step apparently is rate-limiting for plasma removal of retinyl palmitate (18).

Model selection

Plasma disappearance of retinyl palmitate label obeyed biexponential first order kinetics in the majority of previous studies (17–19) (e.g., Fig. 4, left upper panel). The following models were considered to explain the two exponentials k_1 and k_2 of these curves.

1. The two-compartment model for a single class of particles. All retinyl palmitate-labeled particles are cleared by a single kinetic process (slope k_2) from a central compartment, e.g., the blood circulation, but distribute also into a second compartment, e.g., the extravascular plasma volume (k_1 = slope of net exit into the extravascular plasma space).

2. The single compartment model for two serial processes. The first slope k_1 represents transfer of label to $d > 1.006$ g/ml lipoproteins (by lipolysis of chylomicrons to $d > 1.006$ g/ml particles or transfer of retinyl palmitate to $d > 1.006$ g/ml lipoproteins of hepatic origin); the slope k_2, their elimination.

3. The single-compartment model with two clearance processes. A single class of labeled particles is cleared simultaneously by two processes.

4. The single-compartment model for two different particle classes. The two kinetically different retinyl palmitate-labeled particle classes (approximated by $d \leq 1.006$ g/ml lipoproteins and d 1.006–1.063 g/ml lipoproteins) are cleared from a common volume of distribution, the intravascular plasma volume.

Virtually all retinyl palmitate resides in lipoproteins of $d > 1.063$ g/ml and only a minor fraction of it (2–20%) is in d 1.006–1.063 g/ml lipoproteins in the plasma (17–19). Since most (>66%) of the small particles (d 1.019–1.063 g/ml) and nearly all of the $d \leq 1.006$ lipoproteins are confined to the intravascular space (46, 47), the label should distribute almost exclusively in the intravascular compartment (models #2–#4), but not in the extravascular compartment (model #1). The first slope (k_1) of retinyl palmitate disappearance cannot be explained by intravascular processes such as remnant formation or transfer of label (model #2), since *a)* such a process per se would not lower retinyl palmitate concentration; *b)* triglyceride lipolysis (the step of remnant formation) was not rate-limiting for plasma removal of retinyl palmitate (18); and *c)* the label in $d \leq 1.006$ and that in $d > 1.006$ g/ml lipoproteins did not show a kinetic precursor-product relationship during the rapid decrease k_1 (compare Fig. 4). In case of simultaneous clearance of a single class of labeled particles by two different processes (model #3), the first order elimination rates of the two clearance processes would superimpose and yield a monoexponential curve of retinyl palmitate plasma removal.

The single-compartment model with two kinetically different, labeled particle classes (model #4) was accepted for the following reasons. *a)* The intravascular plasma space apparently is the single compartment for virtually all labeled lipoproteins of $d > 1.063$ g/ml (46, 47) during this test (**Fig. 2A**). *b)* Given the presence of a single compartment, only model #4 complies with the kinetic heterogeneity of labeled lipoprotein classes (compare Results, Fig. 3 and 4) and with the associations shown in Fig. 5.

Compartmental model for retinyl palmitate-labeled chylomicron remnants

In accordance with the stated functional definition of chylomicron remnants, their kinetics were estimated from the major, rapid slope of plasma retinyl palmitate elimination (model assumption #1) and from the amount of retinyl palmitate contained in the $d \leq 1.006$ g/ml fraction of the injected lipemic plasma; this amount approximates the retinyl palmitate contained in chylomicron remnants (model assumption #2). The following equations were defined.

Dose of labeled chylomicron remnants (RP,CMR):

$$\text{dose}_{\text{RP,CMR}} = \text{dose}_{\text{RP}} \times \text{fraction}_{\text{RP in } d \leq 1.006} \qquad \textit{Eq. 1)}$$

Apparent volume of distribution of chylomicron remnants:

$$V_d = \frac{\text{dose}_{\text{RP,CMR}}}{RP_0} \qquad \textit{Eq. 2)}$$

Apparent chylomicron remnant clearance (Cl_{CMR}):

$$Cl_{CMR} = V_d \times k_1 \qquad \textit{Eq. 3)}$$

where RP_0 is the y-intercept (nmol/ml) and k_1 the slope (min^{-1}) of the rapid kinetic component of retinyl palmitate decay, dose_{RP} the nmol RP injected, and V_d the apparent volume (ml) of distribution of RP. Blood clearance

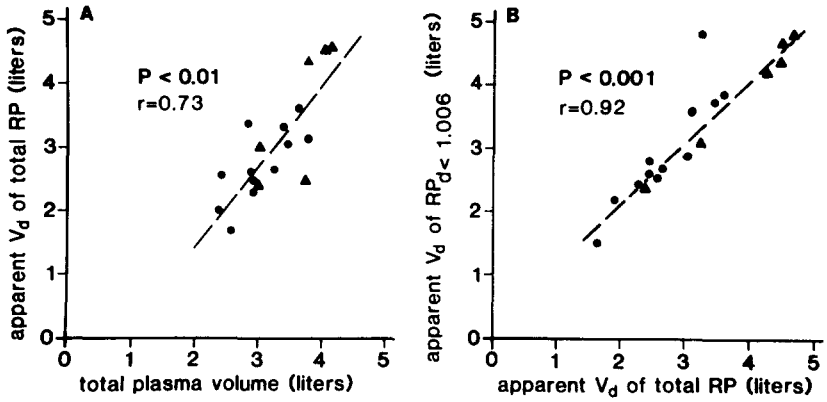

Fig. 2. Correlation of apparent volume of distribution (V_d) of plasma retinyl palmitate (RP) with estimated plasma volume (panel A), and correlation of apparent V_d of retinyl palmitate in d < 1.006 g/ml lipoproteins with apparent V_d of total plasma retinyl palmitate (panel B). Total plasma volume is the sum of estimated (45) and injected plasma volume. Retinyl palmitate in d ≤ 1.006 g/ml lipoproteins reflects retinyl palmitate-labeled chylomicron remnants (compare equation 1). Correlations were as follows: panel A: y = −1.16 + 1.28x, n = 18; panel B: y = 0.15 + 1.0x, n = 18. (▲), Monoexponential studies; (●), biexponential studies.

rates were calculated from plasma clearance rates and the hematocrit determined 30 min after plasma injection (53).

Apparent initial rate of chylomicron remnant elimination (R_{CMR}):

$$R_{CMR} = dose_{RP,CMR} \times k_1, \qquad Eq.\ 4)$$

given in nmol $RP_{d \leq 1.006}$ cleared per min.

The apparent rate of chylomicron remnant elimination of the participants was analyzed for dependence on the injected dose of retinyl palmitate-labeled chylomicron remnants. Apparent maximal elimination rate, V_{max}, and half-saturation constant, K_m, were calculated from the Michaelis-Menten equation (54) by nonlinear least squares regression (55). Quality of fit of the curve to the data was determined by analysis of variance (48).

According to this model, the second slope of biexponential retinyl palmitate disappearance represents elimination of labeled d > 1.006 g/ml lipoproteins, which had probably been injected with the autologous plasma. These could represent a subset of slowly cleared chylomicron remnants or other lipoproteins labeled by transfer of retinyl palmitate. Compartmental analysis is analogous to equations #1-#3 using the retinyl palmitate dose injected in d > 1.006 g/ml lipoproteins.

RESULTS

Composition of injected plasma (Table 2)

The chylomicrons and VLDL fractions contained 89 ± 7% of the retinyl palmitate. In studies with monoexponential retinyl palmitate elimination (n = 6), the proportion of retinyl palmitate in these fractions was even higher (96 ± 3%). In 12 studies with biexponential retinyl palmitate decay, more than 6% of the administered retinyl palmitate (mean, 11 ± 7%) was in the d > 1.006 g/ml

TABLE 2. Composition of the injected plasma

	Plasma Total Dose	Chylomicrons	VLDL A	VLDL B + C	d > 1.006 g/ml
		distribution (%) in lipoprotein fractions			
RP	2.27 ± 1.96 μmol	60 ± 11	17 ± 5	12 ± 4	11 ± 7
TG	511 ± 368 μmol[a]				
Chol[b]	1120 ± 570 μmol				
ApoB-48[c]	2.67 ± 1.79 nmol	69 ± 24	25 ± 17	6.4 ± 12.8	nd
ApoB-100[c]	153 ± 80 nmol	0.8 ± 0.5	2.0 ± 2.5	7 ± 5	90 ± 5

Chylomicron-rich plasma was obtained by plasmapheresis 5–6 h after a liquid test meal (210 μmol retinyl palmitate in 100 ml heavy cream (39% fat) per m^2 body surface area) for endogenous labeling of chylomicrons with retinyl palmitate. After storage at room temperature for 2 days, the sample was taken for analysis of its composition and the plasma was reinjected into the donor to determine plasma decay of retinyl palmitate. Values given as mean ± SD; n = 18. Abbreviations: RP, retinyl palmitate; TG, triglycerides; Chol, cholesterol; nd, not detected.

[a]Corresponding to 411 ± 296 mg (range 110–1150 mg).

[b]Determined in 12 studies.

[c]Determined in 8 studies.

TABLE 3. Kinetic parameters of total plasma retinyl palmitate decay

	RP Dose	Apparent	V_d	k_1	$t_{½}$	k_2	$t_{½}$	$k_1/(k_1 + k_2)$
	mol	*ml*	*%PV*	*min^{-1}*	*min*	*min^{-1}*	*min*	*%*
Monoexponential decay (n = 6)								
Mean	4.15	3642	106	−0.032	29.7			100
SD	2.40	1133	24	0.025	13.4			0
Biexponential decay (n = 12)								
Mean	1.33	2718	88	−0.057	13.3	−0.0070	123	89
SD	0.61	610	15	0.018	4.6	0.0030	62	4
Rapid decay (n = 18)								
Mean	2.27	3026	92	−0.049	18.8			92
SD	1.96	905	19	0.024	11.4			6

Plasma disappearance curves of retinyl palmitate label were obtained as described and analyzed by computer-assisted, nonlinear curve fitting. In addition to a major first order decrease (k_1), a second minor first order decrease (k_2) was observed in 12 of the 18 subjects. The apparent volume of distribution, V_d, was calculated from the retinyl palmitate dose divided by the y-intercept of the plasma retinyl palmitate disappearance curve. The contribution of the rapid kinetic process to total plasma retinyl palmitate clearance is calculated from the slopes as $k_1/(k_1 + k_2)$ and expressed in percent. PV, plasma volume.

lipoproteins (mainly in LDL), which did not contain detectable amounts of apoB-48. The chylomicron and VLDL A fractions contained less than 3% of the apoB-100, but 94 ± 13% (n = 8) of the chylomicron remnant marker apoB-48. The distribution of apoB-48 and retinyl palmitate was similar in the chylomicron and VLDL fractions (Table 2). The concentration of retinyl palmitate was related to that of apoB-48 in chylomicron and VLDL A fractions (RP = 173 + 330 apoB-48 (nM); $P < 0.025$) with a mean molar ratio of 330:1 (range 237:1 to 652:1), which indicates the degree of labeling.

Analysis of retinyl palmitate plasma disappearance

Disappearance of retinyl palmitate in total plasma (**Table 3**) is best described as a monoexponential first order function in 6 subjects and as a biexponential function in the other 12 subjects. The initial rate constant, k_1, represents the major elimination process (92 ± 6% of total clearance; n = 18) under the assumption of a single compartment. The volume of distribution corresponds to the estimated plasma volume (Fig. 2A).

Differential clearances according to class of lipoproteins. In six subjects (#s 12, 14–17, 19) with biexponential decay, 91 ± 5% of the retinyl palmitate in chylomicron and VLDL A fractions, 48 ± 15% in the VLDL B and C fractions, and only 23 ± 7% in the d > 1.006 g/ml fraction were cleared during the rapid, initial slope (from 5 to 75 min after injection). The retinyl palmitate still remaining in the blood by the time of the slow, second exponential (120–210 min) was in the d > 1.006 g/ml lipoproteins, predominantly LDL (93 ± 7%), and in the small VLDL C (7 ± 8%). In these studies and in the two subjects with monoexponential disappearance (#18, #20) whose plasma was analyzed by ultracentrifugation and VLDL precipitation, the half-time of retinyl palmitate removal of the major, rapid kinetic component was similar to the half-time of retinyl palmitate removal from the combined chylomicron and VLDL A fractions (**Fig. 3**). Two independent lipoprotein separation techniques (compare **Fig. 4**) revealed in biexponential (#15, #19) as well as monoexponential studies (#18, #20) that retinyl palmitate is cleared faster in larger size lipoproteins (d ≤ 1.006 g/ml precipitates, chylomicrons, and VLDL A and B) than in the smaller particles (d > 1.006 g/ml infranatant particles, VLDL C, and LDL). There was no kinetic precursor-product relationship (56), since the retinyl palmitate concentration did not increase in the d > 1.006 g/ml fraction during its rapid decrease in the d ≤ 1.006 g/ml fraction. Therefore, the bulk of injected larger particles (d ≤ 1.006 g/ml) apparently was not converted to small particles (d > 1.006 g/ml) before clearance (Fig. 4,

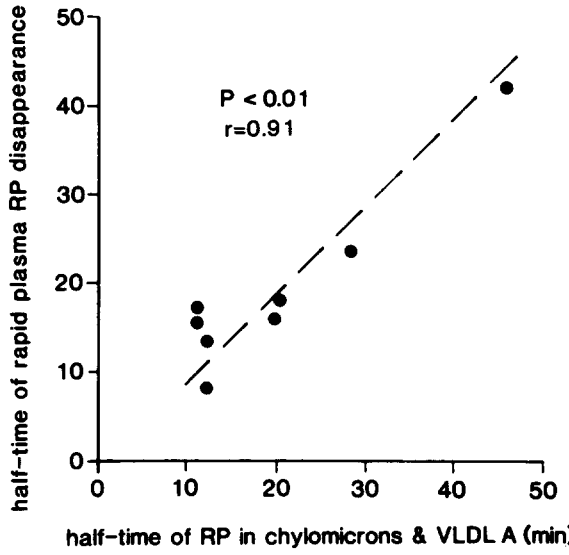

Fig. 3. Half-times (t/2) of retinyl palmitate disappearance. The t/2 of the rapid component in total plasma is plotted against the t/2 in chylomicrons and VLDL A obtained by ultracentrifugation; y = 0.73 + 1.02 x; n = 8.

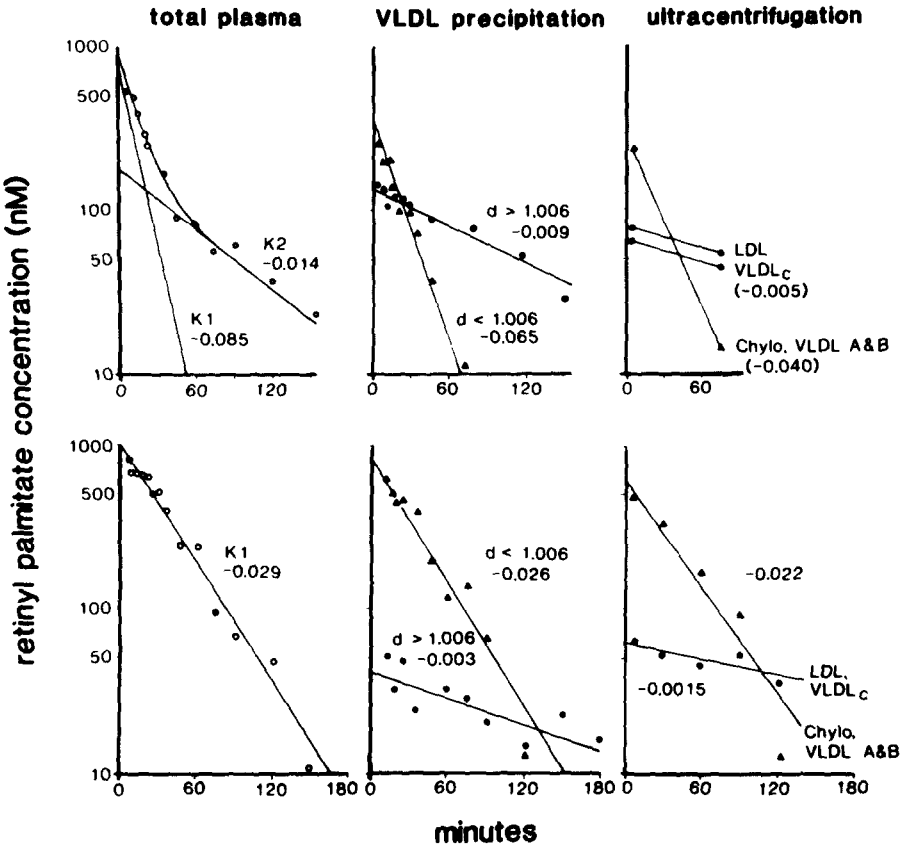

Fig. 4. Retinyl palmitate disappearance in plasma lipoprotein fractions in a subject (#15, taking contraceptive steroids (19)) with apparent biexponential kinetics (upper panels) and a subject (#18) with apparent monoexponential kinetics (lower panels). Analyses of retinyl palmitate were performed on total plasma (○) (left), VLDL precipitates (▲) and d > 1.006 g/ml infranatants (●) (heparin-$MgCl_2$ method) (middle), and fractions derived from ultracentrifugation (right). The kinetic heterogeneity of plasma RP disappearance (upper left) is the result of retinyl palmitate in different lipoprotein fractions. In subject #18 with apparent monoexponential decrease, the minor, slow component was eliminated from the total plasma retinyl palmitate disappearance (lower left) by subtraction of the baseline retinyl palmitate concentration.

middle). Biexponential disappearance, thus, is apparently caused by two labeled particle classes of d ≤ 1.006 and d > 1.006 g/ml, that are cleared at different rates.

Early (6–10 min) after injection of the labeled plasma, the chylomicron fractions were devoid of apoB-100, and the VLDL A fractions contained less than 5% of plasma apoB-100 in the biexponential studies (#s 12, 15, 16, 17, 19). ApoB-48, however, could be detected in VLDL A and B only in one study (#12) 10 min after injection of plasma (containing 4.1 nmol apoB-48), but not in the other four due to the small amount injected (≤2.4 nmol apoB-48).

The percent of plasma retinyl palmitate cleared by the second, slow kinetic component was correlated ($P < 0.02$) with the percent of the injected retinyl palmitate present in apoB-100-rich d > 1.006 g/ml lipoproteins. Conversely, the percent cleared by the major, rapid kinetic component was correlated ($P < 0.02$) with the percent retinyl palmitate in the apoB-48 containing d ≤ 1.006 g/ml fraction of administered plasma (**Fig. 5**).

Model-related analysis for RP-labeled chylomicron remnants

Model analysis. The volume of distribution for retinyl palmitate-labeled chylomicron remnants agrees with that of total plasma retinyl palmitate (Fig. 2B) and corresponds to the estimated plasma volume of the subjects (**Table 4**). Plasma and blood clearance rate for chylomicron remnants derived from this analysis are given in Table 4 and **Table 5**. Plasma clearance rates for labeled chylomicron remnants ranged from 66 to 322 ml/min, and for the slow kinetic process from 6 to 43 ml/min. The apparent V_d of the slow kinetic process also was similar to the estimated plasma volume (Table 5).

Analysis of dose dependence of elimination of labeled chylomicron remnants. In one subject (#20) three different amounts of retinyl palmitate-labeled chylomicron remnants were injected in increasing order (**Fig. 6**). The apparent volume of distribution was the same in each study (4292 ± 97

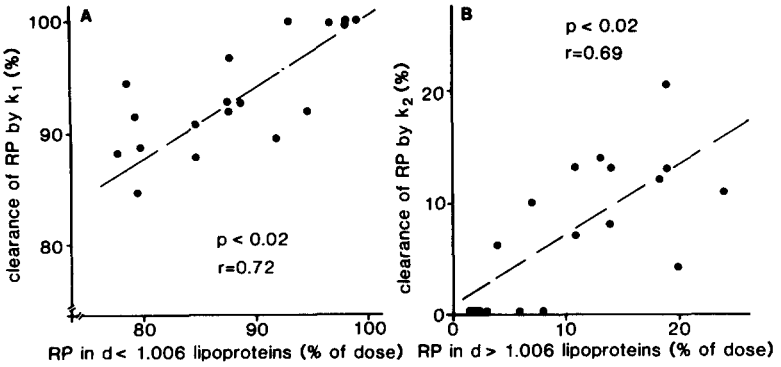

Fig. 5. Plasma retinyl palmitate decay and retinyl palmitate distribution in the injected plasma. Panel A: relationship between the fraction of total plasma retinyl palmitate cleared by the rapid kinetic component, k_1, and the $d \leq 1.006$ g/ml fraction of injected retinyl palmitate ($y = 52 + 0.47x$; $n = 18$). Panel B: correlation between the fraction of retinyl palmitate cleared by the slow kinetic component, k_2, and the fraction of the injected retinyl palmitate dose contained in $d > 1.006$ g/ml lipoproteins ($y = 0.97 + 0.61x$; $n = 18$).

ml), but fractional rate constants and plasma clearance rates of retinyl palmitate-labeled chylomicron remnants decreased as the injected dose increased. The amounts of triglyceride injected were 167, 449, and 784 mg, respectively, which accounted for small increases (5, 12, and 20 mg/dl) of the triglyceride level (102, 92, 122 mg/dl prior to each injection).

The data of all 18 subjects (Table 4) analyzed for dose dependence of the model-derived elimination rate of labeled chylomicron remnants (R_{CMR}) conformed to Michaelis-

TABLE 4. Kinetic parameters of plasma elimination of retinyl palmitate-labeled chylomicron remnants (RP_{CMR}) in healthy subjects (n = 18)

Subject No.	ApoE Isotype	Autologous Fraction of RP in $d \leq 1.006$	Plasma[a] $Dose_{RP,CMR}$	1st Order Function[b] No. of Exponentials	$\frac{k_1}{k_1+k_2}$	k_1	Apparent V_d[c]		Clearance Rate[d] Plasma	Blood
		%	*nmol RP*			*min*$^{-1}$	*ml*	*%PV*	*ml/min*	
1		94	338	1	1.00	0.082	2817	93	231	374
2	4/3	98	3368	1	1.00	0.028	2350	79	66	103
4	3/3	98	5079	1	1.00	0.020	4676	130	94	140
5		96	1048	2	0.94	0.055	3867	106	213	374
7	4/3	93	1278	2	0.90	0.074	2640	75	195	355
8	3/3	98	5406	1	1.00	0.016	4641	113	76	127
9	2/3	86	913	2	0.92	0.066	2692	83	178	274
10	3/3	81	478	2	0.87	0.059	3594	95	212	372
11		87	2246	2	0.86	0.039	3706	130	148	211
12	3/3	76	810	2	0.89	0.094	2833	81	266	291
13		86	1461	2	0.87	0.067	4806	141	322	560
14		81	650	2	0.82	0.043	2175	90	94	146
15	3/3	80	1281	2	0.96	0.068	1476	58	100	171
16	4/2	89	907	2	0.93	0.040	2399	81	96	154
17	4/3	82	653	2	0.88	0.029	2483	98	72	113
18	3/3	92	2775	1	1.00	0.029	2606	71	76	153
19	3/3	89	2006	2	0.87	0.054	2794	95	151	244
20	3/3	97	7110	1	1.00	0.016	4373	108	70	121
Mean		89.1	2100		0.92	0.049	3163	95.4	148	238
SD		7.1	194.5		0.06	0.024	980	22.8	78	127

[a]Chylomicron-rich plasma was obtained by plasmapheresis 5–6 h after subjects drank a standardized amount of retinyl palmitate (RP) homogenized in cream and it was reinjected into the donor after 2 days. The dose of RP label in the plasma was corrected for the amount transferred to $d > 1.006$ g/ml lipoproteins in order to reflect the dose of retinyl palmitate-labeled chylomicron remnants (RP_{CMR}) (compare model equations no. 1–4).

[b]The data were tested for a mono-, bi-, and triexponential decay function using a computer program and analyzed by an F-test to determine whether the fit of the data was significantly improved by introducing an additional exponential. Final fit of the resulting mono- or biexponential function to the data was performed with a nonlinear least squares program as described. The contribution of the slope k_1, the major rapid disappearance component, to the clearance of the total RP is given by the ratio $k_1/(k_1 + k_2)$.

[c]The apparent volume of distribution, V_d, is the dose of RP_{CMR} divided by the y-intercept of k_1. V_d is given in ml and in percent (% PV) of the estimated (45) plasma volume.

[d]Plasma clearance rate of retinyl palmitate-labeled chylomicron remnants was calculated as V_d times k_1, and blood clearance rates were obtained from plasma clearance rates using individual hematocrits after plasma injection and the formula $Cl_{blood} = Cl_{plasma}/(100\% - hct)$.

TABLE 5. Compartmental analysis of total plasma retinyl palmitate removal

	Elimination of RP_{CMR} n = 18			Slow Elimination of $RP_{d>1.006}$ n = 12		
	Apparent V_d		Clearance	Apparent V_d		Clearance
	ml	*%PV*	*ml/min*	*ml*	*%PV*	*ml/min*
Mean	3163	96	148	2093	69	14.3
SD	980	22	78	867	31	9.8

Plasma disappearance curves of retinyl palmitate were obtained as described for Tables 2 and 4. Compartmental analysis was performed according to equations 1-3, and by analogous equations for the slow elimination process using the dose of RP in the d > 1.006 g/ml fraction of the injected plasma. Abbreviations: RP_{CMR}, retinyl palmitate in d 1.006 g/ml lipoproteins; $RP_{d>1.006}$, retinyl palmitate in d > 1.006 g/ml lipoproteins of the injected plasma; PV, plasma volume.

Menten kinetics (**Fig. 7A**) with an apparent K_m of 921 nmol retinyl palmitate and a maximum elimination rate (V_{max}) of 124 nmol retinyl palmitate/min. Saturation occurred in subjects with phenotypes apoE_3/E_3 and E_3/E_4. Assuming an average V_d of 3163 ml (Table 5), an estimated average molar ratio of retinyl palmitate/apoB-48 of 330/1 in these chylomicron remnant particles, and a liver weight of 2.3% of the average body weight (65 kg × 0.023 = 1.50 kg) (53), the following estimates of chylomicron remnant elimination were obtained for normal, healthy adults.

$$\text{Apparent } K_m = 921 \text{ nmol } RP_{CMR}/3.163 \text{ l} \approx 291 \text{ nM } RP_{CMR}$$
$$\text{or} = 0.88 \text{ nM apoB-48}$$
$$\text{Apparent } V_{max} = 124 \text{ nmol } RP_{CMR}/\text{min}$$
$$= 0.38 \text{ nmol apoB-48/min}$$
$$= 0.25 \times 10^{-3} \text{ nmol apoB-48 min}^{-1}\text{g}^{-1} \text{ liver}$$

The range of doses (0.5–5.5 times the K_m equivalent dose) is consistent with pseudo-first order kinetics of retinyl palmitate disappearance (57). Maximum velocity is expected below 10% of the estimated K_m concentration of 291 nM (= 921/3.16) which is below the limit of detection. Superposition of the slow elimination process can conceal increasing rates of removal of the rapid elimination, as the concentration falls into the low range. When the dose of retinyl palmitate-labeled chylomicron remnants was more than 2400 nmol (2.5 times the K_m equivalent dose), the elimination curves were monoexponential in total plasma; but the second, slower decay process could still be shown in the d > 1.006 g/ml fraction (Fig. 4). An increasing fractional rate of removal was observed at high concentrations (subject #8; Fig. 2 in ref. 17). After injection of more than 5 times the K_m equivalent dose, the disappearance of retinyl palmitate obeyed zero order kinetics for 27 min, indicating saturation of the elimination process (53), and apparent first order kinetics for the rest of the curve.

Since the amount of triglyceride injected (110–1159 mg or 1.9–16.1 mg/kg body weight) accounted for only a small increase (4–28 mg/dl) of baseline triglyceride levels (82 ± 29 mg/dl; n = 17), estimated maximum triglyceride levels were less than 150 mg/dl in all studies.

The decay constant k_1 and the clearance rate (= $V_d \times k_1$), but not the apparent volume of distribution V_d, depended on the injected $dose_{RP,CMR}$. Accordingly, model-derived blood clearance rates for chylomicron remnants (Table 4) are consistent with the calculated (53) dose-dependence curve of clearance rate (Fig. 7B), which suggests a theoretical maximum blood clearance rate of 660 ml/min per 1.72 m^2 body surface area. The maximum blood clearance rate determined was 560 ml/min per 1.72 m^2, which is limited not by liver blood flow (1350 ml/min × 1.72 m^2) (53), but by hepatic extraction.

These model-derived saturation parameters apply to actual retinyl palmitate concentrations determined after

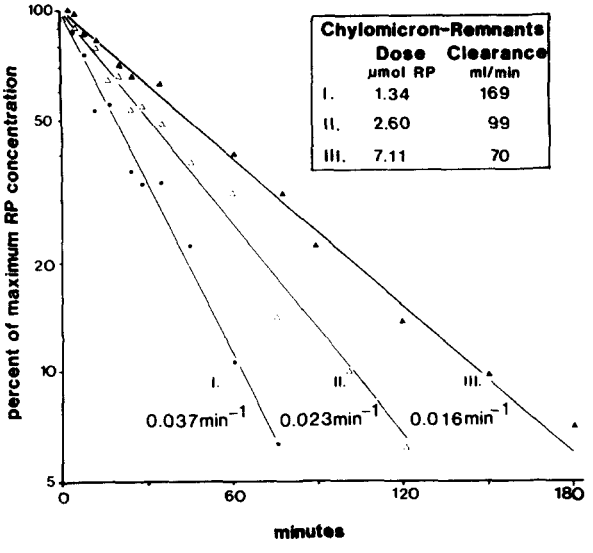

Fig. 6. Dose dependence of retinyl palmitate-labeled chylomicron remnant elimination in a single fasting subject (#20, homozygous for apoE3). Three doses of retinyl palmitate-labeled chylomicron remnants (RP_{CMR}) taken from one bag of pooled, autologous plasma were injected in increasing order on the same day and retinyl palmitate plasma elimination was measured each time. For comparison, retinyl palmitate concentrations were converted to percent of the respective maximum plasma concentrations (57).

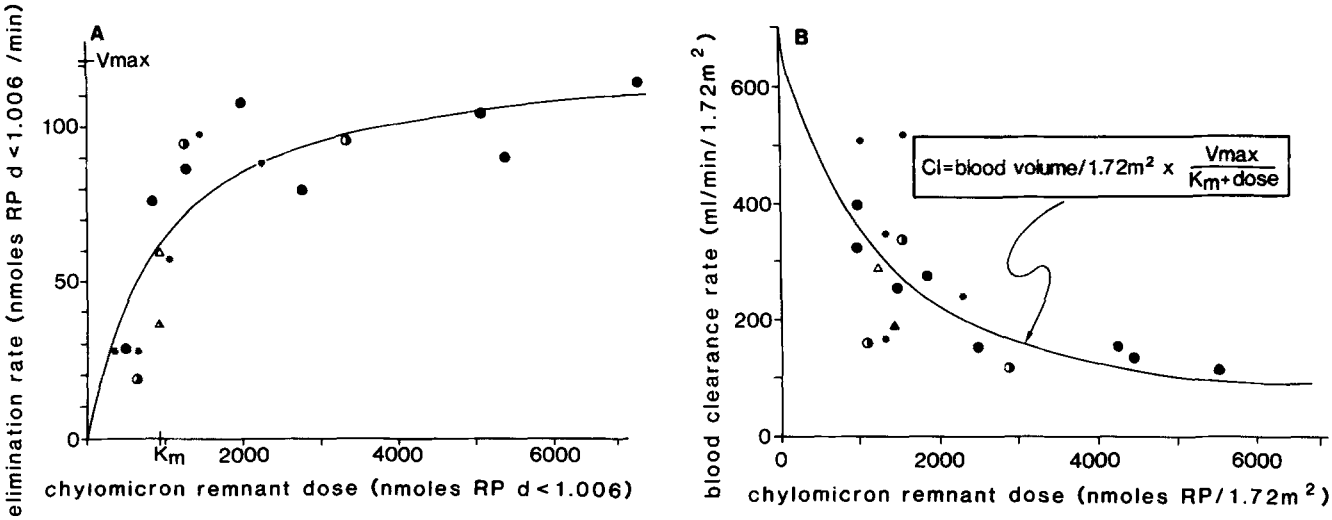

Fig. 7. Dose dependence of chylomicron elimination in 18 healthy adults. Panel A: elimination rates of retinyl palmitate-labeled chylomicron remnants versus dose yield a good fit (F = 3.39; $P = 0.06$) to the Michaelis-Menten equation (55): K_m = 921 ± 305 nmol retinyl palmitate injected in chylomicron remnants of d ≤ 1.006; V_{max} = 124 ± 14 nmol retinyl palmitate label/min. Elimination rate is not limited due to presence of apoE2 (25, 26). Panel B: blood clearance rates of retinyl palmitate-labeled chylomicron remnants (= plasma clearance rate/100% − hematocrit) are plotted versus the administered dose. The theoretical curve of dose dependence of hepatic chylomicron remnant clearance was obtained by the Michaelis-Menten saturation parameters (compare panel A) using the formula in the insert (53). Symbols refer to apoE isotype: △ = E2/E3; △ = E2/E4; ○ = E3/E3; ○ = E3/E4; * = apoE type unknown.

the test meal taken for retinyl palmitate labeling. The intake of 70 g fat as cream apparently leads to chylomicron remnantemia which exceeds saturation (at approximately 1500 nM retinyl palmitate) for more than 7 h (**Fig. 8**, details in legend).

DISCUSSION

This report summarizes the attempt to quantitate chylomicron remnant clearance in humans using chylomicrons endogenously labeled with retinyl palmitate and reinjected intravenously in a bolus of autologous plasma. By analysis of the disappearance of retinyl palmitate in total plasma and in lipoprotein fractions, two major issues have been clarified: *a)* the retinyl palmitate label is rapidly cleared within lipoproteins of d ≤ 1.006 g/ml; these apparently are chylomicron remnants and their plasma elimination kinetics conform to a simple compartmental model; *b)* initial rates of plasma elimination of labeled chylomicron remnants, as calculated by the compartmental model, obey saturation kinetics in healthy human subjects.

Chylomicron remnants had been defined a priori (17) as rapidly cleared serum lipoproteins that had been endogenously labeled with retinyl palmitate. The disappearance of the label from total plasma and its lipoprotein fractions was best described by a mono- or biexponential apparent first order function, which could be analyzed by linear pharmakokinetics (57, 58). The major, rapid kinetic component accounted for 92 ± 6% of retinyl palmitate disappearance from total plasma and was related to the injected labeled lipoproteins of d ≤ 1.006 g/ml. Hence, it appeared to represent elimination of chylomicron remnants. A compartmental model was proposed to estimate this clearance rate (equations 1-4). The validity of this model depends on the correctness of *a)* the general assumptions (see Model Development), *b)* the constraint of the labeled lipoproteins virtually to the intravascular compartment during the elimination phase, and *c)* the presence of two kinetically different, labeled lipoprotein classes that are roughly classified by the hydrated densities of d ≤ 1.006 and d > 1.006 g/ml.

The rationale for the use of retinyl palmitate as endogenous label of the core of chylomicrons and their remnants is widely accepted (17, 21, 23-26); it is stated under General Assumptions. The following requirements were fulfilled for linear pharmacokinetic analysis (53, 57, 58) of plasma disappearance of a single intravenous dose. The plasma bolus containing the retinyl palmitate was distributed in the intravascular space in less than 10 min, as judged by its hemodilution effect, and the retinyl palmitate label was virtually constrained to the intravascular compartment during the elimination phase (Fig. 2A), as would be predicted from the known distribution of low density lipoproteins (46, 47) which have the same density as the smallest labeled particles (compare Model Selection).

The cause for biexponential disappearance of retinyl palmitate was identified as kinetic heterogeneity of labeled lipoprotein classes. The biexponential disappearance curve was separated by a rapid technique of lipoprotein separation into two monoexponential curves—a major, rapid elimination of very low density (d ≤ 1.006

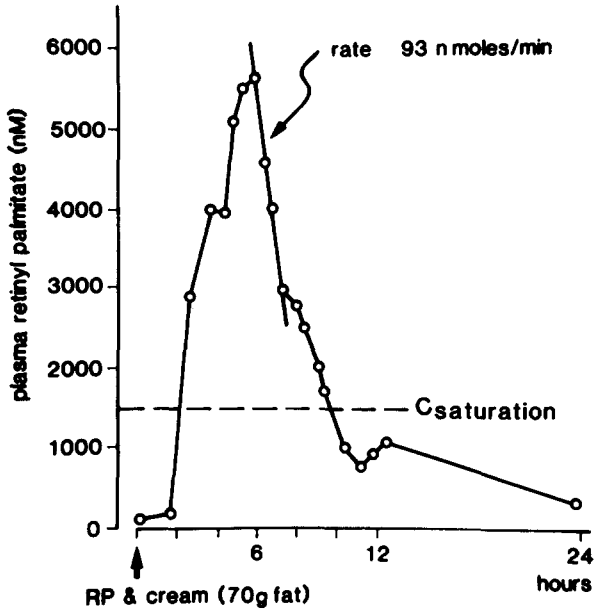

Fig. 8. Saturation parameters (compare Fig. 7A) in relation to postprandial lipemia in a healthy subject (male, 32 years, normolipidemic, apoE3/E3). Typical postabsortive retinyl palmitate concentration (nM) versus time (h) curve after intake of 70 g of fat as cream containing retinyl palmitate (s. study design). Maximum net plasma removal rate of retinyl palmitate is 93 nmol/min calculated from the RP disappearance slope and the plasma volume of 3310 ml. The difference of the net removal rate to V_{max} (124 nmol/min) can be explained by continued input of chylomicrons into plasma. The theoretical saturation concentration (--- $C_{saturation}$) of retinyl palmitate under the applied labeling conditions was estimated as 5 times the K_m dose (921 nmol RP; Fig. 7) divided by the subject's plasma volume. This suggests saturation of hepatic chylomicron remnant removal for approximately 7 h after a meal containing 70 g of fat.

g/ml) lipoproteins and a minor, slow elimination process of higher density (d > 1.006 g/ml) lipoproteins, with a common volume of distribution corresponding to the intravascular plasma volume (Fig. 4). These monoexponential disappearance curves showed no evidence of a kinetic precursor-product relationship (56) between the rapidly eliminated d ≤ 1.006 g/ml particles and the slowly eliminated smaller d > 1.006 g/ml particles. Therefore, the bulk of the retinyl palmitate label is rapidly cleared as d ≤ 1.006 g/ml particles without prior conversion to d > 1.006 g/ml particles; this process is consistent with the clearance of labeled chylomicron remnants (as defined in Model Assumptions).

A mean of 11 ± 7% of the retinyl palmitate in the injected plasma was associated with d > 1.006 g/ml lipoproteins that did not contain detectable concentrations (1 nM) of intestinal apoB-48, but did contain 90% of plasma apoB-100 (Table 2). These retinyl palmitate-containing particles disappear more slowly than label in d ≤ 1.006 lipoproteins (Fig. 4). The half-time of their disappearance (63–346 min) was in the range known for hepatic VLDL and LDL (59, 60). They had accumulated in the d > 1.006 g/ml lipoproteins during the lipemia after the lipid test meal rather than during storage of the plasma (compare Fig. 1 in ref. 17). They might be highly lipolyzed small chylomicron remnants, formed during prolonged residence in the circulation when hepatic uptake is saturated, but it is as likely that the retinyl palmitate was transferred to lipoproteins of hepatic origin prior to reinjection. In either case, this higher density fraction (2–20%) was not included in the analysis of initial rates of chylomicron remnant elimination.

The apparent saturability of plasma elimination of retinyl palmitate-labeled chylomicron remnants was suggested by three lines of evidence. First, in a single subject who was studied three times with different amounts of retinyl palmitate, the apparent k_e and the clearance rate of retinyl palmitate label decreased progressively as the amount injected increased (Fig. 6). Second, in 18 healthy individuals the model-derived initial rates of chylomicron remnant removal after injection of retinyl palmitate-labeled chylomicron remnants in widely varying amounts were consistent with Michaelis-Menten saturation kinetics (Fig. 7A). Third, in one subject who had received more than 5 times the K_m equivalent dose, retinyl palmitate label disappeared in plasma according to a zero order function for the first 27 min (17) indicating saturation of the removal mechanism (57). The saturation phenomenon (Fig. 7A) was not simulated by dose-dependent trends in the degree of labeling, since the molar relationship of RP versus apoB-48 was linear, nor by a dose-related increase in apparent volume of distribution that would have indicated exit of label into an extravascular compartment. Nor can the saturation phenomenon be explained by competition of endogenous chylomicron remnants or VLDL remnants (11), since all subjects were fasting and had low serum triglycerides.

It has been suggested that the plasma half-life of retinyl palmitate label in humans is determined by the rate of lipolysis of chylomicron triglyceride, since it was correlated to the fasting serum triglyceride level (21, 24). However, one study (24) had included patients with endogenous hypertriglyceridemia, in whom abnormally impaired lipolysis (61) and/or competing VLDL remnants could have prolonged the half-life, and the other (21) had included elderly subjects, in whom the retinyl ester half-life as well as the fasting triglyceride level had increased with age. By contrast, in normal young adults the fasting triglyceride concentration is correlated strongly with chylomicron *triglyceride* clearance (62), but not, in the present study, with the half-life of retinyl palmitate label. For two reasons formation of chylomicron remnants by lipoprotein lipase does not appear rate-limiting for remnant removal. *a)* Grundy and Mok (61) had assessed chylomicron triglyceride steady state turnover in 21 normolipidemic subjects during duodenal infusion of triglycerides at a rate of 230 mg/min, which is comparable to the rate injected in the present study intravenously as bolus.

Triglyceride turnover yielded half-times of 4.5 ± 2.9 min (mean ± SD), which is faster than the elimination of retinyl palmitate label (Table 3). Accordingly, enhancing intravascular lipolysis by heparin infusion in 5 subjects (#9–#13) did not enhance plasma clearance of retinyl palmitate label (18). *b)* In the presence of low serum triglycerides (Table 1), lipoprotein lipase activity was not saturated (63). Hallberg (64) had injected thoracic duct lymph chylomicrons intravenously as bolus into humans to saturate intravascular triglyceride lipolysis. The chylomicron load necessary was more than 14 g of triglyceride per 70 kg body weight. The present studies used less than 10% of that dose. When chylomicron remnant removal rates were plotted against the injected dose of triglycerides, there was no suggestion of saturation. In conclusion, the Michaelis-Menten kinetics do not describe triglyceride lipolysis of chylomicrons, but limited plasma removal of chylomicron remnants.

In healthy adults, chylomicron remnant removal could be limited mainly by four mechanisms: liver blood flow, sieving of particles at the sinusoidal fenestrations in the liver, availability of apoE as ligand for the hepatic receptors, or saturation of the receptor-dependent hepatocellular uptake mechanism. The rates of clearance of chylomicron remnants from whole blood (Fig. 7B) did not exceed 50% of estimated (53) liver blood flow. Thus, plasma removal of chylomicron remnants by the liver is not limited by organ perfusion. Sieving of chylomicron remnants by the sinusoidal endothelial fenestrations might delay their entrance to the space of Disse. The diameter of sinusoidal fenestrations is 80–225 nm (65, 66), larger than chylomicron remnants (30–160 nm) (36). Therefore, it appears unlikely that sieving is a major factor accounting for the saturation phenomenon. Since apoE, a ligand for the putative chylomicron remnant receptor (3, 8) and the LDL receptor (67), is rapidly transferred between HDL and chylomicrons and since the plasma pool of apoE is abundant even in alimentary lipemia (68), ligand availability apparently is not limiting. The velocity of elimination of the high doses (Fig. 7A) was not decreased by presence of the apoE2 isotype, which impairs chylomicron remnant elimination (25, 26). Thus, the conformation to Michaelis-Menten kinetics appears to reflect saturation of the hepatocellular uptake mechanism for chylomicron remnants (3–5).

The saturation constant as derived from this compartmental analysis could reflect apparent physiological chylomicron remnant catabolism by the liver. Quantitatively, the half-saturation constant K_m (0.88 nM apoB-48) is similar to the theoretical estimate of a chylomicron remnant concentration of 0.43 nM in alimentary lipemia after intake of 100 g fat, as calculated from particle size by Redgrave and Carlson (36). In fact, their estimate could be too low, since it is based on an assumed half-life of only 5 min for chylomicron remnants in alimentary lipemia. Applying the half-saturation constant to actual retinyl palmitate concentrations determined after the test meal used for endogenous retinyl palmitate labeling indicates that intake of 70 g fat as cream leads to chylomicron remnant concentrations which exceed the putative saturation concentration for more than 7 h in an healthy individual (Fig. 8). The 18 subjects studied with the intravenous test also reached high saturating concentrations of retinyl palmitate at plasmapheresis 4–6 h after intake of this test meal. Furthermore, the lipid composition of postprandial plasma chylomicrons resembles that of chylomicron remnants and is very different from that of lymph chylomicrons (36). Hence, chylomicron remnants appear to accumulate during alimentary lipemia.

This study has methodological, model-related, and statistical limitations. The assumption of fairly uniform labeling of chylomicrons is essential for the interpretation of the data shown in Fig. 8. Provided silver nitrate stains apoB-100 and apoB-48 proportionate to molecular mass, a similar molar ratio of RP/apoB-48 (average 330:1) was obtained by the standardized fat- and vitamin A-rich test meal in the eight subjects studied; this indicates a similar degree of labeling, since each chylomicron particle has a fixed number of molecules of its specific marker, apoB-48 (29–31). A model-related limitation is the approximation that all label in the d ≤ 1.006 g/ml fraction resides in chylomicron remnants. The retinyl palmitate in the VLDL C, approximately 5% of the total label, is classified as chylomicron remnant label, even though it is slowly cleared in VLDL C (Fig. 4). This simplification causes a proportionate small error (of about 5%) in the estimates of chylomicron remnant elimination, but yields a relatively larger underestimate of the slow clearance process. Finally, the absolute estimates of the saturation parameters (K_m, V_{max}) should be interpreted cautiously considering the statistically small sample size.

The present findings give reason for several speculations. First, on a regular Western diet healthy adults would have chylomicron remnantemia for a considerable time of the day, which could be atherogenic in normolipidemic persons. Second, since chylomicron remnants are probably also cleared by the LDL-receptor pathway (6, 7), prolonged chylomicron remnantemia could delay clearance of LDL and VLDL remnants. Third, metabolic and hormonal regulatory influences could regulate the rate of chylomicron remnant clearance, as suggested by some studies (19, 20, 69–71). To further substantiate that chylomicron remnant elimination is saturated in alimentary lipemia, the molar ratio of RP/apoB-48 should remain constant during the lipemia after this vitamin A meal (Fig. 8). Furthermore, equal doses of label should be cleared faster in the fasting state than during the lipemia of a vitamin A-free test meal.

To summarize, the data analysis by the proposed compartmental model (equations 1–4) strongly suggests that

chylomicron remnant uptake by the liver in healthy humans is dose-dependent and saturated by a fat intake of 70–100 g. According to these saturation parameters (K_m, V_{max}) it is not limited by liver blood flow, but probably by liver uptake of chylomicron remnants. Postprandial lipemia seems to be a form of chylomicron remnantemia which lasts for several hours after a lipid meal.

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