

# Plasma clearance of chylomicrons labeled with retinyl palmitate in healthy human subjects<sup>1</sup>

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**Abstract** To estimate hepatic uptake of chylomicron remnants in humans, chylomicrons and intestinal very low density lipoproteins (VLDL) were endogenously labeled with retinyl esters, harvested by plasmapheresis, and pulse-injected into the donor 44 hr after plasmapheresis. Plasma decay of retinyl palmitate was measured in eight healthy volunteers. Retinyl palmitate plasma disappearance obeyed an apparent first order function in seven studies and, in one study, a biexponential function with the second, slow exponential accounting for only 13% of the retinyl palmitate plasma decay. The mean fractional removal rate was  $0.037 \pm 0.037 \text{ min}^{-1}$  (mean  $\pm$  SD) in a one-compartment model. The apparent volume of distribution,  $V_d$ , was  $109 \pm 25\%$  of the estimated plasma volume. Plasma clearance of retinyl palmitate was  $130 \pm 97 \text{ ml/min}$  calculated as  $V_d \times K_e$ . Mean  $T_{1/2}$  was  $29 \pm 16 \text{ min}$ . Both in vitro and in vivo the retinyl palmitate remained largely within chylomicrons and intestinal VLDL. Only 4.3% was transferred from chylomicrons to other lipoprotein classes during in vitro incubation for 5 hr. After plasma was stored for 42 hr, 5% was transferred to higher density lipoproteins. During 12 hr after a test meal containing retinyl palmitate, only  $6.4 \pm 1.5\%$  of the retinyl palmitate absorbed was found in the LDL fraction and  $3.1 \pm 3.8\%$  in the  $d 1.063 \text{ g/ml}$  lipoproteins. We conclude that retinyl palmitate is a useful marker for chylomicrons and their remnants in humans and that the plasma clearance of retinyl palmitate-labeled chylomicrons is probably an estimate of chylomicron remnant plasma clearance in man.—Berr, F., and F. Kern, Jr. Plasma clearance of chylomicrons labeled with retinyl palmitate in healthy human subjects. *J. Lipid Res.* 1984. 25: 805–812.

**Supplementary key words** retinyl palmitate transfer • retinyl palmitate distribution

Hepatic cholesterol synthesis in the rat is regulated, in part, by the size of the bile acid pool in the enterohepatic circulation and by the amount of cholesterol reaching the liver within chylomicron remnants. The inhibition of cholesterol synthesis accompanying expansion of the bile acid pool in rats is mediated through increased intestinal cholesterol absorption and increased hepatic chylomicron remnant uptake (3). In humans, the interrelationships between bile acid pool size, cholesterol absorption, hepatic chylomicron remnant uptake, and hepatic cholesterol synthesis have not been investigated. Validated methods for measurement of bile acid pool size (4) and cholesterol absorption (5) are available, but hepatic chylomicron

remnant uptake has not been quantitated. This study was designed to evaluate a method of estimating hepatic chylomicron remnant uptake in man by measuring plasma disappearance of endogenously labeled chylomicron remnants.

Chylomicron remnants cannot be selectively isolated from human plasma for exogenous radiolabeling of apoproteins. Since esterified cholesterol in the core of chylomicrons and VLDL is exchanged with the cholesterol in other lipoprotein classes during its circulation in the plasma, mediated by cholesteryl ester exchange protein (6), labeled cholesterol cannot be used to measure accurately the hepatic clearance of chylomicrons. It has been suggested that retinyl esters might be an appropriate endogenous label for chylomicron remnants (7–9). In the intestinal mucosa, absorbed retinol is esterified with long-chain fatty acids, primarily palmitic acid, incorporated into the core of chylomicrons and secreted into intestinal lymph (10). Retinyl esters remain largely within the chylomicron remnant particle during triglyceride lipolysis and are completely cleared by the liver (11). In the liver, they are either stored or resecreted as unesterified retinol bound to retinol binding protein (10). After removal of esterified retinol by the liver, it does not recirculate. Melchoir, Mahley, and Buckhold (9) used retinyl esters to study plasma clearance of chylomicron remnants in dogs and found some transfer of esters to higher density lipoprotein fractions, due either to cholesteryl ester exchange protein or possibly to resecretion by the liver. The extent of such a transfer of retinyl esters, if any, in man is not known.

Abbreviations: RP, retinyl palmitate; VLDL, very low density lipoproteins ( $d < 1.006 \text{ g/ml}$ ); IDL, intermediate density lipoproteins ( $d 1.006\text{--}1.019 \text{ g/ml}$ ); LDL, low density lipoproteins ( $d 1.019\text{--}1.063 \text{ g/ml}$ ); HDL, high density lipoproteins ( $d 1.063\text{--}1.21 \text{ g/ml}$ ); HPLC, high pressure liquid chromatography;  $V_d$ , apparent volume of distribution;  $k_e$ , apparent elimination constant; EDTA, ethylene diamine tetraacetic acid.

<sup>1</sup> Part of this work has been previously published in abstract form (1, 2).

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We investigated the following specific questions in healthy human subjects: 1. To what extent are retinyl esters transferred from chylomicrons and intestinal VLDL to LDL and HDL in vitro and in vivo? 2. What are the kinetic characteristics of plasma clearance of intravenously administered chylomicrons labeled with retinyl palmitate (RP)?

## METHODS

### Analytical techniques

**Preparative ultracentrifugation.** Four ml of plasma containing 1.4 mg/ml EDTA and 0.8 mg/ml *p*-chloromercuriphenyl-bis-sulfonic acid was adjusted to density 1.10 g/ml by addition of solid KBr and overlaid with salt solutions of decreasing density (3 ml, *d* 1.065; 3 ml, *d* 1.020; 3.4 ml, *d* 1.006) to form a discontinuous salt gradient. By cumulative rate centrifugation (12) in an SW 40 rotor of a Beckman L5-75 ultracentrifuge, the following lipoprotein fractions were isolated: chylomicrons after 43 min centrifugation at 28,300 rpm ( $4.5 \times 10^6$  g-min); VLDL fraction A after 67 min at 40,000 rpm ( $17.5 \times 10^6$  g-min); VLDL fraction B after 71 min at 40,000 rpm ( $31.2 \times 10^6$  g-min); VLDL fraction C after 18 hrs at 37,000 rpm ( $15.2 \times 10^6$  g-min). After the 18 hr centrifugation, the gradient was fractionated from the top (2 ml, VLDL fraction C; 3 ml, approximate density *d* 1.010–1.020 g/ml; 2.5 ml, visible LDL band; 2 ml, *d* 1.05–1.07 g/ml; 4.0 ml, plasma infranatant). All samples were stored in light-shielded tubes under argon at  $-20^\circ\text{C}$  until analysis.

**Retinyl ester assay.** Eighty pmol of retinyl myristate or 300 pmol of retinyl undecanoate (internal standards) and 2 ml of methanol were added to 0.5 or 0.2 ml of plasma adjusted to a volume of 0.8 ml with water. Total lipids were extracted in 2 ml of chloroform (13), dried under argon, and redissolved in 100  $\mu\text{l}$  of methanol–chloroform 3:1. Retinyl esters were separated by reverse phase HPLC using an analytical C18-bonded silica HPLC column ( $\mu\text{Bondapak}^{\text{R}}$ , Waters Associate, Milford, MA) with 100% methanol as liquid phase at a flow rate of 2 ml/min (14). Retinyl ester peaks were recorded at their absorbance maximum of 326 nm and quantitated by the area ratio method. Intra-assay coefficient of variation was 2.7% (*n* = 10), inter-assay coefficient of variation was 4.6% (*n* = 10). The limit of sensitivity was 20 nM for retinyl palmitate concentration in 0.5 ml of total plasma and 10 nM in chylomicron and VLDL fractions harvested from 4 ml of plasma by ultracentrifugation. The assay had been previously standardized using retinol, retinyl palmitate, and other retinyl esters synthesized from retinol and the corresponding fatty acid anhydride (15) or acyl chloride

(16). The esters were purified by reverse phase preparative HPLC and quantitated by spectrophotometry in hexane using the millimolar extinction coefficient of 52,275 at 326 nm (17). Retinol, retinyl palmitate, undecanoyl chloride, myristic anhydride, oleic anhydride, linoleoyl chloride, and stearic anhydride were purchased from Sigma Chemical Corp., St. Louis, MO.

Cholesterol and triglyceride concentrations in whole plasma, chylomicrons, and VLDL were measured by AutoAnalyzer I methodology (18).

**Retinyl palmitate transfer from chylomicrons to other lipoprotein fractions in vitro.** Lipemic plasma (1.4 mg EDTA/ml of blood) was obtained from five healthy subjects (four male, one female) 5–6 hr after the oral intake of RP (30 mg of retinol equivalent/ $\text{m}^2$  body surface) homogenized in cream, 38% fat (100 ml/ $\text{m}^2$ ). Chylomicrons were harvested from 4 ml of plasma by ultracentrifugation in a salt gradient for  $4.5 \times 10^6$  g-min (12). The total chylomicron fraction of 4 ml of plasma (range, 144–376  $\mu\text{g}$  of triglyceride) suspended in 2 ml of 0.15 M NaCl was incubated with 3 ml of fasting plasma from a different donor for 5 hr at pH 7.4,  $37^\circ\text{C}$ , in the presence of 0.32 mg/ml of HDL-cholesterol and 0.27 mg/ml of LDL-cholesterol. After incubation, the density of the plasma was adjusted to 1.10 g/ml by addition of solid KBr, and chylomicrons, three VLDL subfractions, LDL, and the infranatant (*d* < 1.06) were separated as described above. RP was measured in the individual lipoprotein fractions.

### In vivo studies

**Subjects.** Studies were performed in healthy Caucasian volunteers (four men, four women) 20–35 years old. Their plasma lipid levels were in the normal range. Pregnancy was excluded by menstrual history and a negative pregnancy test (rapid HCG test). This study was approved by the Human Subject Committee of the University of Colorado Health Sciences Center. Informed consent was obtained and the subjects were admitted to the Clinical Research Center for study.

**Transfer of RP to lipoprotein fractions of density less than 1.019 in vivo.** Eight subjects were given the test meal described above. After fasting for 12 hr, they ate a low-fat meal free of vitamin A. Ten ml of blood (in EDTA, 1.4 mg/ml) were taken fasting, at 5 hr (the expected peak of the plasma concentration time curve), 6.5, 8, 12, and 24 hr. At 24 hr the plasma RP had returned to fasting levels. Individual lipoprotein fractions were separated and assayed for RP as described. The area under the concentration time curve of RP in the LDL fraction, the IDL, or the *d* > 1.063 g/ml fraction, respectively, versus the sum of the areas under the curves of RP in all lipoprotein fractions was used to estimate the fraction of absorbed RP transferred to LDL, IDL, and the *d* > 1.063 g/ml infranatant.

TABLE 1. In vitro transfer of retinyl palmitate (RP) from chylomicrons to other lipoprotein fractions

Lipoprotein Fraction	% Net Transfer of RP <sup>a</sup>	
	Mean	Range
Chylomicrons	-4.3	0-7
VLDL A + B	+2.2	0-5.7
VLDL C	+0.10	0-0.24
LDL	+1.2	0-4.0
Plasma infranatant (d 1.063 g/ml)	+0.8	0-1.4

<sup>a</sup> During 5 hr incubation of retinyl palmitate-labeled chylomicrons at 37°C, pH 7.4. Data were derived from five incubation experiments. Recovery of RP from lipoprotein ultracentrifugation was 99.7 ± 6.8%. The fraction harvested from the gradient between VLDL C and LDL (lipoproteins of intermediate density d 1.006-1.019 g/ml) contained 0.11 ± 0.12% of the RP.

*Preparation of RP-rich lipemic plasma for intravenous administration.* After an overnight fast, each subject drank 60 mg of retinol equivalent/m<sup>2</sup> body surface area of RP<sup>3</sup> homogenized in 100 ml of cream/m<sup>2</sup> body surface area and 5-6 hr later 2 units of plasma were obtained by plasmapheresis. The plasma was stored in ACD buffer, pH 7.0, light-shielded at room temperature for 42 hr. The RP concentration in the plasma and its distribution in individual lipoprotein fractions before and after storage were measured.

*Plasma clearance of chylomicrons labeled with RP.* Approximately 42 hr later, after complete clearance of retinyl esters from the circulation, the stored plasma was returned to the donor by a 4-min pulse-injection into a forearm vein, and blood samples for RP assay were obtained prior to the injection, every 3 min for 30 min, and then every 10-30 min for 3½ hr. The infused plasma volumes ranged from 554 to 627 ml in six subjects causing the hematocrit to drop by 5.8 ± 0.9%; it gradually returned to initial values approximately 4 hr after injection. In two subjects only 295 and 317 ml plasma were given. Except for transient (5 min) symptoms of hypocalcemia (hot flushes and peroral paresthesias) due to the citrate injected with the plasma, no adverse reactions to the procedure occurred.

### Calculations

After subtraction of the baseline plasma level, the RP plasma levels of the entire decay period were tested for a mono-, bi-, or triexponential decay function using an exponential stripping computer program (19). The residual sum of squares obtained for each function was compared by an E-test to determine whether the fit of the data improved significantly ( $P < 0.05$ ) by introducing an additional exponential (20). Final fit of the data to the

<sup>3</sup> Vitamin A palmitate, 1,000,000 IU/g in corn oil, was a generous gift from Dr. B. I. Wolkoff, Roche Chemical Division, Nutley, NJ.

resulting mono- or biexponential equation was performed with a nonlinear least squares program (21).

RP plasma clearance was calculated as the apparent first order decay constant,  $k_e$ , multiplied by the apparent volume of distribution,  $V_d$ , defined as the dose divided by the y intercept representing the RP concentration at zero-time.

The apparent volume of distribution for RP was compared to the plasma volume, estimated from age, sex, and body weight (22) to which the infused plasma had been added.

## RESULTS

### In vitro RP transfer

During 5 hr of in vitro incubation of RP-labeled chylomicrons with retinyl ester-free fasting plasma, between zero and 7% of the RP was transferred from chylomicrons to other lipoprotein classes (Table 1). Since none of the listed percentages of retinyl palmitate found in the other lipoprotein fractions was significantly different from zero, they might represent transfer or possibly a technical artifact of the procedures used. Recovery of RP by preparative ultracentrifugation was 99.7 ± 6.8% (n = 5).

After storage of plasma for 42 hr under the conditions described, 91% of the RP was found in chylomicrons and VLDL as compared to 96% in fresh plasma (Table 2).

### In vivo distribution of RP in plasma lipoproteins

The concentration of RP in the plasma lipoprotein fractions during 12 hr after an oral dose is given in Table 3 and the distribution is shown in Fig. 1; at 24 hr the RP levels had returned to baseline. There was a slight increase of RP in the LDL fraction during this time, but when estimated from the area under the concentration time curves, only 6.4 ± 1.5% (n = 8) of the RP absorbed was found in the LDL fraction; 3.1 ± 3.8% was in the

TABLE 2. Retinyl palmitate (RP) distribution in lipoproteins of plasma before and after storage

Lipoprotein Fraction	Fresh Plasma <sup>a,b</sup>	Stored Plasma <sup>a,c</sup>
	percent	
Chylomicrons and VLDL	96 ± 3	91 ± 7
LDL	3 ± 3	5 ± 3
Plasma infranatant (d > 1.063 g/ml)	0	4 ± 5

<sup>a</sup> Percent of total RP recovered from lipoprotein fractionation. Recovery of RP from ultracentrifugation was 84 ± 14%.

<sup>b</sup> Mean ± standard deviation of eight studies. Plasma stored less than 12 hr prior to ultracentrifugation.

<sup>c</sup> Mean ± standard deviation of samples from four plasma units stored for 42 ± 12 hr. Differences are not statistically significant.

TABLE 3. Concentration of retinyl palmitate (RP) in plasma lipoprotein fractions after oral RP dose (105 mol/m<sup>2</sup> body surface area)<sup>a</sup>

Lipoprotein Fraction	Hours after RP Intake				
	0	5	6.5	8	12
	<i>nM</i>				
Chylomicrons	0	1093 ± 376	718 ± 164	482 ± 270	164 ± 221
VLDL A	0, 26 <sup>b</sup>	375 ± 155	271 ± 137	164 ± 91	98 ± 41
VLDL B	0	155 ± 97	97 ± 57	68 ± 44	51 ± 16
VLDL C	9 ± 9 <sup>c</sup>	115 ± 59	132 ± 56	117 ± 41	86 ± 23
IDL <sup>d</sup>	0, 0	90, 109	110, 182	230, 149	120, 93
LDL	15 ± 3 <sup>e</sup>	46 ± 19	53 ± 33	91 ± 47	135 ± 51
Plasma infranatant (d 1.063 g/ml)	0	27 ± 53	31 ± 42	33 ± 56	16 ± 24

<sup>a</sup> Figures represent mean ± SD. Recovery of RP from ultracentrifugation: 88 ± 15%.

<sup>b</sup> In one study, fasting plasma contained 26 nM RP in VLDL fraction A; in all other studies, no RP was detected in VLDL fraction A of fasting plasma.

<sup>c</sup> Five of eight studies had detectable RP levels in VLDL fraction C of fasting plasma.

<sup>d</sup> RP was measured in IDL in only two subjects and the other lipoprotein classes in eight subjects. When the IDL fraction was not studied, recovery was 86 ± 16%, compared to 95 ± 4% recovery with analysis of IDL fraction.

<sup>e</sup> In all eight studies, fasting plasma contained detectable RP levels (10 nM) in the LDL fraction.

fractions of higher density (d > 1.063), and approximately 8% was in the IDL fraction.

The relative distribution of the different retinyl esters (palmitate, linoleate, stearate) circulating in blood remained virtually constant during the period of absorption of an oral dose of RP (Table 4). Serial changes in RP

levels, therefore, directly reflect changes in total retinyl ester concentration ([RP] = 0.71 × [total retinyl ester]). The RP fraction is overestimated by about 5–10%, since the HPLC assay does not separate RP and retinyl oleate; detector response factors at 326 nm wavelength, however, were identical for all retinyl esters as proven by injection of standards.

#### Plasma clearance of intravenously administered chylomicrons labeled with retinyl palmitate

The RP dose infused was 0.36 to 5.5 μmol and the triglyceride dose was 0.5 to 1.5 g. RP was cleared from the circulation in 156 ± 68 min.

In five of the eight studies (Fig. 2, Nos. 1, 2, 4, 5, 6), plasma decay of RP was entirely monoexponential. Two studies (Fig. 2, Nos. 3 and 8) showed deviations from a monoexponential decay, but since a biexponential function did not significantly (*P* < 0.05) improve the fit of the data, they are considered monoexponential. The de-

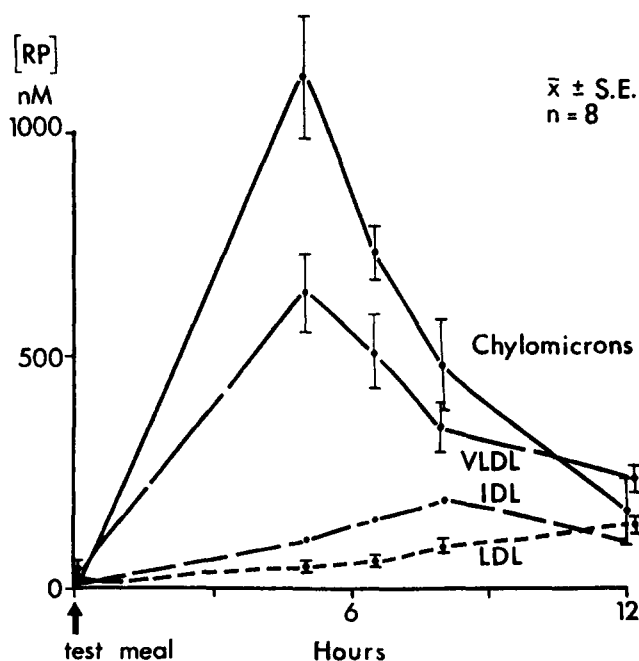


Fig. 1. Distribution of retinyl palmitate (RP) in plasma lipoproteins during 12 hr after a test meal of 30 mg retinol equivalent/m<sup>2</sup> body surface area and 100 ml cream/m<sup>2</sup> body surface area. Data points for IDL represent mean of two studies. All other data points represent mean ± SE of eight studies.

TABLE 4. Distribution of retinyl esters after oral intake of retinyl palmitate (RP) (105 μmol/m<sup>2</sup> body surface area)

Hours after Oral Dose of RP	Retinyl Linoleate <sup>a</sup>	Retinyl Palmitate <sup>a</sup>	Retinyl Stearate <sup>a</sup>
	<i>percent</i>		
5	8 ± 1	71 ± 2	21 ± 1
6.5	8 ± 2	71 ± 2	21 ± 2
8	6 ± 6	71 ± 5	23 ± 4
12	5 ± 5	69 ± 1	26 ± 4

<sup>a</sup> Mean ± standard deviation of  $\frac{\text{individual retinyl ester}}{\text{total retinyl ester}} \times 100$  (%) per sample analyzed. Data were calculated from the chylomicron fractions of eight studies (compare Table 3).

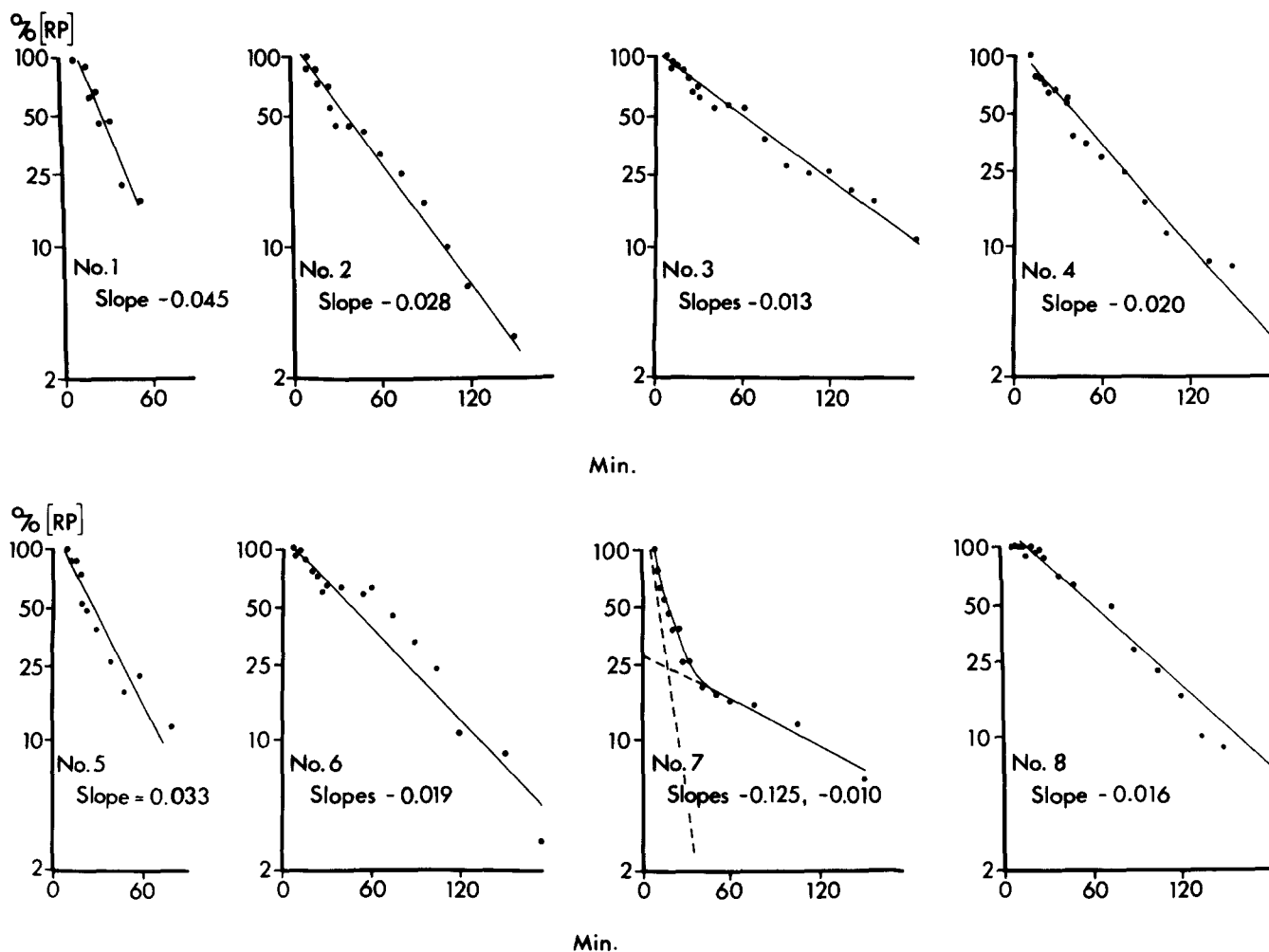


Fig. 2. Plasma retinyl palmitate (RP) disappearance curves after intravenous pulse-injection of plasma containing RP-labeled chylomicrons. Data are expressed in % of peak RP plasma level. Kinetic evaluation and kinetic parameters of all studies are given in Table 5.

cay curve of study No. 3 (Fig. 2) exhibited two unexplained plateaus of 15-min duration. In study No. 8 (Fig. 2), initial saturation is suggested by RP plasma disappearance curve during the first 20 min; in this study the highest triglyceride and RP dose had been infused (Table 5). One study (Fig. 2, No. 7) clearly ( $P < 0.001$ ) demonstrated a slow second kinetic process (apparent  $t_{1/2} = 69$  min) accounting for 13% of the RP plasma decay. In summary then, in seven of eight studies, the decay of RP was monoexponential and in one it was biexponential with a major, rapid elimination process (87% of the RP decay) and a minor, slow elimination process.

The kinetic parameters obtained from least squares fit of the disappearance data are given in Table 5. The apparent elimination constant averaged  $-0.037 \pm 0.037$   $\text{min}^{-1}$  and the half-life  $29 \pm 16$  min. The apparent volume of distribution was  $109 \pm 25\%$  of the estimated plasma volume. Plasma clearance of RP was  $130 \pm 97$  ml/min calculated as  $V_d \times k_e$ .

## DISCUSSION

Retinyl esters, triglycerides, and cholesteryl esters are constituents of the lipid core of chylomicrons and intestinal VLDL (23). When high concentrations of chylomicrons and VLDL are present, there is net transfer of chylomicron core constituents to LDL in animal species with lipid transfer proteins (in the  $d > 1.25$  fraction of plasma) (24). Compared to other vertebrates, man has intermediate activity of cholesteryl ester exchange protein (25). We, therefore, first tested in vitro the extent of transfer from chylomicrons to LDL and found it to be insignificant. Our observations are supported by recent studies of Morton and Zilversmit (26) who found that in vitro transfer of retinyl esters from artificial liposomes to LDL by the purified human cholesteryl ester exchange protein was low (6.6%) during 3 hr of incubation. It was only 20% of the rate of cholesteryl ester transfer and 27% of the rate of triglyceride transfer. The conditions

TABLE 5. Kinetic analysis of plasma retinyl palmitate (RP) disappearance after intravenous injection of RP-labeled chylomicrons<sup>a,b</sup>

Study No.	Subject	Dose of RP Administered	Apparent V <sub>d</sub> (% Plasma Vol)	k <sub>e</sub>	Half-life	Clearance = V <sub>d</sub> × k <sub>e</sub>	No. of Exponentials <sup>b</sup>
		mol		min <sup>-1</sup>	min	ml/min	
1	P.B.	0.36	98	0.045	15.3	148	1
2	L.S.	3.44	81	0.028	24.8	67	1
3	J.M.	2.22	144	0.013	53.3	69	1
4	M.M.	5.18	136	0.020	34.7	95	1
5	M.H.	1.09	126	0.033	21.0	153	1
6	D.K.	2.33	99	0.019	36.3	77	1
7	J.F.	1.37	77	0.125	5.6	355	2 <sup>c</sup>
8	J.J.	5.46	113.7	0.016	42.3	77	1
Mean ± SD		2.68 ± 1.87	109 ± 25	0.037 ± 0.037	29.4 ± 16.0	130 ± 97	

<sup>a</sup> Chylomicrons were harvested by plasmapheresis 5–6 hr after a test meal (100 ml of cream/m<sup>2</sup> body surface area and 60 mg retinol equivalent of retinyl palmitate/m<sup>2</sup>) and after 2 days were reinjected into the donor.

<sup>b</sup> The data from the entire decay period were tested for a mono-, bi-, and triexponential decay function using a computer program (19) and analyzed by an F-test to determine whether the fit of the data is significantly ( $P < 0.05$ ) improved by introducing an additional exponential (20). Final fit of the data to the resulting mono- or biexponential equation was performed with a nonlinear least squares program (21).

<sup>c</sup> In this study, a second slow elimination process ( $k_e = 0.010 \text{ min}^{-1}$ , half-life 69 min) was found, accounting for 13% of the RP plasma decay.

of the assay reported by Morton and Zilversmit and of the in vitro incubations in this study were comparable. Similarly, only about 6% of the retinyl palmitate was transferred from chylomicrons to LDL during storage of plasma for 42 hr (Table 2). During the course of postprandial lipemia, we observed a continuous slight increase of the retinyl palmitate concentration in the LDL fraction which accounted for 6.4% of the total retinyl palmitate of all lipoprotein fractions during 12 hr. In similar studies in man recently reported by Wilson, Chan, and Bell (27) 95% or more of retinyl esters remained in chylomicrons and VLDL.

Since intestinal VLDL and chylomicron remnants cannot be separated from hepatic VLDL by conventional plasma lipoprotein separation methods, we could not study the extent of RP transfer from intestinal VLDL to hepatic VLDL in vivo. In vitro, however, there was no significant transfer of RP from chylomicrons to VLDL: 2% of the label appeared in the combined VLDL fractions A and B. Only 0.1% of RP transferred to the VLDL C subclass containing the bulk of hepatic VLDL (12). Furthermore, the disappearance kinetics of intravenously administered RP-labeled chylomicrons indicate predominantly a single process with a rate constant that is 3–10 times faster than the one reported for the fast component of the plasma decay of radioiodinated VLDL-apoprotein B, representing catabolism of hepatic VLDL (28, 29). Therefore, a major transfer of label to hepatic VLDL did not occur. Transfer of RP to the HDL fraction was not detectable in vitro and only about 3% was detected in vivo. Only one study (no. 7) clearly showed a slower second decay function accounting for 13% of the plasma RP decay, possibly due to RP transferred during storage of plasma to lipoprotein classes with slower turnover. Thus, RP proved to be a stable endogenous label for chylomicrons and their remnants.

Intravenously administered chylomicrons labeled with RP were cleared from the plasma primarily by an apparent first order kinetic process in a one-compartment model. Do these kinetics describe the hepatic uptake of chylomicron remnants in humans that is well characterized in experimental animals (30–32)?

Chylomicron removal kinetics have been assessed in humans by measurement of plasma disappearance of chylomicron triglyceride after bolus injection of chylomicrons (33), or by inducing a steady state of plasma triglyceride concentration by intraduodenal infusion of fat at a constant rate (34). The short half-lives reported, in the range of 4 to 8 min, consequently apply to lipoprotein lipase-mediated lipolysis of chylomicron triglycerides. The longer half-life of RP suggests that this processing step from chylomicrons to remnants is probably not rate-limiting for its plasma clearance.

Lipoprotein lipase-mediated lipolysis is a saturable enzymatic reaction (35). Except for the first 20 min in study No. 8, we did not detect any zero-order kinetic components in the RP disappearance curves. Therefore, the assumption seems justified that the lipoprotein lipase system was not saturated under the conditions studied. Hallberg (33) injected thoracic duct lymph chylomicrons into humans and saturated the lipoprotein lipase system. The chylomicron load necessary for saturation was more than 0.20 g of triglyceride per kg body weight given as a bolus injection. In our study, 10% or less of that triglyceride load was infused.

Similarly, apolipoprotein E transfer from HDL to chylomicrons during alimentary lipemia (36) is faster than the retinyl palmitate disappearance we measured. Therefore, the process involved in chylomicron remnant formation do not appear to be rate-limiting for the plasma clearance of RP-labeled chylomicrons under the conditions studied.

Hepatic chylomicron remnant uptake in animal experiments (30, 32) is 2 to 3 times faster than RP removal from plasma in humans. The model used in such studies does not simulate physiological conditions. Chylomicron remnants are produced by perfusion of chylomicrons in eviscerated rats, separated from the plasma in the intermediate density lipoprotein fraction (d 1.006–1.019 g/ml) by ultracentrifugation and then injected intravenously into intact animals or isolated perfused livers to characterize the kinetics of hepatic chylomicron remnant uptake. Under physiological conditions it is likely that chylomicron remnant particles are taken up by the liver via apoE receptor-mediated endocytosis as a random process involving particles of a wide density range, uptake being favored by decreasing particle size and apoprotein C-III content and increasing apoprotein E content (37, 38). This notion is supported by the fact that during postprandial lipemia only a very small amount of RP is present in the IDL fraction (table 3). During postprandial lipemia in patients with normotriglyceridemic abetalipoproteinemia, only very small amounts of intestinal IDL are found in plasma (39). This rare disorder is characterized by absent hepatic VLDL secretion and hence absent plasma LDL, but normal secretion of chylomicrons and VLDL by the intestine. It is, therefore, conceivable that hepatic chylomicron remnant uptake in man proceeds at a somewhat slower rate than in animal experimental models, which exclusively employ small chylomicron remnants in the IDL density range.

We conclude that retinyl palmitate is a useful endogenous label for chylomicrons in humans and that the plasma clearance of intravenously administered chylomicrons labeled with retinyl palmitate is probably an estimate of chylomicron remnant clearance. ■

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