Kinetics of chylomicron remnant clearance in normal and in hyperlipoproteinemic subjects

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Abstract The kinetics of chylomicron metabolism have been studied by measuring retinyl palmitate in chylomicrons and their remnants for 10–12 hr following oral administration of vitamin A and Lipomul® in three groups of adult male subjects: A) normal plasma triglyceride levels (n = 7); B) endogenous hypertriglyceridemia (n = 12); C) apolipoprotein E (apoE) phenotype E2/2, with Type 3 hyperlipoproteinemia (n = 4) or normal plasma lipids (n = 1). A multicompartmental model was developed using SAAM 27 to characterize the appearance, intravascular metabolism, and clearance from the plasma of retinyl palmitate-labeled dietary lipoproteins. The half-times for retinyl palmitate clearance from the chylomicron remnant fraction ($T_{1/2}$ REMNANT) were 14.1 ± 9.7 min in Group A; they were prolonged in Group B (50.7 ± 20.8 min) and were extremely prolonged for Type 3 subjects in Group C (611.9 ± 419.9 min). One subject with the apoE 2/2 phenotype and normal plasma triglycerides had a $T_{1/2}$ REMNANT of 66.8 min. $T_{1/2}$ REMNANT was highly correlated with fasting plasma triglycerides in Group A and B (r = 0.77, slope = 0.15), and in Group C (r = 0.97, slope = 0.85). These results support the interpretation that delayed chylomicron remnant clearance in subjects with endogenous 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. The subjects with apoE 2/2 have an additional defect in the removal of chylomicron remnants presumably due to the structural abnormality in their apoE.—**Cortner, J. A., P. M. Coates, N-A. Le, D. R. Cryer, M. C. Ragni, A. Faulkner, and T. Langer.** Kinetics of chylomicron remnant clearance in normal and in hyperlipoproteinemic subjects. *J. Lipid Res.* 1987. **28:** 195–206.

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Retinyl esters have been used to label lipoproteins of dietary origin (1–6). The rationale for this approach is based on the metabolic fate of vitamin A (retinol) ingested in the diet. Like cholesterol, retinol is esterified in the intestinal mucosa to long-chain fatty acids (e.g., palmitate) and secreted into the intestinal lymph in the hydrophobic core of chylomicrons. As these lipoprotein particles undergo intravascular metabolism, they become remnants still retaining the retinyl esters in their core (7, 8). These remnants are thought to be removed from the circulaton by hepatic uptake through a cell membrane receptor specific for the apolipoprotein E (apoE) of these remnant particles (9), followed by lysosomal hydrolysis of the cholesteryl esters and retinyl esters to free cholesterol and retinol, respectively. Unlike cholesterol, dietary-derived retinol cannot be recycled into hepatically derived very low density lipoproteins (VLDL), nor can it be synthesized de novo. Instead, it can be stored in the hepatocyte or secreted into the plasma complexed with retinol-binding protein for transport to peripheral target tissues (10).

Hazzard and Bierman (1) described the first human studies in which vitamin A was used to label lipoproteins of dietary origin. More recently, Wilson and co-workers (2–4) and Berr and co-workers (5, 6) have used this approach to investigate the metabolic fate of retinyl palmitate-labeled chylomicrons and their remnants in normal and hypertriglyceridemic human volunteers. With the use of a multicompartmental kinetic model for the analysis of data derived from a protocol in which dietary lipoproteins are endogenously labeled with retinyl palmitate, we have been able to estimate rates of clearance of chylomicrons and their remnants in subjects with normal plasma lipids, endogenous hypertriglyceridemia, and Type 3 hyperlipoproteinemia.

Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; HPLC, high performance liquid chromatography; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; $T_{1/2}$ CHYLO, half-time for retinyl palmitate clearance from the chylomicron fraction; $T_{1/2}$ REMNANT, half-time for retinyl palmitate clearance from the chylomicron remnant fraction; VLDL, very low density lipoproteins.

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METHODS

Subjects

Table 1 briefly summarizes the characteristics of the 24 subjects. The table is arranged into three groups: A) 7 subjects with a fasting plasma triglyceride level below 200 mg/dl were laboratory volunteers or unaffected members of families under study for inherited hyperlipoproteinemias; none had evidence of coronary artery disease; B) 12 subjects with endogenous hypertriglyceridemia, defined as a fasting plasma triglyceride level above 200 mg/dl, with or without hypercholesterolemia; all had a previous history of myocardial infarction or other evidence of coronary artery disease, and a family history of premature coronary artery disease; C) five subjects with the apoE 2/2 phenotype; four of them had Type 3 hyperlipoproteinemia, defined as the presence of β-migrating VLDL and a VLDL cholesterol/total triglyceride ratio of > 0.3; one of the subjects, on numerous occasions, has had plasma levels of total cholesterol and triglycerides within the normal range. None were excessively obese; only subjects #12, 17, 21, and 23 were $> 120\%$ of ideal body weight (11). All of the subjects were white males. In all cases, informed consent for these studies was obtained and the studies were approved by this institution's Committee for the Protection of Human Subjects.

TABLE 1. Clinical and laboratory characteristics of study subjects

Subject	Age	Weight	%IBW[a]	Fasting Lipid Level TG[b]	Chol	HDL	RP	ApoE Type
	yr	*kg*						
A. Normal plasma triglycerides								
1	31	64	82	70	143	42	50	3/3
2	54	73	82	74	192	55	135	4/3
3	41	75	89	94	173	56	193	3/3
4	23	91	101	95	105	30	268	3/2
5	37	59	78	102	141	53	97	3/2
6	22	75	98	108	187	40	375	3/2
7	35	86	100	151	279	25	693	3/3
B. Endogenous hypertriglyceridemia								
8	55	86	116	207	221	15	346	3/3
9	40	96	110	208	338	22	337	4/3
10	36	99	119	218	180	28	461	3/3
11	64	82	105	218	293	41	572	4/4
12	58	116	124	236	144	23	116	3/3
13	33	86	103	256	358	40	1651	3/2
14	47	75	96	267	256	24	1041	4/3
15	56	83	118	279	300	28	988	3/3
16	55	91	101	288	237	23	212	4/3
17	34	115	126	417	212	21	561	3/3
18	67	86	104	456	215	23	351	3/3
19	41	96	98	525	244	18	243	3/2
C. ApoE 2/2 with or without Type 3 hyperlipoproteinemia								
20	39	95	114	272	259	19	179	2/2
21	58	102	120	495	317	32	1096	2/2
22	54	104	118	783	502	36	753	2/2
23	18	93	125	1472	723	28	846	2/2
24	41	64	82	178	234	42	1479	2/2

[a]Percent of ideal body weight (%IBW) calculated according to Metropolitan Life Insurance tables (ref. 11).

[b]TG, triglycerides, mg/dl plasma; Chol, cholesterol, mg/dl plasma; HDL, HDL cholesterol, mg/dl plasma; RP, retinyl palmitate, nmol/l plasma.

Chylomicron remnant clearance protocol

Patients were admitted to the Clinical Research Center in the evening. They were fasting for at least 12 hr prior to the study. At time zero 15 ml of blood was drawn to EDTA, after which the patients ingested one of two test diets: a) low dose, 25 ml of Lipomul® (Upjohn, equivalent to 17 g of corn oil) plus 1.0 ml of Aquasol A® (USV Laboratories, equivalent to 50,000 I.U. of vitamin A); or b) high dose, 50 ml of Lipomul plus 2 ml of Aquasol A (i.e., twice the fat and vitamin A content of the low dose). Our primary concern was to give the minimum dose of fat and vitamin A necessary to provide adequate plasma levels of retinyl palmitate in the later phase of the study. Based on preliminary studies, we chose to administer the low dose to subjects who, by history, had elevated plasma triglycerides and the high dose to subjects with normal plasma triglycerides. Thus, all subjects in Group B and C except for #10, 11, 12, and 24 were given the low dose. In Group A, all subjects were given the high dose; subjects #2 and #5 were also studied using the low dose protocol. Every hour for 10–12 hr after administration of the test dose, 15-ml blood samples were drawn into EDTA. Patients remained fasting except for water until the end of the study, and reported no side effects other than mild headache and gastric upset.

Blood samples were kept on ice in the dark prior to isolation of plasma lipoproteins. One ml of plasma was obtained at each time point for measurement of total retinyl palmitate. The remaining plasma was overlaid with 2 ml of 0.195 M sodium chloride–0.002 M EDTA, pH 7.4, and centrifuged at 20,000 *g* at 4°C for 30 min to obtain chylomicrons. When necessary, a second such centrifugation was performed and the supernatants were pooled. This fraction will subsequently be referred to as CHYLO. The infranatant plasma was used to isolate lipoprotein classes of different densities by sequential density ultracentrifugation using standard techniques (12). The $d < 1.006$ g/ml fraction will subsequently be referred to as REMNANT, although it is recognized that this fraction contains hepatic VLDL and small nascent chylomicrons in addition to chylomicron remnants. All operations involving samples for retinyl palmitate measurement were carried out in low light.

Analytical methods

Retinyl esters were measured by high performance liquid chromatography (HPLC), using a modification of the method described by Ross (13). Plasma samples or isolated

lipoprotein fractions were extracted with 4 volumes of ethanol and then with 12 volumes of hexane containing butylated hydroxytoluene (5 μg/ml). The hexane layer was evaporated under nitrogen; lipid extracts were stored at −70°C under nitrogen. Prior to assay, extracts were resuspended in tetrahydrofuran and injected into a Waters Model 6000 system HPLC with a reversed phase column, C_{18} μBondapak® (Waters), using acetonitrile–tetrahydrofuran–water 90:9:1 as the mobile phase. Flow rate was 1.5 ml/min. Retinoid compounds were detected using a fixed wavelength detector with a 313 nm filter. Retinyl esters were quantitated by measurement of peak height and related to standard curves for retinyl esters. Results were expressed as nmol of retinyl ester per liter of plasma. The major peak, eluting at 14 min, co-eluted with authentic retinyl palmitate and accounted for 75–80% of the total retinyl esters recovered. The limit of detection, with a signal-to-noise ratio of 4, was approximately 10 pmol. Reproducibility of the assay was ± 5% over the usual working range of 25-2000 pmol retinyl palmitate.

ApoE phenotypes were determined by isoelectric focusing (14) using blood collected into EDTA. VLDL was isolated by ultracentrifugation in a Type 50 rotor using a Beckman L2-65B ultracentrifuge at 100,000 *g* for 16–20 hr after overlaying plasma with 0.195 M sodium chloride–0.002 M EDTA. VLDL was delipidated twice using five volumes of acetone–ethanol 1:1 on dry ice. Apolipoproteins from VLDL were resuspended in diethyl ether and centrifuged; residual ether was evaporated under nitrogen, and the pellet was then stored at −70°C. For isoelectric focusing, apolipoproteins from VLDL were resuspended in a solubilization buffer (0.02 M Tris-HCl, pH 8.2, containing 8 M urea). One half of each sample was incubated with 1% (v/v) β-mercaptoethanol (Sigma), the other half with 5 mM β-mercaptoethylamine (cysteamine hydrochloride, Sigma) for 30 min at 37°C. Polyacrylamide gels (7.5%) containing 6 M urea and 1.6% ampholytes (equal volumes of LKB Ampholines, pH 3.5–5.0 and 5.0–7.0) were prefocused at 100 V for 2–4 hr. Samples were applied and focusing was carried out overnight at 200 V at 4°C with an anode solution of 1.5% phosphoric acid and a cathode solution of 0.02 M sodium hydroxide. Gels were stained with 0.04% Coomassie Blue G250 (Sigma) in 3.5% perchloric acid and destained in 7% acetic acid. ApoE phenotypes were designated according to the recommendation of Zannis et al. (15). The frequencies of apoE phenotypes among 441 patients and volunteers studied in our laboratory were: E2/2, 1.4%; E3/2, 22.4%; E3/3, 47.8%; E4/3, 24.5%; E4/4, 2.5%; E4/2, 1.4%.

Plasma total cholesterol and triglyceride levels were measured using enzymatic reagent kits from Boehringer Mannheim Corp. (Indianapolis, IN). Plasma HDL-cholesterol levels were measured following precipitation of apoB-containing lipoproteins with phosphotungstic acid/magnesium chloride (Boehringer Mannheim Corp.). Lipoprotein electrophoresis was carried out in agarose gels (Bio-Rad, Richmond, CA) which were stained with Oil Red O.

Statistical methods

All statistical procedures were performed via computer using the Northwest Analytical Statpak statistical software package.

For each of the variables of interest (e.g., parameters of retinyl palmitate flux), distributions, means and standard deviations were examined for the entire sample and for each sub-group. Homogeneity of variances among groups was evaluated by Bartlett's chi-square statistic or by an F-test of sample variances. For comparisons of means among groups with approximately normal distributions, an overall ANOVA F-test was performed. For distributions departing radically from the normal, the Kruskal-Wallis non-parametric one-way ANOVA-by-ranks test was used. Significance was assigned to comparisons that attained a probability level of 0.05 or less.

Provided that the overall test was significant, pair-wise comparisons between group means were made using the *t*-test for independent groups of unequal size. For comparisons between groups with unequal variances, the *t*-statistic was based on separate estimates of the population variance, while a pooled-variance estimate provided the basis of the *t*-statistic when groups of equal variance were being compared.

In some analyses, Pearson product-moment correlation coefficients were calculated, for example, between fasting triglyceride level and remnant retinyl palmitate clearance.

Compartmental analysis

A multicompartmental model was developed using SAAM 27 (16), in order to describe the metabolic processes that generated the curves for retinyl palmitate appearance and disappearance in lipoproteins of intestinal origin. The kinetic parameters of retinyl palmitate metabolism are defined in **Table 2.** With this approach, the effect of continous appearance of newly formed, retinyl palmitate-labeled lipoproteins in the plasma could be distinguished from the effects of catabolic processes (delipidation and hepatic uptake). The processes which are involved in the generation of the retinyl palmitate curves (**Fig. 1A**) can be described by the model in terms of four subsystems (Fig. 1B).

1) The precursor subsystem. For each individual, a given fraction of the administered dose of retinol will ultimately be recovered in the circulation as retinyl palmitate. This fraction is estimated in the model by the parameter P(1). Note that this reflects only plasma retinyl palmitate and not all of the retinyl esters derived from the ingested vitamin A.

2) The synthesis subsystem. The appearance of absorbed retinol in plasma as retinyl palmitate is not an instantaneous event but occurs over a period of time. This process is reflected in the different degrees of broadness in the peak of the retinyl palmitate curves in different subjects. The

TABLE 2. Kinetic parameters of retinyl palmitate metabolism

Parameter	Definition
P(65)	Fraction of absorbed retinyl palmitate appearing initially in chylomicrons (d < 1.000 g/ml)
1-P(65)	Fraction of absorbed retinyl palmitate appearing initially in chylomicron remnants (1.000 < d < 1.006 g/ml)
P(6)	Fractional turnover (in hr^{-1}) of each chylomicron pool (6, 21, 22, 23)
P(60)	Fraction of the flux through pool 6 (or 21, 22, 23) leaving the plasma by direct removal
1-P(60)	Fraction of the flux through pool 6 (or 21, 22, 23) converted by delipidation to the next pool (21, 22, 23, or 7)
P(7)	Fractional turnover (in hr^{-1}) of the chylomicron remnant pool (7)
P(70)	Fraction of the flux through pool 7 leaving the plasma by direct removal

continuous appearance of newly secreted, retinyl palmitate-labeled material is approximated in our model by a series of four pools (2 through 5) connected in a unidirectional flow, analogous to that previously proposed for VLDL-apoB (17) and VLDL-triglyceride (18) synthesis.

3) The plasma CHYLO subsystem. As chylomicrons are depleted of triglycerides in the circulation, the resulting partially hydrolyzed lipoproteins might either be recovered in a subpopulation of smaller particles or be directly removed in toto from the circulation. These two metabolic fates of the chylomicron must be reflected in the kinetics of retinyl palmitate, which has been shown to remain associated with the particle as it undergoes hydrolysis by lipoprotein lipase (6). Thus for each subpopulation of chylomicron particles, the model can estimate an overall turnover rate, designated as P(6), as well as the fraction of the turnover, P(60), which can be directly removed. The turnover rate associated with direct loss of retinyl palmitate from each subpopulation following delipidation is thus defined as P(6) × P(60). The turnover rate associated with conversion to smaller particles following delipidation, on the other hand, is defined as P(6)[1-P(60)].

While the retinyl palmitate curves from a few of the studies (3 out of 24) could be approximated by the kinetics of a single chylomicron subpopulation, analysis of the majority of the chylomicron retinyl palmitate curves required at least four subpopulations of chylomicron particles differing in size and triglyceride content. Without experimental data on the kinetics of retinyl palmitate in each subpopulation, we must make a number of assumptions to resolve this complex system. These include the assumption that all four subpopulations are kinetically similar. Thus in our model, small and large chylomicrons are metabolized at the same fractional rate; small chylomicrons (i.e., pool 23), however, could leave the CHYLO fraction in one step while the majority of the larger chylomicrons (pool 6) would be

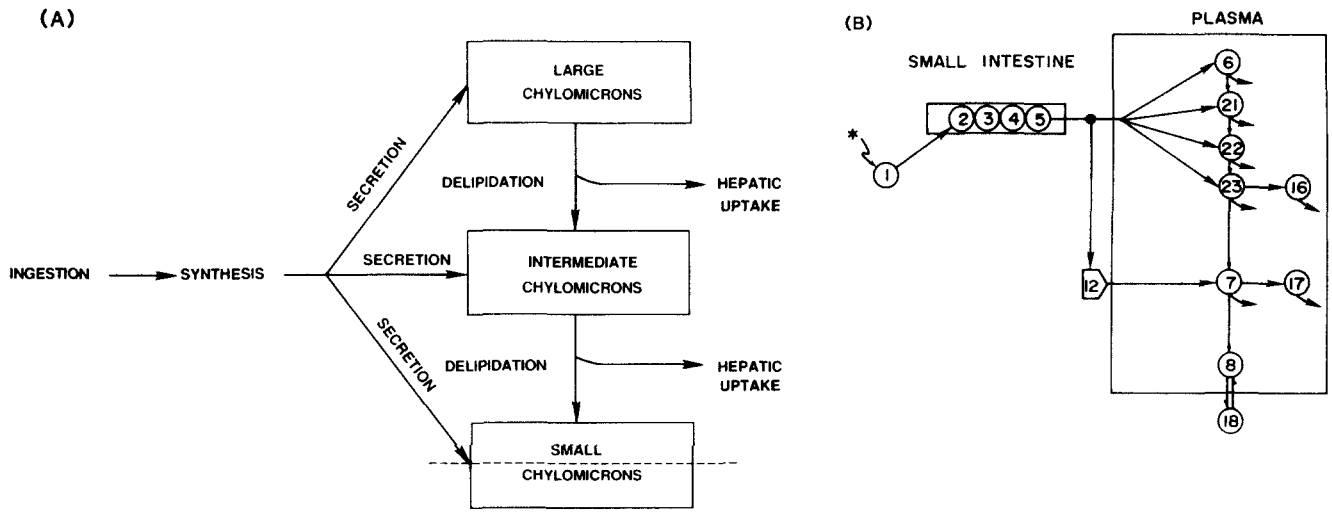

Fig. 1. Schematic representation of: (A) the physiological processes involved in and (B) the components of the multicompartmental model describing the metabolism of retinyl palmitate-containing chylomicrons and their remnants. Dashed line indicates that some small chylomicron particles may appear initially in the circulation in the CHYLO fraction, while others may appear initially in the REMNANT fraction.

sequentially converted to smaller particles within the CHYLO fraction. Another assumption of the model is that the intestine can form and secrete a spectrum of particles differing in size, with the input of retinyl palmitate equally distributed among the four subpopulations.

The final assumption in the CHYLO subsystem involves the slower phase of the chylomicron retinyl palmitate disappearance curve. From the available experimental data, we cannot distinguish between a slow secretory pathway and a slowly catabolized pathway. By analogy with our previous work with VLDL metabolism (19–21), we propose the presence of a slowly catabolized subpopulation (pool 16) of retinyl palmitate-containing particles within the CHYLO fraction. The biexponential disappearance from plasma noted by Berr and Kern (5) upon reinfusion of plasma containing retinyl palmitate-labeled chylomicrons would also support the presence of two catabolic pathways. In any case, the slow pathway accounts for less than 5% of the flux of retinyl palmitate through chylomicrons and would minimally affect our estimates of the clearance of retinyl palmitate from the CHYLO fraction.

4) The plasma REMNANT subsystem. Retinyl palmitate associated with pool 7, the major subpopulation in this density range, may be derived from the hydrolysis of larger particles initially secreted as chylomicrons, or may appear in the plasma for the first time as small chylomicron particles. Since it is physically impossible to separate these two kinds of particles, we must assume that they have similar kinetics. By analogy with the CHYLO subsystem, the metabolism of retinyl palmitate-labeled lipoproteins in pool 7 is characterized by a turnover rate, P(7), and the fraction of that turnover which is accounted for by direct loss is designated as P(70). The turnover rate associated with the direct removal of retinyl palmitate-labeled intestinal lipoproteins from the REMNANT fraction is thus P(7) × P(70).

As in the case of chylomicrons, the slow phase of the retinyl palmitate disappearance curve in the REMNANT fraction is assumed to be associated with a slowly catabolized subpopulation of particles, pool 17. The kinetics of retinyl palmitate in this pool were comparable to those of its counterpart in the CHYLO fraction (pool 16) and thus a single rate was determined for the slowly catabolized subpopulation in each subject.

Although as many as 17 different kinetic parameters were estimated for each set of retinyl palmitate curves (plasma, CHYLO and REMNANT), we were particularly interested in parameters that could characterize the biological lifespan of retinyl palmitate as a marker for chylomicron and chylomicron remnant particles. Half-times for the clearance of retinyl palmitate from the CHYLO and REMNANT fractions were derived, respectively, from the equations:

$$T_{1/2}\ \text{CHYLO, in minutes} = \frac{0.693}{P(6) \cdot P(60)} \times 60$$

$$T_{1/2}\ \text{REMNANT, in minutes} = \frac{0.693}{P(7) \cdot P(70)} \times 60$$

RESULTS

Fasting plasma retinyl palmitate

The distribution of retinyl palmitate in lipoprotein fractions was examined in the fasting plasma of the subjects listed in Table 1. No retinyl palmitate was recovered in the HDL fraction ($1.063 < d < 1.21$ g/ml) or in the $d > 1.21$ g/ml infranatant fraction of plasma in any subject. Among normolipidemic subjects (Group A), most of the retinyl palmitate (75 ± 16%) was recovered in the IDL + LDL fractions ($1.006 < d < 1.063$ g/ml) of fasting plasma, with the remainder (22 ± 15%) present in the REMNANT fraction; virtually none (3.2 ± 5.5%) was present in the CHYLO fraction. Among subjects with endogenous hypertriglyceridemia (Group B), a smaller proportion of retinyl palmitate (49 ± 24%, $P < 0.05$ compared to Group A) was found in the IDL + LDL fractions, with a corresponding increase in the proportion recovered in the CHYLO (8 ± 10%) and REMNANT (44 ± 22%) fractions. Among subjects with apoE 2/2, with or without Type 3 hyperlipoproteinemia (Group C), the distribution of retinyl palmitate was even further disturbed so that only 9 ± 10% ($P < 0.001$ compared to Group A) was recovered in the IDL + LDL fractions, with 72 ± 11% in the REMNANT fraction and 19 ± 13% in the CHYLO fraction. Overall, the fraction of retinyl palmitate recovered in the CHYLO and REMNANT fractions increased as a function of the fasting plasma triglyceride level in the 19 subjects in Group A and B ($r = 0.79$, $P < 0.001$), but was independent of the fasting plasma retinyl palmitate level ($r = 0.20$, not significant).

An experiment was performed to test whether there was transfer of retinyl palmitate from chylomicrons to higher density lipoproteins. Fasting plasma was obtained from subject #2 and contained 229 nmol retinyl palmitate/l, all of which was in lipoproteins of density greater than 1.006 g/ml. The CHYLO fraction was isolated from plasma of subject #5 2 hr after ingestion of vitamin A and Lipomul. One ml of the CHYLO fraction from subject #5, containing 2,413 pmol of retinyl palmitate, plus 4 ml of plasma from subject #2 were incubated at 37°C for 2–7 hr, and lipoproteins were reisolated by centrifugation. Less than 7% of the retinyl palmitate originally present in the CHYLO fraction was recovered in higher density lipoproteins by 2 hr, and this did not increase with longer incubation time.

Chylomicron remnant clearance test

Results from typical studies of a normolipidemic subject, a hypertriglyceridemic subject, and a subject with Type

3 hyperlipoproteinemia are shown in **Fig. 2.** In most subjects in Group A and B, the plasma level of retinyl palmitate peaked 3-6 hr after the ingestion of vitamin A and Lipomul (4.1 ± 1.1 hr in Group A and 4.5 ± 1.2 hr in Group B; difference not significant). This was followed by a rapid decline over a 4-6 hr period, after which the rate of disappearance slowed. The peak for retinyl palmitate appearance in the CHYLO fraction occurred at 3.7 ± 1.2 hr in Group A and at 3.9 ± 0.9 hr in Group B (difference not significant). The peak for retinyl palmitate appearance in the REMNANT fraction was slightly later (4.3 ± 1.6 hr in Group A, 4.9 ± 1.2 hr in Group B; difference not significant). In subjects with the apoE 2/2 phenotype (Group C), with or without Type 3 hyperlipoproteinemia, the peak for retinyl palmitate appearance in plasma was generally later, 8.4 ± 2.1 hr ($P < 0.01$ vs Group A). This delay may be accounted for by the slower rate of removal of retinyl palmitate-containing particles from the circulation of subjects in Group C (see below).

Little change occurred in the retinyl palmitate content of the $1.006 < d < 1.063$ g/ml fraction during the period of study in most subjects. When any change occurred, it was a gradual and moderate (2- to 3-fold) increase; limited studies performed by further separation of this fraction into IDL ($1.006 < d < 1.019$ g/ml) and LDL ($1.019 < d < 1.063$ g/ml) revealed that virtually all of this increase could be accounted for in IDL (data not shown). It should be noted that, even at its peak, the rise in retinyl palmitate in the $1.006 < d < 1.063$ g/ml fractions was only 7.0 ± 6.0% of the rise in total plasma retinyl palmitate levels.

Metabolism of retinyl palmitate

Characteristic curves for the appearance and decay of CHYLO and REMNANT retinyl palmitate, as predicted by the multicompartmental model, are shown in **Fig. 3.** Multicompartmental analysis of data obtained using this labeling protocol allows the estimation of several parameters in the metabolism of dietary lipoproteins. **Table 3, Table 4,** and **Table 5** provide individual as well as mean values of these parameters for subjects in Groups A, B, and C, respectively. The fraction of the ingested dose of vitamin A which appeared in plasma lipoproteins as retinyl palmitate, P(1), was similar in all three groups of subjects, with an overall mean of 0.88 ± 0.13. This value is somewhat higher than that which would be predicted from the proportion of plasma retinyl esters present as retinyl palmitate (75-80% in our experience, and 70% demonstrated by Berr and Kern, ref. 5). As depicted in Fig. 1, a fraction of the absorbed retinyl palmitate, P(65), entered the plasma in the CHYLO fraction and the remainder, 1-P(65), entered as smaller particles which were recovered in the REMNANT fraction. The overall mean for P(65) was 0.54 ± 0.14; there were no significant differences among the values obtained for this parameter in the three groups of subjects.

As the newly secreted chylomicrons undergo hydrolysis,

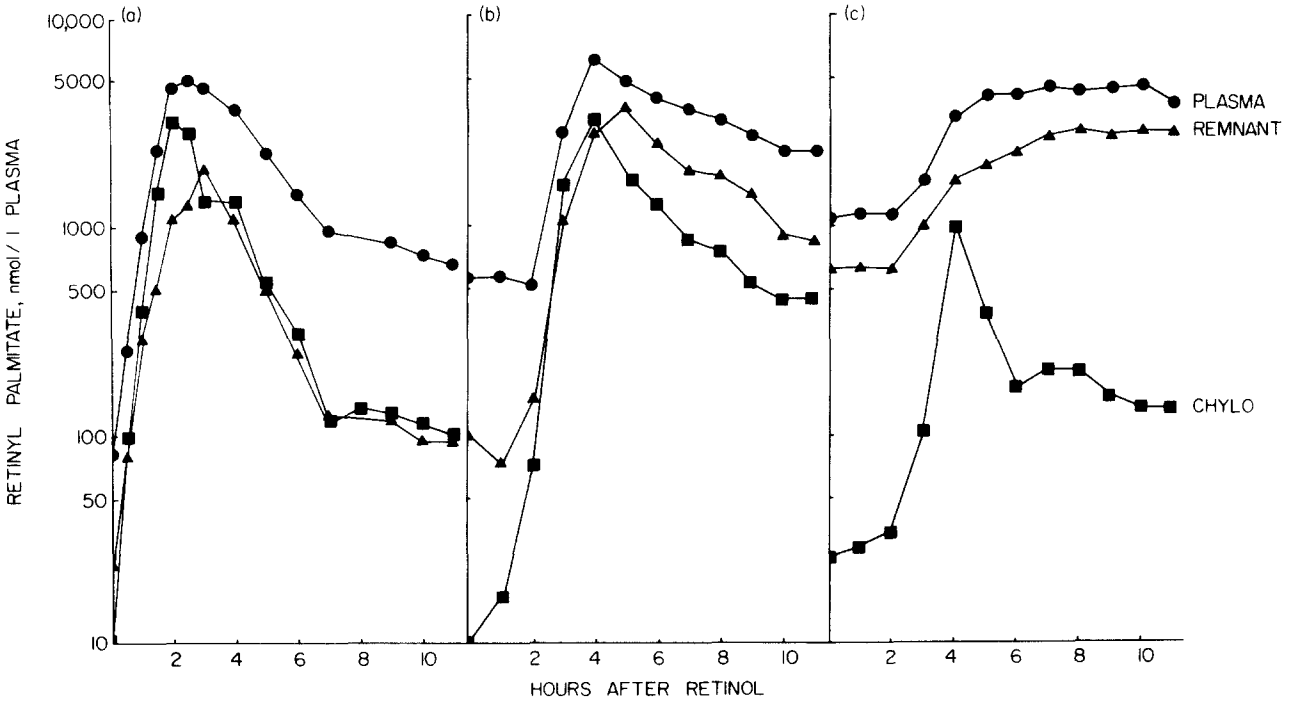

Fig. 2. Retinyl palmitate levels in plasma (●), CHYLO (■). and REMNANT (▲), from zero to 11 hr after ingestion of vitamin A and Lipomul. (a) Normolipidemic subject #5; (b) subject #11 with endogenous hypertriglyceridemia; (c) subject #21 with Type 3 hyperlipoproteinemia.

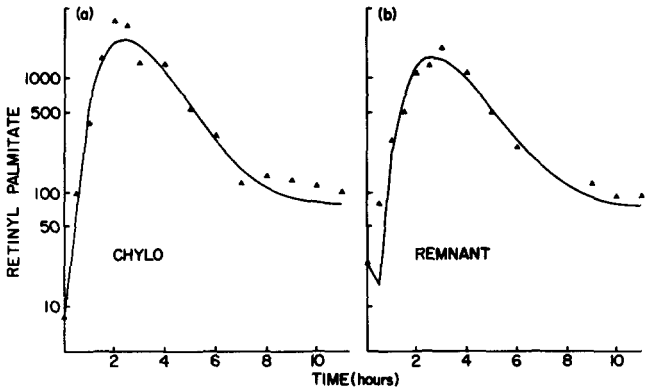

Fig. 3. Decay curves for (a) CHYLO and (b) REMNANT retinyl palmitate (in nmol/liter of plasma) in a normolipidemic subject (#5). (▲) Measured retinyl palmitate levels; (—) values predicted by the model.

the retinyl palmitate label remains with the particles, which are either converted to particles of smaller size or cleared from the circulation via hepatic uptake. In this study, the kinetics of the smaller retinyl palmitate-labeled chylomicrons derived from lipolysis could not be distinguished from those of newly secreted particles comparable in size. For each subpopulation in the CHYLO fraction, that portion of retinyl palmitate-labeled particles cleared by hepatic uptake was defined as P(60). While variability in this parameter could be seen in individual subjects, there were no differences among the three subject groups studied here, with an overall mean for P(60) of 0.46 ± 0.19.

This delipidation cascade yields successively smaller particles until the retinyl palmitate-labeled lipoproteins are recovered in the REMNANT fraction. Another subpopulation of retinyl palmitate-labeled particles in this fraction is derived by direct secretion from the intestine. Within the REMNANT fraction, that portion cleared by hepatic uptake, P(70), was found to be significantly greater than its counterpart, P(60), in the CHYLO subsystem with an overall mean of 0.81 ± 0.10 for the three groups of subjects. Thus, only a small portion of these chylomicron remnant particles is converted to higher density lipoproteins (d > 1.006 g/ml).

Kinetics of CHYLO clearance

The turnover of each CHYLO pool, defined as P(6), was 7.84 ± 2.53 hr^{-1}, and ranged from 4.12 to 11.04 hr^{-1} in normolipidemic subjects (Group A). The resulting half-time for direct removal of retinyl palmitate from this fraction ($T_{1/2}$ CHYLO) was 16.7 ± 9.8 min, ranging from 7.5 to 31.6 min in the seven normolipidemic subjects.

In hypertriglyceridemic subjects (Group B), the turnover of each CHYLO pool, P(6), was significantly reduced to 2.66 ± 1.10 hr^{-1} ($P < 0.01$ compared to Group A). Thus, direct removal of CHYLO retinyl palmitate ($T_{1/2}$ CHYLO) was delayed in this group of subjects, with a mean of 42.5 ± 19.8 min, more than twice that of Group A ($P < 0.01$) (Table 4). It was in the normal range in six of the subjects and was 2–5 times the mean for normals in the other six subjects.

Four subjects with fully expressed Type 3 hyperlipoproteinemia (#20–23) and one normolipidemic individual with the apoE 2/2 phenotype (#24) had delayed clearance of retinyl palmitate from this fraction, with $T_{1/2}$ CHYLO of 35.4 min in subject #24 and 57.9–97.1 min in the other four subjects.

Kinetics of REMNANT clearance

The turnover of the REMNANT pool, P(7), was 4.88 ± 2.98 hr^{-1} in Group A, ranging from 1.60 to 9.47 hr^{-1}. Half-time for direct removal of retinyl palmitate from this fraction ($T_{1/2}$ REMNANT) was 14.1 ± 9.7 min and ranged from 5.2 to 31.3 min in the seven normolipidemic subjects (Table 3), similar to the values for $T_{1/2}$ CHYLO obtained for these subjects.

REMNANT clearance was delayed in Group B, with the mean $T_{1/2}$ REMNANT (50.7 ± 20.8 min) nearly four times longer than in Group A ($P < 0.01$). Only two

TABLE 3. Chylomicron and chylomicron remnant clearance parameters obtained by multicompartmental kinetic analysis in normolipidemic subjects (Group A)

	CHYLO				REMNANT			
Subject[a]	Direct Input P(65)[b]	Fraction Cleared P(60)	Turnover (hr^{-1}) P(6)	$T_{1/2}$ CHYLO (min)	Direct Input 1-P(65)	Fraction Cleared P(70)	Turnover (hr^{-1}) P(7)	$T_{1/2}$ REMNANT (min)
1	0.70	0.15	9.75	28.4	0.30	0.98	8.00	5.3
2	0.40	0.50	11.04	7.5	0.60	0.85	9.47	5.2
3	0.35	0.20	6.58	31.6	0.65	0.90	5.63	8.2
4	0.60	0.44	9.68	9.8	0.40	0.85	1.60	31.3
5	0.50	0.55	4.12	18.4	0.50	0.95	4.32	10.1
6	0.45	0.70	5.50	10.8	0.55	0.75	2.80	19.8
7	0.50	0.50	8.24	10.1	0.50	0.93	2.35	19.0
Mean ± SD	0.50 ± 0.12	0.43 ± 0.20	7.84 ± 2.53	16.7 ± 9.8	0.50 ± 0.12	0.89 ± 0.08	4.80 ± 2.82	14.1 ± 9.7

[a]Numbers refer to subjects in Table 1.
[b]Parameters are defined in Table 2.

hypertriglyceridemic subjects had $T_{1/2}$ REMNANT values which fell in the normal range. This difference in $T_{1/2}$ REMNANT between Groups A and B was a function of the slower turnover of the REMNANT pool, P(7), rather than a reduction in P(70), the fraction of the REMNANT pool which undergoes direct removal (Table 4).

REMNANT clearance in subjects with Type 3 hyperlipoproteinemia (#20–23) was extremely prolonged, with $T_{1/2}$ REMNANT ranging from 216 min to 1120 min. Even the normolipidemic subject with the apoE 2/2 phenotype (#24) had a prolonged REMNANT clearance time (66.8 min) that was nearly five times longer than the mean for Group A. In all subjects with the apoE 2/2 phenotype, this clearance defect resulted from a diminished rate of REMNANT turnover [i.e., P(7)], and not a reduction in the fraction leaving the plasma by direct removal (Table 5).

The half-times for direct removal of retinyl palmitate-labeled lipoproteins do not appear to be dependent on the dose of vitamin A and Lipomul administered. Two normolipidemic subjects were both tested at high and low doses of vitamin A and Lipomul. On the low dose, the values for $T_{1/2}$ CHYLO and $T_{1/2}$ REMNANT were 6.5 and 7.2 min in Subject #2, respectively, very similar to the values shown in Table 3 obtained using the high dose, 7.5 and 5.2 min, respectively. Subject #5, on the low dose, had $T_{1/2}$ CHYLO and $T_{1/2}$ REMNANT values of 20.7 and 16.3 min, again similar to the values obtained using the high dose, 18.4 and 10.1 min, respectively (Table 3).

Correlation of clearance rates and fasting plasma lipid levels

Data from the 19 subjects in Groups A and B were pooled in order to determine whether the calculated rates of CHYLO and REMNANT clearance were correlated with fasting lipid levels. $T_{1/2}$ CHYLO was not correlated with the fasting total cholesterol level ($r = 0.44$, $P > 0.05$), fasting HDL cholesterol level ($r = 0.41$, $P > 0.05$), or fasting retinyl palmitate level ($r = 0.18$, $P > 0.05$). It was modestly correlated with the fasting triglyceride level ($r = 0.54$, $P = 0.016$). $T_{1/2}$ REMNANT was not correlated with the fasting cholesterol level ($r = 0.39$, $P > 0.05$) or fasting retinyl palmitate level ($r = 0.26$, $P > 0.05$). It was highly correlated with the fasting triglyceride level (slope = 0.15, $r = 0.77$, $P < 0.001$), as shown in **Fig. 4.** It was also inversely correlated with the HDL cholesterol level (slope = −1.43, $r = 0.73$, $P < 0.001$).

Fig. 4 (inset) also shows the strong positive correlation (slope = 0.85, $r = 0.97$, $P < 0.01$) between fasting plasma triglyceride level and $T_{1/2}$ REMNANT for the five subjects with the apoE 2/2 phenotype; the delay in their REMNANT clearance was out of proportion to the degree of their fasting hypertriglyceridemia when compared to other hypertriglyceridemic subjects. Even subject #24, with normal plasma lipids, had a $T_{1/2}$ REMNANT at least twice as long as would have been predicted from the regression line drawn for subjects in Groups A and B.

DISCUSSION

The kinetics of chylomicron, and particularly, chylomicron remnant metabolism in man have been little studied. Grundy and Mok (22) instituted a constant fat infusion protocol to raise the level of plasma chylomicron triglycerides to a new steady state and reported that the half-time for chylomicron triglyceride removal in normotriglyceridemic subjects was 4.5 ± 2.9 min. This half-time

TABLE 4. Chylomicron and chylomicron remnant clearance parameters obtained by multicompartmental kinetic analysis in hypertriglyceridemic subjects (Group B)

	CHYLO				REMNANT			
Subject[a]	Direct Input P(65)[b]	Fraction Cleared P(60)	Turnover (hr^{-1}) P(6)	$T_{1/2}$ CHYLO (min)	Direct Input 1-P(65)	Fraction Cleared P(70)	Turnover (hr^{-1}) P(7)	$T_{1/2}$ REMNANT (min)
8	0.75	0.45	1.65	55.4	0.25	0.70	0.82	72.7
9	0.40	0.55	1.37	55.2	0.60	0.95	0.89	49.2
10	0.56	0.45	4.68	19.7	0.44	0.86	2.91	16.6
11	0.50	0.25	2.76	60.4	0.50	0.92	1.12	40.4
12	0.75	0.45	4.69	19.7	0.25	0.80	2.24	23.2
13	0.80	0.40	3.61	28.8	0.20	0.85	1.41	34.7
14	0.75	0.25	2.26	73.5	0.25	0.90	0.70	65.7
15	0.35	0.60	2.55	27.2	0.65	0.80	1.11	46.7
16	0.55	0.70	2.12	28.0	0.45	0.85	0.94	52.2
17	0.55	0.70	2.07	28.6	0.45	0.75	0.88	63.3
18	0.45	0.45	2.20	42.0	0.55	0.60	0.77	90.2
19	0.65	0.30	1.94	71.4	0.35	0.68	1.15	53.3
Mean ± SD	0.59 ± 0.15	0.46 ± 0.15	2.66 ± 1.10[c]	42.5 ± 19.8[c]	0.41 ± 0.15	0.81 ± 0.11	1.25 ± 0.67[c]	50.7 ± 20.8[c]

[a]Numbers refer to subjects in Table 1.
[b]Parameters are defined in Table 2.
[c]Mean is significantly different from corresponding mean for normolipidemic subjects in Table 3 ($P < 0.01$).

TABLE 5. Chylomicron and chylomicron remnant clearance parameters obtained by multicompartmental kinetic analysis in subjects with apoE 2/2 (Group C), with Type 3 hyperlipoproteinemia (#20-23) or normal plasma lipids (#24)

	CHYLO				REMNANT			
Subject[a]	Direct Input P(65)[b]	Fraction Cleared P(60)	Turnover (hr^{-1}) P(6)	$T_{1/2}$ CHYLO (min)	Direct Input 1-P(65)	Fraction Cleared P(70)	Turnover (hr^{-1}) P(7)	$T_{1/2}$ REMNANT (min)
20	0.50	0.40	1.60	64.8	0.50	0.75	0.26	215.7
21	0.30	0.20	3.59	57.9	0.70	0.75	0.17	324.2
22	0.45	0.95	0.45	97.1	0.55	0.80	0.07	787.5
23	0.60	0.45	1.56	59.2	0.40	0.80	0.05	1120.2
Mean ± SD	0.46 ± 0.13	0.50 ± 0.32	1.80 ± 1.31[c]	69.8 ± 18.5[c]	0.54 ± 0.13	0.78 ± 0.03	0.14 ± 0.10[c]	611.9 ± 419.9[c]
24	0.40	0.55	2.14	35.4	0.60	0.76	0.82	66.8

[a]Numbers refer to subjects in Table 1.
[b]Parameters are defined in Table 2.
[c]Mean is significantly different from corresponding mean for normolipidemic subjects in Table 3 ($P < 0.01$).

for chylomicron triglyceride clearance, however, reflects lipolytic activity and not the kinetics of uptake of dietary lipoproteins by the liver (23). The latter process would be better evaluated using a marker for the core of chylomicron and chylomicron remnant particles. There is considerable rationale (1–6) for the use of oral vitamin A to place a safe "tag" (as retinyl palmitate) into the core of human chylomicrons. Since retinyl palmitate remains in the core of the chylomicron until its removal by the apoE receptor-mediated process, the rate of retinyl palmitate disappearance from the plasma should supply an estimate of chylomicron remnant clearance. Hazzard and Bierman (1), using vitamin A as a marker for the metabolic fate of chylomicron particles, have reported that there is dissociation between the kinetics of vitamin A and triglyceride removal, especially in subjects with Type 3 hyperlipoproteinemia.

Berr and Kern (5) used oral vitamin A to endogenously label chylomicrons with retinyl palmitate in normolipidemic volunteers, following which plasma was harvested by plasmapheresis, stored at room temperature for 42 hr, and then pulse-injected back into the donors. The decay of retinyl palmitate from plasma was then followed and the half-time of chylomicron remnant clearance was found to be 29 ± 16 min (assuming first-order kinetics) in eight normolipidemic subjects. However, their method has the disadvantages of removal, storage, and reinjection of plasma containing a mixture of chylomicrons and chylomicron remnants.

We wished to develop a method for the kinetic analysis of chylomicron remnant clearance which would: *1)* be safe for use in children; *2)* be appropriate for repeated use to ascertain the effect of therapeutic intervention in adults and children; and *3)* employ endogenous labeling of dietary lipoproteins in order to avoid the disruption that can occur from removal, isolation, exogenous labeling, and reinjection of lipoproteins.

In order to accurately estimate the rate of clearance of chylomicrons and their remnants from the plasma, no substantial amount of exchange of retinyl palmitate can occur between lipoprotein classes. Our data demonstrate that there is less than 7% transfer in vitro of retinyl palmitate from large triglyceride-rich postprandial lipoproteins to higher density lipoproteins present in fasting plasma. This may have occurred due to exchange of retinyl palmitate from one class of lipoproteins to another, or might have resulted from the formation of lipolytic products during prolonged incubation at 37°C in vitro (24). In any case, the findings are consistent with the results obtained by Wilson and co-workers (2, 24) and Berr and Kern (5), whose in vivo and in vitro studies demonstrated little transfer of retinyl palmitate from chylomicrons to other lipoprotein classes in man. The small rise in IDL and LDL retinyl palmitate levels that we observed during the course of some chylomicron remnant clearance studies could have resulted from exchange of retinyl palmitate from chylomicrons or their remnants, or by the progressive delipidation of chylomicron remnants to particles recovered in the d > 1.006 g/ml fraction. In either case, however, the extent of this rise was so minute compared to the dramatic changes in retinyl palmitate content of particles in the CHYLO and REMNANT fractions that it is extremely unlikely to have had a significant effect on our interpretation of chylomicron remnant clearance.

The retinyl palmitate curves obtained in plasma (Fig. 2) are the result of many metabolic processes. When first-order kinetics were used to analyze the data obtained in the present study, the $T_{1/2}$ values obtained for the disappearance of retinyl palmitate from the CHYLO fraction were 74.4 ± 21.6 min for the normotriglyceridemic subjects, compared with values of 16.7 ± 9.8 min obtained by multicompartmental analysis. Similarly, prolonged $T_{1/2}$ values were obtained for retinyl palmitate clearance from the REMNANT fraction using first-order kinetics for the same subjects (86.4 ± 27.0 min). Simple approximation of these kinetics using only the initial decay phase of the curve would be expected to overestimate half-times, since the continuous input of newly secreted, retinyl palmitate-labeled chylomicrons would not be accounted for. Results from sub-

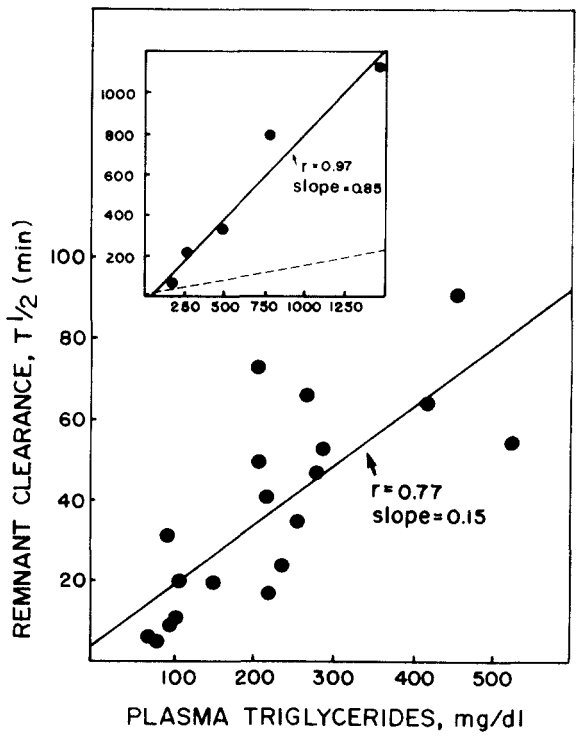

Fig. 4. Chylomicron remnant clearance ($T_{1/2}$ REMNANT) as a function of fasting plasma triglyceride level in 19 subjects in Groups A and B. Inset: $T_{1/2}$ REMNANT as a function of triglyceride level in five subjects in Group C; dotted line indicates the regression line for the data for Group A and B subjects.

jects with Type 3 hyperlipoproteinemia could not be analyzed assuming first-order kinetics, because these subjects did not clear retinyl palmitate from the REMNANT fraction during the 10–12 hr of study. An alternative method for analyzing these data would be to use the area under each retinyl palmitate curve as a composite estimate of the rate of formation and the rate of removal of retinyl palmitate-containing particles. This approach, however, would not be able to distinguish between the irreversible loss of particles from the circulation by hepatic uptake and the conversion of large triglyceride-rich particles to smaller particles recovered in a higher density fraction.

In the present work, by developing a multicompartmental model we can assign rates to each of the various processes involved in the generation of the observed retinyl palmitate curves in plasma. For each subject, these rates represent the best set of parameters that can explain the kinetics of retinyl palmitate from three independent experimental curves (i.e., plasma, chylomicrons, and chylomicron remnants). The proposed model was based on previously reported observations including: *a)* continuous fat absorption and formation of chylomicrons after a meal (25, 26) *b)* heterogeneity in size of newly secreted (27, 28) and circulating (25, 26) chylomicrons; *c)* heterogeneity in metabolic fate of the particles (29, 30); and *d)* ability of the hepatic uptake system to remove chylomicron remnants of a wide range of particle size (31, 32). Thus, a unique feature of this study is the ability to estimate rates of clearance of chylomicrons and of chylomicron remnants via hepatic uptake, reported here as $T_{1/2}$ CHYLO and $T_{1/2}$ REMNANT, respectively.

Following a fat-containing meal, the intestine secretes a spectrum of chylomicrons of different densities due primarily to variable content of triglyceride, which is dependent on the amount of fat ingested (28). Our data suggest that approximately half of the retinyl palmitate was initially secreted in lipoprotein particles recovered in the CHYLO fraction and half in the REMNANT fraction. In the circulation, these lipoprotein particles then undergo lipolysis by lipoprotein lipase, a rapid process with a half-time reported to be 4.5 ± 2.9 min in normolipidemic subjects (22). The resulting particles would also vary in their density as a function of their residual lipid composition; some would be recovered in the CHYLO fraction and other more dense particles would be recovered in the REMNANT fraction. Following lipolysis and the loss of other constituents (apoA-I, apoA-IV, apoE, some phospholipids), chylomicron remnants are formed. Their irreversible removal from the circulation is mediated by hepatic apoE receptors (28). Our $T_{1/2}$ CHYLO and $T_{1/2}$ REMNANT values, therefore, are estimates of the half-lives of these particles from intestinal secretion to hepatic uptake.

The $T_{1/2}$ CHYLO was found to be 16.7 ± 9.8 min and the $T_{1/2}$ REMNANT was found to be 14.1 ± 9.7 min in normolipidemics. These values are probably not different from the value of 29 ± 16 min obtained by Berr and Kern (5) following the reinfusion of plasma containing retinyl palmitate-labeled lipoproteins. The estimates of retinyl palmitate clearance obtained by Berr and Kern and in the present study, however, are substantially shorter than the value of approximately 50 min obtained by other investigators who have followed the decay of ^{125}I-labeled apoB-48 from these particles (33, 34). Whether this difference in kinetics between apoB-48 and retinyl esters represents distinct metabolic fates of these two moieties or is the result of artifacts in the isolation and radioiodination of the lipid-laden chylomicrons in the studies of Haffner et al. (33) and Malloy and Kane (34) cannot be determined.

As a group, subjects with endogenous hypertriglyceridemia had prolonged values for both $T_{1/2}$ CHYLO and $T_{1/2}$ REMNANT; however, $T_{1/2}$ REMNANT was a more discriminating value, with only two of twelve hypertriglyceridemic subjects having $T_{1/2}$ REMNANT values within the normal range. When the data for Groups A and B were pooled, there was a high correlation between $T_{1/2}$ REMNANT and fasting triglyceride level. These findings are consistent with the recent observation of Wilson et al. (3) that plasma retinyl ester levels 12–15 hr after ingestion of a vitamin A-containing meal were correlated with fasting plasma triglyceride levels. They can be readily explained by the fact that, as the concentration of liver-derived VLDL

increases in the circulation, either as a result of overproduction of VLDL (35, 36) or decreased plasma lipolytic activity (37, 38), their remnants compete with retinyl palmitate-labeled chylomicron remnants for a common removal process (39). It is evident, however, from Fig. 4 that there are individuals in Groups A and B with approximately the same fasting plasma triglyceride level who have very different rates of chylomicron remnant clearance. For example, subjects #8–11 have plasma triglyceride levels between 207 and 218 mg/dl, but $T_{1/2}$ REMNANT values between 16.6 and 72.7 min (Table 4). These data suggest that those individuals with relatively fast clearance (e.g., subject #10) may have a defect in the hepatic synthesis of VLDL, leading to overproduction of endogenous triglyceride-rich lipoproteins associated with normal receptor-mediated removal. Others with relatively slow clearance (e.g., subject #8) may be hypertriglyceridemic because they have a primary abnormality in remnant formation and/or clearance.

In four subjects with the apoE 2/2 phenotype and Type 3 hyperlipoproteinemia, $T_{1/2}$ CHYLO was in the same range as the six Group B subjects with abnormal values (57.9 to 97.1 min and 42.0 to 71.4 min, respectively). The $T_{1/2}$ REMNANT values, however, were extraordinarily prolonged in these four subjects (215.7 to 1120.2 min). It has been known for some time that chylomicron remnant clearance is prolonged in patients with Type 3 hyperlipoproteinemia, reflecting the failure of hepatic receptor-mediated uptake due to inherited defects in their apoE (e.g., the Arg_{158} →Cys substitution) (40). The results obtained for our four subjects with Type 3 hyperlipoproteinemia and the apoE 2/2 phenotype illustrate the ability of this method to quantitate, for the first time, this delay in clearance of chylomicron remnants.

It is known from population and family studies that most people with the apoE 2/2 phenotype have normal plasma lipids, despite their apparently defective apoE (41, 42). The results obtained for subject #24 with the apoE 2/2 phenotype and a fasting plasma triglyceride level of 178 mg/dl, illustrate that his $T_{1/2}$ REMNANT was nearly five times longer than the mean rate for normolipidemic individuals with other apoE phenotypes. As a group, the five subjects with the apoE 2/2 phenotype had $T_{1/2}$ REMNANT values that were highly correlated with their plasma triglyceride levels ($r = 0.97$), but with a slope of 0.85. This is significantly different from the slope of 0.15 obtained for the correlation between plasma triglyceride level and $T_{1/2}$ REMNANT clearance among normolipidemic subjects and subjects with endogenous hypertriglyceridemia who had apoE phenotypes other than apoE 2/2. We can conclude that $T_{1/2}$ REMNANT in subjects with normal plasma triglycerides and those with endogenous hypertriglyceridemia would be prolonged by approximately 15 min for every 100 mg/dl increase in their fasting triglyceride level. By contrast, $T_{1/2}$ REMNANT in subjects with apoE 2/2 would be prolonged by 85 min for every 100 mg/dl increase in plasma triglycerides.

We have thus validated a method which allows the determination of removal rates for chylomicrons and chylomicron remnants via hepatic receptor-mediated uptake. The method reported herein has advantages over conventional tracer studies since it does not require the isolation of the chylomicrons for labeling. Also, since no radioactive label is used, this protocol may be carried out in a wider population of subjects, including children.

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