Chylomicron-Retinyl Palmitate Clearance in Type I Hyperlipidemic Families

Dennis L. Sprecher,*‡ Shari L. Knauer,* Donald M. Black,§ Lawrence A. Kaplan,|| Ann A. Akeson,¶ Mary Dusing, David Lattier,** Evan A. Stein,§|| Michael Rymaszewski,* and Dan A. Wiginton‡****

Lipid Research Clinic, General Clinical Research Center, and Departments of Medicine and Pediatrics,‡ University of Cincinnati, Cincinnati, Ohio 45267; Children's Hospital Medical Center,** Cincinnati, Ohio 45229; The Christ Hospital Cardiovascular Research Center,§ Cincinnati, Ohio 45219; Medical Research Laboratories,|| Cincinnati, Ohio 45219; and Marion Merrell Dow Research Institute,¶ Cincinnati, Ohio 45215*

Abstract

Our primary aim was to determine the extent to which intraplasmic retinyl palmitate (RP) transfers to other lipoprotein particles when chylomicron remnants are not produced and/or the plasma RP residence time is increased. The study was conducted on three familial type I hyperlipoproteinemic patients, four lipoprotein lipase (LpL)-deficient heterozygotes, and three controls on a metabolic research unit. To each subject, a fat load was administered containing 16% of total daily calories in type I patients, 40% in heterozygotes and controls, plus 60,000 U/m^2 vitamin A. Triglyceride (TG) and RP levels were evaluated in chylomicron and nonchylomicron fractions.

Delay in the clearance of chylomicron fraction RP and the marked deficiency in nonchylomicron-RP (presumed lack of remnant production) in all three type I patients suggests that RP does not demonstrate significant intraplasmic transfer from chylomicrons to existent apolipoprotein B100 particles. In contrast to noncoincident TG and RP peaking in the normal subject, heterozygotes were found to demonstrate coincident plasma TG and RP curves, which is consistent with a common catabolic pathway for both TG and RP and inconsistent with intraplasmic RP transfer. This corroborates reports on compromised chylomicron clearance in heterozygotes. We conclude that RP is an appropriate representative marker for intestinally derived particles in LpL-deficient or partially deficient individuals. (*J. Clin. Invest.* 1991. 88:985–994.) Key words: chylomicron remnant • compound heterozygote • lipoprotein lipase • mutation • vitamin A

Introduction

Retinyl palmitate (RP)[1] has been used as a marker for chylomicron metabolism because RP is secreted with intestinal particles, and presumably remains highly associated with the chylomicron–chylomicron remnant apolipoprotein B48 (apo B48) particle until the remnant's removal from the plasma (1–3). This occurs primarily by an apolipoprotein E (apo E)–mediated hepatic remnant receptor (4–6). However, the recently suggested intraplasmic transfer of RP from chylomicrons and remnants to denser particles having longer half-lives in the plasma (containing apolipoprotein B-100 [apo B-100] or apolipoprotein A-I [apo A-I]) could lead to incorrect assumptions concerning the presence of chylomicrons or remnants (7). In the totally or partially lipoprotein lipase (LpL)-deficient individual, the delay in chylomicron clearance should give adequate opportunity for RP intraplasmic transfer. Study of type I patients and their parents (obligate heterozygotes) could provide some insight into the validity of the RP method.

Type I hyperlipoproteinemia is associated with a deficiency of functional LpL, measured in plasma in vitro after heparin administration (postheparin lipolytic activity [PHLA], exclusive of hepatic lipase activity) (8–10). Chylomicrons are processed to remnants through the action of LpL. Thus, it would be expected that type I patients have a delay in chylomicron clearance and a marked reduction in remnant production (11). Recent evidence indicates that partial deficiency in LpL activity (heterozygous for a defect at amino acid residue 188) may predispose individuals to hypertriglyceridemia (12). The elucidation of a valid method to study chylomicron metabolism would permit correlations to be drawn between LpL-deficient phenotypes and modifications in metabolic pathways related to triglyceride (TG)-rich particles of intestinal origin.

Many of the aforementioned partially LpL-deficient heterozygotes defective at amino acid residue 188 and predisposed to high TG are, in fact, normotriglyceridemic (12). Variables including gender, hormone levels, diet, alcohol use (13), alternate chylomicron clearance routes (6), remnant receptors (4), and apo E isoforms (14, 15) may modify the chemical and/or biochemical expression of any specific LpL defect. The recent cloning and sequencing of the LpL cDNA (16), the elucidation of the LpL gene structure (17), and the continued characterization of LpL gene defects (18–23) should further clarify the connection between specific mutations and clinical expression.

We investigated the validity of the RP marker method for studying chylomicron and chylomicron remnant catabolism in subjects with reduced LpL function. In addition, using orally administered vitamin A, we describe the in vivo clearance of chylomicrons and remnant formation in LpL-deficient and partially deficient individuals.

Methods

Subjects. The type I subjects studied include two male siblings (DM, the proband, and SM [24]) and a male (MP) from a separate previously unreported family. Diagnosis of type I was based on markedly reduced or absent LpL activity in postheparin plasma, and a lipoprotein pheno-

Address reprint requests to Dr. Sprecher, University of Cincinnati, 231 Bethesda Avenue, ML No. 540, Cincinnati, OH 45267.

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1. *Abbreviations used in this paper:* AUC, area under the curve; LpL, lipoprotein lipase; PHLA, postheparin lipolytic activity; RP, retinyl palmitate; S_f, ultracentrifuge flotation rate; TG, triglyceride.

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type characteristic of type I, i.e., hyperchylomicronemia, low HDL-cholesterol and low LDL-cholesterol.

Family 1. DM was found to have a TG level of 16,000 mg/dl at birth; became ill with gastrointestinal pain, nausea, and vomiting at age 8 wk; and was diagnosed with familial hyperchylomicronemia after a lengthy clinical investigation. His brother, SM, aged 3 yr, was diagnosed at the same time, after xanthomata was noted on his buttocks at age 2½ yr and TG levels were well above 10,000 mg/dl. SM had numerous hospitalizations for severe abdominal pain when he was young, but over the last few years has few, if any, gastrointestinal complaints. DM continues to have approximately yearly episodes of pancreatitis. The father of SM and DM (HM) was diagnosed with type V hyperlipidemia ~ 8 yr ago, whereas the mother (LM) has always had a normal lipoprotein profile.

Family 2. MP presented at 5 wk of age with colicky abdominal pain and grossly bloody stools. A nonfasting lipid profile revealed a triglyceride level of 25,000 mg/dl and he was eventually diagnosed with familial hyperchylomicronemia. MP has done well on a severely restricted diet (< 5–10 g of fat per day) and is able to maintain a fasting TG level of 200–300 mg/dl. With even minor dietary indiscretion, this value quickly increases. He has a sister with familial hyperchylomicronemia (1 yr of age), who presented with TG levels of 10,000 mg/dl at 4 wk of age. She is also able to maintain fasting triglyceride levels of < 300 mg/dl on a very strict diet. The father, MiP, has slightly decreased HDL levels and slightly elevated triglycerides, whereas the mother, MoP, has always been completely normolipidemic.

DNA was isolated by a modification of the method of Bell et al. (25). Southern analysis (26) and sequencing (27) of exonic fragments resulting from polymerase chain reaction (28) demonstrated that DM, SM, and HM are heterozygous for the previously reported mutation at residue 188 (18), and MP and MoP are heterozygous for the previously reported mutation at residue 194 (29). Preliminary data indicates the two remaining allelic defects to be neither the 188 nor the 194 defects, and to be different from each other.

Controls included three healthy adult male volunteers with normal lipid values. Using children as controls was not allowed by the Institutional Review Board. Recruiting children for these studies would be very difficult even if permission was granted. Heterozygotes and normal siblings of type I patients may be potential subjects in the future. No subject was taking any medication with a known lipid-altering effect. Baseline data on each subject are presented in Table I.

The protocol was approved by both the University of Cincinnati and the Cincinnati Children's Hospital Institutional Review Boards. The subjects and the parents of each subject under the age of 18 yr read and signed an informed consent. Blood drawing and heparin administration met guidelines established by the Children's Hospital.

Study design. The studies were conducted at the University of Cincinnati's General Clinical Research Center (GCRC) or the Children's Hospital Clinical Research Center (CRC). Type I patients, heterozygotes, and normal controls were admitted to the metabolic research unit for a 9-d study period. Upon admission, they were placed on isocaloric diets which were based on diet records and questionnaires and provided no more than 5% of the subject's daily caloric needs as fat. Each subject consumed his or her special diet for the entire study period. Fasting blood samples were obtained each morning. Subjects were permitted to move about the research unit freely. Heights were taken on the first day of the study and weights, blood pressures, and temperatures were taken daily.

PHLA. All subjects were tested for LpL activity at least 2 full d before the fat load described below. After a 12-h fast, blood was drawn just before and 15 min after administration of heparin (50 U/kg) (Elkins-Sinn, Inc., Cherry Hill, NJ). Assessment of lipase activity followed the method of Goldberg et al. (30). The substrate emulsion (31) included triolein, glycerol tri [9,10 $^{-3}$H] oleate, and bovine serum albumin (BSA). LpL activity was determined by inhibiting hepatic TG lipase activity with anti-hepatic TG lipase antiserum.

Mass assays. LpL mass was assayed in 0- and 15-min postheparin serum aliquots by the enzyme-linked immunosorbent assay (ELISA) method of Goers et al. (32). Specific activity was calculated by dividing LpL activity (micromoles per milliter per hour) by the mass (micrograms per milliliter), giving units of micromoles per hour per micrograms.

Lipid and apolipoprotein analysis. Plasma cholesterol, TG, and HDL-cholesterol levels were measured. After a 12-h fast, samples were collected, and plasma was separated as for PHLA determinations described above. For lipoprotein fractionation, plasma was ultracentrifuged at 1.006 g/ml for 18 h at 4°C, 39,000 rpm; for determination of HDL-cholesterol plasma was subjected to heparin-manganese precipitation. Cholesterol and TGs were determined in whole plasma, as well as in the infranatant and supranatant fractions. LDL and VLDL were calculated by difference (33). Apo B100 was performed by ELISA (antibody specific for B100) in chylomicron and nonchylomicron fractions with a coefficient of variation of 7% (34, 35). Apo E isoforms were performed with isoelectric focusing gels according to Sprecher et al. (36).

RP and TG clearance. For the first 4 d of the in-house stay, patients were maintained on a low-fat, high-carbohydrate diet. On the fifth day, after a 12-h fast, subjects received a meal that provided 40% (controls and heterozygotes) or 16% (type I) of daily caloric needs as fat. This was done to maximally "stress" the lipolytic system of the normal patient as well as give a substantial load to the type I patient without risking the development of pancreatitis. The fat load was in a milkshake consisting

Table I. Background Data on Subjects

Subject	Age	WT	HT	Quetelet	E form	TG	TC	HDL	LDL
	yr	*kg*	*cm*	*wt/ht²*			*mg/dl*		
Homozygotes									
MP	3	13.4	89.2	1.68	3/3	234*	80	15‡	18
SM	13	58.6	158.0	2.35*	3/4	5,070*	484	9‡	—
DM	11	27.3	130.0	1.62	3/4	4,870*	461	7‡	—
Heterozygotes									
MiP	23	73.9	171.2	2.52	3/3	233*	180	40	93
MoP	22	54.3	157.0	2.20	3/4	65	191	53	111
HM	46	95.0	173.0	3.17*	4/4	468*	221	22‡	—
LM	39	57.9	158.0	2.32	3/3	83	200	47	136
Controls (mean values, n = 3)	29	76.2	168.8	2.67		72	176	56	117

Value in upper decile (*) or lower decile (‡), based on Lipid Research Clinics distributions (see reference 67). TC, total cholesterol.

of a standard nutritional supplement (Ensure), corn oil, and aqueous vitamin A (60,000 U/m^2 body surface area) (Aquasol A, Armour Pharmaceutical Co., Kankakee, IL) (37). Subjects were required to consume the beverage in < 10 min. Blood samples were obtained before and at 2, 4, 6, 8, 12, 18, and 24 h after the meal. Subjects were provided with a fat-free meal after the blood draw at 8 h, but no other food or liquid, except water, was given for 24 h. In addition, blood samples were drawn in type I patients for RP on the mornings of days 7, 8, and 9. Heterozygotes and controls received an identical fat load on day 7; type I children were given the single fat load on day 5 only.

Blood was drawn in tubes containing EDTA, centrifuged, and separated. Plasma was refrigerated until analysis. 1 ml of plasma was overlayed with 4 ml of a solution of NaCl (d = 1.006 g/ml) and centrifuged at 30,000 rpm for 17 min at 20°C in a rotor (model 50.4, Beckman Instruments, Inc., Fullerton, CA) (38, 39). The top milliliter of plasma from each tube was overlaid with 2.5 ml of saline and washed under the centrifugation conditions described above. Both top chylomicron and bottom nonchylomicron fractions were diluted to 5 ml with 0.15 M NaCl and stored at either −20°C or −70°C before analysis. Small samples were taken from the whole plasma and from the top and bottom fractions for TG assay by standard methods (40).

RP was also measured in whole plasma and in the top and bottom fractions. All extractions for RP were performed in a dark room. 0.5–1.0 ml of sample was placed in an extractor tube and 1 ml of ethanol was added while mixing. 3 ml of hexane was added and the mixture was vortexed for 20 min. After 5 min of centrifugation at 2,500 g, 2 ml of the hexane layer was transferred into a test tube; 2 ml of hexane was added to the remaining fluid, and samples were vortexed for 2 min and centrifuged as above. The hexane layer was removed and combined with the first 2 ml of extract. The combined hexane extracts were evaporated to dryness at 37°C under a stream of N_2. The residue was then reconstituted with 200 μl of mobile phase and 25–100 μl of each sample was injected into a high-performance liquid chromatography (HPLC) system (38–40).

The HPLC system consisted of an automated injector (WISP 710B, Waters Associates, Milford, MA), a pump, a detector (model 490, Waters Associates), and an integrator-recorder (Hewlett-Packard Co., Palo Alto, CA). The mobile phase consisting of acetonitrile/chloroform/2-propanol/water (78:16:3.5:2.5 vol/vol) was pumped at 2 ml/min through the HPLC system. Effluent from the 5 μm particle C-18 (octadecyl) Biophase ODS column (Bioanalytical Systems, Inc., W. Lafayette, IN) was monitored at 292 nm (40).

The concentration of RP in each sample was determined by proportional comparison of the RP peak height of the sample (PHs) with the peak height of an external standard (Phx) (retinyl palmitate, Sigma Chemical Co., St. Louis, MO). The RP concentration of the standard was determined spectrophotometrically using the molar absorptivity value of 50,390 $l \cdot mol^{-1} \cdot cm^{-1}$. The appropriate dilution fraction (DF) was used to correct for dilution of plasma RP during ultracentrifugation so that the RP levels in the $d > 1.006$ g/ml and $d > 1.006$ g/ml (i.e., top and bottom fractions) are reported as plasma concentrations for each fraction. The calculation was as follows (40):

$$\frac{\text{PHx (ng standard injection) } 0.2 \text{ ml (DF)}}{\text{PHs (injection volume X) (sample volume)}} = \text{concn RP mg/ml.}$$

Curve evaluation. Assessment of the RP curves was performed in three ways. First, the peak level was recorded. Secondly, the area under the chylomicron RP curve was assessed by drawing the curves for each subject on standard weight paper and weighing the cutout curve on an analytical balance scale. Finally, the time delay within the clearance curve was defined as the difference between time at peak and time when value returned to one-half the difference between peak and baseline (C1/2).

Results

Subjects and curve characteristics. Controls, whose mean baseline TG value was 72 mg/dl (Table I), demonstrated a TG increase twice that of the baseline after the fat load, whereas RP values in whole plasma increased to approximately three times the baseline level (Fig. 1). While on the high-carbohydrate, low-fat diet for 4 d, the mean TG in the three controls rose from 72 to 110 mg/dl before the fat load, carbohydrate induction (41). Chylomicron-RP rose sharply, until hour 6, to approximately

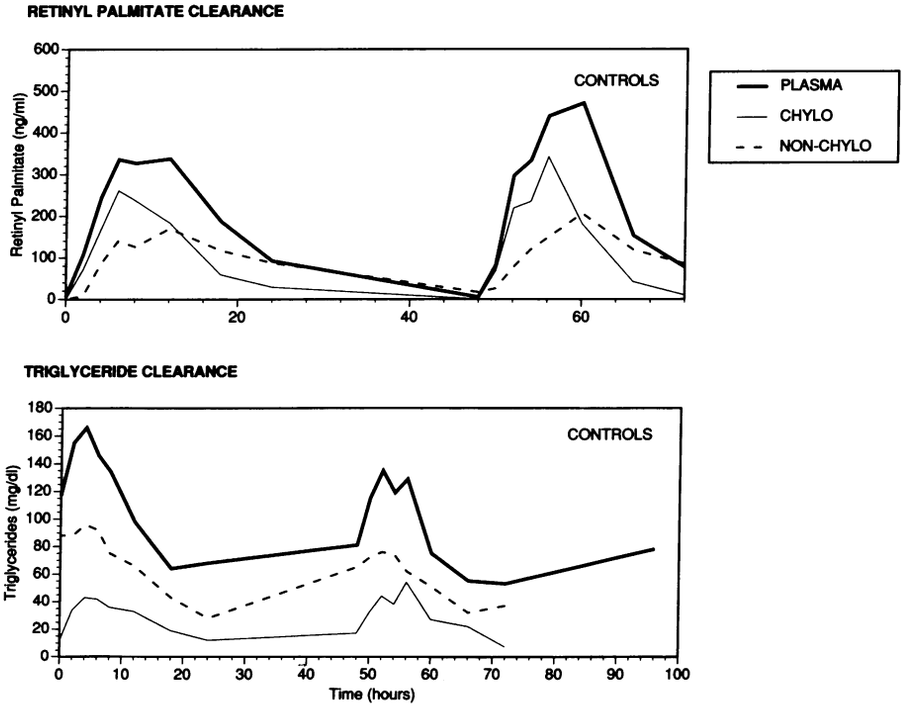

Figure 1. TG and RP chylomicron clearance curves for control patients. Whole-plasma RP and TG curves (*thick line*) demonstrate a similar pattern, but the chylomicron-RP (*thin line*) and nonchylomicron-RP (*dashed line*) curves intersect at hour 12, suggesting a precursor–product relationship. The two sequential fat loads result in very similar curves.

two-thirds the RP value for whole plasma, then returned to baseline within 24 hours. Nonchylomicron-RP began virtually at zero, lagged behind the chylomicron-RP, intersecting it and peaking at approximately hour 12. This finding is consistent with the known precursor–product relationship between chylomicrons and the denser nonchylomicron fraction remnants (42). Results for the controls were fairly consistent for the first and second fat loads, thus demonstrating reproducibility.

The three type I subjects were admitted to the metabolic ward with TG levels ranging from 200 to 5,000 mg/dl; MP is extremely well controlled on a < 5% fat diet at home, thus his level on the ward was only 234 mg/dl. SM and DM are poorly controlled at home and, therefore, had levels of 5,070 and 4,870 mg/dl, respectively (Table I). These levels decreased significantly during the 4-d dietary lead-in period. Values at time 0, immediately preceding the fat load, can be discerned in Fig. 2. DM experienced emesis of 86 ml of milky fluid 2 h and 5 min after ingestion of the fat load. MP was able to ingest only 60% of his fat load. Thus, two of the three type I patients had approximately one-half of the fat load originally proposed. All heterozygotes demonstrated gradual elevation in TG levels on the low-fat, high-carbohydrate diet, except MiP, who demonstrated a decrease. This resulted in only HM being upper decile TG at the time of the fat challenge. Values observed before this study are listed in Table I.

The type I patients showed clear deviations from normal in both TG and RP postprandial clearance (Fig. 2). This is the impression based on any of three different approaches for quantifying the chylomicron RP curve: area under the curve (AUC; 19–55 times greater than for controls), C1/2 (time necessary for values to return to half of maximum level, and three to four times above the mean control value), and actual peak value (Table II). All RP values had returned to baseline by 48 h, however, suggesting an active chylomicron removal system independent of LpL processing. Though SM's plasma TG and RP values were approximately twice those of other type I subjects, he ingested twice the amount of fat that they did. Plasma-RP and chylomicron-RP curves were virtually identical and thus were overlapping in SM as well as DM, indicating relatively little or no nonchylomicron (i.e., remnant) production (Fig. 2). There was some difference between plasma and chylomicron-RP in the RP curves for MP. This difference was not

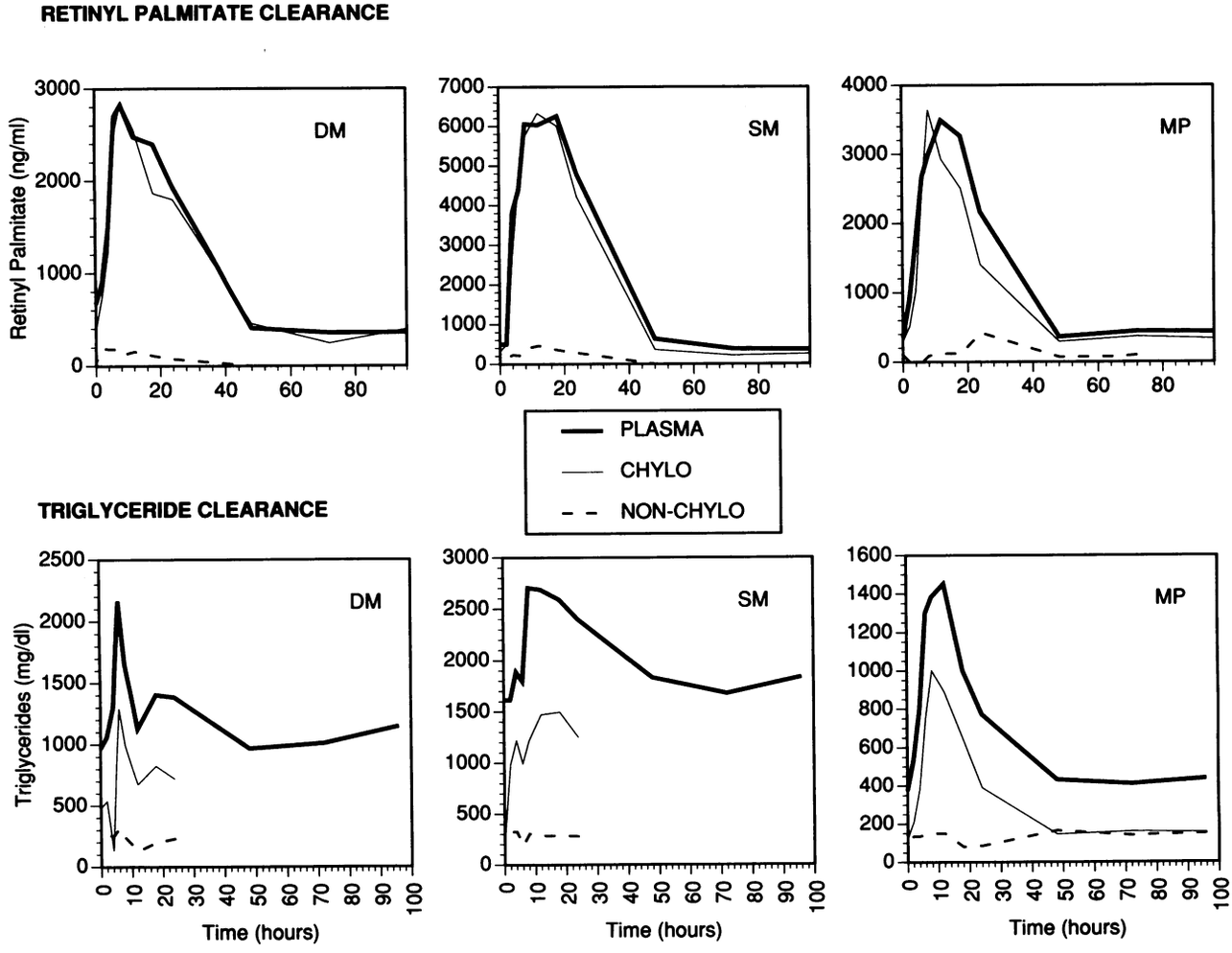

Figure 2. TG and RP chylomicron clearance curves for type I probands SM, DM, and MP. Whole plasma RP and TG curves are coordinated in their rise and decay, while chylomicron-RP is virtually identical to total RP, suggesting the lack of remnant formation. This is supported by low RP levels in the nonchylomicron fraction. TG levels in chylomicron and nonchylomicron fractions were not measured after 24 h in SM and DM. (Note the differences in the scales of the vertical axes.)

Table II. Three Quantifications of Chylomicron RP Clearance Curves

	Area under curve		C 1/2 time		Peak value	
	1st fat load	2nd fat load	1st fat load	2nd fat load	1st fat load	2nd fat load
	μg/ml · h		*h*		*ng/ml*	
Homozygotes						
MP	14,922	—	13	—	3,641	—
SM	41,117	—	14	—	6,330	—
DM	14,273	—	23	—	2,855	—
Heterozygotes						
MiP	4,162	5,870	4	6	2,170	2,660
MoP	643	571	2	4	366	519
HM	6,279	7,240	10	7.5	1,950	2,410
LM	1,955	1,974	5.5	2.5	844	1,175
Controls						
(mean, n = 3)	747	857	4.2	5.7	237	342

totally accounted for in the nonchylomicron fraction. Variation in the assay or processing of the sample could account for this data. Virtually identical RP curves, with an equivalent delay in chylomicron-RP clearance for a 26-yr old type I patient (data not shown) suggest that lack of pediatric controls does not compromise the interpretation of the results.

TG and RP curves in the heterozygotes were heterogeneous (Fig. 3). In three of the four peak values and AUC were clearly

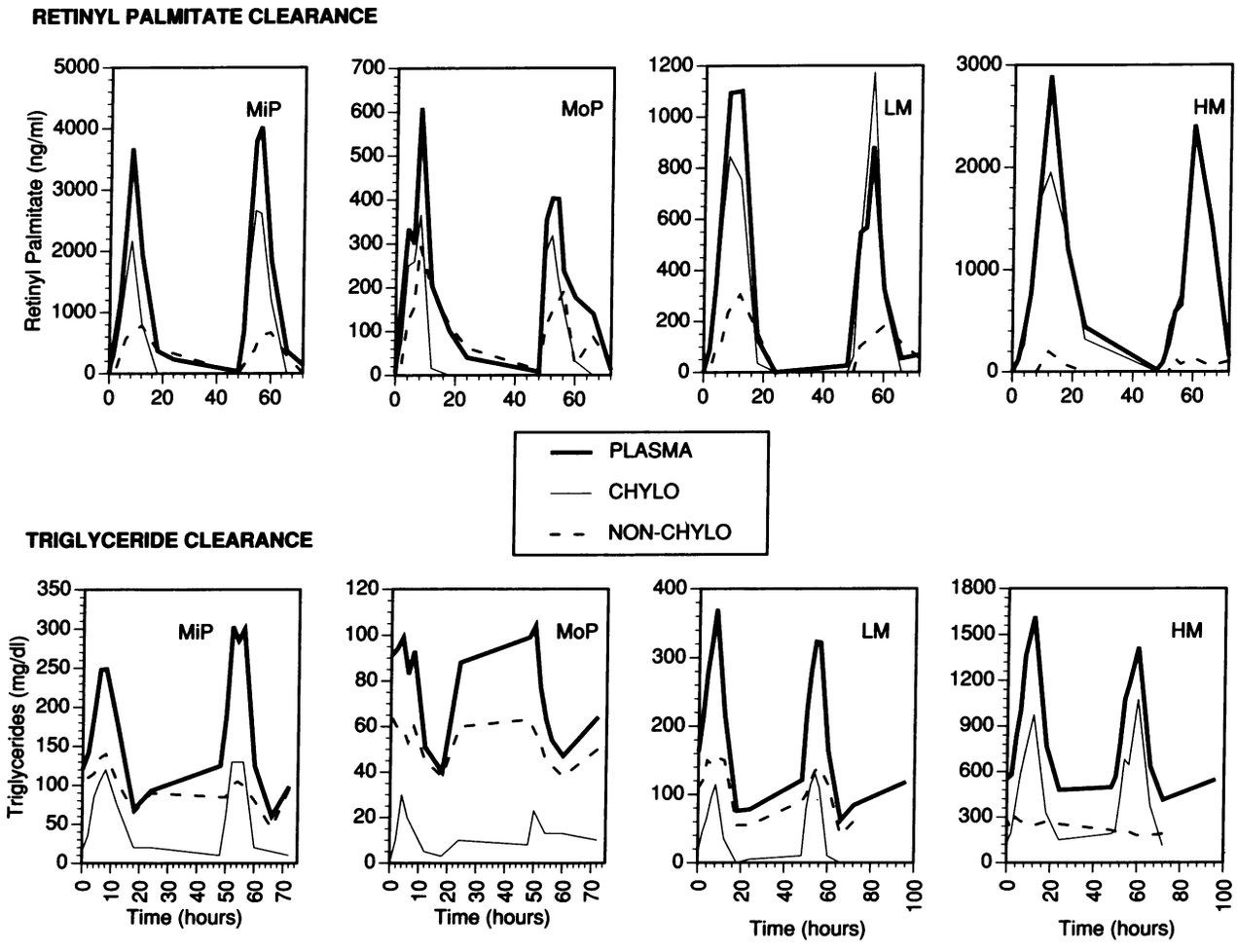

Figure 3. TG and RP chylomicron clearance curves for LpL-deficient heterozygotes, MiP, MoP, HM, and LM. Peak RP levels for all except MoP are elevated above normal. MoP has a reduction in chylomicron-TG immediately after the fat load, which subsequently resumes baseline. HM and LM also demonstrate absent bottom fraction RP 24 h after the fat load.

greater than controls. The fourth subject had fairly normal RP curves, but an abnormal TG response to the fat load. HM was unique in that his lipoprotein profile is historically consistent with a type V pattern, in addition to expressing an E4/4 phenotype. His plasma and chylomicron-RP curves were fairly equivalent on both fat loads, with little or no remnant formation. His C1/2 was increased, also supporting a delay in clearance. MiP has an E3/3 phenotype and TG values usually < 50% of HM, but whose RP response was equivalent to HM's after a fat load. LM expressed a mild elevation in TG at time 0, but had a peak RP value and AUC two to three times control. MoP had an early-peaking RP curve, and a marked decrease in chylomicrons after each of the two fat loads (Figure 3).

LpL mass and activity for each subject are presented in Table III. Activity was done with normal apo C-II added to the assay. Normal intensity and position of apo C-II bands were observed for SM, DM, and MP on isoelectric focusing gels. All three type I subjects had an incremental increase in LpL immunoreactive mass (class II defect) (43) which was, nonetheless, significantly decreased from normal 15 min after the administration of heparin; LpL activities were < 5% of control values. The specific activity was also markedly reduced. MP had higher mass levels than the other type I subjects, which corresponded to MoP, his mother, whose mass was two to three times normal. Otherwise, heterozygotes had low LpL activity and mass, 25–50% of normal. LpL activity among heterozygotes did not correlate with peak TG or RP values after the fat load.

Chylomicron/chylomicron remnant TG/RP correlation. In control subjects, the mean plasma RP peak occurs later than the mean plasma TG peak (Fig. 4). In contrast, the peak level for both plasma RP and TG are attained at the same time in the LpL-affected subject, here represented as the mean of the three type I or four heterozygous patients in Fig. 4. From the control curves, this represents a shift of the TG curve to the right with the RP curve remaining similarly positioned. This can be interpreted for the heterozygotes as consistent with a decrease in lipase function, with a similar rate of remnant uptake as in controls.

In the type I patients, the RP levels remain high for up to 10 h after the TG levels have begun to decrease (Fig. 4). This is ample time for RP to mobilize to other denser lipoprotein particles. Further, chylomicron-RP and TG curves are coincident for type I and heterozygous patients, as well as in controls (Fig. 5). These latter data suggest a lack of intraplasmic RP transfer from chylomicrons regardless of LpL function. The RP values observed in the bottom fraction throughout the study for each individual are presented in Figs. 1–3. It is important to note that the levels, for example in DM, peak from hours 2 through 6 and do not increase as one might expect if the RP was increasingly associated with LDL or HDL, but rather gradually decrease over the next 24–48 h. HM and LM have absent RP levels in the bottom fraction at time points 0, 24, and 48 h on the first fat load.

Apo B-100 levels obtained at the 0-, 8-, and 24-h points of the study were not detected in the chylomicron top fraction; thus, all plasma apo B-100 was located in the bottom, which represents a combination of VLDL, IDL, LDL, and HDL. Within the error of the assay, no clear reproducible change or trend was observed in any group of subjects for apo B-100 levels (Table IV).

Table III. LpL Mass and Activity after 12-h Fast

Subject	LpL activity	LpL mass		Specific activity
		0 min	15 min	
	μM/ml·h	*ng/ml*		*μM/h·μg*
Homozygotes				
MP	0.2	26	250	0.8
SM	0.0	17	54	0.0
DM	0.3	27	82	3.7
Heterozygotes				
MiP	4.9	48	125	39
MoP	3.9	52	1,330	3.0
HM	3.5	14	207	17
LM	3.2	17	186	17
Controls				
(mean, n = 3)	4.87–12.43	349–866		17.2–31.5

Discussion

Our data suggest that RP secreted on TG-rich intestinally derived lipoprotein particles does not rapidly nor significantly migrate to other intraplasmic particles, specifically in LpL-deficient and partially deficient subjects. This conclusion was reached because: (*a*) RP is found almost exclusively and for prolonged periods on chylomicrons in LpL-deficient subjects, with relatively little to none found on higher density lipoprotein particles; (*b*) partial LpL-deficient subjects can demonstrate low to absent RP concentrations on nonchylomicron particles 24–48 hours after an oral load of fat and vitamin A; and (*c*) plasma TG and RP curves in heterozygous LpL-deficient subjects are coincident after a fat load suggesting concurrent removal of both moieties, as would be expected if RP catabolism is based primarily on remnant receptor uptake.

The method used in this report of using RP to label chylomicron and nonchylomicron fractions has been previously applied to patients with type II, III, and IV hyperlipidemia in order to assay their in vivo metabolism of chylomicrons and chylomicron remnants (37). As reported, chylomicron-RP levels in normals were approximately back to baseline within 12 h (type IV baseline achieved in 24 h), whereas in the current study, type I patients had elevated chylomicron-RP values at hour 24 (> 50% peak value) with baseline reached by hour 48. This significant delay would be expected from data reported on TG levels in this type of patient (8, 44–46). A correlation between area under the chylomicron clearance curve and LpL activity by PHLA was reported by Weintraub et al. (37). The profound increase in area under the chylomicron clearance in type I patients in the present study, therefore, reflects the marked decrease in LpL activity. Further, all type II, III, or IV patients developed significant levels of nonchylomicron-RP after a fat load. In our study, type I patients had, in comparison, vanishingly small amounts of nonchylomicron-RP, as would be predicted from the literature (8, 47, 48).

These conclusions on the physiology of chylomicron processing assumes that RP is a valid marker for the chylomicron-related particles. This appears to be the case in rabbits and dogs (49, 50), and has been reported for humans even though there

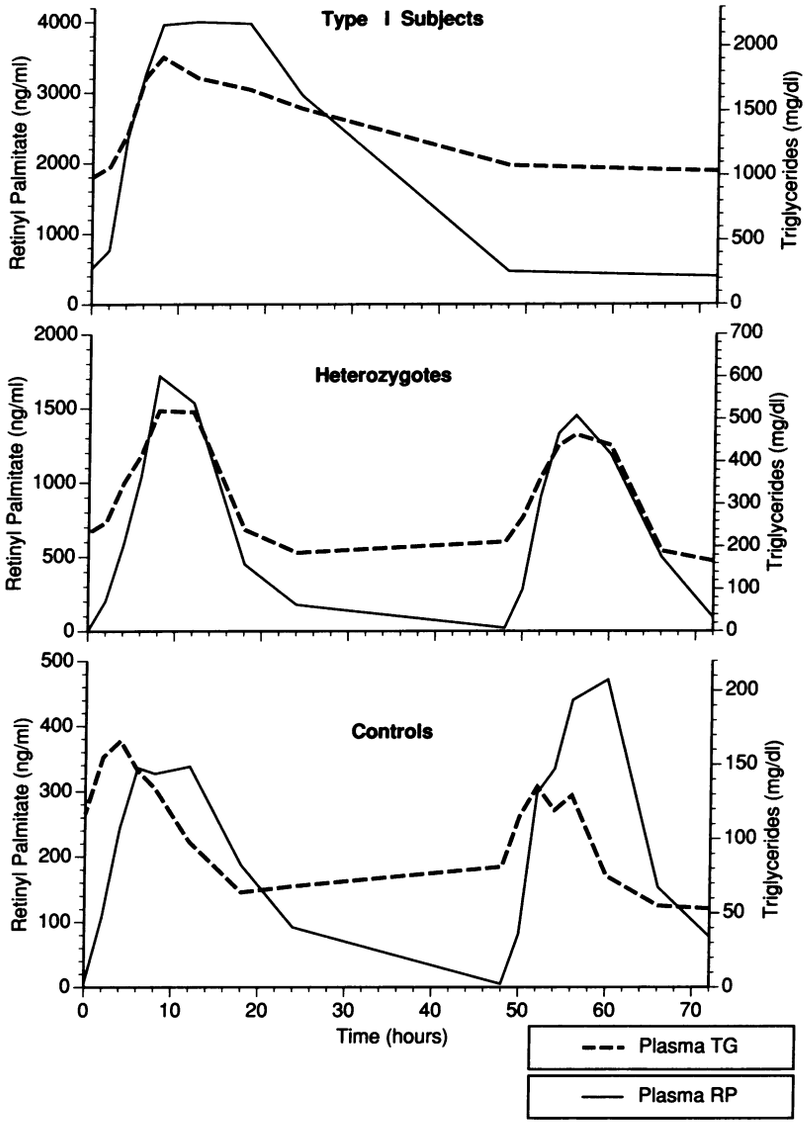

Figure 4. Total mean plasma TG and RP levels for each group of patients: type I, heterozygotes, and controls. Peak levels are not attained coincidentally in the control group. Similar coincident curves for TG and RP in the LpL-deficient or partially deficient subjects suggest that RP appropriately labels these intestinally derived particles. RP is sustained in the type I subjects after peak level is attained, whereas the TG values immediately begin a downward trend.

was an observed minimal transfer of RP from chylomicrons to LDL (39, 50, 51). However, a recent report has determined that RP, once secreted with intestinal particles, is progressively and irreversibly transferred to denser apo B-100 and apo A-I particles (7) resulting in at least 20% of the plasma RP localized to the LDL and/or HDL fraction at hour 9 after fat load, and an even greater percentage at hour 12. Enhanced LDL concentrations in familial hypercholesterolemia has been reported to induce an even more substantial transfer of RP to LDL (52). Thus, the apparent "decay" in plasma RP over time would actually represent intraplasmic transfer of RP to other particles rather than the catabolism of the chylomicron or its remnant.

However, the striking parallelism noted between chylomicron-RP and chylomicron-TG curves for type I, LpL heterozygous, and control subjects in the current study (Fig. 5) suggests that RP does not significantly transfer from chylomicrons to other plasma lipoprotein particles. In addition, RP clearance observed for the chylomicron fraction in type I patients is markedly delayed as compared to controls, although reasonably rapid transfer of RP off of chylomicrons and onto LDL should have been noted by first, lack of any delay, and secondly, by observing increasing amounts of RP in the bottom fraction. Rather, minimal amounts of RP were found in the bottom fraction, and the levels were not increasing in any subject group. Further, LpL-deficient heterozygotes LM and HM demonstrate virtually absent flotation rate (S_f) < 1,000 fraction RP 24 h after fat load, a finding inconsistent with significant transfer of RP to LDL or HDL. Alternatively, the dramatically reduced concentration of LDL observed in the type I patient (53), and/or abnormal VLDL or LDL from lack of LpL processing (11, 53) could result in inadequate levels of acceptor for the RP. Such considerations could influence any extrapolation to the normal patient as it relates to RP and its association with nonintestinally derived lipoprotein particles. In addition, the significant mass of chylomicrons (for type I or heterozygous subjects) may cause the RP to have a relatively higher affinity for chylomicrons than other less concentrated lipoprotein species (54). Further, the effective transfer of RP between particles, thought to be mediated by cholesterol ester transfer protein (55), may require active LpL enzyme working in some coordinated fashion with cholesterol ester transfer protein itself (56).

A discrepancy in the timing for the plasma RP and TG peak

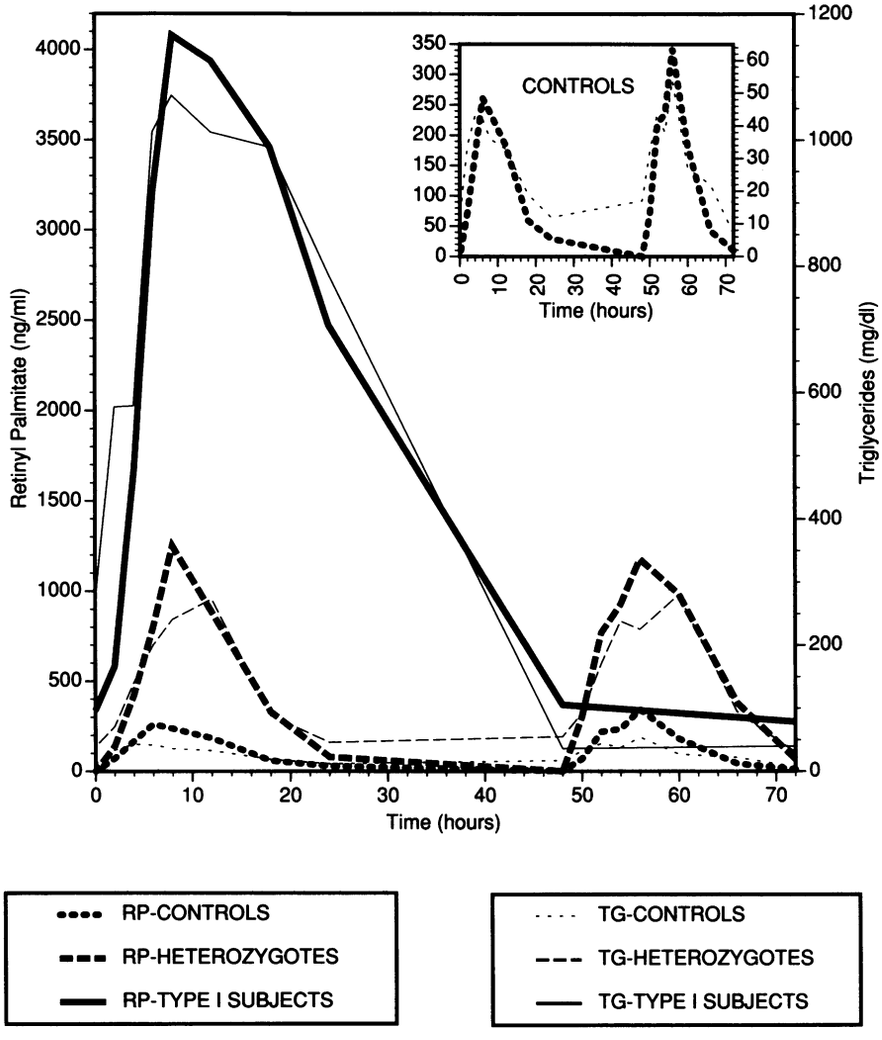

Figure 5. Chylomicron TG and RP mean values in the three subject groups: type I, heterozygotes, and controls (latter enlarged on *inset*). The amplitude and duration of the curves suggest the relative efficacy of chylomicron clearance among groups. TG and RP are coincident in all subjects recommending RP as an appropriate marker for chylomicrons (data points at 48 and 72 h only represent TG values for MP in the chylomicron TG curve).

in normal controls leads one to suspect the RP marker system as inadequate (7). However, the marked reduction in the time interval between peaks for LpL partially deficient subjects, producing coincident curves (Fig. 4), suggests that the lipoprotein lipase activity is the critical factor related to the presence of these intervals. The coordinated peaks for chylomicron TG and RP in LpL-deficient subjects suggests that disjoint absorption or secretion of fat and vitamin A from the gastrointestinal tract is probably not the basis for any disjoint post fat challenge TG and RP peaks in normal subjects. The aforementioned coordinate chylomicron TG and RP curves in subjects, regardless of LpL activity, suggest that RP does not transfer significantly from chylomicrons. Thus, the plasma TG-RP discrepancy when present in a normal control probably develops during the remnant phase of the particle. A rightward shift of the TG curve from controls to heterozygotes in Fig. 4, demonstrated previously by Harlan et al. (45), but now in this report producing coincident RP-TG curves, is consistent with a slower rate of decline in TG from remnants than the decline of the RP level through receptor uptake of remnant particles (7). Such a mechanism substantiates the in vivo reduction in LpL function in the heterozygous state (45). In addition, it suggests that the intestinal particle is removed with TG and RP still adjoined, allowing for little isolated transport of RP to other particles.

Table IV. Plasma Apo B-100 Values

Time (*h*)	0	8	24	48	56	72
Homozygotes						
MP	76	64	90	—	—	—
SM	73	74	70	—	—	—
Dm	79	81	86	—	—	—
Heterozygotes						
MiP	94	89	92	78	66	90
MoP	120	96	113	133	100	104
LM	81	74	111	93	71	93
HM	196	144	173	134	112	116
Controls						
(n = 3)	92	90	95	80	79	95

A small but significant amount of RP was still found in the bottom $S_f < 1{,}000$ fraction in type I subjects. Minimal conversion of chylomicrons to remnants is possible, but unlikely, in light of a stable, unchanging bottom fraction TG level. Liver-derived apo B-100 makes a contribution to the postprandial TG response, suggesting the potential for the production of apo

B100-RP particles (57, 58). Significant levels of such particles would be inconsistent with the stable apo B levels in the bottom fraction of the patients in the current report and contrary to data demonstrating a lack of liver derived RP in rabbits (59). Further, apo B-100–associated RP (hepatic or intestinally derived [60]) should accumulate in the bottom fraction given a presumed LDL-like 2-d half-life. This is inconsistent with the observed RP levels of $S_f < 1{,}000$ fraction in our type I and heterozygous patients. Thus the mechanism underlying bottom fraction RP in type I subjects remains unclear.

One of the patients (HM) in this present study was proven to have the same allelic defect (amino acid residue 188) as in the study suggesting heterozygotes to be predisposed to hypertriglyceridemia (12), while yet another heterozygote (MoP) had a proven physiologically relevant allelic defect at amino acid residue 194 (29). These unequivocal heterozygotes, as well as the presumed heterozygotes MiP and LM, had substantially different clinical expressions and RP clearance parameters. HM presented with the most abnormal clinical phenotype, expressing type V hyperlipidemia. However, HM is the oldest of the four heterozygotes, has the greatest body mass index, and the highest apo B level. The overlay of age-related LpL functional decreases (12, 61) as well as increases in VLDL synthetic rates, possibly due to enhanced body mass (61), may critically reduce the threshold for LpL saturation (62). Hepatic lipase activity may represent another variable related to TG hydrolysis (53, 63). In addition, the apo E4/4 phenotype could contribute to elevated lipids and low levels of RP in the bottom fraction, resulting in a modified clinical presentation (64–66).

In summary, intraplasmic transfer of RP is not generally evident from chylomicrons regardless of LpL function, an observation most clearly demonstrated in type I patients. The apparent lack of or, at best, minimal transfer of plasma RP from intestinally derived particles to denser lipoprotein particles recommends RP as an appropriate marker for the chylomicron–chylomicron remnant pathway in LpL-deficient and partially deficient subjects. In addition, type I subjects had a marked delay in chylomicron clearance which was complete, nonetheless, in 48 h. This suggests that, without LpL activity, chylomicron remnants are not made, and that another active processing mechanism must be available for chylomicron clearance. LpL-deficient heterozygotes demonstrated an abnormal chylomicron-RP curve and a general rightward shift in plasma TG distribution leading to coincident plasma RP and TG curves. This indicates reduced LpL function in heterozygous subjects, which may predispose to hypertriglyceridemia, but most likely results in heterogeneous phenotypic expression owing to numerous secondary variables.

Note Added in Proof. Since this paper was submitted, it has been determined that DM, SM, and LM are heterozygotes for the recently reported defect at amino acid residue 207 (68).

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