Different Patterns of Postprandial Lipoprotein Metabolism in Normal, Type IIa, Type III, and Type IV Hyperlipoproteinemic Individuals

Effects of Treatment with Cholestyramine and Gemfibrozil

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Abstract

To study exogenous fat metabolism, we used the vitamin A-fat loading test, which specifically labels intestinally derived lipoproteins with retinyl palmitate (RP). Postprandial RP concentrations were followed in total plasma, and chylomicron ($S_r > 1,000$) and nonchylomicron ($S_r < 1,000$) fractions. In normal subjects postprandial lipoproteins were present for more than 14 h, and chylomicron levels correlated inversely with lipoprotein lipase activity and fasting high density lipoprotein (HDL) cholesterol levels and nonchylomicron levels correlated inversely with hepatic triglyceride lipase activity. The main abnormality in type IV patients was a 5.6-fold increase in the chylomicron fraction, whereas in type III patients it was a 6.4-fold increase in nonchylomicrons. Type IIa patients had abnormally low chylomicron fractions. In type IV patients gemfibrozil decreased, whereas in type IIa patients cholestyramine increased the chylomicron fraction 66 and 88%, respectively.

This study demonstrates an unexpectedly large magnitude and long duration of postprandial lipemia in normal subjects and patients. These particles are potentially atherogenic, and their role in human atherosclerosis warrants further study.

Introduction

Chylomicrons, the vehicle in plasma for lipids of exogenous origin, are responsible for the transport of the largest quantity of lipid from sites of absorption to sites of storage and utilization. Chylomicron metabolism is considered to occur in two steps: hydrolysis of triglycerides at extrahepatic tissues followed by uptake and degradation of remnant particles, predominantly in the liver (1-3). Numerous studies have been published on postprandial lipemia in humans following a fat meal and examining the structure and composition of the chylomicrons and the fate of their lipid and protein constituents (4-7). These studies have shed light on processes involved with the first stage of chylomicron metabolism (6, 8, 9). Less is known about remnant metabolism. This lack of knowledge reflects the difficulties of differentiating chylomicron remnants from endogenous very low density lipoprotein (VLDL), two particle populations of similar physical and chemical properties (10); the only difference being

the presence of apolipoprotein (apo) B-48¹ in the former and apo B-100 in the latter (11). Yet, although apo B-48 determination may allow the study of remnant particles, specific and accurate assays of this protein are unavailable. This limited information on the metabolic fate of chylomicron remnants in humans is particularly unfortunate as abnormalities of postprandial lipoprotein metabolism may play an important role in the development of atherosclerosis (12). Such abnormalities, however, may remain undetected when plasma triglycerides are determined.

In 1976, Hazzard and Bierman introduced a sensitive and specific method for investigating the metabolism of postprandial lipoproteins in humans (13). The method is based on earlier studies in animals, carried out by Zilversmit (14) and utilize the feeding of a vitamin A-fat meal and determinations of vitamin A ester levels in plasma. The vitamin A is absorbed, becomes esterified in the intestinal absorptive cells (much like cholesterol) and is secreted with chylomicrons (15, 16). The retinyl ester (predominantly retinyl palmitate [RP]) circulates with the chylomicrons and chylomicron remnants, and finally is taken up with the remnant particles by liver cells. Unlike cholesterol, the vitamin A enters a storage pool and does not recycle in VLDL (14, 17). The appearance of RP in plasma lipoproteins, therefore, can be used for studying the metabolism of chylomicrons and their remnants with high specificity. Indeed, that method has recently been applied for measuring some parameters of chylomicron metabolism in human subjects (18-22).

In the present investigation, we report our studies on the pattern of chylomicron and chylomicron remnant metabolism, using the vitamin A-fat meal method, in normo- and hyperlipidemic (types IIa, III, and IV) human subjects, and the effects of lipid lowering drugs on these metabolic patterns.

Methods

Subjects. The studies were done on 15 normal subjects, 11 type IV patients, 7 type IIa patients, and 4 type III patients. The clinical characteristics of the subjects and patients are outlined in Table I.

All 15 normal subjects had normal fasting lipids. They had no diseases and were on no medications. All were on an average American diet and only one was obese. Their ages were between 19 and 72 yr.

Of the seven type IIa patients, five had a known positive family history of coronary heart disease, xanthomas, and hypercholesterolemia, and were presumed to have familial hypercholesterolemia, whereas the other two had little family history of coronary heart disease, no xanthomas, and probably had type IIa on another basis. One suffered from coronary heart disease, and none were on any medications. All patients were on a low fat, low cholesterol diet. Their ages were between 28 and 62 yr.

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1. Abbreviations used in this paper: apo B-48, apolipoprotein; HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; RP, retinyl palmitate.

Table I. Characterization of Study Subjects and Patients

Group	N	Sex			Age	Height	Weight	Fasting lipids and lipoprotein				
		F	M					TC	TG	VLDL-C	LDL-C	HDL-C
						cm	kg	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
Normal subjects	15	6	9	Mean	31	174	76	175	79	19	106	51
				(SD)	(17)	(11)	(15)	(23)	(29)	(5)	(24)	(8)
				Range	19–72	156–190	49–105	133–214	50–126	10–27	66–139	38–62
Type IIa HLP	7	3	4	Mean	47	171	71	376	109	41	272	63*
				(SD)	(15)	(8)	(9)	(82)	(48)	(33)	(82)	(33)
				Range	25–62	160–183	55–83	287–502	65–185	7–95	201–437	35–134
Type III HLP	4	2	2	Mean	48	170	70	424	428	249	137	39
				(SD)	(19)	(9)	(5)	(142)	(121)	(138)	(25)	(8)
				Range	28–65	157–175	66–75	275–601	252–511	85–401	112–170	30–50
Type IV HLP	11	4	7	Mean	52	171	82	269	808 [‡]	119	117	34
				(SD)	(17)	(13)	(24)	(39)	(679)	(36)	(29)	(8)
				Range	28–82	146–189	62–130	183–324	315–2758	62–180	67–152	21–53

* In this group, there was one patient with hyperalpha, and if one deletes these values, the mean HDL is 51±9 with a range of 35 to 60. [‡] In this group, there was one patient with severe hypertriglyceridemia, and if one deletes these values, the mean triglyceride is 613±218 with a range of 315 to 970.

One of the four type III patients suffered from mild peripheral vascular disease. One had mild diabetes controlled by diet. The two others did not suffer from any other diseases. None of the patients were on medications. Their ages were between 28 and 65 yr. Three of these patients had the E2/2 phenotype. The fourth had an E3/2 phenotype, but had the classical lipid abnormality of type III as well as palmar and tuberous xanthomas.

Of the 11 type IV patients, four suffered from stable angina pectoris. Three of these were on medications, two on beta blockers and nitrites, and the other on a beta blocker plus a calcium channel blocker and nitrites. The other seven type IV patients did not suffer from other diseases and were on no medications. All of them were on a low fat, low cholesterol diet and only one of them was obese. Their ages were between 28 and 82 yr. One of these patients has triglyceride levels above 2,000 mg/dl and might be considered to have type V, but his response to the vitamin A-fat loading test was consistent with the other patients in this category.

The weight of all the subjects was stable and none had a significant weight loss in the month before the study. Six of the normal subjects were recruited from the outpatient clinic of the Laboratory of Biochemical Genetics and Metabolism at The Rockefeller University, and nine were college students participating in diet studies. The vitamin A-fat loading test was done while they were stabilized on a metabolic diet that mimics the average American diet. All subjects gave informed consent for the study.

Vitamin A fat loading test. Subjects were admitted to the The Rockefeller University Hospital, and after an overnight 12-h fast, they were given a fatty meal plus 60,000 U of aqueous vitamin A/m² body surface. Vitamin A (Aquasol A, 50,000 U/ml) was purchased from Armour Pharmaceutical Co., Kankakee, IL. The fatty meal contained 50 g of fat/m² body surface, consisting of 65% of calories as fat, 20% as carbohydrate, and 15% as protein. It contained 600 mg cholesterol/1,000 calories, and the P/S ratio was 0.3. This was given as a milkshake, scrambled eggs, bread, and cheese, and was eaten in 10 min. Vitamin A was added to the milkshake. After the meal, subjects fasted for 24 h, but as much drinking water was allowed as desired. Blood samples were drawn before the meal and every hour after the meal until 6 h, then every 2 h until 14 h, and at 24 h. Seven of the type IV patients, and five of the type IIa patients had two tests: the first on no lipid lowering medications; the second for the type IV patients after 5–6 wk of gemfibrozil 1,200 mg/d

treatment, and for the type IIa patients after the same period of cholestyramine 24 g/d treatment. The subjects tolerated the meal well, and no one had diarrhea or other symptoms of malabsorption.

Hydrogen breath test. To exclude a possible effect of differences in gastric emptying rates on the results, we measured the gastrointestinal transit time in eight normal subjects and seven patients using the hydrogen breath test (23): 10 g of Lactulose diluted in 100 ml of water was given together with the fatty meal. Breath samples were collected by blowing into bags every 10 min after the meal. Hydrogen concentrations were measured using instrument gas chromatography (Quintron Instrument Co., Inc., Milwaukee, WI).

Analysis of samples. Venous blood was drawn from the forearm and transferred to a tube containing sodium EDTA. Samples were immediately centrifuged at 1,500 g × 15 min and 0.5 ml of plasma was stored wrapped in foil at –20°C for retinyl ester assay. Another 0.5 ml was stored at 4°C for cholesterol and triglyceride determinations. An aliquot of 2.5 ml of plasma was transferred into a ½ × 2 in. cellulose nitrate tube and overlaid with 2.5 ml sodium chloride solution (*d* = 1.006 g/ml). Tubes were subjected to preparative ultracentrifugation for 1.6 × 10⁶ g-min in a rotor (Beckman SW-55, Beckman Instruments, Inc., Fullerton, CA) to float chylomicron particles of *S*_f > 1,000 (6, 24, 25). The chylomicron-containing supernatant was removed and brought to a total volume of 2 ml with saline. The infranatant was brought to a volume of 5 ml with saline. 0.5-ml aliquots of supernatant and infranatant were wrapped in foil and assayed for retinyl ester. Additional aliquots were assayed for cholesterol and triglyceride concentration. The cholesterol levels in the *S*_f > 1,000 fraction were too low to be reliably distinguished from background in our assay.

Retinyl ester assay. The assays were carried out in subdued light with high performance liquid chromatography (HPLC) grade solvents. Retinyl acetate was added to the samples as an internal standard. The samples were then mixed with 4 ml ethanol, 5 ml hexane, and 4 ml water with vortexing between each addition. Two phases were formed and 4 ml of the upper (hexane) phase was removed and evaporated under nitrogen (26). The residue was dissolved in a small volume of benzene, and an column (Beckman Instrument Inc., Altex Division, San Ramon, CA). 100% methanol was used as the mobile phase at a flow rate of 2 ml/min. The effluent was monitored at 340 nm, and the retinyl ester peaks were quantitated by the area ratio method (27). The efficiency of extraction

of retinyl esters was > 95%, and the variance of triplicate assays was < 5.4% of the mean.

Lipid and lipoprotein determination. Cholesterol and triglycerides were measured enzymatically using the reagents Cholesterol 236691 and Triglyceride 126012 (Boehringer Mannheim Biochemicals, Indianapolis, IN). Total and HDL cholesterol measurements were standardized by the Lipid Standardization Program of the Center for Disease Control, Atlanta, GA. HDL cholesterol was determined after precipitation of whole plasma with dextran sulfate-magnesium (28). HDL plus LDL cholesterol was measured on the infranatant after a 2-h spin in the Beckman airfuge to float the VLDL. Appropriate corrections were made for dilution factors, and VLDL, low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol levels were calculated.

Postheparin plasma lipolytic activities. Lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) were released into the circulation by intravenous heparin injection at a dose of 60 U/kg body weight. After 15 min blood was drawn into tubes containing 4 mM EDTA. The plasma was immediately separated at 4°C by centrifugation at 2,700 rpm for 12 min and promptly frozen at -70°C. The assay itself is a modification of the method of Krauss (29). 20 μ l of postheparin plasma is added to freshly sonified substrate, containing radiolabeled and unlabeled triolein and Triton in a Tris HCl buffer pH 8.6, mixed, and incubated for 20 min at 37°C. The reaction is stopped by a mixture of chloroform, methanol, and heptane, and a base in the form of K₂CO₃ is added. After mixing and centrifugation, an aliquot of the aqueous phase is counted and the total lipolytic activity is given as micromoles of FFA liberated per milliliter plasma per hour. Another aliquot of 20 μ l of postheparin plasma is incubated for 1 h at 4°C with 25 μ l of rabbit anti-human LPL antiserum before the lipase assay described above. The antiserum was prepared in our laboratory by Dr. Rudolf Zechner by injecting rabbits with LPL purified from fresh human breast milk. LPL activity is the difference between total and antiserum-inhibited activity. The latter is taken to be the HTGL activity. Lipolytic activities were determined in most subjects participating in the study, at least 1 wk prior to the vitamin A-fat load test.

Statistical analysis. The differences between hyperlipidemic and normal subjects, and patients before and after drug treatment were analyzed for significance using unpaired and paired Student *t* tests, respectively. Correlations between all measured variables and the RP area in chylomicron and nonchylomicron fractions were calculated by linear regression analysis using the least squares method. Calculations were performed on the The Rockefeller University Hospital CLINFO system.

Results

Plasma and lipoprotein RP response to the vitamin A-fat loading test. Before initiating studies in patients, several investigations were carried out to test the validity of the vitamin A-fat loading technique. Individual variation in gastrointestinal transit time was determined by the lactulose breath test. In 15 subjects (8 normo- and 7 hyperlipidemic subjects), lactulose hydrogen peaked in the expired air after a mean period of 198 $\pm$ 28 min. The range was small, 160 to 225 min and was similar in normals and patients, 197 $\pm$ 37 and 198 $\pm$ 20 min, respectively. These values are similar to those found in the literature when lactulose is given with a solid meal (30).

The reproducibility of the vitamin A-fat loading test was examined by studying two subjects twice at 3-wk intervals. In each individual both plasma RP concentration curves were almost identical (Fig. 1). To be sure that the test did not exceed the ability of the intestine to absorb vitamin A, increasing doses of vitamin A were given to two normal subjects with a fixed amount of fat, and plasma RP concentration curves derived. Increasing the vitamin A dose from 25,000 to 100,000 U resulted in a proportionate and linear increase in the plasma RP peak height and the area below the RP curve (Fig. 2).

Figure 1. Reproducibility of the vitamin A-fat loading test. After a vitamin A fat meal, plasma concentrations of RP were determined over a 24-h period (open circles) and the test was then repeated 3 wk later (closed circles). Two subjects were studied, the results are shown in (A) and (B), respectively.

Plasma, chylomicron, and nonchylomicron RP responses were determined for 15 normolipidemic subjects, 7 type IIa, 4 type III, and 11 type IV hyperlipidemic subjects. The results for each group of subjects were averaged and are presented in Fig. 3. In normal subjects, total plasma RP was measurable 1 h after the vitamin A-fat meal. These levels increased rapidly between 1 and 3 h, remained high until 7 h, declined rapidly between 7 and 10 h, were ~ 20% of peak levels at 12 h, and continued to fall at a slower rate to levels that were low but detectable even at the end of 24 h.

Total plasma RP was separated into chylomicron and nonchylomicron fractions, which appeared to behave differently. During the first 6 h, chylomicron RP levels closely paralleled those of total plasma RP; the peaks of both occurred at 6 h. Chylomicron RP concentration then even more rapidly declined to reach a level < 10% of its peak level at 12 h. By 24 h, chylomicron RP levels were barely detectable. Although lower than chylomicron RP levels, nonchylomicron RP levels increased to peak levels between 3 and 6 h, and then slowly disappeared. At

Figure 2. Linear dependence of the vitamin A-fat loading test on the oral vitamin A dose. Increasing amounts of vitamin A were given to two normal subjects (closed and open circles) with a fixed amount of fat per square millimeter body surface area, and plasma RP concentration curves derived. (A) shows the peak height RP concentration values. In (B) the area under the RP concentration curves were correlated with the oral dose of vitamin A. Linear regression analysis showed all correlations to be 0.98 or greater.

Figure 3. RP concentration curves in normal and hyperlipidemic subjects. Total plasma (solid line), chylomicron (dotted line) and non-chylomicron (dashed line) RP responses were determined for normal individuals ($n = 15$), and type IIa ($n = 7$), type III ($n = 4$) and type IV ($n = 11$) hyperlipidemic patients. For each group, the levels at each time point were averaged. The scale is lower for normal and type IIa subjects than it is for type III and type IV subjects.

9 h, their concentration exceeded the chylomicron RP concentration and this continued for the duration of the study with measurable levels present even at 24 h.

Plasma, chylomicron, and nonchylomicron RP responses were distinctly abnormal in the three groups of patients. In type IIa patients' chylomicron RP peak levels were significantly lower than normal ($P < 0.05$), and at 12, 14, and 24 h, the nonchylomicron RP levels tended to be higher than normal, but the difference was not statistically significant. The abnormal early behavior of the chylomicron fraction and late behavior of the

nonchylomicron fraction caused the concentration of RP in the nonchylomicron fraction to exceed that in the chylomicron fraction at 6 h, which is significantly earlier than the crossover point in normal subjects. In type IV patients, total plasma, chylomicron and nonchylomicron RP levels are severalfold higher than normal. The most impressive differences are found in the total plasma and chylomicron RP peak levels and areas. The peak levels are 3.6- and 4.1-fold higher, respectively, and the areas are 4.8- and 6.3-fold higher, respectively (Table II). Nonchylomicron RP peak levels and areas were 2.5- and 2.9-fold, respectively, above normal ($P < 0.01$). Due to the very slow disappearance of chylomicron RP, its level exceeded those in the nonchylomicron fraction during the entire study. In type III patients, the vitamin A-fat loading test was unusual in several respects. Total plasma RP levels were elevated to a similar extent as in type IV patients. However, in type III patients, these remarkably elevated levels mainly reflected the nonchylomicron RP fraction. While chylomicron RP levels were elevated two to threefold above normal, they were only half the levels observed in type IV patients (Table II). Both the peak and area of nonchylomicron RP in the type III patients were 6.4-fold higher than in the normolipidemic group (Table II). Throughout the study the behavior of nonchylomicron RP fraction was very abnormal. After the first 6 h, this fraction contained most of the plasma RP. The slow clearance of nonchylomicron RP is indicated by a large amount of this material remaining in plasma at 24 h.

Plasma and lipoprotein triglyceride response to vitamin A-fat load. Conventional studies of exogenous fat metabolism have examined the postprandial rise in triglyceride levels. To compare these with our vitamin A-fat loading test, we measured triglycerides in total plasma, and chylomicron, and nonchylomicron fractions in all studies. The results for each group of subjects were averaged and the data presented in Fig. 4. No adjustments were made for the initial (fasting) plasma and lipoprotein triglyceride levels. It can be seen that increases of total plasma, chylomicron, and nonchylomicron triglyceride levels are observed. However, many of the specific responses of the patient groups seen with the RP measurements are obscured by only measuring triglycerides. For example, after the fat meal, the nonchylomicron triglycerides transiently increase, but then rap-

Table II. Plasma, Chylomicron, and Nonchylomicron RP Peak Levels and Areas in Normal Subjects and Hyperlipidemic Patients

Group	RP peak levels			Area below RP curves		
	Plasma	Chylomicron fraction	Nonchylomicron fraction	Plasma	Chylomicron fraction	Nonchylomicron fraction
	µg/liter	µg/liter	µg/liter	µg/liter per h	µg/liter per h	µg/liter per h
Normal subjects	2,132 (767)	1,388 (579)	767 (348)	15,487 (4,273)	8,983 (3,454)	6,506 (2,193)
Type IIa HLP	1,519 (705)	847* (417)	729 (354)	12,722 (4,691)	5,978 (2,575)	6,819 (3,083)
Type III HLP	7,565‡ (1,782)	3,650* (1,320)	4,899‡ (1,367)	77,281‡ (22,575)	35,663* (13,690)	41,619‡ (8,889)
Type IV HLP	7,635‡ (3,093)	5,751‡ (2,823)	1,918‡ (698)	75,214‡ (33,254)	56,263‡ (30,255)	18,934‡ (4,930)

Mean values and (SD) of RP peaks and areas below the curves of plasma, chylomicron, and nonchylomicron fractions: * $P < 0.05$; ‡ $P < 0.001$.

Figure 4. Triglyceride concentration curves in normal and hyperlipemic subjects. Total plasma (solid line), chylomicron (dotted line), and nonchylomicron (dashed line) triglyceride responses were determined for normal individuals ($n = 15$), and type IIa ($n = 7$), type III ($n = 4$), and type IV ($n = 11$) hyperlipidemia patients. For each group, the levels at each time point were averaged. The scale is lower for normal and type IIa subjects than it is for type III and type IV subjects.

idly return to baseline or below starting levels. Specifically, beyond 6–9 h in normals and patients the RP measurements indicate the presence of considerable amounts of exogenous fat containing particles in the nonchylomicron fraction, whereas this would be totally missed by only measuring triglyceride levels. In fact, the nonchylomicron RP and triglyceride peaks and areas were not correlated. In the case of the chylomicron fraction, triglyceride measurements more accurately reflect the RP levels. However, presumably due to the contribution of large VLDL, especially in some of the patients, the correlation of chylomicron RP and triglyceride peak levels was only $r = 0.559$, which was barely significant ($P = 0.047$).

Correlations between the vitamin A-fat loading test and post-heparin lipase, insulin, lipid, and lipoprotein levels. To indicate factors that might play a role in exogenous fat metabolism, correlations were sought between the chylomicron and nonchylomicron RP responses and postheparin lipases, insulin, lipid, and lipoprotein levels (Table III). With a P value of < 0.01 , in normal subjects, significant negative correlations were found between chylomicron RP area and LPL activity ($r = -0.726$) and HDL cholesterol levels ($r = -0.647$), and between nonchylomicron RP area and HTGL activity ($r = -0.620$). These correlations were specific for the respective lipoprotein fractions and the respective lipases, since nonchylomicron response was not correlated with LPL activity, and chylomicron response was not correlated with HTGL activity. These correlations were not due

Table III. Correlation Between Chylomicron and Nonchylomicron Fractions Area to Lipolytic Activity, Insulin Levels and Fasting Lipids

Variable	RP area	Normal subjects	Type IIa HLP	Type IV HLP
LPL	Chylomicron	$-0.726 (0.002)^*$	$-0.622 (0.132)$	$-0.255 (0.486)$
	Nonchylomicron	$0.133 (0.641)$	$0.408 (0.368)$	$0.080 (0.828)$
HTGL	Chylomicron	$0.258 (0.863)$	$0.242 (0.603)$	$0.478 (0.193)$
	Nonchylomicron	$-0.620 (0.009)^*$	$-0.724 (0.060)$	$-0.173 (0.636)$
Fasting insulin	Chylomicron	$0.098 (0.746)$	$0.512 (0.296)$	$-0.006 (0.999)$
	Nonchylomicron	$0.529 (0.052)$	$-0.252 (0.635)$	$-0.260 (0.472)$
2° PP insulin	Chylomicron	$-0.490 (0.074)$	$0.217 (0.681)$	$0.314 (0.386)$
	Nonchylomicron	$0.110 (0.712)$	$-0.377 (0.461)$	$0.187 (0.615)$
Δ Insulin	Chylomicron	$-0.597 (0.039)$	$0.107 (0.824)$	$0.295 (0.419)$
	Nonchylomicron	$-0.149 (0.682)$	$-0.090 (0.853)$	$0.262 (0.472)$
TC	Chylomicron	$0.33 (0.918)$	$-0.350 (0.442)$	$0.435 (0.181)$
	Nonchylomicron	$-0.070 (0.816)$	$0.435 (0.331)$	$0.288 (0.394)$
TG	Chylomicron	$0.336 (0.227)$	$0.574 (0.180)$	$0.765 (0.006)^*$
	Nonchylomicron	$-0.153 (0.594)$	$0.082 (0.864)$	$0.199 (0.563)$
VLDL-C	Chylomicron	$-0.026 (0.931)$	$0.619 (0.141)$	$0.576 (0.060)$
	Nonchylomicron	$0.107 (0.709)$	$0.104 (0.826)$	$0.159 (0.644)$
LDL-C	Chylomicron	$0.199 (0.480)$	$-0.417 (0.359)$	$-0.164 (0.632)$
	Nonchylomicron	$-0.414 (0.143)$	$0.253 (0.587)$	$0.142 (0.681)$
HDL-C	Chylomicron	$-0.647 (0.008)^*$	$-0.479 (0.286)$	$-0.477 (0.134)$
	Nonchylomicron	$0.438 (0.116)$	$0.377 (0.402)$	$-0.040 (0.917)$

Correlation coefficients between RP area in chylomicron and nonchylomicron fractions and lipoprotein lipase (LPL), hepatic TG lipase (HTGL), insulin levels (fasting, postprandial and the difference between them), cholesterol, triglycerides, VLDL, LDL, and HDL cholesterol, respectively, are shown. The P values for the significance of these correlations are in parentheses. Correlation with P values < 0.01 are indicated by an asterisk. The groups consisted of 15 normal, 7 type IIa, and 11 type IV subjects.

to outliers and appeared to pertain to the entire range of results obtained (Fig. 5 A-C).

In the type IIa patients, a qualitatively similar pattern was observed. However, perhaps because of the fewer number of patients studied, the results did not reach significance. Type IV patients show a different pattern from normal and type IIa subjects. In such patients, perhaps because of a fundamental disturbance in the lipase system, the inverse correlations of chylomicron RP area and LPL activity and nonchylomicron RP area and HTGL activity were very weak. The inverse correlation of chylomicron RP area and HDL cholesterol was stronger and similar to that observed in type IIa patients, but not significant. In type IV patients, a significant ($P < 0.01$) correlation was observed between chylomicron RP area and fasting triglyceride levels ($r = 0.765$). There were no highly significant ($P < 0.01$) correlations between the vitamin A-fat loading test and insulin determinations in the fasting state, postprandially, or the difference between these two measurements (Δ insulin). In normal subjects, there was a less significant ($P < 0.05$) inverse correlation between chylomicron RP area and Δ insulin levels ($r = -0.597$).

Effects of drug therapy on the response to vitamin A-fat load.

To better understand the vitamin A-fat loading test as well as to gain insight into how two different types of lipid lowering drugs might affect exogenous fat transport, the effects of drug interventions on the response to the vitamin A-fat loading test were investigated in patients with type IV and type IIa hyperlipidemia. Seven type IV patients were studied before, and 5-6 wk after the initiation of gemfibrozil therapy. The pre- and post-treatment total cholesterol, triglyceride, VLDL, LDL, and HDL cholesterol levels and total plasma, chylomicron, and nonchylomicron RP responses are shown in Fig. 6 A and Table IV. Mean plasma triglyceride levels decreased 65% on therapy. Gemfibrozil therapy also caused the RP response in the patients to be more like normal. After therapy, the peak chylomicron RP levels occurred earlier and were reduced by over 60%. The chylomicron RP area was decreased by 71%. On drug treatment, by 24 h only minimal amounts of chylomicron RP were identified in plasma, whereas the same patients untreated had high levels at this time point. Gemfibrozil also improved the nonchylomicron RP response, but to a lesser extent. In comparison

Figure 5. Significant correlations between lipases and HDL cholesterol levels and the results of the vitamin A-fat loading test in normal subjects. In (A) the chylomicron retinyl palmitate area is plotted against postheparin plasma lipoprotein lipase levels. In (B) the nonchylomicron retinal palmitate area is plotted against postheparin plasma hepatic lipase levels. In (C) the chylomicron retinyl palmitate area is plotted against HDL cholesterol levels.

Figure 6. Effect of drug treatment on vitamin A-fat loading test. Total plasma (solid lines), chylomicron (dotted line), and nonchylomicron (dashed line) RP responses were determined before and after drug treatment. (A) shows the results for seven type IV patients treated with gemfibrozil, and (B) for five type IIa patients treated with cholestyramine. For each group, the levels of each time point were averaged. The scale is lower for type IIa than for type IV subjects.

to pretreatment values, the peak nonchylomicron RP level occurred earlier and was decreased by 20%. The nonchylomicron RP area was decreased 31% (derived from Table IV).

In a second study, five type IIa patients were investigated before and 5-6 wk after initiation of cholestyramine treatment (Fig. 6 B and Table IV). Therapy decreased LDL cholesterol levels by 23%, with triglyceride levels unchanged. Although cholestyramine is used as a lipid-lowering drug, it increased peak chylomicron RP levels 89% and chylomicron RP area 86%, without significantly changing fasting total plasma triglyceride levels (Table IV). Cholestyramine treatment was also associated with a significant ($P < 0.05$) decrease in LPL activity (data not shown).

Discussion

The aim of the present investigation was to determine the metabolic behavior of exogenous fat in normo- and hyperlipidemic subjects. The effects of lipid lowering drugs on exogenous fat metabolism were also studied. Previously, two methods have been employed for studying exogenous fat metabolism. These involved either determining postprandial increments in total plasma and chylomicron triglycerides (6, 7), or administering a vitamin A-fat meal and following plasma and lipoprotein RP levels in the postprandial period (13, 19-22). The vitamin A-

Table IV. Effect of Gemfibrozil and Cholestyramine on Total Plasma, Chylomicron and Nonchylomicron RP Responses in Type IV and Type IIa Patients, Respectively

Group	Treatment	Fasting lipids and lipoproteins					RP peak levels			Area below RP curves		
		TC	TG	VLDL-C	LDL-C	HDL-C	Plasma	Chylomicron fraction	Nonchylomicron fraction	Plasma	Chylomicron fraction	Nonchylomicron fraction
		md/dl	md/dl	md/dl	md/dl	md/dl	μg/liter	μg/liter	μg/liter	μg/liter h	μg/liter h	μg/liter h
Type IV HLP	—	271 (31)	1,018 (816)	126 (38)	115 (32)	31 (6)	8,172 (3,533)	6,389 (2,942)	1,783 (643)	82,430 (37,528)	63,367 (33,963)	19,040 (6,029)
<i>n</i> = 7	Gemfibrozil	234 (27)	367 (255)	78 (19)	121 (34)	37 (7)	3,895* (1,265)	2,467* (806)	1,428 (581)	31,526* (11,987)	18,145* (7,465)	13,212* (5,164)
Type IIa HLP	—	398 (73)	145 (59)	53 (42)	295 (100)	51 (11)	1,474 (715)	932 (501)	613 (216)	11,929 (6,367)	6,197 (3,445)	5,807 (3,427)
<i>n</i> = 5	Cholestyramine	315 (95)	144 (77)	40 (24)	226 (108)	53 (13)	2,388 (1,084)	1,764* (862)	623 (217)	16,828 (8,859)	11,523* (7,340)	5,319 (2,337)

Mean values and (SD) of RP peaks and areas below the curves of plasma, chylomicron, and nonchylomicron fractions before and 6 wk after medical treatment were * $P < 0.05$, † $P < 0.005$.

RP method has an advantage in that it specifically traces particles of intestinal origin, while absorbed triglycerides cannot be discerned from triglycerides of endogenous (hepatic) origin. The data reported here clearly supports the view that the vitamin A-RP method is more sensitive and reliable for studies of the metabolism of postprandial lipoproteins than the triglyceride method. Moreover, we show that the method is highly reproducible, when tested repeatedly in the same individual, and is very sensitive to the amount of vitamin A included with the fat meal.

Chylomicron metabolism is considered to occur in two stages. Initially, the particles interact with lipoprotein lipase in extrahepatic tissues resulting in triglyceride hydrolysis and delivery of free fatty acids to the tissues (1, 2, 31). After most triglycerides are hydrolyzed, remnant particles are formed (3, 32) that are removed from the circulation by hepatocyte receptors that recognize apo E (33–37). The exact stage when chylomicrons become remnants is not known, and there are no methods currently available to physically separate the two. In the present investigation, it was decided to follow the protocol of Mok and Grundy (6), which uses a centrifugation step to separate large and less dense chylomicrons of $S_f > 1,000$ from smaller and denser particles. It was hoped that with this procedure a predominantly chylomicron population would be separated from a predominantly remnant population. As presented in Results and discussed here, this was achieved, since it appears that the metabolic behavior of RP in the chylomicron fraction ($S_f > 1,000$) was very different from the RP in the nonchylomicron fraction ($S_f < 1,000$) fraction. Of course, it must be recognized that these are operational definitions and do not correspond exactly with chylomicron and chylomicron remnant particles, respectively.

The rate of increase in chylomicron RP and triglyceride levels and the peak level attained reflects a balance between rates of absorption of vitamin A and triglycerides from the gastrointestinal tract and rates of clearance of chylomicron particles and their remnants from plasma. Several lines of evidence strongly suggest that the different RP curves described for the patient groups and after drug treatment reflect differences in rates of

clearance rather than rates of absorption. (a) In the present investigation, there were no marked differences between individuals in gastrointestinal transit time as measured by the lactulose breath test. (b) None of the subjects showed signs of fat malabsorption. (c) In normal and type IIa subjects, chylomicron RP clearance correlated directly with LPL activity, which is the key enzyme of chylomicron triglyceride hydrolysis (38, 39). (d) As shown in Fig. 4, in spite of the marked differences in the RP responses of normal subjects, type IIa, type III, and type IV subjects, the initial rate of increase in plasma RP levels was remarkably similar. (e) Cholestyramine treatment, which if anything should decrease absorption, actually resulted in a significant increase in the chylomicron RP peak and area responses.

In studies of postprandial fat metabolism, measurements of triglyceride levels do reflect to some degree the behavior of chylomicrons, but as we show, no information is obtained about the nonchylomicron fraction. The metabolic behavior of the latter can only be followed by the vitamin A-RP method. Such particles, in what we are calling the nonchylomicron $S_f < 1,000$ fraction, could arise by either direct synthesis and secretion from intestine, or conversion of large, less dense chylomicrons to remnant particles, or both. Two observations suggest that most of the nonchylomicron particles originate in the plasma compartment and are derived from secreted large chylomicrons. The first is the crossover of chylomicron and nonchylomicron RP concentration curves observed for normals and type IIa and type III patients. The second is the relationship of chylomicron and nonchylomicron RP levels between 10 and 14 h in type III patients. In these patients during this time period, the chylomicron RP levels fell, indicating a postabsorptive phase, whereas nonchylomicron RP levels rose a proportionate amount. Type III patients have a basic defect in remnant removal (40, 41) and this indicates that indeed the major source of nonchylomicron RP is the chylomicrons themselves.

Chylomicron and nonchylomicron responses were distinctly different between normal and type IV subjects. In the latter, clearance of chylomicron RP and triglyceride, as estimated by peak height and area, was considerably delayed. In type IV pa-

tients, a significant positive correlation was found between the chylomicron RP response and fasting plasma triglyceride levels. Mok and Grundy also reported delayed chylomicron triglyceride clearance in hypertriglyceridemic subjects (6) and other investigators have demonstrated negative correlations between chylomicron clearance and fasting plasma triglyceride levels (42, 43). In our study, nonchylomicron RP clearance was also delayed in type IV patients. Whether delayed clearance of chylomicrons or nonchylomicron RP is due to defective triglyceride hydrolysis, or merely reflects competition between absorbed exogenous fat and triglycerides of endogenous origin, is not known. Regardless of mechanism, it is obvious that the magnitude of postprandial lipemia is greatly increased in patients with high fasting plasma triglyceride levels.

The fact that the chylomicron and nonchylomicron RP curves do not cross over in type IV patients implies something unique about exogenous fat metabolism in these subjects compared with normal, type IIa, and type III subjects. Namely, that most of the chylomicrons are cleared from plasma in the $S_r > 1,000$ fraction, and only a few become particles of $S_r < 1,000$. The metabolic process by which large-sized chylomicrons are directly cleared from plasma is not known. However, it may very well be different from the normal process of degradation of chylomicrons to remnants with subsequent receptor-mediated clearance of small-size particles. The direct clearance of large-size chylomicrons from plasma may preclude the normal transfer of surface lipids and proteins to HDL that occurs with normal chylomicron degradation. This could conceivably account, at least in part, for the low HDL levels consistently found in hypertriglyceridemic patients (44).

In type III patients, the nonchylomicron RP response was quite different from all other groups studied in that the removal of these particles was greatly delayed. This can be inferred from the rate of nonchylomicron RP disappearance 14–24 h after the fatty meal, when absorption was complete and chylomicron RP concentrations were low. This is compatible with the current concept that type III is a chylomicron remnant removal disorder, due to the presence of a form of apo E, E2, on the surface of these particles that is not recognized by hepatic receptors. In type III individuals, chylomicron RP (and triglyceride) peak height and area were intermediate between normal and type IV subject. This could either reflect an intrinsic block in type III patients in the conversion of chylomicrons to remnants, or inhibition of the conversion by high concentrations of remnants. In fact, Chait et al. have shown that β -VLDL in type III patients is not as susceptible to lipolysis as normal VLDL (45) and Ehnholm et al. claim that the E2 in β -VLDL of type III patients inhibits its conversion to LDL (46).

The results of the vitamin A-fat loading test in type IIa patients were unexpected. These patients had a significantly lower chylomicron RP response, indicating accelerated clearance of chylomicrons. This result did not correlate with any of the biochemical parameters measured (Table III) and remains unexplained. Nonchylomicron RP response of type IIa patients was normal.

Highly significant ($P < 0.01$) negative correlations were found between chylomicron RP responses and LPL activity ($r = -0.726$) and fasting HDL cholesterol levels ($r = -0.647$) in normal subjects. These observations are in line with current concepts on the relationships between rates of chylomicron metabolism and transfer of surface remnants to the HDL system (43, 47).

In contrast to chylomicron clearance, the nonchylomicron RP response was independent of LPL activity in all subjects (even normolipidemics). This observation indicates that this enzyme is not involved in the clearance of smaller particles ($S_r < 1,000$) of exogenous origin. However, of great interest is the finding that in normolipidemic subjects, a significant ($P < 0.01$) negative correlation existed between nonchylomicron RP response and HTGL activity ($r = -0.620$) (Table III, Fig. 5). The same trend was also seen in type IIa patients, with a correlation of $r = -0.724$, but was not quite significant. These correlations suggest that the hepatic enzyme plays a role in the process of chylomicron remnant removal from plasma. It has been established that chylomicron remnants avidly interact with specific receptors on hepatocytes, and that this interaction is followed by rapid uptake and degradation of the particles (3, 32, 34, 36). Our data suggest that before their catabolism via the receptor-mediated pathway that the chylomicron remnant particles interact with HTGL. This could result in the removal of excess triglycerides and/or phospholipids and be a requirement for receptor-mediated catabolism. A role for the HTGL in VLDL remnant metabolism has recently been suggested (48–52). This enzyme, evidently, may also be involved in chylomicron remnant metabolism. In a recent study, Bensadoun has found that inhibition of HTGL delays removal of intestinally derived particles in rats (53).

The results of the vitamin A-fat loading test in type IV and IIa patients after treatment with gemfibrozil and cholestyramine, respectively, support the concepts discussed above. Gemfibrozil effectively reduced the elevated fasting plasma triglyceride levels in the type IV patients studied. This was associated with approximately a two-thirds reduction in the chylomicron RP response and a one-fourth reduction in the nonchylomicron RP response. However, this was not associated with a significant increase in LPL activity. Previous workers have studied the effect of gemfibrozil on endogenous fat metabolism in type IV patients (54–58). They found that gemfibrozil decreases production of VLDL-triglyceride and accelerates VLDL-triglyceride clearance. The latter is probably due to stimulation of LPL, although this has not been consistently demonstrated (56, 57). The current study demonstrating an effect of gemfibrozil on chylomicron metabolism is compatible with the previous studies showing its effect on VLDL metabolism. Similar observations were previously reported for clofibrate (6). In type IIa patients, cholestyramine therapy is frequently associated with an increase in fasting plasma triglyceride levels (59, 60). In the present study, such an increase was not observed. However, the chylomicron RP response was about twice that observed before initiation of therapy without any change in the nonchylomicron RP response. Thus, it appears that the dietary fat load unmasks metabolic abnormalities that remain unnoticed without a specific challenge. Therefore, this method may be useful in other instances, even where there are no obvious differences in fasting plasma triglyceride levels. The increase in chylomicron RP response was associated with a significant ($P < 0.05$) decrease in LPL activity. This may explain the observed effect of cholestyramine on chylomicron and VLDL metabolism. Alternatively, the increased RP response during cholestyramine therapy may be due to the reported increase of triglyceride transport (61). How this drug could change LPL activity and/or triglyceride transport is not known.

Do the studies described in the present report have any relevance to atherogenic processes? It has been suggested that chy-

lomicron remnants are formed on endothelial surfaces of large arteries, and that their cholesterol becomes incorporated into the artery wall and stimulates formation of the atherosclerotic lesion (12, 62, 63). The studies reported here demonstrate that both chylomicrons and their remnants are present in the plasma of "normal" people for a prolonged period of time after fat ingestion. As suggested by Fig. 3, normal subjects have significant circulating levels of chylomicron remnants for at least 12–24 h, which was not at all appreciated by following postprandial triglyceride levels. The duration and magnitude of the exposure to postprandial particles in hypertriglyceridemic patients, as shown by our studies, is much greater than in normals. Thus, in some circumstances, it appears that arteries may become exposed to high concentrations of remnants for prolonged periods of time, perhaps throughout an entire 24-h period. This situation can be dramatically improved by gemfibrozil treatment. Apart from hypertriglyceridemic subjects, it is intriguing to speculate that in a subset of patients with premature coronary artery disease and normal fasting lipids, that a defect in exogenous fat clearance may exist and play a role in the etiology of this disease. The vitamin A-RP fat loading test as presented here might be ideal for identifying such patients.

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