

Evidence for a Common, Saturable, Triglyceride Removal Mechanism for Chylomicrons and Very Low Density Lipoproteins in Man

JOHN D. BRUNZELL, WILLIAM R. HAZZARD, DANIEL PORTE, JR., and EDWIN L. BIERMAN

From the Department of Medicine, University of Washington School of Medicine, and the Veterans Administration Hospital, Seattle, Washington 98108

ABSTRACT Hypertriglyceridemic subjects were fed diets in which dietary fat calories were held constant, but carbohydrate calories were varied. Three subjects with fasting chylomicronemia (Type V) were given less carbohydrate and four subjects without fasting chylomicronemia (Type IV) were fed diets with more calories as carbohydrate. The restricted carbohydrate intake led to disappearance of chylomicronemia in those subjects who had chylomicronemia on a normal diet (Type V to IV). In those subjects without chylomicronemia, chylomicronemia appeared in response to increased carbohydrate intake (Type IV to V). Thus chylomicron concentrations in plasma were altered even though fat intake and presumably chylomicron input into plasma was kept constant. These findings provide evidence for saturation of chylomicron removal mechanisms by alteration of endogenous triglyceride-rich lipoprotein concentrations. They suggest that chylomicrons compete with very low density lipoproteins for similar removal mechanisms. The relationship between endogenous triglyceride concentration and the lipolytic activity in plasma following heparin was then evaluated with the use of long-term heparin infusions to release and maintain lipolytic activity in the circulation. 10 subjects were placed on fat-free diets to remove circulating dietary fat. The plasma lipolytic rate during the heparin infusion was measured consecutively on different days in individuals whose triglyceride concentrations were varied by either increasing

or decreasing calories. The lipolytic rate was curvilinearly related to the plasma triglyceride concentrations. This curvilinear relationship followed Michaelis-Menton saturation kinetics over a wide range of triglyceride concentrations on fat-free, high-carbohydrate diets, in multiple studies in a group of individuals. These studies suggest that endogenous and exogenous triglyceride compete for a common, saturable, plasma triglyceride removal system related to lipoprotein lipase.

INTRODUCTION

In recent years evidence has accumulated to suggest that plasma triglyceride (TG)¹ removal mechanisms are saturable in man. For example, when TG levels are artificially raised by intravenous fat loads of artificial emulsions or chylomicrons, plasma triglyceride disappearance rates are dependent on the size of the load (1-3). Some studies of endogenous TG transport support this concept and suggest that TG removal approaches saturation at triglyceride levels found in hypertriglyceridemic man (4-6), while in other studies a conclusion concerning saturation of TG removal could not be made, since no relationship was found between TG transport rates and concentration among groups of normal and hypertriglyceridemic subjects (7-10).

Since in these studies no consistent evidence for saturation of TG removal at TG concentrations found in man emerged, additional approaches were sought using different methodological assumptions to examine this question. First, to test whether removal of exogenous and endogenous TG was related and saturable, the presence

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¹Abbreviations used in this paper: PHLA, post-heparin lipolytic activity; PVP, polyvinylpyrrolidone; TG, triglyceride.

TABLE I
Characterization of Subjects

Subject	Age	Sex	Wt	% Ideal body wt*	Basal diet		Standard PHLA
					TG‡	Presence of chylomicrons	
					mg/100 ml		
			kg				$\mu\text{eq FFA/ml/min}$
1	41	M	69.0	114	449	—	0.412
2	58	M	96.6	141	556	—	0.512
3	59	M	63.3	117	613	—	0.326
4§	53	M	88.0	142	317	—	0.341
5	62	M	70.7	107	334	+	0.453
6§	49	M	76.7	118	480	+	0.426
7§	45	M	82.3	113	1,812	+	0.422
8	31	M	83.2	112	94	—	0.516
9	50	M	99.0	163	166	—	0.529
10	51	M	66.2	104	188	—	0.522
11	50	M	69.4	108	255	—	0.583
12	34	M	101.0	133	428	—	0.650
13§	49	M	77.3	119	945	+	0.426
14§	45	M	85.0	116	1,126	+	0.422
15	54	F	76.6	145	3,080	+	0.331
16	39	M	83.0	117	3,820	+	0.486
17§	52	M	88.1	142	333	—	0.341

* Metropolitan Life Insurance Tables.

‡ Mean of last two plasma TG determinations on basal diet.

§ Some subjects were included in both sections of the study.

|| Hypothyroidism treated with thyroxin.

of chylomicrons in fasting plasma was measured after changing endogenous TG levels by controlled manipulation of the quantity of carbohydrate in diets containing a constant amount of fat. Then, the relationship between the endogenous TG concentration and its rate of hydrolysis (the plasma lipolytic rate) was examined during a constant infusion of heparin (11).

METHODS

14 subjects with a spectrum of plasma triglyceride levels and normal post-heparin lipolytic activity were studied while eating constant composition, liquid formula diets and hospitalized on a metabolic ward. These subjects had no evidence of an abnormality in thyroid, hepatic, or renal function, no fat malabsorption; and were taking no drugs known to affect lipid metabolism, except as noted (Table I).

All subjects initially ingested a basal formula diet for at least 2 wk consisting of 40% of calories as fat, 45% as carbohydrate, and 15% as protein. The fat employed in these diets was a mixture of 50% butter fat and 50% corn oil, the carbohydrate was either dextrose or a mixture of dextrans and maltose (dextrimaltose), and the protein source was skim milk powder. Supplements of multivitamins (Unicap-M; Upjohn Co., Kalamazoo, Mich.), iron, and folic acid were added. The diet was given as five equal feedings at 8 and 11 a.m. and 2, 5, and 8 p.m. The calories were adjusted, if necessary, during the basal period to maintain constant body weight (± 1 kg). Plasma for analysis was separated from

blood samples collected in EDTA (1 mg/ml) obtained prior to the 8 a.m. feeding at least three times per week.

In seven hypertriglyceridemic subjects (Table I, subjects 1-7) after the basal period, the amount of carbohydrate in the basal diet was varied for a subsequent 6-27 day period. If the subject had chylomicronemia in fasting plasma (Type V lipoprotein pattern) (12, 13), dietary carbohydrate content was reduced (-23% of basal calories); if the subject had no chylomicronemia in fasting plasma (Type IV pattern) (12, 13), he was overfed with carbohydrate (+20 to 30% of basal calories). In each case the absolute intake of fat and protein was maintained constant (identical with that on the basal diet).

In 10 subjects (Table I, subjects 8-17), the basal diet period was followed by a 10-14 day period of a fat-free, high carbohydrate, weight-maintaining diet (0% fat, 85% carbohydrate, and 15% protein). At the end of the fat-free dietary period a measurement of the plasma lipolytic rate was obtained (see below). These subjects were then overfed or underfed the same fat-free diet (+30 to -75% of basal calories) for periods of approximately 7-10 days and repeat determinations of the plasma lipolytic rate were obtained.

Plasma glucose was measured by the AutoAnalyzer (Technicon Corp., Tarrytown, N. Y.) ferricyanide method and plasma TG by an automated modification of the method of Carlson as previously described (14). The presence of chylomicrons was determined by retention of lipid staining material at the origin after 1% agarose electrophoresis (15) by an independent observer and by polyvinylpyrrolidone (PVP) flocculation. The latter was quantified by tube slicing and

analysis of top particle triglyceride (14, 16) and reported as percent of total TG in chylomicrons.

Post-heparin lipolytic activity (PHLA) after rapid intravenous injection of heparin (380 U/m²) was measured on an artificial TG emulsion by the method of Fredrickson, Ono, and Davis (17), and a mean value was calculated from samples taken at 6, 8, and 10 min. PHLA was also determined during a prolonged (3-5 h) heparin infusion at 60 min and again in triplicate 2-4 h later. For these measurements, heparinized blood samples were collected in ice, and the plasma was promptly separated, frozen, and assayed at a later date.

The plasma lipolytic rate, that is, the rate of hydrolysis of endogenous plasma TG, was measured by a previously described method during a prolonged heparin infusion (18). These studies were done after a minimum of 10 days on the fat-free formula to exclude any contribution from dietary (exogenous) TG. The assessment of the plasma lipolytic rate is based on the property of intravenous heparin to release lipolytic activity into plasma where it acts on TG-rich lipoproteins at a rate that can be measured in vitro. Heparin is given as a loading dose (2,280 U/m²) and then as a constant infusion (1,985 U/m² per h) designed to keep circulating enzyme levels constant. Plasma triglyceride levels fall as lipolysis occurs in the circulation, until a new equilibrium level is reached. At that time (180-360 min) with enzyme and substrate levels constant (18) the plasma lipolytic rate was measured by in vitro incubation without added substrate. 15 ml of blood were drawn into heparinized tubes (20 U/ml) in triplicate 30 min apart; the plasma was separated rapidly (less than 1 min) by high-speed centrifugation, and incubation begun at 37°C in a Dubnoff shaker in an Erlenmeyer flask with an atmosphere of 5% CO₂ and 95% O₂. The rate of lipolysis was obtained by measurement of fatty acids produced during the incubation over 2-22, 22-42 min

averaged for the three samples. This plasma lipolytic rate was then expressed as microequivalents of free fatty acids formed in vitro per minute per milliliter of plasma.

RESULTS

Dietary studies on fat-containing diet. On the weight-maintaining, basal 40% fat diet, four hypertriglyceridemic subjects (Table I, Subjects 1-4) who had no detectable chylomicrons present in plasma after an overnight fast either by agarose electrophoresis or by PVP flocculation (Type IV lipoprotein pattern) were studied. With dietary fat intake held constant they were overfed with carbohydrate. With this maneuver plasma triglyceride levels increased, and the subjects developed chylomicronemia in fasting plasma (Type V pattern) (Table II, Fig. 1). One subject sustained an increase in plasma TG while on the basal diet with development of chylomicronemia detectable by agarose electrophoresis prior to being overfed. When overfed, the amount of plasma TG present as chylomicrons increased from 4 to 43% of total plasma TG as quantified by PVP flocculation.

To test the possibility that there was a decrease in PHLA on the diet with excess carbohydrate calories that could explain the appearance of chylomicrons, PHLA was measured in two of the subjects and found to be essentially unchanged (in $\mu\text{eq FFA/ml per min}$: no. 1, basal 0.412, high calorie 0.452; no. 2, basal 0.512, high calorie 0.650). Furthermore, carbohydrate overfeeding

TABLE II
Influence of Diet on Detection of Chylomicrons (Type IV Subjects)

Subject	Days of basal diet					Days of carbohydrate overfeeding							
	0	1-3	4-6	7-9	10-12	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24
No. 1													
TG*	401	428	530	470	457	516	575	622	663	673			
PVP†		0		0	0	0	0	0	2	6			
Agarose‡		-	-	-	-	-	-	±	+	+			
No. 2													
TG	306	368	357	567	595	873	852						
PVP		0	0	1	4	43	15						
Agarose		-	-	+	+	+	+						
No. 3													
TG	548	610	522	708		895	1,302	1,255					
PVP			0	0		1	4	2					
Agarose		-	-	-		-	+	+					
No. 4													
TG	334	274	256	270	324	418	372	342	373	654	474	730	666
PVP		0	0	0	0	0	0	0	0	8	1	15	2
Agarose		-	-	-	-	-	-	-	-	+	±	+	+

* Milligrams per 100 ml.

† Chylomicrons as detected by PVP flocculation (% of total TG) or agarose electrophoresis.

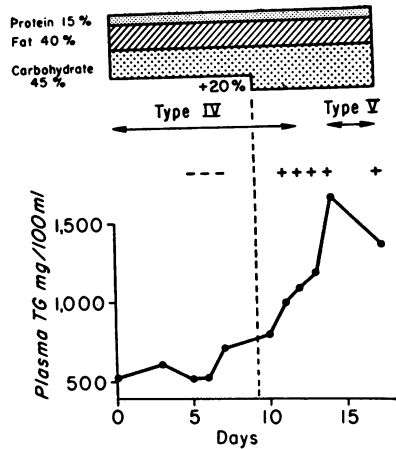


FIGURE 1 Effect of carbohydrate overfeeding on plasma triglyceride in a subject with a Type IV pattern (no. 3, Table I) on constant fat diet. Presence of chylomicrons in fasting plasma indicated by PVP flocculation (- not present, + present).

on a totally fat-free diet does not change PHLA from the level on the weight-maintaining, fat-free diet (see below).

Conversely, three subjects (Table I, Subjects 5-7) with chylomicronemia in fasting plasma (Type V pattern), detectable both by agarose electrophoresis and by PVP flocculation, while maintained on the eucaloric diet of regular composition, cleared their chylomicronemia with caloric restriction (-23%), accomplished by removing only the carbohydrate from the diet, again while dietary fat calories were maintained constant (Table III, Fig. 2).

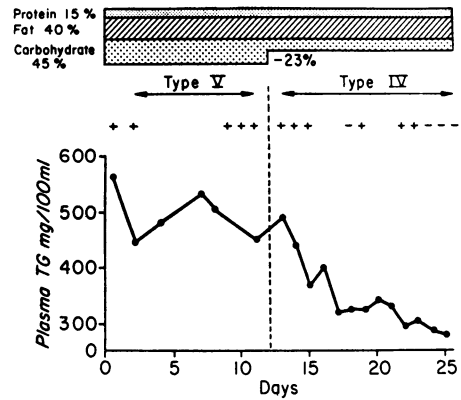


FIGURE 2 Effect of carbohydrate restriction on plasma triglyceride in a subject with a Type V pattern (no. 6, Table I) on constant fat diet. Presence of chylomicrons in fasting plasma indicated by PVP flocculation (+ present, - not present).

Dietary studies on fat-free diet. The plasma lipolytic rate during a constant heparin infusion was measured in 10 subjects on a fat-free, high carbohydrate, weight-maintaining formula diet. These subjects were then restudied following periods of greater than 7 days of caloric (carbohydrate) excess or restriction, which changed endogenous plasma triglyceride levels (Table IV). Inspection of the relation between lipolytic rate and TG concentration at equilibrium at the end of the heparin infusion (Fig. 3) suggests a nonlinear function. This was confirmed ($P < 0.001$) by statistical analysis of nonlinearity (19) with the plasma TG concentration ranked in groups of three in ascending order. Further-

TABLE III
Influence of Diet on Detection of Chylomicrons (Type V Subjects)

	Days of basal diet				Days of carbohydrate restriction								
	0	1-3	4-6	7-9	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27
No. 5													
TG*	288	268	288	381	297	228	189						
PVP†	11		5	26	14	1	0						
Agarose‡	+	+	+	+	+	±	-						
No. 6													
TG	568	445	483	520	450	419	348	332	300	283			
PVP	6	6	7			6	1	0.3	0.3	0			
Agarose		+	+	+	+	-	-	-	-	-			
No. 7													
TG	1,655	1,478	1,630	1,748	1,745	1,568	1,053	778	908	629	595	551	419
PVP	18		15	11		10		5	4	2	2	4	0
Agarose	+		+	+		+	+			±	±	±	-

* Milligrams per 100 ml.

† Chylomicrons as detected by PVP flocculation (% of total TG) or agarose electrophoresis.

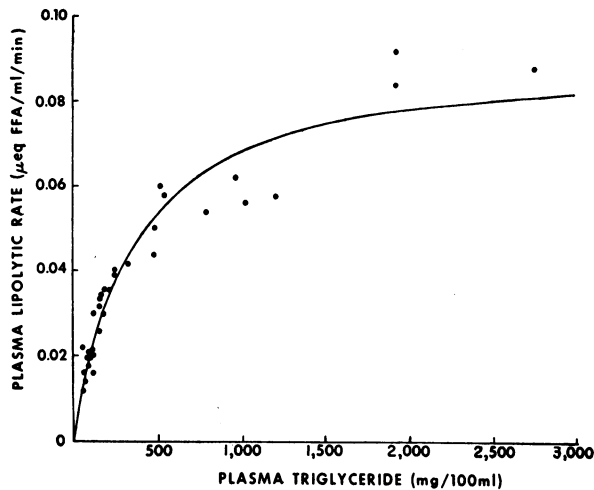


FIGURE 3 Plasma lipolytic rate and plasma triglyceride concentration at equilibrium during heparin infusion obtained from studies in multiple subjects (8-17, Table I) studied sequentially before and after caloric manipulation on a fat-free diet. Hyperbola calculated as best fit by least squares after Woolf linear transformation.

more, if the plasma lipolytic enzymatic system is saturable, this relationship should be a hyperbolic function. With the Woolf transformation of the Michaelis-Menton

kinetics, s/v vs. s (20), a significant linear relationship was found ($P < 0.001$) consistent with a hyperbolic function in a system following saturation kinetics. Statistical analysis for nonlinearity of the transformed function was not significant. Multiple studies in each individual (Table IV) closely follow this curve describing the relation between plasma lipolytic rate and TG concentration at equilibrium (Fig. 3).

Changes in lipoprotein lipase activity did not account for this nonlinear relationship, since PHLA measurements after 60 min of the constant heparin infusion (18) were not changed between studies (Table V).

DISCUSSION

The dietary studies indicate that an interaction exists between the removal of chylomicrons and endogenous triglyceride-rich lipoproteins as previously suggested by Havel (21). Thus, the presence of chylomicrons in fasting plasma of these hypertriglyceridemic subjects was related to the carbohydrate content of the diet, when dietary fat input into plasma was kept constant. Feeding excess calories as carbohydrate increased the concentration of endogenous plasma triglyceride and chylomicrons appearing in plasma after an overnight fast, whereas caloric restriction of carbohydrate decreased endogenous plasma triglyceride levels and chylomicrons were cleared.

TABLE IV
Influence of Diet on Plasma Lipolytic Rate

Subject	Basal diet			Hypercaloric diet			Hypocaloric diet		
	Lipolytic rate*	Plasma†	TG‡	Lipolytic rate	Plasma†	TG‡	Lipolytic rate	Plasma†	TG‡
8	0.0165	188	113	0.0397	358	248			
9	0.0304	246	123				0.0212	133	60
10	0.0204	390	106				0.0135	281	49
11	0.0448	853	485	0.0530	1,382	766			
12	0.0418	654	341				0.0193	178	86
13	0.0509	902	481	0.0583	1,340	528	0.0188	348	78
				0.0597	1,136	517			
14	0.0561	1,756	1,051	0.0577	1,912	1,200	0.0263	397	148
15	0.0916	3,215	1,915				0.0404	632	246
16	0.0836	2,905	1,907	0.0876	3,755	2,757	0.0626	1,545	972
17	0.0207¶	218	87	0.0305	495	185	0.0128	129	47
	0.0167	243	72	0.0353	559	189	0.0197	200	84
	0.0203	232	96	0.0361	723	218			
	0.0333	428	158						
	0.0334¶	462	160						
	0.0325	490	151						

* Microequivalents FFA released in vitro per milliliter plasma per minute.

† TG at zero time heparin infusion, milligrams per 100 milliliters.

‡ TG at equilibrium heparin infusion, milligrams per 100 milliliters.

|| Subject no. 17 studied over period of 2 yr at different stable body weights interspersed with hypercaloric and hypocaloric feeding, ranked by body weight.

¶ Studies performed on clofibrate and phenformin therapy, respectively.

TABLE V
Influence of Diet on Plasma Post-Heparin Lipolytic Activity

Subject	Basal diet (60 min PHLA)	Hypercaloric diet		Hypocaloric diet	
		60 min PHLA	Change	60 min PHLA	Change
	$\mu\text{eq FFA/ml/min}$	$\mu\text{eq FFA/ml/min}$	%	$\mu\text{eq FFA/ml/min}$	%
8	0.349	0.354	+1.4	—	—
9	0.787	—	—	0.806	+2.4
10	0.600	—	—	0.673	+12.2
11	0.522	0.654	+25.3	—	—
12	0.662	—	—	0.723	+9.2
13	0.650	0.647	-0.5	0.823	+26.6
14	0.726	0.569	-21.6	0.679	-6.5
15	0.494	—	—	0.606	+22.7
16	0.622	0.489	-21.4	0.905	+45.5
17	a)*	0.479	-4.8	—	—
			-8.4	—	—
	b)*	0.490	—	0.464	-5.3
				0.422	-13.9
Mean % change			-5.2	+10.3	
P			NS	NS	

* Subject no. 17 studied several months apart.

The effect of perturbation of endogenous lipoprotein levels are probably mediated through changes in the plasma TG pool size which would then affect the time required for clearance of dietary TG. Thus, an increase in the endogenous TG plasma pool would prolong chylomicron removal sufficiently that they are present after the twelve hour overnight fast when plasma was sampled. These chylomicrons do not appear to be very large endogenous very low density lipoproteins (22), since the latter behave differently with PVP flocculation (14) and do not remain at the origin after electrophoresis on agarose. Furthermore, in the subjects tested after 10 days on fat-free diets (Table I, subjects 8-17) no chylomicronemia was detectable by either method, despite plasma TG levels in excess of 3,000 mg/100 ml.

One possible explanation for these findings is that chylomicron TG and very low density lipoprotein TG are processed by a common mechanism. Whether the mechanism of removal of these two lipoproteins is identical or an interaction at one site in a complex removal system is unknown at this time. Although these studies do not provide information concerning the rates at which each is processed, it is not necessarily at the same rate. Carbohydrate overfeeding does not decrease PHLA, which would argue against the possibility that chylomicrons appear simply because of a change in removal capacity associated with a change in the diet. It is also unlikely that in these studies changes in dietary carbohydrate altered fat absorption since it has been shown that the relative amounts of dietary carbohydrate and fat do not affect

the absorption of neutral dietary fat in subjects who do not have fat malabsorption (23).

Results of studies of exogenous particulate fat removal in man are compatible with those in the present study. Increasing amounts of intravenously injected chylomicrons were cleared from plasma such that the fractional removal rate was inversely related to load in a nonlinear fashion (1, 24). Using artificial fat emulsions Boberg, Carlson, and Hallberg (3) also demonstrated that TG removal was a nonlinear function of TG concentration, but approached saturation only at levels above those found in hyperlipemia in man. However, the removal of emulsions may not be a valid index of removal of native chylomicrons (25).

The question of saturation of removal of endogenous TG-rich lipoproteins in man has been more difficult to assess. Studies of TG transport rates have been made in subjects with endogenous hypertriglyceridemia (4, 5, 7-10). In some of these studies (4, 5) the relationship between TG concentration and transport rate among subjects suggested that plasma triglyceride removal was saturable. However, in a given individual studied once at a point in time when the plasma TG concentration is stable, no conclusions can be derived regarding the question of saturation of removal.

In this study an independent method that could be readily applied in repetitive testing in an individual under a variety of conditions was sought to test whether saturability of lipolysis of endogenous substrate could be demonstrated. A previous report (6) with the pres-

ent heparin infusion method indicated a nonlinear relation between TG concentration and lipolytic rate which, after correction for body weight, was interpreted as TG transport rate. At present there are limitations in the use of the plasma lipolytic rate as a quantitative estimate of TG production or removal since it is necessary to assume that sources of TG removal other than lipoprotein lipase are negligible and that the entire lipoprotein lipase system that is operative in TG removal is released into plasma by very large heparin infusions. However, even without these assumptions the lipolytic rate can be directly measured and is useful in determining whether or not the relation between endogenous TG substrate and lipolytic activity after heparin is saturable.

The post-heparin lipolytic activity (PHLA) is a measure of the hydrolytic activity in post-heparin plasma upon an excess of exogenous substrate. The plasma lipolytic rate is a measure of the interaction of this enzymatic activity with endogenous TG substrate. Since plasma lipoprotein lipase has not yet been completely purified and studied, it can only be assumed that either measure of lipolysis reflects tissue lipoprotein lipase(s). The curvilinear relationship between the plasma lipolytic rate and triglyceride levels on a fat-free diet is consistent with the concept of saturability of the lipoprotein lipase-related component of the TG hydrolytic system. It is probable that the rate of hydrolysis of plasma TG at low plasma TG levels is limited by both the amount of substrate and enzyme, but that as the TG concentration increases the rate of hydrolysis is primarily limited by enzyme activity only. This curvilinear relationship was obtained when a group of individuals was studied at different triglyceride concentrations produced by caloric perturbation with varying amounts of carbohydrate. Even though PHLA levels vary greatly from individual to individual, the relation between plasma lipolytic rate and TG concentration appears to be constant in the group of subjects in this study. Possibly, TG removal related to lipoprotein lipase is fairly uniform, despite variations in PHLA, in individuals who have no apparent reason to have an abnormality in lipoprotein lipase.

If exogenous TG and endogenous TG interact during the removal process and this process is related to lipoprotein lipase, then subjects with PHLA (lipoprotein lipase) deficiency and chylomicronemia should have higher endogenous plasma TG levels than normal subjects when eating a fat-free, high carbohydrate diet. Review of published experiments in such subjects eating a fat-free diet do in fact show TG concentrations ranging from the upper limit of normal to a fourfold elevation (26). Thus, it is not necessary to postulate separate removal mechanisms for endogenously and exogenously derived TG-rich lipoproteins.

These results may help to explain the observation that in a single individual, serial assessment of lipoprotein patterns by electrophoresis may yield patterns that vary from time to time, particularly with regard to the detection of chylomicronemia. A similar explanation may account for those studies that have demonstrated that individuals in a family may have chylomicrons present in fasting plasma (Type V) while others in the same family have no circulating chylomicrons (Type IV) (27). Rather than postulate two distinct genetic diseases in the same family, those results are compatible with different degrees of saturation of the plasma triglyceride removal system possibly related to different environmental factors superimposed upon the same genetic substrate.

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