Lipid composition of fat particles from normal man and patients with idiopathic hypertriglyceridemia

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ABSTRACT Lipemic plasma from normal and hyperlipemic patients has been fractionated on columns of 3% (w/v) aqueous polyvinylpyrrolidone (PVP) and the lipid composition of the separated fat particles ($S_f > 400$) measured. Plasma from patients with carbohydrate-induced lipemia on fat-free diets contained particles with a greater percentage of cholesterol and phospholipid than either normal primary particles (which float to the top of 3% PVP columns) or secondary particles (which remain concentrated just above the plasma layer at the bottom of the 3% PVP column). These "hyperlipemia" particles remained in the lower half of 3% PVP columns, which allowed easy separation from primary (top) particles. In the same hypertriglyceridemic patients primary (top) particles with the usual lipid composition were isolated from plasma 8 hr after ingestion of 200 g of corn oil, but 24 hr after the meal, primary (top) particles isolated in the same way contained a higher percentage of cholesterol than normal primary particles. High-cholesterol primary (top) particles were isolated from the patients mentioned and also from patients with fat-induced lipemia after an overnight fast on a regular fat-containing diet.

It is concluded that the lipid composition of primary particles is variable and reflects the length of time these particles have been in the general circulation.

KEY WORDS plasma · fat particle · lipid composition · hyperlipemia · lipoprotein · polyvinylpyrrolidone · man · fat load

THE INVESTIGATION of fat particles (lipoproteins of $S_f > 400$) has been accelerated by the introduction of flocculation in polyvinylpyrrolidone columns (1–3) and zone electrophoresis (4) as techniques to separate this heterogeneous group of macromolecules. The use of constant-density (3%, w/v) polyvinylpyrrolidone columns for studying particles is described elsewhere (5). In this report the lipid composition and behavior of the particles isolated by this method from plasma of hyperlipemic patients is compared with that from normal subjects.

Abbreviation: PVP, polyvinylpyrrolidone.

METHODS

Blood samples, anticoagulated with 1 mg of EDTA per ml, were obtained from 8 hyperlipemic subjects (7 male, 1 female) and 10 normal subjects (7 male, 3 female). The hyperlipemic patients were studied after an overnight fast and also after the ingestion of 200 g of corn oil. Each hyperlipemic subject had previously been hospitalized for studies of, among other things, his lipid response to a two-week fat-free diet (85% of calories as carbohydrate, 15% of calories as protein), determination of the post-heparin lipolytic activity (6) while on a diet containing 40% of calories as fat, and a standard oral glucose tolerance test. Two male subjects (Guz, San) presented the features of fat-induced hyperlipemia (6–8), but the rest were considered to have a carbohydrate-induced hyperlipemia (7, 8).

Blood samples from the normal subjects were obtained 4, 6, and 8 hr after ingestion of 2 g of corn oil per kg body weight. The sample with maximum visible lipemia was analyzed on 3% (w/v) polyvinylpyrrolidone columns. Lipemic plasma was layered under columns of 3% PVP in 10% NaCl to separate lipoproteins of $S_f > 400$ into two classes. "Top particles" were concentrated into a zone near the top of the tube, "bottom particles" just above the plasma layer. Both fractions were freed from any contaminating lipoproteins of $S_f < 400$, by centrifugation for 10^6 *g*-min through 0.85% NaCl. Total plasma

TABLE 1 LIPID COMPOSITION OF FAT PARTICLES ISOLATED FROM PLASMA* OF NORMAL SUBJECTS BY USE OF 3% PVP COLUMNS AND CENTRIFUGATION

	Particle TG	Top			Bottom			Centrifugation ($S_f > 400$)		
	TG	Chol†	PL	TG	Chol†	PL	TG	Chol†	PL
	mg/100 ml	*wt %*			*wt %*			*wt %*	
Ken	278	94	4	2	80	10	10	84	9	7
Bee	174	87	6	7	87	7	6	85	7	8
Nor	86	93	5	2	87	9	4	93	5	2
Hutch	76	85	8	7	83	14	3	83	10	7
Harb	75	no top particles			90	4	6	91	3	6
Dob	75	no top particles				nd‡		87	8	5
Hark	65	no top particles			90	5	5	88	7	5
Mc	51	no top particles			79	12	9	80	11	9
Uye	29	no top particles				nd		85	9	6
Jen	14	no top particles			81	12	7	83	11	6
Mean ± SD		89 ± 5	6 ± 2	5 ± 3	85 ± 4	9 ± 4	6 ± 3	86 ± 4	8 ± 3	6 ± 2

* Plasma was taken at the peak of alimentary lipemia, 4 or 6 hr after 2 g of fat per kg of body wt.
† Total sterol × 1.5.
‡ Not done.

particles ($S_f > 400$) were obtained by direct centrifugation of plasma for 10^6 *g*-min for comparison. These particle classes are thus defined operationally; however, "top particles" appear to be identical with primary particles separated by zone electrophoresis (4) and "bottom particles" appear to be identical to secondary particles separated by zone electrophoresis (4). Details of the PVP column and ultracentrifugation techniques used for particle separation, and methods of lipid analysis are described in reference 5.

RESULTS

Normal Subjects

Six of the ten normal subjects had no top particles, despite the large fat load (Table 1). In general the lipid compositions of the primary (top) and secondary (bottom) particles isolated were similar to those obtained using starch block electrophoresis (4, 9). However, in the four samples which could be directly compared there was a small but significantly higher cholesterol and lower triglyceride content in the bottom particle ($P < 0.05$). Similar results were found when duplicate aliquots were analyzed on PVP *gradient* columns.

Hypertriglyceridemic Subjects

The turbid plasma from hyperlipemic subjects eating fat-free diets for at least 1 week imparted a bluish opalescence to gradient and 3% PVP columns without forming visible aggregates. When lipemia was slight, the opalescent portion was immediately above the plasma layer, and was present whether or not the native plasma contained 5% PVP–10% NaCl. When samples of plasma freed from $S_f > 400$ lipoproteins by centrifugation were layered at the bottom of the column, any faint turbidity always remained within the plasma layer even if this particle-free plasma contained high concentrations of S_f 20–400 lipoproteins. When marked lipemia was present in the lipemic patients while they were ingesting fat-free diets, the bluish opalescence from these plasma samples extended almost to the top of the column. For the same sample, the opalescence tended to rise higher in the 3% than in the gradient PVP column. The particles causing this opalescence could then be packed at the top of the column by centrifugation for 10^6 *g*-min in an angle rotor. The cholesterol content of this particle fraction (Table 2) was significantly higher ($P < 0.001$) than that of particles from normal plasma (Table 1) and was similar to the value found for the "hyperlipemia" particle isolated by starch block electrophoresis (9). Therefore, the blue opalescence above the plasma layer was considered to be due to the presence of "hyperlipemia" particles.

The cholesterol content of the top particles in the plasma of hyperlipemic subjects on regular meals after an overnight fast was significantly higher (mean 17%, $P < 0.001$) than that of the primary particles from their own plasma 8 hr after the fat load (Table 2) or from the plasma of normal subjects (Table 1). The cholesterol content of the top particle fraction was not reduced by slicing the tube immediately under the top band or by centrifugation of the particles through 0.85% NaCl for 10^6 *g*-min.

Eight hours after the ingestion of 200 g of corn oil by five carbohydrate-induced hyperlipemic subjects, the lipid composition of their top particles closely resembled that of normal primary particles, although the bottom particles retained the higher cholesterol content (Table 2). The lipid composition of the $S_f > 400$ fraction from centrifugation then appeared to represent a mixture of top and bottom particles. The middle of the PVP col-

TABLE 2 LIPID COMPOSITION OF FAT PARTICLES ISOLATED FROM PLASMA OF HYPERLIPEMIC SUBJECTS 3% PVP COLUMNS AND CENTRIFUGATION

	Particle TG	Top			Bottom			Centrifugation ($S_f > 400$)		
	TG	Chol	PL	TG	Chol	PL	TG	Chol	PL	
	mg/100 ml	*wt %*			*wt %*			*wt %*		
Fat-free diet*										
Ran (CI)†	344			78	15	7	74	15	11	
Wal (CI)	130			73	14	13	72	19	13	
Sex (CI)	160			76	15	9	74	15	11	
Sal (CI)	94			70	18	12	67	20	13	
Pea (CI)	89			76	15	9	77	15	8	
Mean ± SD				75 ± 3	15 ± 2	10 ± 2	73 ± 4	16 ± 2	11 ± 2	
8 hr after 200 g fat										
Sal (CI)	1305	90	6	4	70	17	13	89	6	5
Sex (CI)	1264	86	6	8	75	14	11	83	9	8
Di (CI)	1152	93	4	3	72	14	14	86	6	8
Ran (CI)	510	90	5	5	80	12	8	77	10	13
Pea (CI)	473	85	6	9	74	15	11	84	8	8
Mean ± SD		90 ± 3	5 ± 1	6 ± 3	74 ± 4	14 ± 2	11 ± 2	84 ± 4	8 ± 2	8 ± 3
Overnight fast on regular meals										
Guz (FI)	1280	78	16	6	75	18	7	79	14	7
Ran (CI)	535	70	17	13	70	17	13	74	16	10
Sex (CI)	413		nd‡			nd		77	14	9
Wal (CI)	292	72	17	11	72	20	8	75	15	10
Sal (CI)	23		nd			nd		79	14	7
San (FI)	2200	80	12	9	82	13	5		nd	
Mean ± SD		75 ± 5	16 ± 2	10 ± 3	75 ± 5	17 ± 3	8 ± 3	77 ± 2	15 ± 1	9 ± 2

* Only hyperlipemic particles present.
† CI, carbohydrate-induced hyperlipemia; FI, fat-induced hyperlipemia.
‡ Not done.

umn from these samples often appeared opalescent, unlike the middle zone of columns containing lymph or plasma from normal subjects, which was relatively clear or contained a few small visible aggregates. This middle opalescence was not due to overloading the column with alimentary particles since it was not found in lymph samples containing higher particle-triglyceride loads. It probably reflected the presence of "hyperlipemia" particles in addition to the primary and secondary particles normally present during alimentary lipemia. Samples of plasma from three of these patients with carbohydrate-induced lipemia were placed on PVP columns 24 hr after the corn oil load. There were no top particles prior to the fat load, and a "normal" top particle was present after 8 hr, but 24 hr after the fat was ingested, these particles continued to increase in cholesterol content (Table 3) until they resembled top particles obtained after an overnight fast on regular meals (Table 2).

A similar phenomenon was observed in one patient with fat-induced lipemia (San) who was fasted for 5 days. At the beginning of this period total plasma triglyceride was 3528 mg/100 ml and the top particles had a cholesterol content of 12% (ad libitum diet, Table 2). At the end of the 5 day fasting period total plasma triglyceride had decreased to 800 mg/100 ml. Top particles were still present, but the cholesterol content had increased from 12 to 23%, while the triglyceride had decreased from 80 to 72% and phospholipid from 9 to 6%.

DISCUSSION

Previous data on the lipid composition of primary, secondary, and "hyperlipemia" particles, isolated by starch block electrophoresis (9), are in general agreement with the lipid composition revealed after PVP separation. However, while PVP will separate primary from secondary or "hyperlipemia" particles and zone electrophoresis will separate secondary particles from primary

TABLE 3 LIPID COMPOSITION OF FAT PARTICLES ISOLATED AT TOP OF 3% PVP COLUMN EARLY AND LATE AFTER FAT LOAD IN HYPERLIPEMIC SUBJECTS

Subject	8 hr after Fat Load*			24 hr after Fat Load		
	TG	Chol	PL	TG	Chol	PL
	wt %			*wt %*		
Di (CI)†	93	4	3	84	10	6
Ran (CI)	90	5	5	85	7	8
Sal (CI)	90	6	4	82	10	8

* 200 g of corn oil.
† CI, carbohydrate-induced hyperlipemia.

or "hyperlipemia" particles, both techniques are required to elucidate the composition of unknown mixtures of lipoproteins $S_f > 400$.

After the ingestion of a large fat load by hyperlipemic subjects, the plasma contains top particles with composition similar to that of "normal" primary particles, while the composition of the bottom particles reflects that of secondary particles plus the imprint of the "hyperlipemia" particles, which are also concentrated at the bottom of the tube. Of considerable interest is the finding of top particles with an increased cholesterol content in the plasma of these patients when fasted overnight after regular meals. This change in lipid composition of the "primary" particles appears to be an additional effect of interactions between the primary particles and plasma [and(or) tissue] during their prolonged circulation in the plasma of hyperlipemic subjects. It seems less likely that this change reflects contamination with "hyperlipemia" particles at the top of the tube since primary particles with a "normal" cholesterol composition were isolated on PVP columns from the plasma of patients with carbohydrate-induced lipemia 8 hr after corn oil, at a time when the concentrations of "hyperlipemia" particles were known to be high. This same phenomenon was also observed in a patient with fat-induced lipemia. There is increasing evidence, therefore, that fat particles do not remain fixed, homogeneous entities, but undergo major alterations as they circulate in plasma.

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