EARLY EFFECTS OF FAT INGESTION ON LIPIDS AND LIPOPROTEINS OF SERUM IN MAN

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The mechanisms of fat transport in the blood are still poorly understood. Recent studies have shown that all lipids in blood plasma exist as lipoprotein complexes, that a broad spectrum of such complexes exists, and that groups of these complexes can be separated from each other by various physical means. Application of these separation methods to the study of chemical changes within lipoprotein classes should provide greater understanding of fat transport.

The chylomicronemia produced by ingestion of fat in man is accompanied chiefly by an increased concentration of triglycerides, the chief components of chylomicra (1, 2) in the serum. Phospholipid concentration also increases significantly (3), but free and ester cholesterol concentrations increase slightly (4) or not at all (5, 6). No changes have been reported in the concentrations in serum of high density and S_f 0-30 classes of lipoproteins after ingestion of fat, but the concentration of the higher S_f classes increases. There appears to be a progressive shift with time in the concentrations of these very low density lipoprotein classes in the direction of greater density (7). Chylomicra and lipoproteins of high S_f rates consist chiefly of triglycerides with lesser quantities of phospholipids, cholesterol esters, unesterified cholesterol, and protein (8). Thus, the serum lipid changes found after ingestion of fat might be explained exclusively by increased concentrations of chylomicra and lipoproteins having S_f rates exceeding 30.

To define further the effects of ingestion of fats on serum lipids and lipoproteins, alterations in various lipid constituents of ultracentrifugally separated lipoprotein classes of serum were measured in healthy young adults following the ingestion of high fat meals. These studies have shown that the concentrations of major chemical constituents of both high and very low density lipoproteins in serum are increased after ingestion of fat.

METHODS

Experimental subjects. Healthy young adults, who had undergone thorough physical and laboratory examinations and who had no apparent systemic disease, were hospitalized in a metabolic ward. Other subjects were professional and technical personnel who were not examined but who had no known systemic disease.

Diets. Hospitalized subjects were given measured diets containing varying quantities of fat. After a 12 to 15-hour fast, they were given high fat breakfasts containing 1.5 gram of fat per kgm. of body weight as calculated from standard tables. The composition of these meals is listed in Table I. Occasional transient nausea was the only apparent adverse effect of eating this quantity of fat. The subjects were allowed water but no food during the next 8 hours. Between the eighth and tenth hour they were given a supper supplying 10 calories per kgm. of body weight which contained practically no fat. Only water was permitted thereafter until completion of the test.

Blood samples and analytical procedures. Blood samples were taken from an antecubital vein and allowed to clot. The serum was extracted with ethanol-acetone (1:1 v/v) or chloroform-methanol (2:1 v/v). Two ml. of serum contained in a calibrated hypodermic syringe was forcefully ejected into about 45 ml. of solvent through a 23-gauge needle, and additional solvent added to give a final volume of 50 ml. Analyses for cholesterol (9), lipid phosphorus (10), and total lipid (11) were done directly on the ethanol-acetone extract after the precipitated proteins had been centrifuged down. The chloroform-methanol extract was treated further by a modification of the procedure described by Sperry and Brand (12). The extract was separated into two phases by the addition of 10 ml. of distilled water. The two phases were mixed by inversion and allowed to separate overnight. The upper phase contains the crystalloids of serum, and the lower phase the lipids. The final volume of the lower phase was 36 ml.

Direct comparison of results obtained by the two extraction procedures demonstrated that the yields of total and free cholesterol and of lipid phosphorus were identical,

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TABLE I

COMPOSITION OF MEALS USED TO PRODUCE ALIMENTARY LIPEMIA

Meal No.	Composition per Kgm. Body Weight		Preparation
1	36% cream	4.0 ml.	homogenized
	skimmed milk	4.0 ml.	
	cocoa	0.12 gm.	
	sucrose	0.25 gm.	
2*	whole egg	4.5 gm.	egg beaten in melted oleomargine and fried
	oleomargarine	0.8 gm.	
	bacon, limp fried	0.5 gm.	
3	corn oil	1.5 gm.	homogenized
	skimmed milk powder	0.5 gm.	
	sucrose	0.15 gm.	
	water	3.0 ml.	
	vanilla (to flavor)		

* Contains only about 0.05 gm. carbohydrate

whereas the calculated triglyceride content (11) was about 100 mg. per 100 ml. of serum higher in the ethanol-acetone extract. This results from the fact that certain crystalloids, principally chloride ion, contaminate the ethanol-acetone extract and contribute to the apparent total lipid concentration. Consequently, 100 mg. has been subtracted from all triglyceride values obtained by this method. Crystalloids are practically absent from the chloroform-methanol extract. Unesterified fatty acids are distributed between the two phases of this extract (13), but virtually no other lipids are present in the polar phase. "Triglyceride" values given for the chloroform-methanol extract therefore represent glyceryl esters of fatty acids (chiefly triglycerides [14]) plus a portion of the unesterified fatty acids.

Separation and analysis of lipoprotein fractions. Lipoprotein fractions were separated from serum as described previously (15). The densities and nature of the fractions are given in Table II. Aliquots of Fraction I + II were extracted in chloroform-methanol. The other fractions were extracted in ethanol-acetone. Since Fraction I + II contains about 50 per cent triglycerides and practically no unesterified fatty acids (13), triglyceride values for this fraction are considered to be more accurate than those obtained on whole serum. Analyses were done as on whole serum.

RESULTS

Serum lipids

The initial studies were carried out on subjects in a metabolic ward to determine the effects of previous diet on the nature and duration of hyper-

TABLE II

ULTRACENTRIFUGALLY SEPARATED LIPOPROTEIN FRACTIONS

Fraction	Nomenclature	Density	Electrophoretic Mobility (starch)
I + II	Very low density lipoproteins, including chylomicrons (Sf >10)	<1.019	Alpha-2 globulin
III	Low density lipoproteins (Sf 0-10)	1.019-1.063	Beta-1 globulin
IV	High density lipoproteins	1.063-1.21	Alpha-1 globulin
V	Unesterified fatty acids	>1.21	Albumin
	+		+
	Residual serum proteins		All components

lipemia following ingestion of fat. Four subjects were given, in succession, isocaloric diets which contained 1.0, 0.1 and 2.0 grams of fat per kgm. body weight. The first diet was maintained for 2 weeks, and the second and third for 1 week each. Fat breakfast No. 1 was given at the end of each week of study. The results, tabulated in Table III, show that the magnitude of the increment in serum triglyceride concentration was not related consistently to the nature of the diet during the week preceding the test; however, the hyperlipemia frequently persisted longer when the subjects were given the high and low fat diets than when they received the moderate fat diet. Of further interest is the fact that the serum triglyceride concentration was usually lower 12 hours after the fat meal than just before or 24 hours after its ingestion, regardless of the fat content of the diet. For all diets the mean triglyceride concentration at 12 and 24 hours, respectively, was 56 and 79 mg. per 100 ml. of serum, with a probability of less than 0.01 that the difference observed was the result of

TABLE III

ALTERATIONS IN WHOLE SERUM LIPID CONCENTRATIONS FOLLOWING INGESTION OF 1.5 Gm. CREAM FAT PER Kg. BODY WEIGHT

Patient	Test No.*	Total Cholesterol				Phospholipids				Glycerides			
		1	2	3	4	1	2	3	4	1	2	3	4
	Hours												
G.W. Male 20	0	134	122	93	135	166	175	143	175	60	75	95	45
	4	128	129	94	135	177	190	163	199	225	200	175	290
	8	132	130	93	145	197	212	159	228	150	190	110	210
	12	134	120	103	132	179	190	156	180	20	60	45	60
	24	129	121	98	136	173	170	143	169	55	90	40	65
G.C. Male 20	0	193	154	121	164	205	183	159	195	80	75	100	50
	4	182	160	125	182	231	218	191	226	290	200	190	190
	8	198	153	126	170	229	214	195	231	55	100	145	140
	12	181	159	121	174	199	210	172	211	40	60	75	45
	24	182	140	114	182	199	180	159	211	90	90	110	95
F.B. Female 24	0	151	153	111	135	185	179	162	175	75	50	120	85
	4	170	167	128	145	229	216	200	210	145	120	235	280
	8	167	170	132	150	218	230	218	217	90	85	245	170
	12	157	169	111	158	198	205	175	226	65	50	40	100
	24	166	---	109	140	190	---	155	170	75	--	85	80
P.M. Female 19	0	174	175	129	157	189	185	180	188	65	45	75	100
	4	189	175	147	178	225	212	209	235	105	90	155	205
	8	178	181	155	178	218	225	228	230	70	65	155	70
	12	183	175	138	166	205	200	202	206	50	20	70	55
	24	175	---	134	158	200	---	166	182	70	--	85	75

* Test No. 1: On isocaloric diet containing 1 gm. fat per kg. body weight for 1 week

Test No. 2: On isocaloric diet containing 1 gm. fat per kg. body weight for 2 weeks

Test No. 3: On isocaloric diet containing 0.1 gm. fat per kg. body weight for 1 week

Test No. 4: On isocaloric diet containing 2 gm. fat per kg. body weight for 1 week

chance. The concentration of phospholipids rose uniformly and significantly during fat absorption. The increment in phospholipid concentration was not related consistently to the increment in concentration of triglycerides, and phospholipid concentration usually remained elevated after triglyceride concentration had returned to baseline, or lower, values. Twelve hours after the ingestion of fat, triglyceride concentration was below the baseline value 13 times in 16 tests, whereas phospholipid concentration was more than 12 mg. per 100 ml. of serum higher than the baseline value 14 times in 16. The concentration of total cholesterol in the serum usually showed no consistent increase in the two male subjects. In the females cholesterol concentration rose at least 15 mg. per 100 ml. of serum following 7 of the 8 fat meals.

Lipoprotein fractions

Five additional subjects who were not on measured diets were studied after the ingestion of a single high fat meal. Meals No. 1 and 2 were

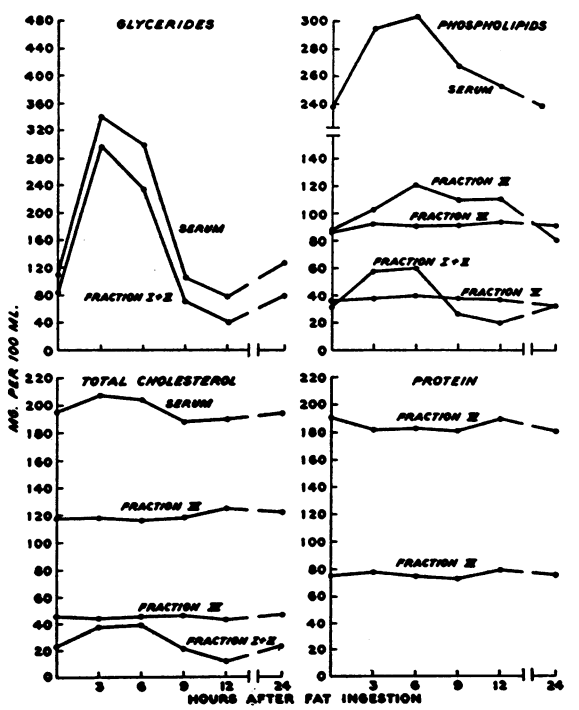

FIG. 1. ALTERATIONS IN THE CONCENTRATIONS OF SERUM LIPIDS AND CHEMICAL CONSTITUENTS OF SERUM LIPOPROTEIN FRACTIONS FOLLOWING INGESTION OF 1.5 GRAM OF FAT PER KGM. BODY WEIGHT IN A HEALTHY ADULT

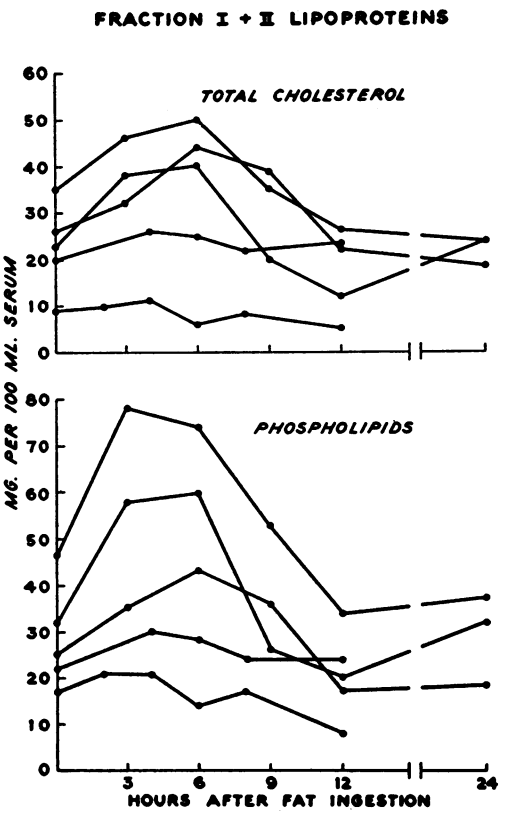

FIG. 2. ALTERATIONS IN THE CONCENTRATIONS OF CHEMICAL CONSTITUENTS OF SERUM LIPOPROTEIN FRACTIONS FOLLOWING INGESTION OF 1.5 GRAM OF FAT PER KGM. BODY WEIGHT IN FIVE HEALTHY ADULTS

used twice, and meal No. 3 once. The design of the experiments was the same as in the 4 subjects studied previously. Lipoprotein fractions were separated from aliquots of each serum sample and analyzed for lipid and protein constituents. Similar alterations in the various fractions were observed with the three types of fat-rich meal. Detailed results of one study are shown in Figure 1. The significant features are: 1) Alterations in serum triglyceride concentration reflected solely changes in triglycerides of Fraction I + II; 2) changes in serum total cholesterol concentration were relatively slight, but the small quantity of cholesterol in Fraction I + II was almost doubled; 3) changes in serum phospholipid concentration reflected the sum of changes in Fractions I + II and IV, and the elevation of Fraction IV persisted longer than that of Fraction I + II; 4) changes in protein concentration in Fractions III and IV were slight; 5) changes in the three major lipid

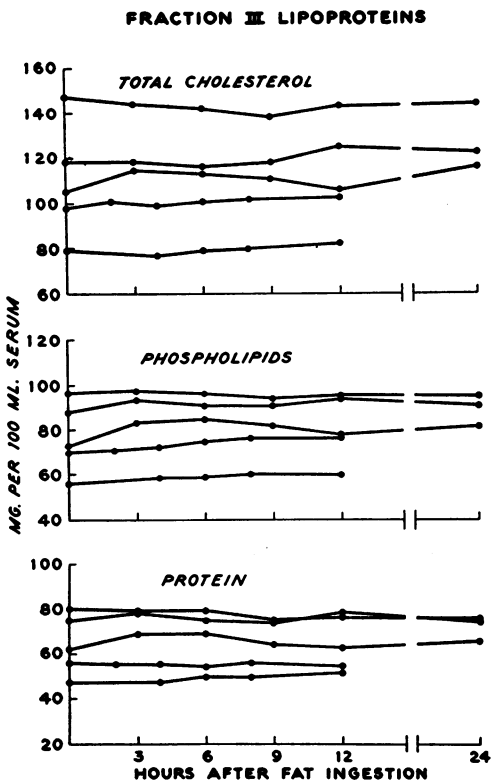

FIG. 3. SEE LEGEND, FIGURE 2

components of Fraction I + II tended to parallel each other, but the fall in the concentration of triglycerides preceded that of cholesterol and phospholipids; 6) the concentrations of Fraction I + II components were lower after 12 hours than after 24 hours. Alterations in measured components of the lipoproteins of the five subjects are shown in Figures 2 to 4. Although not shown in the figures, the magnitude of the increments in serum and Fraction I + II triglyceride concentrations tended to parallel that of the other lipid constituents of this fraction. Alterations in the concentrations of components of Fraction III were slight. In Fraction IV, phospholipid concentration rose consistently and tended to remain elevated 12 hours after the ingestion of fat, whereas total cholesterol and protein concentrations rose in only 3 of the 5 tests. The ratio of cholesterol to phospholipid in this fraction fell during fat absorption.

DISCUSSION

The demonstration that the increment in serum triglyceride concentration after ingestion of fat is contained entirely in chylomicra and/or very low density lipoproteins is in accord with current concepts of exogenous fat transport. It has been shown previously in dogs by Swank and Wilmot (16) and in man by van Eck, Peters, and Man (17) that the bulk of the increment in serum triglyceride concentration after the ingestion of fat can be layered on the surface of the serum by relatively gentle centrifugation. In their studies no layering of cholesterol or phospholipids was demonstrated, although later studies have shown that equivalent centrifugation layers some of the cholesterol and phospholipids of human chyle (18), a result to be expected from the known composition of chylomicra (8). The present results neither support nor contradict the concept that chylomicra are degraded in blood plasma with the production of progressively higher density lipoproteins (7), but the fact that the concentration of cholesterol and of phospholipids in Fraction I + II sometimes fell later than that of the triglycerides is consistent with such an interpretation.

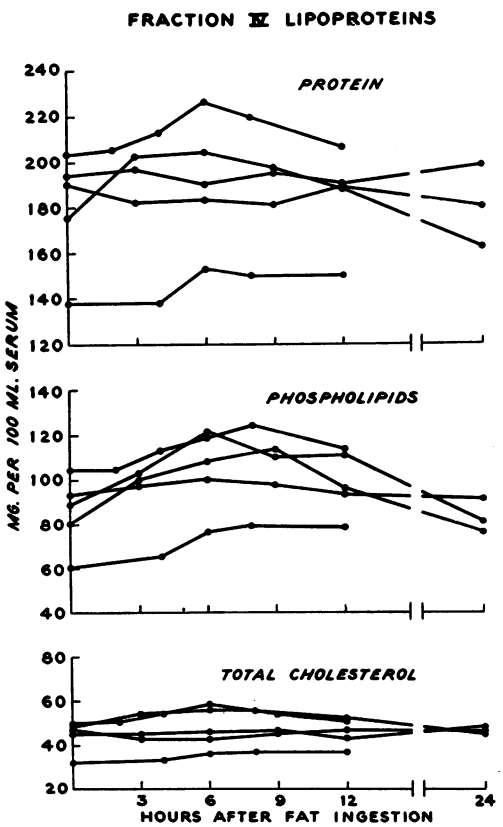

FIG. 4. SEE LEGEND, FIGURE 2

The delayed fall in the concentration of serum phospholipids following ingestion of fat is clearly the result of increased phospholipid concentration in high density lipoproteins (*cf.* Figure 1). This finding is not in accord with current concepts of lipoprotein alterations during transport of exogenous fat in the blood, although Swank and Wilmot found that the increased concentration of phospholipids in the serum of dogs absorbing fat remained in the infranatant serum after flotation of chylomicra (16).

The mechanism of the rise in phospholipid concentration in high density lipoproteins is unknown. Net transfer of phospholipid from Fraction I + II appears unlikely since there was no similar increase in phospholipid of Fraction III lipoproteins. That the increment resulted from an augmented rate of transfer of high density lipoproteins from thoracic duct lymph also appears unlikely because the other constituents of this fraction frequently showed no increase. It seems most probable that the changes observed are related to the further metabolism of exogenous fatty acids after they first leave the blood stream. It has been shown previously in dogs transfused with chylomicra obtained from lymph of dogs fed palmitic acid-1-C^{14} that radioactivity in phospholipids of high density lipoproteins continues to increase after the disappearance of the chylomicra from the plasma (19).

The data presented suggest that previous diet may have some effect on the rate of absorption of ingested fat or on the rate of its removal from the blood; however, no firm conclusions can be drawn from the limited data available. The striking reduction in the concentration of serum cholesterol and phospholipids observed in four young adults after one week on an isocaloric diet containing practically no fat confirms previous observations (20–22). Unfortunately, data regarding the participation of the various lipoprotein fractions in this change were not obtained.

The lowest concentrations of triglycerides and very low density lipoproteins observed in this study (12 hours after ingestion of fat) occurred 3 to 4 hours after a fat-free meal. This raises the possibility that the availability of energy from sources other than fat may influence the concentration of these lipoproteins. This problem is the subject of the succeeding paper.

SUMMARY

1. The effects on serum lipids and ultracentrifugally separated lipoprotein fractions of the ingestion of fat-rich meals were studied in healthy young adults.

2. The increment in the concentration of triglycerides in the serum following ingestion of fat was entirely the result of an increase in their concentration in very low density ($S_f > 10$) lipoproteins. The cholesterol and phospholipid concentrations in this lipoprotein fraction also increased significantly and tended to parallel the triglyceride concentration. These changes were usually maximal 4 hours after the ingestion of fat.

3. Increments in the concentration of cholesterol in the serum after ingestion of fat were usually slight because the increment in the very low density lipoproteins represents only a small fraction of the serum value and the cholesterol concentration of the other fractions usually changes very little.

4. Serum phospholipid concentration increased significantly following ingestion of fat and frequently remained elevated after the serum triglyceride concentration had returned to baseline, or even lower, levels. The increase reflected changes in both the very low density and high density lipoprotein fractions. The delayed fall was the result of the persistence of the increased concentration of high density lipoprotein phospholipids after the visible lipemia had disappeared. Lesser increases in the concentration of high density lipoprotein cholesterol and protein may also occur after ingestion of fat.

5. These findings are discussed in relation to current concepts of exogenous fat transport.

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