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## ALTERATIONS IN THE FATTY ACID COMPOSITION OF HUMAN LYMPH AND SERUM LIPOPROTEINS BY SINGLE FEEDINGS \*

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Dole and co-workers (1) reported that the fatty acid composition of serum chylomicrons and other serum lipid classes was remarkably stable despite the feeding of fats of widely different composition. Blomstrand and Dahlbäck (2) analyzed the fatty acid composition of human thoracic duct chyle in two subjects after the feeding of fat formulas and observed that lymph triglycerides closely resembled the pattern of the dietary fat. Cholesterol esters of the chyle were also found to be markedly influenced by the dietary fat, but the FFA and phospholipids were relatively unaffected. Fernandes, van de Kamer, and Weijers (3) studied the pattern of fatty acids in the chyle of a child with chylothorax and observed significant differences between the composition of the fed fat and that of the chylous fluid. Bragdon and Karmen (4) noted that the fatty acid composition of lymph and serum chylomicrons in the rat reflected dietary feeding to a marked extent, and reported that serum chylomicrons in humans also changed to reflect the ingested lipid.

In this study, the acute effect of single feeding of fats on the composition of human thoracic duct lymph and serum has been studied. The thoracic duct chyle and serum were fractionated into 5 lipoprotein classes, and the fatty acid compositions of the lipids were analyzed to study the process of fat absorption and the extent of alterations in lipoprotein composition that occur after feeding of fats of different composition.

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### METHODS

The thoracic duct was cannulated at the level of the supraclavicular space in two subjects. The operation was carried out under local anesthesia, and polyethylene tubing was used to obtain a free flow of lymph as described by Linder and Blomstrand (5). The first patient was a 64-year-old man with inoperable carcinoma of the lung. The second patient was a 54-year-old man with a long history of chronic alcoholism and intermittent bouts of pancreatitis who had a gastrotomy tube at the time of the study. In a third patient, a 39-year-old male convalescing from lobar pneumonia, thoracic duct cannulation was not carried out, and only serum samples were obtained. Cannulation had been carried out in Patient A 2 days and in Patient B 1 day before the day of the study. After an overnight fast, a test meal of 100 g of corn oil or coconut oil mixed in 250 ml of fruit juice was given. Chyle was continuously collected, and blood samples were taken intermittently for the subsequent 48 hours. Clear fluids and fruit juices were permitted for 8 hours after the test meal, and the patient was then allowed to have his regular diet. No attempt was made to control the dietary intake of the patients before or during the study. At the end of the study period, the cannula was withdrawn, and normal lymph flow re-established without difficulty.

The lymph was strained through gauze to remove fibrin strands. Suitable samples were layered under buffered saline, pH 7.4 (.01 M sodium phosphate with .05% disodium salt of ethylenediaminetetraacetic acid) and spun in the Model L Spinco centrifuge at  $26,000 \times g$  for 30 minutes at room temperature. The chylomicrons were concentrated into a thick layer at the top of the tube; they were removed from the lusteroid tube by a tube slicer and capillary pipettes. The chylomicrons were emulsified in buffered saline by repeated drawing through a fine needle. The suspended chylomicrons were again separated in the ultracentrifuge at the same gravitational field. This procedure of emulsification and centrifugation was repeated 3 times. The infranatant fluids of the washed chylomicrons were discarded. Higher density lipoprotein fractions of the lymph were obtained by successive ultracentrifugation of the original infranatant fluid at  $105,000 \times g$  for 20 hours at specific gravities of 1.019, 1.063, and for certain studies 1.21, with potassium bromide in appropriate concentration to

obtain the desired specific gravity. Serum samples were handled in similar fashion. Although no layering developed in the tube during the initial spin of the fast-ing serum samples, the top fractions were removed and washed by being suspended in buffered saline and cen-trifuged twice without emulsification.

The lipids were extracted from lipoproteins by a modi-fied Folch procedure using chloroform and methanol, 2:1, vol/vol, and washing this extract with water (6). The chloroform extracts were dried *in vacuo* and re-extracted into chloroform. Samples were taken for the individual lipid analyses as well as for the preparation of methyl esters for gas chromatography. Separation of cholesterol esters, triglycerides, cholesterol, and phos-pholipids was carried out by silicic acid column chroma-tography in stages as follows: Activated silicic acid 1 to 2 g, prepared according to the method of Sweeley and Moscatelli (7), was made into a slurry with chloro-form methanol, 4:1, vol/vol, and poured to form a col-umn 7 mm wide. The column was washed with pure chloroform, and the sample (in chloroform) was placed on the column. The initial eluant was 20 ml of 100% chloroform. This eluate contained cholesterol esters, tri-glycerides, and free cholesterol. This fraction was evaporated under nitrogen and re-extracted into petroleum ether (bp, 60° to 62° C). The petroleum ether extract of the initial eluate (100% chloroform) was rechromato-graphed on a column of silicic acid supercel in the ratio of 1:1, with nonactivated silicic acid. The slurry was made with chloroform, poured into a syringe barrel, and washed with petroleum ether. Cholesterol esters were eluted with 10% chloroform in petroleum ether, and tri-glycerides with 100% chloroform as described by Van Handel and Zilversmit (8).

The lipid fractions were then dried under a stream of nitrogen. Methyl esters of the fatty acids were pre-pared by incubating these extracts at 65° C with 1 ml of a mixture of 98% methanol and 2% sulfuric acid in a sealed tube for 16 hours. Five ml of water was then added to each tube, and the lipids were extracted into petroleum ether. The petroleum ether extracts were carefully dried by using nitrogen, isooctane was added,

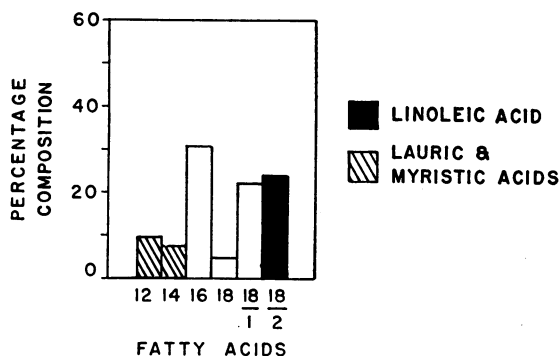


FIG. 1. PERCENTAGE OF FATTY ACID COMPOSITION OF HUMAN THORACIC DUCT LYMPH CHYLOMICRONS IN THE FASTING STATE. Patient A.

TABLE I  
Fatty acid composition of human thoracic duct lymph lipo-proteins in the fasting state and after corn oil feeding\*

Density class	Carbon no.	Fast-ing	After corn oil			
			2-4 hrs	8-10 hrs	11-23 hrs	35-47 hrs
<1.005	10:0	2	0	0	0	0
	12:0	3	0	0	0	2
	14:0	10	1	0	0	4
	16:0	37	16	10	14	29
	18:0	8	3	1	3	8
	18:1	32	31	26	34	38
	18:2	8	49	63	49	19
1.005-1.019	10:0	1	1	0	0	0
	12:0	1	1	0	0	1
	14:0	5	0	1	1	2
	16:0	32	21	16	16	32
	18:0	14	6	4	6	10
	18:1	35	31	37	33	35
	18:2	12	40	42	44	20
1.019-1.063	10:0	0	0	0	0	0
	12:0	1	1	0	0	1
	14:0	1	1	1	1	1
	16:0	26	27	25	24	26
	18:0	7	6	5	6	8
	18:1	36	27	27	31	35
	18:2	29	38	42	38	29
>1.063	10:0	1	0	0	0	0
	12:0	2	1	0	0	1
	14:0	5	1	1	1	2
	16:0	26	20	18	18	31
	18:0	8	5	4	5	9
	18:1	38	29	26	28	31
	18:2	20	44	51	48	26

\* Fatty acid composition of corn oil—16:0, 11%; 18:1, 27%; 18:2, 61%.

and samples of the isooctane solution, 2 to 3  $\mu$ l, were used for gas-liquid chromatography. Gas-liquid chromatography columns were 6 feet long and filled with Chromosorb-W<sup>1</sup> coated with an ethylene glycol adipate, polyester coating approximately 10% of the weight of the Chromosorb. The column temperature was 196° C. The detector was a modified argon ionization detector (9). The detector system was calibrated for quantitative accuracy by analyzing serial dilutions of the methyl esters of corn oil in isooctane. To minimize the effect of small differences in the relative response of the detector for the different fatty acid esters, and to facilitate comparison, fractions obtained from each experiment were analyzed on the same column consecutively. The components of the mixture were identified by comparing retention times with those of known fatty acid esters.<sup>2</sup>

Gas-liquid chromatography was carried out through the 18-carbon fatty acid esters. The quantity of longer-chain fatty acids present represented relatively small fractions of the total and did not materially affect the results. Neither diet contained fatty acids longer than 18 carbons.

<sup>1</sup> Johns-Manville Co., New York, N. Y.

<sup>2</sup> Obtained from The Hormel Institute, Austin, Minn.

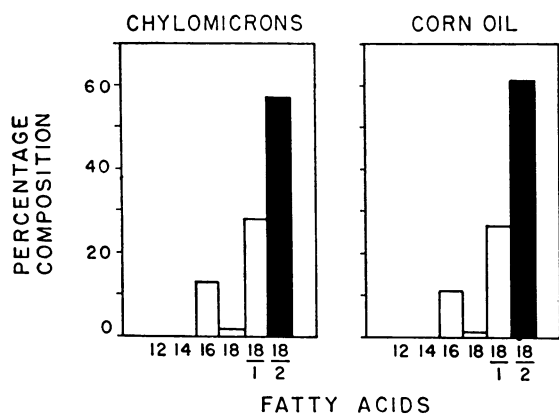


FIG. 2. FATTY ACID COMPOSITION OF HUMAN THORACIC DUCT LYMPH CHYLOMICRONS AFTER CORN OIL FEEDINGS AND THE FATTY ACID COMPOSITION OF CORN OIL. Patient A.

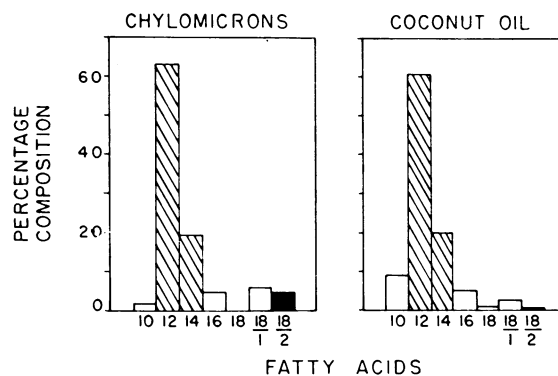


FIG. 3. FATTY ACID COMPOSITION OF HUMAN THORACIC DUCT LYMPH CHYLOMICRONS AFTER COCONUT OIL FEEDING AND THE FATTY ACID COMPOSITION OF COCONUT OIL. Patient A.

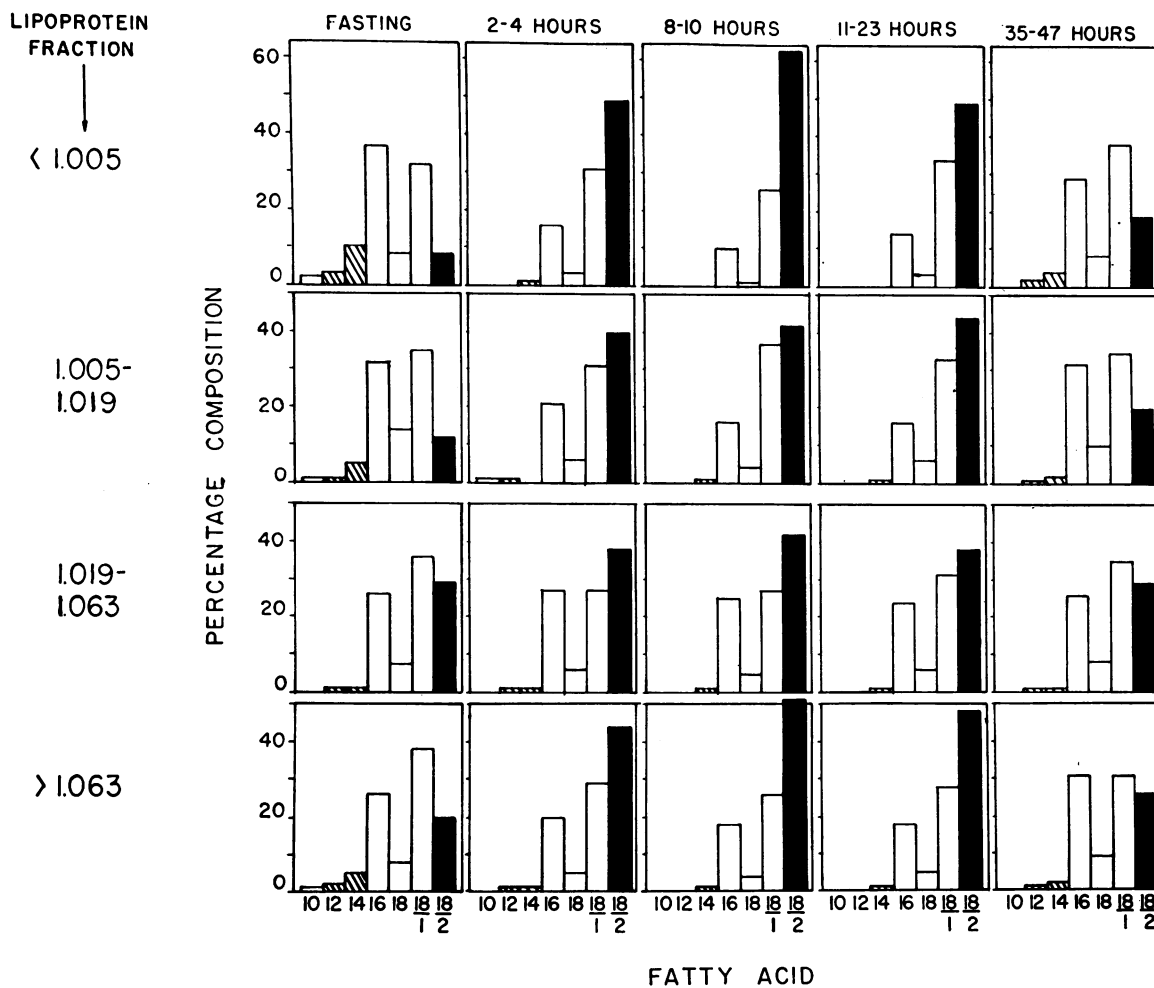


FIG. 4. FATTY ACID COMPOSITION OF HUMAN THORACIC DUCT LYMPH LIPOPROTEINS AFTER 100 G CORN OIL. Patient B.

## RESULTS

The fatty acid composition of the lymph chylomicrons of Patient A after an overnight fast is shown in Figure 1. The major components are 31% palmitic acid (16:0), 22% oleic acid (18:1), and 24% linoleic acid (18:2). Figure 2 compares the composition of lymph chylomicrons in thoracic duct chyle 8 hours after 100-g corn oil feeding with the composition of corn oil itself. This highly unsaturated oil was found to be composed of 11% palmitic acid, 27% oleic acid, and 61% linoleic acid. The gas chromatographic patterns of the chylomicrons and of corn oil are almost identical. The lymph sample 24 hours after the original feeding was still identical with that of corn oil. At this time, 100 g of coconut oil, a highly saturated oil containing predominantly fatty acids 10:0 (9%), 12:0 (61%), 14:0

(20%), and 16:0 (7%) was given. Figure 3 shows the fatty acid composition of the chylomicrons of chyle 8 hours after coconut oil feeding and of coconut oil itself. The lower concentration of 10:0 saturated fatty acid in thoracic duct chyle agrees with the observations that shorter-chain fatty acids are absorbed directly into the portal system. The appearance of lauric and myristic acids was first noted about 2 hours after ingestion of the coconut oil.

In Patient B, the lymph was continuously collected for a 48-hour period after the administration of 100 g of corn oil by gastrotomy tube. The patient had fasted for 12 hours before ingestion of corn oil and did not receive any food until after the 10-hour collection. The gas-liquid chromatographic analysis of the fatty acids in the total lipid extracts of the lymph lipoprotein fractions—i.e., the chylomicrons, density classes 1.005

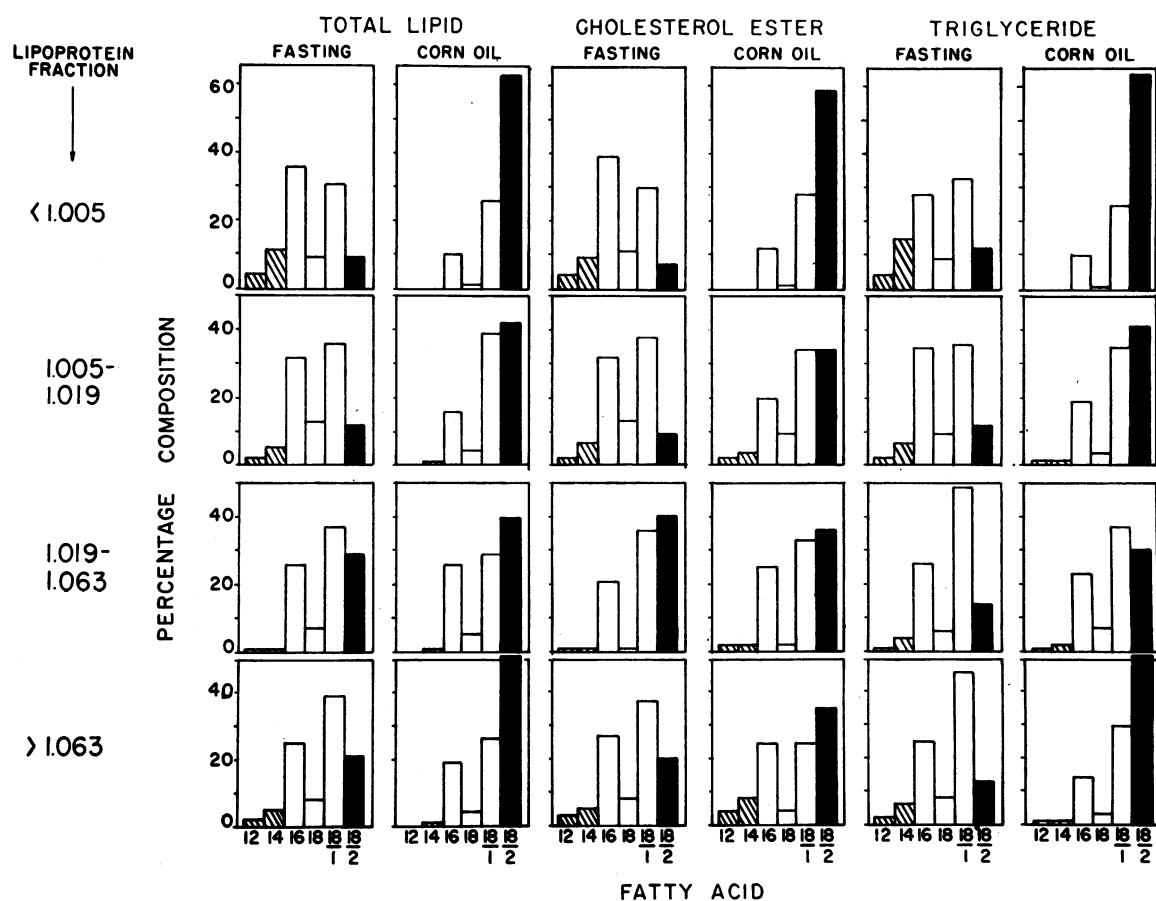


FIG. 5. FATTY ACID COMPOSITION OF TOTAL LIPID EXTRACTS OF HUMAN THORACIC DUCT CHYLE LIPOPROTEINS AND OF THEIR CHOLESTEROL ESTER AND TRIGLYCERIDE FRACTIONS IN FASTING STATE AND 8 TO 10 HOURS AFTER 100 G CORN OIL. Patient B.

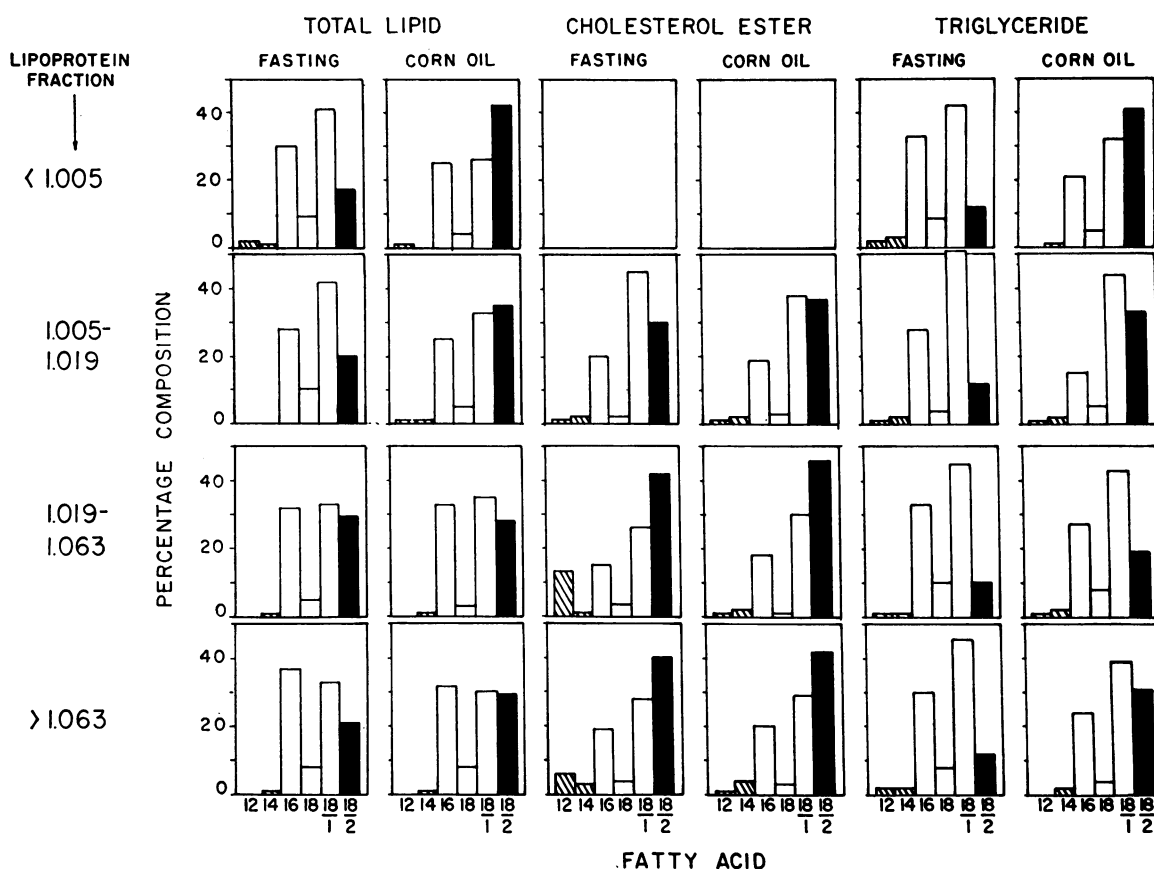


FIG. 6. FATTY ACID COMPOSITION OF TOTAL LIPID EXTRACTS OF HUMAN SERUM LIPOPROTEINS AND OF THEIR CHOLESTEROL ESTER AND TRIGLYCERIDE FRACTIONS IN THE FASTING STATE AND 8 HOURS AFTER 100 G OF CORN OIL. Patient B.

to 1.019, 1.019 to 1.063, and greater than 1.063—is depicted in Figure 4 and Table I. The successive panels in the figure represent the composition of the fasting lymph and the specimens collected 2 to 4 hours, 8 to 10 hours, 11 to 23 hours, and 35 to 47 hours after the corn oil feeding. The relative concentration of linoleic acid, the solid bar, is increased in each of the lipoprotein classes, but the maximal concentration reached during the 48-hour collection period was different in each lipoprotein class. The chylomicron fraction contained 63% linoleic acid, but in the other lipoprotein fractions the peak levels of linoleic acid are only 44 to 51% of the total fatty acids in each fraction. At the end of the 48-hour collection period, the composition of the chylomicrons and other lipoproteins was similar to the values obtained during the fasting state, except for an increase in linoleic acid concentration of from 6 to 11%.

The lipoprotein fractions of the lymph collection of the 8- to 10-hour period after ingestion of corn oil were separated into cholesterol ester, triglyceride, and phospholipid fractions. In Figure 5 the fatty acid composition of the whole lipid extract and of the constituent cholesterol ester and triglyceride fraction is shown. The composition of the cholesterol ester fraction of the various lipoproteins varied. The linoleic acid content of the cholesterol ester fraction in the fasting state was 7% and 9% in the chylomicron and 1.005 to 1.019 classes, respectively, but made up 41% of the 1.019 to 1.063 class. Cholesterol ester represents from 3 to 6% of the total lipid weight in the lipoprotein fractions of fasting chyle. The chylomicron fraction of lymph after a test meal contained about 90% of the total lipid of the lymph.

The fatty acid distribution of both the cholesterol ester and triglyceride moieties in the lipo-

proteins of the 1.019 to 1.063 density class of lymph showed little change after the ingestion of corn oil, i.e., despite linoleic acid concentration of 61% and oleic acid concentration of 27% in corn oil, the oleic acid percentage in triglyceride fraction fell to 37%, and linoleic acid concentration rose to only 30%. In contrast, the changes in the cholesterol ester and triglyceride fractions of the high density lipoprotein with respect to linoleic acid were from 20 to 35% and from 13 to 51%.

In Patient B, the thoracic duct cannula was withdrawn 24 hours after the corn oil study, and normal drainage re-established into the venous system. A coconut oil test meal was given by gastrotomy tube, and blood samples were drawn at specified intervals. Two days later, the corn oil feeding was repeated, and blood samples were drawn.

The fatty acid composition of serum lipopro-

teins in the fasting state and 8 hours after 100 g of corn oil is shown in the first two panels of Figure 6, and after 100 g of coconut oil in the first two panels of Figure 7. The fatty acid composition of the total lipid extract of the serum lipoprotein fraction  $d < 1.005$ , "serum chylomicrons," resembles the dietary fat to a lesser extent than the thoracic duct chylomicrons. The relative insensitivity of the composition of the lipoprotein class 1.019 to 1.063 to either the coconut or corn oil feeding is reflected by the uniformity of fatty acid distribution in all four samples. There are changes in fatty acid distribution in the fraction 1.063 to 1.21, but these are not so striking as those seen in the less than 1.005 or 1.005 to 1.019 fraction.

The numerical values for the percentage distribution of the fatty acids of the cholesterol ester and triglyceride fractions of each class of serum lipoproteins in the fasting state and after corn and

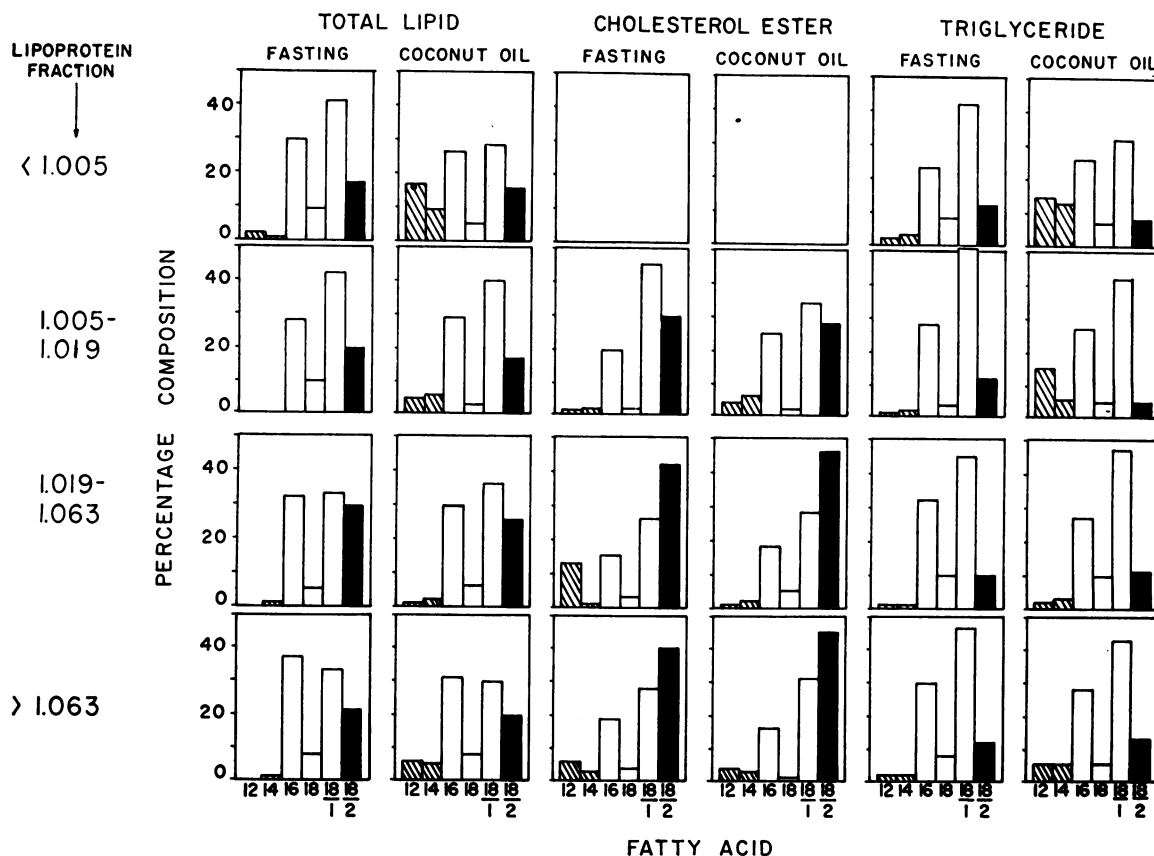


FIG. 7. FATTY ACID COMPOSITION OF TOTAL LIPID EXTRACTS OF HUMAN SERUM LIPOPROTEINS AND OF THEIR CHOLESTEROL ESTER AND TRIGLYCERIDE FRACTIONS IN THE FASTING STATE AND 8 HOURS AFTER 100 G OF COCONUT OIL. Patient B.

TABLE II  
*Fatty acid composition of total lipid extract of human serum lipoproteins and their cholesterol ester and triglyceride fractions\**

Density class	Carbon no.	Total lipid			Cholesterol ester			Triglyceride		
		Fast-ing	After corn oil	After coconut oil	Fast-ing	After corn oil	After coconut oil	Fast-ing	After corn oil	After coconut oil
<1.005	12:0	2	1	17				2	0	15
	14:0	1	0	9				3	1	12
	16:0	30	25	26				33	21	26
	18:0	9	4	5				8	5	7
	18:1	41	26	28				42	32	32
	18:2	17	42	16				12	41	8
1.005-1.019	12:0	0	1	5	1	1	5	1	1	15
	14:0	0	1	6	2	2	7	2	2	6
	16:0	28	25	29	20	19	25	28	15	27
	18:0	10	5	3	2	3	2	4	5	5
	18:1	42	33	40	45	38	34	53	44	42
	18:2	20	35	17	30	37	28	12	33	5
1.019-1.063	12:0	0	0	1	2	1	1	1	1	2
	14:0	1	1	2	1	2	2	1	2	3
	16:0	32	33	30	18	18	18	33	27	27
	18:0	5	3	6	3	1	5	10	8	10
	18:1	33	35	36	33	30	28	45	43	47
	18:2	29	28	25	42	46	46	10	19	11
>1.063	12:0	0	0	6	6	1	4	2	0	6
	14:0	1	1	5	3	4	3	2	2	6
	16:0	37	32	31	19	20	16	30	24	28
	18:0	8	8	8	4	3	1	8	4	5
	18:1	33	30	30	28	29	31	46	39	42
	18:2	21	29	20	40	43	45	12	31	13

\* Fatty acid composition of corn oil—16:0, 11%; 18:1, 27%; 18:2, 61%. Coconut oil—10:0, 9%; 12:0, 61%; 14:0, 20%; 16:0, 7%.

coconut oil are given in Table II. The distribution is shown graphically in the last four panels of Figures 6 and 7. The changes in the fatty acid composition of the triglycerides appear to vary from density class to density class. The triglycerides of the 1.019 to 1.063 class showed less change after either corn oil or coconut oil than did the triglycerides isolated from the 1.005 to 1.019 class. The cholesterol ester fraction of each lipoprotein class was much less subject to change after dietary feeding than was the triglyceride fraction, but the changes that did occur were of a different extent in each lipoprotein class.

The third patient, Patient C, received a corn and a coconut oil test meal, and only serial blood samples were drawn. The changes in the fatty acid composition of his lipoprotein fractions were similar to those found in Patient B.

#### DISCUSSION

Chylomicrons, the least dense class of lipoproteins, are harvested from serum or chyle and

purified by several washings and centrifugation at specific gravity 1.005. In the studies described here, human chyle chylomicrons 6 to 8 hours following the feeding of a fat meal have been shown to have a fatty acid composition similar to that of the fatty meal. The chylomicron fraction is not alone in this respect, since the fatty acid composition of other fractions of the chyle separated by ultracentrifugation also changes to resemble the fatty meal fed. Quantitatively, the changes in the classes other than chylomicrons were most marked in the 1.005 to 1.019 specific gravity class and in the high density 1.063 to 1.21 lipoprotein class. The changes in these fractions were neither so marked nor was the amount of fat contained so high that it would be reasonable to conclude that these fractions carry a large portion of recently ingested fat in the chyle.

Although the composition of the lymph chylomicrons after the fatty meal eventually (6 to 8 hours after ingestion) became identical with that of the meal, in the early postprandial state (2 to 4



hours and 4 to 6 hours) after the fat content of the chyle had already increased, the fatty acid composition of the chylomicrons did not completely resemble that of the fed fat. It was also apparent that 10 to 12 hours after the meal, even though the fat content of the lymph was still markedly elevated above that of the fasting sample, the fatty acid composition was once again different from that of the meal. The chylomicrons during the early and late postprandial periods were composed of the same fatty acids as were in the fat meal, but in different relative proportions, and also of fatty acids not in the test meal. Although the presence of different amounts of the same fatty acids as in the meal might represent selective or sequential absorption of these fatty acids, the presence of the other fatty acids strongly suggests a contribution to the lymph chylomicrons from a source within the organism. It is possible that the intestinal mucosa may synthesize chylomicrons from circulating plasma lipids as well as from the dietary fat in the lumen.

The lymph collected from the thoracic duct cannula is composed of lymph from other sources as well as the intestine. Hepatic lymph enters the main abdominal lymph channel at the level of the cisterna chyli, where it becomes the main thoracic duct. The contribution of hepatic lymph to the thoracic duct lymph may be appreciable. In hepatic cirrhosis, the thoracic duct lymph flow rate may reach 10 ml per minute, the major contribution being from hepatic lymph (10). The liver is the major organ for synthesis of all plasma lipoproteins, and these lipoproteins are also present in the thoracic duct lymph. It may well be that the stability in fatty acid composition of the low-density lipoproteins (1.019 to 1.063) observed during these fat absorption studies reflects the continuing synthesis of these lipoproteins from lipid sources other than the newly absorbed fat. On the other hand, the change in fatty acid composition of the other lipoprotein classes (1.005 to 1.019 and 1.063 to 1.21) may reflect the incorporation of newly absorbed fatty acids into the lipoproteins in the intestine.

The fractionation of the lipoproteins into their constituent lipids of triglycerides, cholesterol esters, and phospholipids was carried out by silicic acid column chromatography. The fatty acid composition of each of these lipids in each lipoprotein class was altered to a different extent.

The triglyceride fraction in each lipoprotein class showed the greatest change for that class, with the phospholipids and cholesterol esters showing lesser but definite changes. These variations in the changes of each of the lipids in the lipoprotein classes as well as within a given lipoprotein group imply that each lipid (triglyceride, cholesterol ester, phospholipid, etc.) is synthesized by a different mechanism with a different specificity during the process of absorption, or that they are the results of a mixing of lipids from different sources, or both. At the peak of absorption, the triglyceride content of the chylomicrons so closely resembled the fed fat that the effect of contribution of fat from other sources may be masked. In other lipids, the effect of dietary fat is less prominent, and the increase in lipid cannot be ascribed solely to the diet.

#### SUMMARY

1) The fatty acid composition of human thoracic duct chyle lipoproteins has been analyzed by gas-liquid chromatography in two men in the fasting state and after the feeding of a highly unsaturated oil, corn oil, and after the feeding of a highly saturated oil, coconut oil. Serum lipoproteins have also been studied in similar fashion in two subjects.

2) The fatty acid composition of thoracic duct chylomicrons was almost identical with that of the test meal 6 to 8 hours after ingestion.

3) Serum lipoproteins  $d < 1.005$ , "chylomicrons," also reflected the composition of the test meal, but the change in composition was not so complete as that observed in thoracic duct chylomicrons.

4) Other lymph and serum lipoprotein classes also showed alterations in composition after the single test meal. The density class 1.019 to 1.063 was the most stable group of the lipoprotein classes.

5) Within each density class, the triglycerides were altered to the greatest extent, with less change noted in the cholesterol ester fraction.

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