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Research Article

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Studies on the Lipid Composition of Human Small Bowel Mucosa *

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Until recent times there have been no techniques enabling the gastrointestinal physiologist to investigate *in vivo* the intracellular phase of fat absorption in man. With a hydraulic biopsy tube (2), the sequence of morphological changes in the small bowel absorptive cell was described after giving fatty meals to normal subjects (3, 4). However, there has been no thorough study of the composition of the lipids of human small bowel mucosa in the fasting state or during absorption. In the present study, multiple mucosal biopsies were obtained with a hydraulic biopsy tube (2) from the distal duodenum in man. Their lipid composition was determined by microanalytical techniques. A larger group of normal subjects and a smaller group of patients with idiopathic hyperlipemia were studied. The influence of certain dietary manipulations on intestinal absorptive cell lipids was also investigated.

Methods

Normal subjects. The 16 males and one female, aged 21 to 43 years, had no history suggestive of small bowel disease, and the small bowel biopsies were morphologically normal.

Specimens of jejunum, 4 to 8 cm in length, were obtained at laparotomy from three patients who had no evidence of small bowel disease. After being washed in buffered salt solution, the mucosa was separated from the underlying tissue with a glass slide.

Patients. The three patients with carbohydrate-induced hyperlipemia were characterized by the following criteria: hypertriglyceridemia that was maintained on a fat-free diet, normal levels of plasma lipoprotein lipase, impaired carbohydrate tolerance, and the presence of "hyperlipemic" particles in plasma on a fat-free diet (5).[1]

Dietary control. Seventeen normal subjects were biopsied after an overnight fast without attempting prior dietary control (ad libitum diet). Three of these subjects were subsequently biopsied after 2 weeks of a formula diet containing 40% fat calories (20% from butter and 20% from corn oil), 15% protein calories,[2] and 45% carbohydrate calories. Each subject received sufficient calories from this 40% fat diet to maintain body weight.

In another experiment, fasting biopsies were taken from six normal subjects and the three patients with hyperlipemia who had been on ad libitum diets. They were then given a fat-free formula diet containing 85% carbohydrate calories (as dextrose or maltose) and 15% protein calories for 2 weeks. After subjects had fasted overnight, a second series of biopsies was obtained.

Biopsy techniques. Under fluoroscopic control, the biopsy tube was passed to the ligament of Treitz. As it was withdrawn through the distal duodenum, 4 to 18 (mean, 9) biopsies were taken (2). After recovery of a biopsy in buffered salt solution, it was blotted lightly on a paper towel, weighed to an accuracy of ± 0.05 mg, and placed in methanol.

In some experiments, corn oil (1.5 ml per kg body weight), mechanically emulsified in 2 vol of water, was infused through the biopsy tube into the first portion of the duodenum. With this technique, there were no uncertainties about gastric emptying, yet mixing of the corn oil with biliary and pancreatic secretions could oc-

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[1] We are indebted to Drs. Bierman, Porte, and O'Hara for providing us with these patients.

[2] Meritene, Doyle Pharmaceutical Co., Minneapolis, Minn.

cur in the proximal duodenum. The biopsy tube was then advanced to the ligament of Treitz and a second series of biopsies obtained during its withdrawal.

Extraction and analysis of lipid. After grinding the biopsies in 5 ml of methanol in a manual glass homogenizer, we mixed in an equal volume of chloroform. The mixture was then transferred to a centrifuge tube and spun at 1,500 rpm for 10 minutes; the supernatant was removed by decanting. This method of extraction was repeated twice on the sediment, and the combined supernatants were evaporated to dryness *in vacuo*. The lipid residue was then dissolved in 24 ml of chloroform: methanol (2:1, vol:vol) and 4.6 ml of 0.1 M KCl added. After the mixing, and cooling to 4° C, the mixture was returned to room temperature, whereupon a clean separation into two phases occurred. After the lower phase had been removed, the upper phase was washed with 10 ml of chloroform, and the combined lower phases were evaporated to dryness *in vacuo*. Total lipid weight was determined by evaporating the entire sample in a small (1- to 2-g) tared tube which was then dried *in vacuo* over silica gel for 2 hours; there was no further reduction in weight after drying over phosphorus pentoxide for 8 to 12 hours. The lipid was then dissolved in 1 to 2 ml of benzene from which portions were taken for further analysis.

When portions of a solution of pure cholesterol were placed in a tared tube, the solvent was removed, and the residue was dried over silica gel, the mean error [(observed weight − theoretical) ÷ theoretical weight] was + 2.1% for 16 samples of 2 to 5 mg.

The distribution of individual phospholipids was determined by paper chromatography (6) or by combined column and paper chromatography (7).

Neutral lipids were separated into fractions of free cholesterol, free fatty acids (FFA), triglycerides (TG), and cholesterol esters by applying 0.8 to 2.0 mg of total lipids as a streak to a glass plate covered with a 0.25-mm layer of silica gel. We found that a mixture of silica gel H [3] and 10% (by weight) calcium sulfate [4] (previously washed with chloroform, hexane, and diethyl ether) gave a uniform coating on the glass plates as well as low blanks in the microanalytical procedures. The silica gel H had been previously washed with methanol: chloroform: formic acid (2:1:1, vol:vol:vol), then methanol, and finally distilled water, and dried in an oven at 100° C for 48 hours. The thin layer plates were activated at 110° for 1 hour and stored in a dessicator over silica gel. The plate was developed in *n*-hexane (redistilled): diethyl ether: acetic acid (90:15:1, vol:vol:vol), and the separated bands of lipids were identified by determining the position of authentic standard materials run in guide lanes at either side of the plate. The guide lanes were sprayed with a solution of 0.2 g of recrystallized 2′,7′-dichlorofluorescein in 100 ml of redistilled 95% ethanol and scanned under ultraviolet light. The bands of cholesterol, TG, FFA, and cholesterol esters were scraped into small glass columns (6 mm i.d.) containing plugs of glass wool. TG, FFA, and cholesterol ester were eluted with methanol in diethyl ether (1:9, vol:vol), and the cholesterol band was eluted with chloroform. This was shown to yield quantitative recoveries when known amounts of pure cholesterol, TG, and FFA were applied to thin layer plates.

FFA was determined quantitatively as the copper salt in chloroform. Duncombe's method (8) was modified in that the sample of FFA was dissolved in 2 ml of chloroform. One ml of the copper reagent was added, and the absorbance at 400 mμ of the copper palmitate in a 1-ml cuvette was measured in a Zeiss spectrophotometer. The mean and SD of the absorbance when six samples of 98 μg of palmitic acid were analyzed were 0.368 ± 0.014.

TG was measured by two methods. In earlier experiments the microequivalent of fatty acid ester was determined in a sample of total lipid by measuring its absorbance at 5.80 μ on a Perkin-Elmer infrared spectrophotometer and calculating from the absorbance of appropriate standards. We assumed that 20% of the phospholipids were plasmalogens and sphingomyelin, so that 80% of the phospholipids contributed as diacyl compounds to the measurement of total fatty acid ester. The small amount of cholesterol ester, and mono- and diglyceride was disregarded. The average molecular weight of mucosal TG was calculated from a separate analysis of TG fatty acids by gas chromatography. The milligrams of TG was then estimated by multiplying the nonphospholipid fatty acid ester (in microequivalents) by the average molecular weight of TG and dividing by 3.

In later experiments, TG, eluted from the thin layer plate, was dissolved in 0.2 ml chloroform. Two ml of 0.5 M KOH in absolute methanol [prepared according to Vogel's method (9)] was added and the mixture shaken intermittently over 20 minutes at room temperature. Two-tenths ml 6 N HCl, 2.0 ml chloroform, and 1.4 ml water were added successively with mixing. After it had been centrifuged at 1,500 rpm for 5 minutes, the chloroform phase was taken for analysis of the methyl esters of fatty acids. One ml of the upper phase was mixed with 0.5 ml of 0.2 N H_2SO_4 in a glass tube and the methanol removed by heating in a boiling H_2O bath. The glyceride glycerol was then determined by the chromotropic acid technique (10).

The results obtained by these two methods were compared on three occasions. Three different samples of mucosal lipid were calculated to contain, from the infrared data, 19.6, 13.5, and 7.9% of lipid weight as TG. The corresponding values by measuring glyceride glycerol were 18.9, 13.6, and 5.3%, respectively.

Cholesterol was measured by the method of Sperry and Webb (11) or the ferric chloride method (12). The latter was modified so that the final volume of the reaction mixture was 1.0 ml and the absorbance read at 500 mμ in a Zeiss spectrophotometer.

To assess the error of the analytical procedures, we separated 2.0-mg samples of mucosal lipid on six thin layer plates. The coefficient of variation of the FFA as per cent of total lipid weight was ± 11.1%; of cholesterol, ± 4.8%; and of TG, ± 8.0%.

[3] Brinkmann Instruments, Great Neck, N. Y.

[4] Applied Science Laboratories, State College, Pa.

TABLE I

Lipid composition of duodenal mucosa obtained after an overnight fast from 17 normal subjects without prior dietary control

	Total lipid as per cent of biopsy weight	Per cent of total lipid found in			
		Phospho-lipid	Cho-lesterol	Triglyc-eride	Free fatty acid
Mean	4.2	49.6	7.7	22.8	3.2*
SD	0.9	8.6	1.9	8.5	2.9*
Range	3.0–6.3	30.0–73.0	4.3–11.8	10.0–38.8	0.9–8.6*

* Six subjects.

The phospholipids, remaining at the origin of the neutral lipid plate, were transesterified in sulfuric acid, methanol, and benzene as described previously (7).

The cholesterol esters, eluted from the neutral lipid plate, were also transesterified by treatment with sulfuric acid, methanol, and benzene. Subsequently, the free cholesterol and fatty acid methyl esters were separated by thin layer silicic acid chromatography, and the isolated free cholesterol was measured by the ferric chloride technique. Only 0.7% (mean of five groups of biopsies) of the total lipid weight was cholesterol ester, although there was evidence on the thin layer plate that a variable, but small, portion of the cholesterol ester had not been hydrolyzed.

Gas-liquid chromatography was performed on a 6-foot column (i.d., 5 mm) of 12% ethylene glycol succinate on Chromosorb W (60 to 80 mesh)[4] at 178° C with a H_2 flame ionization detector (Barber-Coleman, series 5000). The distribution of fatty acids (moles per 100 moles of fatty acids) was calculated from the peak height, the width at half height, and the molecular weight. Quantitative results with standards of methyl esters of fatty acids (mixtures KA, KB, KD, and KF)[4] agreed with the stated composition with a mean relative error[5] of less than 7% for all components except myristic acid (where the mean relative error was as high as 25.7% when myristic acid was < 5% of the total mixture). In seven determinations with the KD standard the largest coefficient of variation was ± 3.1% for myristic acid.

[5] At least five samples of each mixture were analyzed.

Results

Size and appearance of mucosal biopsies

The average wet weight of a single biopsy was 12.2 mg, and 50 to 60 mg of tissue was required for lipid analysis. It was not possible to estimate how much of a biopsy specimen consisted of the actively absorbing intestinal epithelium at the tips of the villi, because unpredictably variable amounts of lamina propria and muscularis mucosa and submucosa were included in these suction biopsies (2).

Lipid composition of normal fasting duodenal mucosa

In Table I are shown the mean, standard deviation, and range of the per cent composition by weight of the major lipid classes obtained from 21 determinations on pooled biopsies from 17 normal fasting subjects. There was a wide variation in values with the following means as per cent of lipid weight: phospholipid, 49.6%; triglyceride, 22.8%; cholesterol, 7.7%; free fatty acids, 3.2%.

The distribution of the various phospholipids is shown in Table II. Lecithin was the predominant phospholipid, with phosphatidyl serine and phosphatidyl ethanolamine comprising an additional 33.9%. In most of the paper chromatograms, sphingomyelin and phosphatidyl inositol ran as a single spot and together averaged 16.8% of the total phospholipid. From two of the surgical specimens enough lipid was obtained to carry out a preliminary separation of the phospholipids on a silicic acid column. The relative amounts of sphingomyelin and phosphatidyl inositol were then assessed by paper chromatography. By this technique, phosphatidyl inositol was found to be

TABLE II

Composition of duodenal mucosa phospholipids obtained after an overnight fast from normal subjects without prior dietary control

	Per cent of total phospholipids found in				
	Phosphatidyl inositol + sphingomyelin	Lecithin	Phosphatidyl serine	Phosphatidyl ethanolamine	Per cent of lipid phosphorus recovered
Mean	16.8	43.0	13.5	20.4	104.8
SD	1.2	3.4	2.5	3.1	13.2
Range	14.4–19.0	38.4–47.9	10.0–16.0	17.5–26.5	88.2–138.0
No. subjects	11	11	6*	6*	11

* In five subjects phosphatidyl serine and phosphatidyl ethanolamine were not separated.

TABLE III

Distribution of fatty acids within triglycerides and phospholipids of duodenal mucosa obtained after an overnight fast from 17 normal subjects without prior dietary control

Fraction	16:0	16:1	18:0	18:1	18:2	20:4
	moles/100 moles fatty acids					
Triglycerides						
Mean	31.8	3.7	11.9	35.3	13.7	
SD	4.4	1.1	1.2	4.8	6.2	
Range	22.4–39.6	1.8–5.6	7.8–18.4	26.6–47.1	4.9–27.7	
Phospholipids						
Mean	23.9	1.0	22.4	16.8	26.2	7.6
SD	3.3	0.4	2.3	2.4	3.2	2.6
Range	18.6–29.2	0.5–1.9	19.2–29.4	12.9–22.7	18.8–32.4	3.9–12.8
t^* =	8.09†	2.603†	19.357†	16.840†	8.622†	

* t test (13*a*). The comparison is between the moles per 100 moles of a fatty acid in the two lipid fractions.
† $p < 0.01$.

approximately 10% of the total phospholipid and sphingomyelin approximately 6%.

In Table III the distributions of the fatty acids within the triglyceride and the phospholipid fractions of mucosa from normal fasting subjects are compared. The triglycerides contained more palmitic (16:0), palmitoleic (16:1), and oleic acid (18:1), whereas the phospholipids contained relatively more stearic (18:0) and linoleic acid (18:2). The phospholipids also contained arachidonic acid (20:4), which was found only in trace amounts in the triglycerides. There was considerable variation in the amount of some fatty acids, especially linoleic acid in the triglyceride fraction.

The values given above for the lipid composition of duodenal mucosa from normal fasting subjects were similar to those found in lipid isolated from jejunal mucosa, obtained at laparotomy from three patients.

Studies on possible causes of variability in mucosal fatty acids

Differences in mucosal lipids from one site of the duodenum to another. In three normal fasting

TABLE IV

Distribution of fatty acids in mucosal lipids from three sites in the duodenum of normal fasting subjects without prior dietary control

Patient	Site in duodenum*	Phospholipid fatty acids						Triglyceride fatty acids				
		16:0	16:1	18:0	18:1	18:2	20:4	16:0	16:1	18:0	18:1	18:2
		moles/100 moles fatty acids						*moles/100 moles fatty acids*				
Ke	1	26.7	0.8	19.7	14.4	26.4	8.5	34.3	3.2	11.6	31.9	9.3
	2	22.9	1.6	19.2	17.9	24.4	8.6	32.5	3.6	13.1	33.6	9.6
	3	23.1	1.1	20.7	15.8	23.2	9.7	34.3	3.8	11.3	31.3	9.8
Cz	1	20.2	0.9	25.2	18.9	26.2	7.0	34.1	4.7	14.6	32.9	4.9
	2	22.4	0.9	21.2	20.2	25.9	4.5	35.7	4.1	14.0	34.7	5.1
	3	22.6	Trace	22.7	21.1	26.2	4.8	36.5	4.2	11.8	36.7	6.0
Sy	1	20.3	0.5	21.4	15.1	29.7	9.8	35.0	4.3	13.1	29.2	11.9
	2	24.0	0.7	22.8	14.5	26.8	7.9	31.3	5.6	15.0	29.6	12.0
	3	22.1	0.9	21.0	15.5	28.3	9.0	36.2	4.9	12.6	26.6	11.7
	F, among sites†	= <1 NS		<1 NS	1.38 NS	2.61 NS	1.87 NS	2.13 NS	<1 NS	5.28 NS	<1 NS	1.31 NS
	F, among subjects†	= 1.15 NS		3.21 NS	15.66‡	9.57‡	14.61‡	1.10 NS	5.69 NS	3.55 NS	9.71‡	253.40§

* Site 1 is in the second portion of the duodenum; site 3 is at the ligament of Treitz; site 2 is between sites 1 and 3.
† Analysis of variance (13*b*).
‡ $p < 0.05 > 0.01$.
§ $p < 0.01$.

TABLE V

The effect of a 40% fat diet on the lipid composition of normal fasting duodenal mucosa

Experiment*	Total lipid as per cent of biopsy weight	Per cent of total lipid found in			Phospholipid fatty acids					Triglyceride fatty acids				
		Phospholipid	Cholesterol	Triglyceride	16:0	18:0	18:1	18:2	20:4	16:0	16:1	18:0	18:1	18:2
					moles/100 moles fatty acids					*moles/100 moles fatty acids*				
A	4.2 ±0.6	49.9 ±10.8	8.6 ±0.2	25.4 ±6.2	24.9 ±4.8	23.4 ±0.7	17.1 ±1.8	27.1 ±1.7	6.2 ±1.5	31.6 ±3.8	4.0 ±1.3	11.2 ±2.2	36.4 ±4.6	13.3 ±10.4
Lipid of 40% fat diet		0.4		81.0						36.6	1.9	9.8	22.3	28.2
B	3.6 ±1.0	53.3 ± 7.9	9.1 ±2.9	25.0†	23.8 ±3.5	20.4 ±3.4	19.4 ±6.4	28.9 ±2.2	5.6 ±2.6	26.1 ±1.1	3.0 ±0.8	9.0 ±1.9	32.6 ±0.9	28.3 ±1.7
C	4.9	38.3	8.1		22.4	21.9	13.4	29.5	6.6	25.8	1.5	8.4	26.7	27.5
Analysis of variance, A and B‡														
	F =0.375 NS	1.750 NS	0.0034§		1.820 NS	0.0486‖	0.0787 NS	0.640 NS	0.362 NS	11.000 NS	2.630 NS	1.380 NS	24.81‖	35.61‖

* After biopsies had been obtained without prior dietary control (A), a second series of biopsies was taken after 2 weeks of a formula diet containing 40% fat calories (B). One of the subjects continued the diet for an additional 4 weeks (C). All groups of biopsies were taken after an overnight fast. The results in experiments A and B are expressed as the mean ± standard deviation of three subjects.

† Mean of two subjects.

‡ Two-sided test (14).

§ $p < 0.01$.

‖ $p < 0.05 > 0.01$.

subjects, seven to twelve mucosal biopsies were taken from each of three areas (the second portion of the duodenum, the ligament of Treitz, and between these two) and analyzed separately (Table IV). There was no difference among sites in the same individual, but considerable difference in the fatty acid composition among different individuals —specifically for oleic, linoleic, and arachidonic in the phospholipids, and oleic and linoleic in the triglycerides.

The effect of previous diet on the lipid composition of fasting duodenal mucosa. To ascertain whether the variations in mucosal fatty acids were a function of dietary lipid and might be minimized by dietary control, we performed the following experiments. After initial biopsies, three normal subjects were given, for 2 weeks, a 40% fat formula diet. After subjects had fasted overnight, duodenal biopsies were again obtained. The results are shown in Table V. The variances of the moles per 100 moles of oleic and linoleic acids in the triglycerides were reduced by the 40% fat diet. Surprisingly, the variances of cholesterol as per cent lipid weight and the moles of stearic acid per 100 moles of phospholipid fatty acids were increased after 2 weeks of the formula diet. There were important differences between the distribution of fatty acids in the triglyceride and phospholipid fractions of mucosal lipid and the composition of the triglyceride fed for 2 weeks. These differences persisted in one subject even after 6 weeks of the 40% fat diet.

A similar experiment was conducted with the fat-free diet (Table VI). In this instance there was no significant difference in the variances of the lipid components except that on the formula diet the variances of the lipid as per cent biopsy weight and the triglyceride as per cent lipid weight were reduced.

When the data in the latter experiment were analyzed by comparing each individual before and after the fat-free diet (Table VI), mucosal cholesterol was found to be increased, and there were changes in the distribution of fatty acids in the phospholipids (a decrease in stearic and linoleic and a rise in oleic and arachidonic) and triglycerides (a rise in palmitoleic and a decrease in oleic). The absence of a statistically significant fall in triglyceride linoleic acid was due to an anomalous rise in one individual. When the other five pairs

of observations were considered, a decrease of 4.1% was found ($p < 0.01$). In absolute amounts, there was a net decrease in triglyceride linoleic acid after the fat-free diet in the anomalous subject.

The acute effects of corn oil on the composition of mucosal lipids

Duodenal biopsies were obtained 28 to 73 minutes after three normal subjects had been given corn oil intraduodenally (Table VII). There was an increase in mucosal triglycerides, and in this fraction there was a fall in the relative amounts of palmitic and stearic acids with an increase in linoleic. In striking contrast was the absence of change in the distribution of the phospholipid fatty acids.

The possibility that the effect on mucosal triglyceride was due to adsorption of corn oil to the mucosal biopsies was excluded by finding morphological evidence of fat only within the absorptive cells (3, 4). Furthermore, there was no radioactivity in mucosal lipids when biopsies were taken in the usual way from a segment of rat jejunum that had been incubated with tripalmitin-^{14}C emulsified in corn oil for 4 minutes.

The lipid composition of duodenal mucosa of patients with carbohydrate-induced hyperlipemia

The lipid composition of the duodenal mucosa of these fasting patients was similar to that of normal subjects also on ad libitum diets (Tables I and VIII).

After these three patients had received a fat-free diet for 2 weeks, no significant changes in fasting mucosal lipids were found compared with the values previously obtained in patients on ad libitum diets.

Discussion

Analysis of the lipids in the duodenal mucosa of fasting man demonstrated that phospholipids, triglycerides, and cholesterol were the principal components, and free fatty acids and cholesterol esters were minor ones. However, these five classes combined accounted for only 80% of the total lipid weight (Table I). The possibility of error in the weight of the total lipid owing to incomplete drying or the presence of proteinaceous material was excluded. As determining glyceride

TABLE VI

The effect of a fat-free diet on the lipid composition of duodenal mucosa from six normal subjects

Experiment*	Total lipid as per cent of biopsy weight	Per cent of total lipid found in			Phospholipid fatty acids				Triglyceride fatty acids			
		Phospholipid	Cholesterol	Triglyceride	16:0	18:1	18:2	20:4	16:0	16:1	18:0	18:1	18:2
					moles/100 moles fatty acids				*moles/100 moles fatty acids*				
A	4.8±1.1	44.2±10.5	5.4±1.0	17.4±8.8	26.3±1.2	22.9±1.6	15.1±1.3	24.1±1.9	7.5±1.8	32.7±4.2	3.9±1.2	10.3±1.2	35.6±3.0	14.5±4.9
B	3.9±0.3	51.4±10.6	7.4±1.0	8.9±3.8	26.3±1.1	18.9±2.0	20.3±0.9	16.3±4.3	11.2±1.5	33.6±4.0	7.0±1.8	9.3±1.9	31.9±3.6	12.4±6.2
Analysis of variance†														
$F =$	12.83‡	0.946 NS	1.06 NS	5.41§	1.10 NS	0.655 NS	1.89 NS	0.191 NS	1.49 NS	1.11 NS	0.43 NS	0.40 NS	0.675 NS	0.614 NS
Difference between means‖														
$t =$	1.124 NS	0.717 NS	4.076‡	2.43§		5.671‡	6.118‡	4.263‡	2.927§	0.116 NS	2.818§	0.936 NS	4.625‡	1.090 NS

* Biopsies were taken without prior dietary control (A) and after 2 weeks of a fat-free formula (B). Both groups of biopsies were obtained after an overnight fast. The results are expressed as the mean ± standard deviation.
† Two-sided test (14).
‡ $p < 0.01$.
§ $p < 0.05 > 0.01$.
‖ Paired data (13b).

TABLE VII

Acute changes in the composition of mucosal lipids after giving corn oil to three normal subjects

Experiment*	Total lipid as per cent of biopsy weight	Per cent of total lipid found in		Phospholipid fatty acids				Triglyceride fatty acids			
		Phospholipid	Triglyceride	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
				moles/100 moles fatty acids				*moles/100 moles fatty acids*			
A	4.4	52.4	21.4	22.3	19.8	21.8	25.0	34.4	10.6	34.5	13.7
Corn oil fatty acids								11.6	2.1	27.6	58.7
B	6.3	34.8	36.2	24.7	18.8	19.7	27.5	21.3	4.2	32.8	38.7
Difference between means (A and B)†											
t =	1.955 NS	2.825 NS	15.920‡	1.188 NS	0.568 NS	0.250 NS	1.947 NS	20.092‡	11.405‡	1.111 NS	5.910§

* Biopsies were obtained after an overnight fast without prior dietary control (A). A second series of biopsies was taken 28 to 73 minutes after instilling corn oil (1.5 mg per kg body weight) intraduodenally (B). The results of experiments A and B are expressed as the mean of the three studies.

† Paired data (13*b*).

‡ $p < 0.01$.

§ $p < 0.05 > 0.01$.

glycerol gave values for the amount of triglycerides similar to those obtained by the indirect method of measuring total fatty acid ester, it is unlikely that glycerol ethers and vinyl ether phospholipids (which would not contribute to the total fatty acid ester) were present in sufficient amounts to give low calculated values for triglyceride. Total carbohydrate-reacting material was determined by the anthrone reaction. The results suggested that glycolipids might have accounted for 10 to 20% of the total lipid weight. A more precise estimate is impossible until the relative proportions of the various glycolipids are known.

There are few figures in the literature for the phospholipid composition of intestinal mucosa. As in the dog (15) and the rat (16), lecithin is the predominant phospholipid in mucosa of human small intestine, although there seem to be differences among species in the distribution of the other phospholipid components.

The distribution of fatty acids in mucosal phospholipids and triglycerides obtained from subjects on an ad libitum diet (Table II) is similar to that reported for lower animals (17) and for lymph lipid in fasting humans (18, 19). Oleic acid occurred in higher concentration in triglycerides than phospholipids, whereas stearic and linoleic acids were concentrated in the latter. Of the minor fatty acids, palmitoleic was found mainly in the triglycerides, but arachidonic occurred in the phospholipids.

The effect of a fat-free diet on the composition of tissue lipids has been studied extensively. Verdino, Blank, and Privett found an increase in palmitoleic and oleic with a decrease in linoleic acid in both the phospholipid and triglyceride fractions of thoracic duct lymph in fasting rats given a fat-free diet compared with rats fed a corn oil diet (20). Similar changes were observed in human duodenal mucosa when a fat-free formula was substituted for the ad libitum diets, with the exception that the proportion of oleic acid in triglyceride fatty acids decreased (Table VI). These mucosal fatty acids might have been formed within the absorptive cell or synthesized elsewhere and transported to the intestine. Synthesis of long chain fatty acids from a two carbon precursor, acetate-^{14}C, has been shown to occur in rat small intestine *in vitro,* although phospholipid palmitic and stearic were the chief fatty acids formed (21). The *in vivo* capacity of the human small intestine for synthesizing long chain fatty acids from nonlipid sources appears to be small, for, contrary to the increase in triglyceride fatty acids found in human plasma (22, 23) and in rodent liver (24), mucosal triglycerides decreased when fat was removed from the diet (Table VI). Supporting this contention are the results of the experiments in patients with hypertriglyceridemia, which is maintained or enhanced by a fat-free diet. If the intestinal mucosa of these patients were a site where long chain fatty acids were extensively synthesized from nonlipid sources, differences in the fatty acid composition of mucosal triglycerides from that of normal subjects might have been detected, and these abnormalities should have been exag-

gerated by a fat-free diet. In fact, the triglyceride fatty acids of mucosal lipids of three fasting patients with carbohydrate-induced hyperlipemia did not differ from normal values, and no abnormality was induced by removing fat from the diet (Table VIII).

When mucosal lipids were analyzed before and after giving corn oil to fasting normal subjects, an increase in mucosal triglycerides was observed. In addition, the fatty acids of the mucosal triglycerides reflected the composition of the corn oil fatty acids. These changes in mucosal lipids, occurring 28 to 73 minutes after intraduodenal instillation of corn oil, correspond well with the morphological observations on fat absorption in man (3, 4).

Contrary to the changes in triglyceride fatty acids, there was no alteration in the fatty acid distribution of the phospholipids. Several investigators have been unable to demonstrate a phospholipid intermediate during triglyceride synthesis in rodent intestinal mucosa (16, 25), and recent data support the direct acylation of monoglyceride as the predominant pathway for synthesizing mucosal triglyceride (26). These combined observations suggest that there may be little interrelation between phospholipid and triglyceride metabolism during fat absorption in man.

Recently, it has been reported that endogenous gut lipid contributes significantly to chylomicron lipid in intestinal lymph, even after a fatty meal (18, 19, 27, 28). The present studies demonstrate the influence of endogenous fat on the lipid composition of the intestinal mucosa—the site of chylomicron formation. The variation in the composition of fatty acids in mucosal lipid among fasting normal subjects, while on ad libitum diets, could not be reduced by a fat-free diet for 2 weeks (Table VI). The contribution of endogenous fat to mucosal lipid in fasting patients was evident after the 40% fat diet (Table V). Although the mucosal concentration (moles per 100 moles of fatty acids) of linoleic acid in phospholipids and linoleic and stearic in triglycerides was similar to that of the diet, considerable disparity existed between the other mucosal and dietary fatty acids. This disparity was still present in the subject who was given the formula diet for a total of 6 weeks. As the turnover time of human duodenal mucosa is 5 to 6 days (29), these persistent variations in mucosal fatty acids probably result from individual

TABLE VIII

The lipid composition of duodenal mucosa obtained from patients with carbohydrate-induced hyperlipemia

Experiment*	Total lipid as per cent of biopsy weight	Per cent of total lipid found in			Phospholipid fatty acids				Triglyceride fatty acids				
		Phospholipid	Cholesterol	Triglyceride	16:0	18:0	18:1	18:2	20:4	16:0	16:1	18:0	18:1	18:2
					moles/100 moles fatty acids					*moles/100 moles fatty acids*				
A	6.0	42.8	4.5	17.0	28.7	19.0	15.7	22.0	9.2	30.9	4.7	9.3	34.3	18.7
B	5.3	43.2	5.8	10.9	28.0	18.7	16.7	17.7	11.8	32.7	6.6	7.5	30.6	16.6
Differences between means†														
t =	0.665 NS	0.102 NS	2.013 NS	1.685 NS	0.361 NS	0.244 NS	1.406 NS	1.100 NS	1.677 NS	0.436 NS	1.056 NS	1.147 NS	1.850 NS	0.311 NS

* After an overnight fast, biopsies were obtained from 3 patients without prior dietary control (A), and again after 2 weeks of a fat-free formula diet (B). The results are expressed as the mean of the 3 studies.

† Paired data (13*b*).

differences in lipid synthesis by intestinal mucosa, or from differences in the composition of endogenous lipid delivered to the mucosa via the plasma or the intestinal lumen.

The data, obtained during the absorption of corn oil, also indicated that there was considerable dilution of the dietary fatty acids by endogenous fat (Table VII). This was particularly striking in the mucosal phospholipids, but even in the triglyceride fraction, where the influence of the corn oil fatty acids was apparent, endogenous fat contributed to triglyceride fatty acids. These results, at the level of the intestinal absorptive cell, are in accord with the recent data of Blomstrand, Gürtler, and Werner, who studied thoracic duct lymph during fat absorption in man (*27*).

Summary

1) The composition of mucosal lipids, obtained by suction biopsy of the distal duodenum of normal subjects, was examined with and without prior dietary control. The results demonstrate the influence of endogenous fat on mucosal lipids after diets of known lipid composition and during the absorption of fat.

The variances of mucosal phospholipid and triglyceride fatty acids, obtained after an overnight fast from subjects on ad libitum diets, were not reduced by a fat-free formula diet for 2 weeks.

After 2 to 6 weeks of a formula diet containing 40% fat calories, there was considerable disparity between the composition of mucosal fatty acids and that of the dietary fat.

Mucosal biopsies were obtained before and 28 to 73 minutes after giving corn oil intraduodenally. There was an increase in mucosal triglycerides whose fatty acid composition changed towards that of the corn oil. However, the influence of endogenous fat on triglyceride fatty acids was still apparent and was especially striking on the mucosal phospholipid fatty acids.

2) The data obtained from normal subjects and patients with carbohydrate-induced hyperlipemia suggest that human intestinal mucosa does not synthesize excessive amounts of triglyceride fatty acids from nonlipid sources.

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