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Fatty acid esterification and chylomicron formation during fat absorption: 1. Triglycerides and cholesterol esters*

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SUMMARY

Experiments were conducted to study in vivo the over-all fatty acid specificity of the mechanisms involved in chylomicron cholesterol ester and triglyceride formation during fat absorption in the rat. Mixtures containing similar amounts of two, three, or four C^{14}-labeled fatty acids (palmitic, stearic, oleic, and linoleic acids), but with varying ratios of unlabeled fatty acids, were given by gastric intubation to rats with cannulated thoracic ducts. The chyle or chylomicron lipid so obtained was chromatographed on silicic acid columns to separate cholesterol esters and glycerides (the latter being 98.2% triglycerides). After assaying each lipid class for total radioactivity, gas-liquid chromatography was employed to measure the total mass and the distribution of mass and of radioactivity in the individual fatty acid components of each lipid fraction. The specific radioactivity of each fatty acid in each fraction could then be calculated. The data provided quantitative information on the relative specificity of incorporation of each fatty acid into each chylomicron lipid class and on the relative extent to which each fatty acid in each lipid fraction was diluted with endogenous fatty acid. With the exception of a slight discrimination against stearic acid, the processes of fatty acid absorption and chylomicron triglyceride formation displayed no specificity for one fatty acid relative to another. In contrast, chylomicron cholesterol ester formation showed marked specificity for oleic acid, relative to the other three fatty acids. This specificity was not significantly altered by varying the composition of the test meal, by including cholesterol in the test meal, or by feeding the animal a high-cholesterol diet for several weeks preceding the study. Considerable dilution of the dietary fatty acids with endogenous fatty acids was observed. In one experiment, 43% of the chylomicron triglyceride fatty acids was of endogenous origin. Relatively more (54%) of the cholesterol ester fatty acids was of endogenous origin.

It is now well established that long-chain fatty acids absorbed by the intestine are almost exclusively transported in the chylomicron fraction of the lymph, mainly as triglycerides, but also to a lesser extent as phospholipids, cholesterol esters, and free fatty acids. There is, however, little precise information comparing the extent to which different fatty acids are incorporated into the various lipid classes. Several studies have been reported in which a single labeled fatty acid has been fed and its distribution measured among the lipid classes of the chylomicron. These studies have suggested that the common long-chain fatty acids, with the exception of stearic acid, are similarly absorbed from the intestine. Thus, in both rats (2, 3) and humans (4, 5), labeled palmitic, oleic, and linoleic acids given singly were absorbed in similar fashion, since in each instance 90% or more of the labeled fatty acid recovered in lymph was present as triglycerides, and 2–6% as phospholipid. Stearic

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acid was incorporated less into triglycerides and more into phospholipids in both rats (6) and humans (5). There has, hitherto, been no similar study using two or more labeled fatty acids simultaneously, in order to make direct comparisons in a single experiment.

Cholesterol esters have received less attention in these studies but have been noted to contain about 1–2% of the labeled fatty acids in chyle after feeding stearic acid to rats (6), or stearic and linoleic acids to humans (5). On the other hand, the possibility of intestinal specificity towards particular fatty acids for cholesterol esterification has been strongly suggested by in vitro studies. Murthy et al. reported that homogenates of rat intestine incorporated polyunsaturated fatty acids into cholesterol esters much more rapidly than saturated fatty acids, whereas pancreatic homogenates utilized oleic acid more rapidly than the other fatty acids (7). Others have considered the intestinal esterifying activity to be derived from the pancreas (8, 9) and have demonstrated a relative specificity for unsaturated fatty acids with pancreatic cholesterol esterase (10, 11).

In addition, studies of the fatty acid composition of the lymph chylomicrons after the ingestion of fat have suggested that the constituent fatty acids are not exclusively derived from the recently fed fat but are derived from other sources within the animal as well (4, 12–14). During the period following a meal of corn oil, the fatty acid composition of the lymph chylomicrons rapidly changed toward the composition of the corn oil and eventually became almost identical with it (12, 13). However, in the early stages of this period, after the fat content of the chyle had already increased significantly, the fatty acid composition of the chylomicrons did not closely resemble that of the fed fat and neither did that of the lymph samples collected in the later stages when the fat content of the lymph was still high. Both these samples contained substantial amounts of fatty acids not present in the diet. The presence of endogenous fatty acids in the chylomicron lipid greatly complicates studies of the metabolism of individual lipids in the lymph and makes it necessary to distinguish the effects of the relative specificities of the esterification mechanisms from the effects of varying contributions from recently fed fat and from fatty acid pools in the animal.

The experiments described herein were designed to examine these several questions through the use of labeled dietary fatty acids.

EXPERIMENTAL METHODS

The animals employed were male Sprague-Dawley rats weighing 250–300 g each and previously fed Purina laboratory chow and tap water. In addition, the animals for Experiment III had been maintained for 7 weeks on a diet of chow with 10% (by weight) additional oil (corn oil–olive oil, 3:1) and 1% additional cholesterol. Polyethylene cannulae were tied into the thoracic ducts by a modification of the method of Bollman, Cain, and Grindlay (15), under light ether anesthesia. The rats were subsequently kept in restraining cages and allowed to drink freely of a solution of 5% glucose in half-strength Krebs-Ringer saline. The lymph drained into containers standing in ice. At least 24 hr were allowed for recovery from the operation before test feeding was begun. Each test meal was given by gastric intubation under light ether anesthesia, followed by collection of chyle for 24 hr. Subsequent test meals were given only when the chyle had visibly cleared.[1]

Fatty acids labeled with C^{14} in the carboxyl group were purchased from Nuclear–Chicago Corp. (palmitic acid-1-C^{14}), the Volk Radiochemical Corp. (oleic acid-1-C^{14}), Orlando Chemical Co. (linoleic acid-1-C^{14}), and from New England Nuclear Co. (stearic acid-1-C^{14}). Purification was effected as follows: Each labeled fatty acid was first dissolved in a solution of *n*-hexane–glacial acetic acid 1:1 (v/v); $^1/_{10}$th volume of water was then added, followed by removal of the acetic acid phase and washing of the hexane phase with water to remove any short-chain fatty acids present. The hexane solution was then vigorously shaken with an equal volume of 0.1 N KOH in 50% ethanol, to extract all fatty acids. The ethanolic KOH was acidified and extracted with hexane to separate the labeled fatty acid from nonacidic impurities. Analysis for radioactive impurities by gas-liquid chromatography (GLC) indicated each fatty acid to be at least 99% pure.

Diets: Experiment I. The first study employed labeled palmitic and linoleic acids added in tracer amounts to two triglyceride mixtures. In one diet, equal amounts of radioactivity as palmitic and as linoleic acid were added to commercial olive oil; in the second diet, the same labeled fatty acid mixture was added to commercial corn oil. Approximately 0.5 ml of each diet was administered to each of two cannulated rats. Rat #1 received the olive oil (O) test meal first, followed by the corn oil (L) meal; rat #2 received L then O.

Experiment II. In the second study, two different dietary mixtures (A and B) of three unlabeled and

[1] The clear lymph was not assayed for radioactivity. It can be estimated, however, from the specific radioactivity data of Experiment II (in which two very different diets were alternately fed) that the preceding day's test meal contributed a maximum of 2–3% of the fatty acids of any given sample.

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labeled free fatty acids (palmitic, oleic, and linoleic acids) were used. The unlabeled fatty acids were obtained from the Hormel Foundation (Austin, Minn.) and were each more than 99% pure as determined by GLC analysis. Each diet contained approximately equal amounts of radioactivity as palmitic, oleic, and linoleic acids. The mass distribution differed, however, in that oleic acid predominated in diet A, whereas linoleic acid predominated in diet B. The standard dose used was 0.3 ml, slightly warmed to produce a uniform solution. For the later experiments with rat #4, about 50 mg of cholesterol was added to each dose of the primary diets (thereafter designated as test meals AC and BC). During the study, rat #3 was given diet A first, but the subsequent collection of lymph was discarded because of a technical mishap; feedings with B and A followed in that order. Rat #4 was given, in order, A, B, BC, and AC.

Experiment III. The test meal employed in the third study also consisted of a mixture of labeled and unlabeled free fatty acids. Stearic acid was included as a fourth fatty acid in this study, as well as the linoleic, oleic, and palmitic acids used in Experiment II. Two diets (D and DC) were fed. The fatty acid composition of the two was the same, but the second diet (DC) had 50 mg cholesterol added to the 0.3 ml test meal administered. The composition of the diet resembled that of the fat that the rats (#5 and 6) had been accustomed to eating.

Lymph Lipid Extraction: Experiment I. After collection, each whole lymph sample was buffered to pH 7.4 with potassium phosphate buffer (final concentration about 0.02 M), and sucrose was added to a final concentration of 5% (w/v). The volume was then reduced to about 10 ml by lyophilization, and the remaining mixture extracted with 250–300 ml of $CHCl_3$–MeOH 2:1 (v/v). After addition of 50 ml of 0.04 N H_2SO_4 to split the extract into two phases, the $CHCl_3$ phase was collected, evaporated to dryness under a stream of nitrogen, and the residue, representing the total lipid extract of the lymph sample, dissolved in benzene and stored at −20° under nitrogen.

Experiments II and III. In these experiments, chylomicron lipid was analyzed rather than the total lipid extract of the lymph. For collection and washing of chylomicra, the lymph was layered under isotonic saline and centrifuged for 30 min at 25,000 rpm (62,000 × *g*, average) in the 40.0 rotor of a Spinco model L ultracentrifuge. The centrifuge tubes were sliced below the packed chylomicra, and the chylomicra redispersed with isotonic saline. The concentrated chylomicron collections were then extracted with 25 volumes of $CHCl_3$–MeOH 2:1 (v/v) as described above, and the total chylomicron lipid was finally dried under nitrogen, weighed, and stored in benzene.

In Experiment III, the remaining lymph, from which the chylomicrons had been centrifugally harvested, was also extracted with $CHCl_3$–MeOH to obtain its total lipid content.

Lipid Analyses. Aliquots of the benzene solutions were used for chemical estimation of total lipid (16); total, free, and esterified cholesterol (17); and, in the second and third experiments, lipid phosphorus (18) and triglycerides by difference. The gravimetric and chemical estimates were used as guides to subsequent procedures and as checks on the more definitive measurements derived from GLC.

Silicic acid chromatography was employed to separate the lipid samples into cholesterol ester (E), glyceride (G), and phospholipid (P) fractions. The critical separation of cholesterol esters from glycerides was effected by using columns of 10 mm i.d. loaded with 5 g silicic acid ("Unisil," 100–200 mesh, Clarkson Chemical Co., Inc., Williamsport, Pennsylvania). The sterol ester fraction was eluted with 16% benzene in redistilled *n*-hexane (v/v), a method similar to that of Horning, Williams, and Horning (19).

Since chyle contains a vast preponderance of glycerides over cholesterol esters, the efficiency of the separation and recovery of cholesterol esters from mixtures containing a much larger amount of triglycerides was tested. Separation of 0.8 mg of a 1:1 mixture of cholesteryl oleate and cholesteryl linoleate from 25 mg of corn oil and analysis of the fatty acids by GLC showed that the contribution of corn oil to the sterol ester fraction at most was 10% of the fatty acids of the sterol ester fraction. Small (0.5 mg) quantities of the same mixture of cholesteryl oleate and cholesteryl linoleate were put through the silicic acid column alone, without corn oil, to test for selective loss of polyunsaturated fatty acid. The ratio of linoleic acid to oleic acid in the effluent from the silicic acid column was the same as in the original mixture.

Separation of H^3-labeled cholesteryl linoleate (synthesized as in [20]) from purified tripalmitin or triolein present in as much as 50 times the concentration of cholesteryl linoleate showed that leakage of triglyceride into the sterol ester fraction was very slight, as demonstrated by GLC, while recovery of radioactivity was excellent (Table 1).

When the lymph or chylomicron lipids were chromatographed, the load put on a 5-g column was always less than 75 mg lipid. The sterol ester fraction (E) was eluted with 100 ml 16% benzene in hexane, followed by elution of all nonphospholipid lipids with 60 ml benzene and then 60 ml $CHCl_3$. This latter fraction

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TABLE 1. Effectiveness of Separation of H^3-Cholesteryl Linoleate from Triglyceride by Silicic Acid Chromotography

Volume of Eluant	Recovery of H^3	Contamination with Triglyceride
ml	%	%
Separation of Cholesteryl Linoleate from Tripalmitin*		
70	80	4.0
80	92	3.7
90	97	4.0
100	99	4.3
Separation of Cholesteryl Linoleate from Triolein*		
70	81	2.4
80	91	2.1
90	95	2.1
100	97	2.0

* See text for experimental details.

will be called the glyceride (G) fraction. In Experiments II and III, phospholipids (P fraction) were then eluted with 75 ml methanol (see accompanying paper [26]). With each column, the last 10 ml of the sterol ester fraction was separately collected and assayed for radioactivity to ensure that an effective separation of sterol esters and glycerides was being achieved. In all instances, the concentration of radioactivity in this 10 ml was less than 20% of the average concentration of radioactivity in the first 75 ml. In order to chromatograph relatively large amounts of material, some of the samples of chylomicron lipid were subjected to two separate silicic acid chromatographies, the first on a 20-g column, followed by rechromatography of the sterol ester fraction on a 5-g column.

After chromatography the various fractions were evaporated to dryness under nitrogen, and the residues were weighed, dissolved in benzene, and stored under nitrogen at −20°. The total recovery of radioactivity in Experiments II and III, in the cholesterol ester + glyceride + phospholipid fractions compared to the total chylomicron lipid, was quantitative in every case (96.3–103.7%).

Thin-layer silicic acid chromatography was used to check the composition of the chylomicron G fractions in Experiment III. The solvent system used was *n*-hexane–ethyl ether–acetic acid 70:30:2, and standard spots were included to assist in the location of some components. The several components were visualized with rhodamine 6G under ultraviolet light and were clearly separated from each other. They were then scraped on to filter paper and eluted with $CHCl_3$. Radioactivity measurements showed a constant composition of the four samples, with the average distribution of radioactivity being triglycerides 98.1%, free fatty acids 1.2%, diglycerides 0.5%, and monoglycerides 0.1%.

Gas-Liquid Chromatography. The total fatty acid content, the fatty acid composition, and the radioactivity in each fatty acid were determined by gas-liquid chromatography.

The total lipid in each sample was estimated from the results of the chemical analysis. A known amount of margaric acid, approximately $^1/_{10}$ of the estimated amount of the total fatty acid mass, was added as an internal standard. The methyl esters were then prepared by incubating less than 10 mg of lipid with 1 ml of a 2% (v/v) solution of sulphuric acid in methanol overnight at 65° in a screw-top test tube with a tetrafluoroethylene gasket. Following incubation, 1 ml of water was added, and the methyl esters were extracted with light petroleum ether. The petroleum ether was taken to dryness under nitrogen, and the residue was taken up in isooctane. The recovery of radioactivity was routinely checked and was never found to be less than 95% of the quantity taken for esterification. Aliquots of these solutions were separately analyzed for mass and radioactivity by GLC. Although the equipment used for the radioassay was equipped with a mass detector as well, it was considered less difficult and more accurate to perform a separate mass analysis using a smaller sample, approximately 30–50 μg of material. The amount of material taken to analyze for radioactivity was decided from the counting rate of the sample.

Analyses were performed using columns containing Chromosorb W coated with ethylene glycol adipate polyester, 20% of the weight of the Chromosorb, at 200°. An argon ionization detector (21) was used for mass detection. The detector cell was constructed as previously described (22). The detection system was calibrated for quantitative accuracy by injecting 3-μl aliquots of various dilutions of the methyl esters of the fatty acids of corn oil and demonstrating that, at the voltages and other conditions used, the response of the detector to each ester was linearly related to the concentration over the current range tested. The results for fatty acid composition reported here were taken directly from the areas under the curve without any correction for possible variation in the response of the detector for different molecular species. The quantity of each fatty acid present was then determined by comparing its area with the area under the peak of the known amount of margaric acid (23). The "total fatty acids" was computed from the area under all the curves for the duration of the analysis, and thus did not take into account any very long-chain methyl esters present (of retention time greater than methyl arachidonate).

Radioassay was performed by either of two methods previously described (24, 25). In the first method, the

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TABLE 2. Composition of the Diets, as Determined by GLC and by Radioassay of the GLC Effluents

		Mass					Radioactivity				
		Total	Distribution				Total	Distribution			
Expt. No.	Diet		16:0	18:0	18:1	18:2		16:0	18:0	18:1	18:2
		mg	%				*cpm × 10⁻⁶*	%			
I	O	440	14.6	1.2	68.4	12.8	2.85	45	—	—	54
I	L	400	13.0	1.0	27.0	58.1	2.71	46	—	—	53
II	A	276	12.6	0.3	77.0	10.0	17.31	40	~0.3	32	28
II	B	269	11.3	0.6	12.2	76.0	17.61	40	~0.4	30	30
III	D	285	7.1	7.1	43.3	42.5	28.60	26.6	29.2	24.9	19.4

effluent of the column was fractionated by passing it through a series of cartridges containing anthracene crystals coated with silicone oil. The fatty acid esters condense in these cartridges in close proximity to the anthracene, and radioactivity in each cartridge is then measured directly by scintillation counting. An automatic fraction collector was used, the changing mechanism of which was triggered by an electric timer without regard for the mass analysis. The record of radioactivity obtained was then compared with the mass record.

In the operation of this method of radioassay, several precautions were followed. The integrity of the gas connections was checked daily by injecting a known quantity of a C^{14}-labeled methyl laurate and collecting it in a single cartridge. The amount injected was always quantitatively collected, allowing for a 12% loss to the mass detector. The over-all performance of the system was also evaluated by performing repeated analyses of the same mixture of two methyl esters and showing that the portion of the total radioactivity present in each ester was consistent and reproducible.

In the first experiment, it was assumed that no significant radioactivity was present in fatty acids other than linoleic and palmitic. The total effluent of the column was therefore collected in only two equal fractions, one of which contained methyl palmitate and the other methyl linoleate.

In the second experiment, fractions were collected at 1-min intervals. The temperature of the column was adjusted so that the radioactivity in methyl linoleate could easily be distinguished from that in methyl oleate.

The average specific radioactivity of the fatty acids in Experiments II and III was sufficiently high so that 15,000 to 20,000 dpm could be injected into the column without overloading it. This made possible the use of the second method of radioassay (25), in which the column effluent was subjected to combustion to carbon dioxide and then passed through a transparent scintillation detector cell containing anthracene crystals. The counting rate of the anthracene was recorded on one channel of a two-channel strip chart recorder. The output of the mass detector was recorded on the other. The area under a peak in the radioactivity record was proportional to the quantity of radioactivity present in the peak just as the area under the mass peak was proportional to the amount of mass present.

Aliquots of some of the methyl ester samples from Experiment II were analyzed this way, and the results of the analyses were compared with those obtained using the fraction collector. No differences were observed. Since the lipids collected during Experiment III had even higher specific radioactivity, the second method of radioassay was used exclusively.

Radioassay of lipid fractions other than the GLC effluents was performed by liquid scintillation counting, using a Packard scintillation spectrometer. Samples were taken to dryness in the counting vials, and 15 ml of a solution of 0.6% diphenyloxazole in toluene was added.

RESULTS

The fatty acid composition and distribution of radioactivity in the several diets employed are listed in Table 2. The complete analyses of the glyceride and cholesterol ester fractions of the total lymph and chylomicron lipids from Experiments I, II, and III are presented in Tables 3, 4, and 5, respectively. These tables include the total fatty acid mass and radioactivity, as well as the distribution of mass and radioactivity, in each collection from each rat. In Experiment II, traces of radioactivity were also found in stearic acid in all G and E samples; this usually was of the order of 1% of the total radioactivity, and in all cases was definitely less than 2%. This stearic acid-C^{14} probably originated in the diets, which were found to contain trace quantities of stearic acid-C^{14}. The additional possibility that some of the stearic acid-C^{14} was derived from one of the other labeled fatty acids is discussed in the accompanying paper (26).

In Experiment II, three samples of chylomicron total lipid were also analyzed before silicic acid column chromatography. As with Experiment I (see Table 3),

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TABLE 3. Recovery and Distribution of Mass and Radioactivity in the Fatty Acids of The Lymph Lipids in Experiment I

Rat No.	Diet	Mass Total	Mass Distribution 16:0	18:0	18:1	18:2	Radioactivity Total	Radioactivity Distribution 16:0	18:2
		mg	%				*cpm* $\times 10^{-6}$	%	
Total Lipids of Lymph									
1	O	474	18.9	4.7	56.2	17.5	1.74	45	54
2	O	351	15.4	2.4	67.2	12.3	1.72	45	54
1	L	415	19.5	4.8	30.0	43.8	2.09	48	51
2	L	296	18.5	5.0	25.9	49.5	1.67	46	53
Glycerides (G)									
1	O	352	19.8	3.1	59.0	15.5	1.38	48	51
2	O	288	15.3	1.7	66.8	13.9	1.48	47	52
1	L	298	18.8	3.5	30.7	45.2	1.70	48	51
2	L	252	17.2	3.5	27.1	51.8	1.36	47	52
Sterol Esters (E)									
1	O	5.09	28.6	9.2	40.8	15.7	0.0106	50	49
2	O	2.76	23.7	10.2	46.2	15.8	0.00795	47	52
1	L	6.82	29.6	12.9	31.3	20.5	0.0176	48	51
2	L	3.89	32.3	12.2	25.6	24.6	0.0139	40	59

the results of these analyses were nearly identical with those obtained for the G fractions. This verified that there was no alteration of fatty acid pattern during silicic acid chromatography and provided a further check on the validity of the analytic techniques.

The average recovery of radioactivity in the lymph lipids of Experiment I was 65% of the administered radioactivity (range 60–79%). In Experiment II, the recovery of radioactivity in the chylomicra averaged 53% of that administered, but varied widely, in parallel with variations in the total recovery of lipid mass. This variability occurred in both rats and affected both diets and each of the dietary fatty acids without any apparent bias. The chylomicrons contained an average of 54% of the administered radioactivity in Experiment III (range 39–67%).

The data in Tables 2 to 5 indicate that the glyceride fractions had fatty acid compositions resembling those of the fed fat in every instance. The fatty acid compositions of the cholesterol ester fractions were less similar to those of the diets than were the glycerides, although a general resemblance to the diets did exist. These relationships are clearly set forth in Table 6, which summarizes the average values of the distribution of mass and radioactivity in the chylomicron glycerides and cholesterol esters after diets A, B, and D. The values listed in Tables 6 and 7 were obtained by summing the total mass and total radioactivity recovered in each fatty acid in each individual sample and then determining the distribution of mass and radioactivity in the combined sums for all samples after each diet. The tabulated values thus represent an average of individual values that have been "weighted" in proportion to their total mass and radioactivity. Very little variation existed in the distribution data for the individual collections after each diet.

In each glyceride fraction analyzed, the distribution of radioactivity was very similar to that in the test meal. In Experiment I, the distribution of radioactivity in the cholesterol esters was also similar to that in the test meal, with similar amounts of radioactivity found in each of the two labeled fatty acids (palmitic and linoleic) being studied. In Experiments II and III, however, the distribution of radioactivity in the cholesterol esters differed from that in the test meal and from that in the glycerides. In every sterol ester sample in both these experiments, relatively more radioactivity was found in oleic acid than in any of the other fatty acids being studied. These relationships are also summarized in Table 6.

The average values of the ratio of the specific radioactivity of each fatty acid in each of the two chylomicron lipid fractions (in Experiments II and III) compared to that of the same fatty acid in the diet is shown in Table 7. These data show that each dietary fatty acid was diluted to a different extent. From the data of Experiment III, the contributions of the dietary fat to the glyceride and cholesterol ester fractions were calculated. These calculations showed that 55–60% of the glycerides were synthesized from the dietary fat, while only 40–47% of the fatty acids in the cholesterol esters were derived from the diet.

Analyses of the lymph lipids remaining after removal of the chylomicrons, in Experiment III, showed that an

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TABLE 4. Recovery and Distribution of Mass and Radioactivity in the Fatty Acids of the Chylomicron Glycerides and Sterol Esters in Experiment II

Rat and Diet	Mass									Radioactivity			
	Total	Distribution								Total	Distribution		
		16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4		16:0	18:1	18:2
	mg	%								*cpm × 10⁻⁶*	%		
Glycerides (G)													
3 A	322.4	13.8	1.4	1.3	57.0	23.2	0	2.7	0.5	14.745	37	32	30
4 A	49.7	16.4	1.1	4.4	56.0	16.9	0.6	1.6	1.6	1.378	35	30	33
4 AC	445.8	16.3	0.9	1.8	44.3	35.0	Tr	1.5	0.2	20.239	39	31	29
3 B	83.5	20.0	4.0	4.6	20.9	42.2	0	4.1	2.9	2.924	39	28	30
4 B	228.5	16.9	1.3	2.4	24.5	50.1	Tr	4.0	0.9	8.964	38	30	29
4 BC	115.8	19.6	3.0	3.5	15.8	53.2	0	2.3	1.8	4.622	38	31	30
Sterol Esters													
3 A	3.69	16.2	3.1	2.8	56.8	15.6	2.6	2.3	0.5	0.1458	38	39	21
4 A	0.77	21.4	3.7	7.8	51.8	13.0	1.3	1.0	Tr	0.0143	28	49	20
4 AC	9.75	14.8	3.3	2.8	52.9	23.8	1.8	0.7	Tr	0.3125	28	48	23
3 B	1.62	25.8	12.7	7.8	27.1	23.1	0	2.0	1.4	0.0319	26	46	25
4 B	2.96	20.0	4.0	3.9	32.7	34.8	1.0	2.4	1.2	0.1044	29	45	24
4 BC	3.44	20.0	7.5	4.5	24.0	42.7	Tr	0.7	0.7	0.1112	25	46	27

average of 9% of the total radioactivity of the lymph resided in this "1.006 bottom" fraction. The complete analysis of the glyceride and sterol ester fractions from one of these "bottom" samples (5DC) is presented in Table 8. Comparison with the data for collection 5DC in Table 5 shows that the E fraction of the "1.006 bottom" had relatively more arachidonic and palmitic acids and contained relatively more radioactivity in palmitic and less in stearic acid, than did its counterpart in the chylomicrons.

DISCUSSION

The experiments described here were designed primarily to study in vivo the over-all fatty acid specificity of the mechanisms involved in chylomicron cholesterol ester formation during fat absorption. The thoracic ducts of rats were cannulated, and the animals were fed meals containing mixtures of C^{14}-labeled fatty acids. The lipids of the chyle were separated, the fatty acids analyzed, and the quantity of, and radioactivity in, each fatty acid in the triglycerides and cholesterol esters were determined. A variety of test diets containing different proportions of the various fatty acids was studied.

The data obtained provide two kinds of information. In the first place, the specific radioactivity of each acid compared with the specific activity of the same acid in the fatty meal (Table 7) indicates the portion of that fatty acid contributed by the fat meal. In the experiments in which each of the fatty acids of the diet was

TABLE 5. Recovery and Distribution of Mass and Radioactivity in the Fatty Acids of the Chylomicron Glycerides and Sterol Esters in Experiment III

Rat and Diet	Mass									Radioactivity				
	Total	Distribution								Total	Distribution			
		16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4		16:0	18:0	18:1	18:2
	mg	%								*cpm × 10⁻⁶*	%			
Glycerides (G)														
5 D	264.7	14.9	0.8	6.6	35.3	38.5	Tr	2.2	1.7	14.778	29	23	28	20
6 D	181.0	13.4	1.0	6.6	37.5	39.8	Tr	1.1	0.7	10.454	27	22	29	23
5 DC	328.6	15.5	0.9	8.3	33.8	37.5	Tr	2.1	1.9	17.309	27	28	26	20
6 DC	229.2	13.2	0.8	7.0	37.1	38.9	Tr	1.3	1.7	14.061	28	25	27	21
Sterol Esters (E)														
5 D	3.06	18.5	3.1	9.2	40.2	27.7	0	1.3	Tr	0.1030	17	25	42	17
6 D	1.74	17.3	2.3	8.8	43.0	27.8	Tr	0.7	Tr	0.0619	16	23	43	18
5 DC	9.53	15.7	2.3	8.5	44.6	27.6	0	1.3	Tr	0.3795	18	27	37	17
6 DC	6.17	11.5	1.7	7.0	46.5	31.9	Tr	1.3	Tr	0.2505	17	23	39	21

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TABLE 6. Average Values for the Distribution of Mass and Radioactivity Among the Fatty Acids of the Diets and of all the Chylomicron G and E Fractions Obtained after each Diet

	Mass Distribution							Radioactivity Distribution			
	16:0	16:1	18:0	18:1	18:2	20:1	20:4	16:0	18:0	18:1	18:2
				%					%		
Diet: A + AC	12.6	—	0.3	77.0	10.0	—	—	40	0.3	32	28
Glycerides	15.3	1.1	1.8	50.0	29.2	2.0	0.4	38.0	1.0	31.4	29.6
Sterol Esters	15.5	3.2	3.0	53.9	21.1	1.0	<0.1	31.3	1.0	45.2	22.3
Diet: B + BC	11.3	—	0.6	12.2	76.0	—	—	40	0.4	30	30
Glycerides	18.2	2.3	3.1	21.4	49.4	3.5	1.5	38.2	1.7	29.9	29.5
Sterol Esters	21.2	7.2	5.0	27.8	35.8	1.5	1.0	26.8	1.1	45.6	25.5
Diet: D	7.1	—	7.1	43.3	42.5	—	—	26.6	29.2	24.9	19.4
Glycerides	14.3	0.9	6.6	36.2	39.0	1.8	1.3	28.1	22.5	28.5	20.9
Sterol Esters	18.1	2.7	9.0	41.2	27.7	1.0	Tr	16.7	24.0	42.2	17.2
Diet: DC	7.1	—	7.1	43.3	42.5	—	—	26.6	29.2	24.9	19.4
Glycerides	14.5	0.9	7.8	35.2	38.1	1.8	1.8	27.3	26.3	26.2	20.2
Sterol Esters	14.1	2.0	7.9	45.4	29.3	1.3	Tr	17.9	25.4	38.0	18.8

labeled, one could thus calculate the fraction of the entire chylomicron lipid that originated in the diet.

Secondly, the distribution of radioactivity in each lipid class can be compared to that in the diet. During fat absorption and chylomicron formation, several processes take place. These include (1) absorption of labeled fatty acids from the gut lumen into the intestinal mucosa; (2) admixture of these dietary fatty acids with unlabeled, endogenous fatty acids (in the lumen and/or mucosa, *vide infra*) to form a mixed pool that is available for the formation of chylomicron esters; and (3) utilization of fatty acids from this pool to make glycerides, sterol esters, and phospholipids, and the parcelling of these together as chylomicrons. As long as all fatty acids are equally absorbed, the distribution of radioactivity among the different fatty acids will be the same in the mixed pool as in the diet. The same distribution will also be found in a given chylomicron ester if the mechanisms involved in formation of that chylomicron lipid do not discriminate among the several labeled fatty acids. On the other hand, if the mechanisms involved in formation of a chylomicron ester do display specificity for one fatty acid relative to another, then relatively more of this fatty acid will be incorporated into that ester, and the final distribution of radioactivity will reveal a bias in this direction.

Table 9 compares the distribution of radioactivity among the chylomicron lipid fatty acids with the distribution in the diet, relative to palmitic acid taken as 1.0. These values were derived by expressing the distribution of radioactivity in each fatty acid as a percentage of the total, dividing by the corresponding figure from the diet, and then dividing by the value for palmitic acid.

Despite marked differences in the diets, the results of all experiments were similar in that the distribution of radioactivity in the triglyceride fraction was very similar to that in the diet. This therefore indicates that the processes of fatty acid absorption and chylomicron triglyceride formation were nonspecific and did not distinguish among the various fatty acids fed. The only minor exception to this conclusion was observed with stearic acid, which was absorbed and incorporated into triglycerides a little less effectively than the other three fatty acids. On the average, the total recovery of stearic acid-C^{14} in lymph (Experiment III) was 85% as much as that of the other three fatty acids. It should be noted that this conclusion would not be valid if absorption of the fed fatty acids had been complete, since complete absorption would necessarily give the same distribution of radioactivity in the triglycerides and the diet, regardless of any possible specificity of the triglyceride-forming mechanisms. Partial and variable absorption of the administered labeled fatty acids was observed in these experiments. The additional possibility that varying specificity occurred during the 24-hr collection period is most unlikely, since the data would require that the patterns of specificity during different parts of the collection period be exactly opposite. Information about the incorporation of fatty acids into different positions of the lymph triglycerides will be presented in a subsequent paper.

In Experiment I, the distribution of radioactivity in palmitic and linoleic acids in the cholesterol ester fraction did not significantly differ from the distribution in the diet. This suggested that the process of chylomicron cholesterol ester formation showed no specificity for either palmitic or linoleic acids during fat absorption. The results of Experiment II support this conclusion. Thus, the distribution of radioactivity in

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TABLE 7. THE AVERAGE VALUES FOR THE SPECIFIC RADIOACTIVITY OF EACH FATTY ACID IN EACH LIPID CLASS DIVIDED BY THE CORRESPONDING VALUE FOR THAT FATTY ACID IN THE DIET

	Relative Specific Radioactivity			
	16:0	18:0	18:1	18:2
Diet A + AC	1.00	—	1.00	1.00
Glycerides	0.55	—	1.07	0.26
Sterol Esters	0.34	—	1.07	0.20
Diet B + BC	1.00	—	1.00	1.00
Glycerides	0.35	—	0.33	0.89
Sterol Esters	0.17	—	0.31	0.85
Diet D	1.00	1.00	1.00	1.00
Glycerides	0.30	0.47	0.77	0.66
Sterol Esters	0.08	0.22	0.61	0.46
Diet DC	1.00	1.00	1.00	1.00
Glycerides	0.28	0.46	0.72	0.65
Sterol Esters	0.14	0.31	0.58	0.56

palmitic, oleic, and linoleic acids in diet A was in the ratio of 100:80:70; in the chylomicron glycerides recovered after this diet, it was 100:83:78 (average values, see Table 6); and in the sterol esters, 100:144:71 (Table 6). The similarity of glyceride and dietary distributions is obvious as is also the palmitic and linoleic relationship between diet and sterol ester. Labeled oleic acid, on the other hand, was relatively almost twice as abundant in the sterol esters as would be expected from the diet (144/80). The corresponding distribution in the three fatty acids of diet B was 100:75:75; the average values for the glycerides, 100:78:77; and sterol esters, 100:170:95. Again there was a disproportionate loading of the sterol ester with labeled oleic acid as compared with the available dietary supply (170/75), and with this linoleic-rich diet, there was a slight preferential incorporation of linoleic acid relative to palmitic acid.

The results of Experiment III, in which the rats were fed a high cholesterol diet for several weeks prior to the experiment, were similar to those of Experiment II, in showing a distinct relative specificity for oleic acid in chylomicron cholesterol ester formation. In addition to the more than twofold specificity for oleic acid (see Table 9), this experiment also demonstrated a slight preferential incorporation of stearic and linoleic acids, relative to palmitic, into cholesterol esters.

It is clear, therefore, that the over-all process of chylomicron cholesterol ester formation during fat absorption, in the intact rat, displayed a strong specificity for exogenous oleic acid compared to the other fatty acids studied. This relative specificity was almost equally apparent with all the rats, regardless of whether oleic or linoleic acid was predominant in the diet and whether or not cholesterol was included in the diet.

TABLE 8. THE DISTRIBUTION AND RECOVERY OF MASS AND RADIOACTIVITY IN THE FATTY ACIDS OF THE GLYCERIDE AND STEROL ESTER FRACTIONS OF THE "1.006 BOTTOM" OF COLLECTION 5 DC

Fraction	Total	Distribution						
		16:0	16:1	18:0	18:1	18:2	20:1	20:4
Mass	*mg*				%			
G	32.64	23.9	1.6	9.5	25.3	31.8	4.4	3.3
E	1.07	27.2	3.0	5.9	25.3	23.7	Tr	14.9
Radioactivity *cpm* $\times$ *10*$^{-3}$								
G	889.40	26.3	—	31.6	26.0	16.3	—	—
E	54.00	26.6	—	15.6	42.5	15.4	—	—

Addition of cholesterol to the test diet increased the amount of cholesterol esters found in the chylomicrons by several milligrams. Because the quantity of lipid in this fraction was limited in all of the experiments, addition of cholesterol to the test meal was a useful experimental maneuver. This addition caused no significant change in the apparent pattern of specificity. In addition, prior feeding of a high cholesterol diet for several weeks seemed to have very little effect (Experiment III compared to Experiment II). The percentage of the chylomicron fatty acids present in cholesterol esters was similar in both Experiments III and II, and similar patterns of specificities were observed in the two experiments.

The endogenous component of chylomicron lipid was surprisingly great. In Experiment III, an average of 43% of the chylomicron triglyceride came from a source within the animal, and the mass of chylomicron triglyceride of endogenous origin varied from 76 mg (collection 6D) to 155 mg (collection 5DC). This amount of endogenous lipid is vastly in excess of that observed with rats on fat-free diets. Thus, in separate experiments in which cannulated rats were fed 10% glucose in half-strength saline alone, the 24-hr lymph collections contained only 5–20 mg of total lipid, and only 10–20% of this was found in the chylomicron fraction. Furthermore, the data of Tables 4 and 5 indicate that there was a rough proportionality between the amounts of exogenous and endogenous lipid in the different collections. Thus, in every instance, the greater the amount of exogenous lipid (radioactivity) recovered, the greater

TABLE 9. THE RELATIVE SPECIFICITY OF INCORPORATION OF DIETARY FATTY ACIDS INTO CHYLOMICRON TRIGLYCERIDE AND STEROL ESTER*

	Triglyceride				Cholesterol Ester			
	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
Diet A + AC	1.00	—	1.03	1.11	1.00	—	1.81	1.02
Diet B + BC	1.00	—	1.04	1.03	1.00	—	2.27	1.27
Diet D + DC	1.00	0.81	1.05	1.02	1.00	1.30	2.35	1.43

* These are the average values after each diet, and the individual values being averaged were "weighted" in proportion to their mass.

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TABLE 10. The Average Distribution of Endogenous Fatty Acids Among the Different Fatty Acids of the Glyceride and Sterol Ester Fractions of Experiment III

	16:0	16:1	18:0	18:1	18:2	20:1	20:4
Glycerides	25.2	2.5	9.5	22.2	32.6	4.3	3.9
Sterol Esters	26.0	4.3	11.4	36.2	26.4	2.4	—

was the associated amount of endogenous lipid. The explanation for this phenomenon is not clear and it warrants further study.

The composition of the endogenous fatty acids incorporated into the glyceride and sterol ester fractions in Experiment III was calculated from the total minus the exogenous fatty acid mass and distribution. This pattern was the same whether or not cholesterol was added to the diet, and the average distributions are shown in Table 10. As with the exogenous fatty acids, oleic acid was prominent in the sterol esters. This suggests that most of the esters containing endogenous fatty acid were synthesized de novo, along with the incorporation of exogenous lipid, so that the same over-all specificities were operative. The origin of the endogenous fatty acids is not known; possible sources include circulating free fatty acids (27) and acids newly synthesized in the mucosa. Some endogenous fatty acids may have come to the chyle as preformed esters from the plasma, cell contents, or even from the bile and other secretions absorbed from the intestine. Endogenous dilution within the gut lumen probably also occurred to some extent.

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