Hepatic uptake of chylomicrons and triglyceride emulsions in rats fed diets of differing fat content

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Abstract The hepatic removal of plasma chylomicrons was determined for rats fed the following diets: *a*) containing no triglyceride, *b*) regular chow diet with 4.5% of its mass as lipid and, *c*) a corn oil-supplemented chow with triglyceride accounting for 20% of the mass. The fractional hepatic uptake of either radiolabeled chylomicrons or a triglyceride emulsion was reciprocally related to the amount of lipid in the diet. The animals receiving only carbohydrate and protein calories had the most active hepatic uptake of particulate triglyceride and were observed to have a significant decrease in the plasma concentration of the C apolipoproteins. The addition of either C-I, C-II, or C-III apoproteins to the triglyceride emulsion prior to intravenous injection produced a significantly lower hepatic triglyceride recovery of emulsions containing apoC-III. When the plasma of animals fed a fat-free diet was supplemented with human C-III-1 apolipoprotein, the distribution into the liver of either enterally administered fatty acid or parenteral triglyceride was diminished. The triglyceride content in the liver of the rats fed fat-free or corn oil-supplemented diets was significantly greater than that of the control rats and composition was somewhat similar to that of lymph triglyceride. The studies indicate an important influence of dietary lipid on both the partition of plasma triglyceride into the liver and the steady state hepatic triglyceride content.—**Kortz, W. J., B. D. Schirmer, C. M. Mansbach II, F. Shelburne, M. R. Toglia, and S. H. Quarfordt.** Hepatic uptake of chylomicrons and triglyceride emulsions in rats fed diets of differing fat content. *J. Lipid Res.* 1984. **25:** 799–804.

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The influence of C apoproteins on the hepatic uptake of triglyceride rich lipoproteins and emulsions has been documented in vitro by a number of laboratories (1–3). In recycling liver perfusions, all C apolipoproteins were observed to decrease the hepatic uptake of triglyceride-rich lipoproteins (2, 3). Single pass perfusions of the liver demonstrated significant inhibition for only the C-III apolipoproteins (1). The E apoprotein has been shown to increase the hepatic uptake of triglyceride emulsions and chylomicrons (1).

The in vivo significance of the C apoprotein inhibition of hepatic lipoprotein uptake is unclear at this time. The amount of these apoproteins in chylomicrons and chylomicron remnants (4) correlates well with the respective hepatic clearance (5) of both and suggests an important in vivo function in normal plasma triglyceride metabolism. A diminished content of these apoproteins may be influential in the enhanced hepatic uptake of triglyceride rich emulsions and lipoprotein by the fasting liver (6). The studies described here indicate a role for C apolipoproteins particularly apo-C-III in the differing hepatic uptake of both synthetic and natural triglyceride particles in rats on diets with and without lipid.

MATERIALS AND METHODS

Animals and diet

For all studies, male albino Sprague-Dawley rats weighing 200–500 g were employed. Animals were fed one of three diets. The first was regular Purina rat chow containing 4.5% triglyceride; the second a regular chow diet supplemented to a triglyceride level of 20% with corn oil; and the last, a fat-free diet containing sucrose and casein as calories (ICN Nutritional Biochemicals, Cleveland, OH). Rats on all three dietary regimens were monitored in terms of their calorie intake as well as their weight gain over the intervals they were fed the diet. Diets were usually administered for 2 weeks before the animals were studied. Experimental procedures on these animals were all begun between 10:00–11:00 AM after the diet had been withdrawn at 7:00 AM. Five minutes after administering 5 mg/100 g pentobarbital intraperitoneally an intravenous injection of 2 mg of either emulsion or chylomicron triglyceride was given by the way of the femoral vein. At appropriate time intervals (usually 30 min), the animals were exsanguinated by an aortic puncture and the liver was cleared of blood by a perfusion through the portal vein of 50 ml of saline. In studies evaluating the hepatic recovery of lipid when administered via an enteral route, the rats were given 5 μCi of

[9,10^{3}H]oleic acid (New England Nuclear, Boston, MA) in 1.2 g of triolein. The oil was given via gastric lavage or constant jejunal infusion. The absorption of the radiolabeled lipid was determined by subtracting the total radioactivity remaining in the entire intestinal succus from that administered. Infusions of human apoC-III into the rat were via femoral venous catheters at a rate of 1 ml of saline per hr containing 1 mg of apoC-III per ml.

Apoprotein, emulsion, and chylomicron isolation

Human C-I, C-II, and C-III apolipoproteins were prepared from normal humans or patients with hyperprebetalipoproteinemia by previously published methodology (7). The purity of the preparation was defined by polyacrylamide gel electrophoresis in both sodium dodecyl sulfate (8) and urea (9) systems. Rat chylomicrons were obtained by cannulation of a mesenteric lymphatic by an established method (10) after intestinal infusion of 20 μCi of [U-^{14}C]glycerol (9 mCi/mmol) and 2 μCi of [9,10-^{3}H]oleic acid (4 Ci/mmol) (New England Nuclear). The synthetic emulsion was prepared as published (1) with trioleoyl-[2-^{3}H]glycerol and tri-[1-^{14}C]oleoylglycerol (Amersham, Arlington Heights, IL). The chylomicrons and emulsions were purified by multiple centrifugations at 0.8×10^6 *g*-min. The incubation of a commercial triglyceride emulsion, Liposyn (Abbott Labs, Chicago, IL), with rat plasma, to determine the association of plasma apolipoproteins with the emulsion, was performed by the method of Robinson and Quarfordt (11).

Chemical determination

The plasma and hepatic homogenates were extracted both by the procedures of Dole (12) and/or Folch, Lees, and Sloane Stanley (13). Radioactivity was assayed in an Intertechnique liquid scintillation spectrophotometer (Fairfield, NJ). Quenching corrections were made by additions of appropriate internal standards. The mass of triglyceride was assayed by a standard spectrofluorometric technique (14). Triglyceride was isolated from both the chylomicron and the liver extract by thin-layer chromatography on plates developed in diethyl ether–petroleum ether–glacial acetic acid 80:20:1. Greater than 90% of both radiolabels in the hepatic lipid extract were in triglyceride 30 min after the emulsion was injected. Triglyceride was transmethylated by established methods (15). The methyl esters of triglyceride fatty acids were quantitated on a Packard (Des Plains, IL) gas–liquid chromatograph model #417 employing a 10% EGSSX column and integrating the effluent peaks and known fatty acid standards.

Apoprotein content of plasma lipoproteins was qualitatively assayed using both SDS (8) and urea (9) polyacrylamide electrophoresis. The gels were stained with Coomassie blue. Molecular sieve chromatograph of total apolipoproteins obtained from equal amounts of pooled plasma from rats fed fat-free and fat-supplemented diets was performed by a standard method (7). The C apolipoprotein peak was recovered, assayed for protein (16), electrophoresed in a urea system (9), and scanned by an LKB Laser model 2202 densitometer (Bromma, Sweden).

RESULTS

The hepatic recovery of endogenously labeled chylomicron triglyceride after an intravenous injection differed and depended on the diets ingested over the preceding 2 weeks (**Table 1**). Animals on a fat-free diet demonstrated significantly more labeled triglyceride glycerol and fatty acid in the liver than animals fed the regular or high-fat diets. The hepatic recoveries observed for the regular chow and high-fat animals were similar. When a synthetic double-labeled emulsion was employed rather than chylomicrons, significantly more triglyceride glycerol and fatty acid radiolabel was recovered in the liver of the animal eating regular chow compared to the animal supplemented with corn oil (**Table 2**). The rats fed a fat-free diet had the most avid hepatic uptake of radiolabeled triglyceride of all the dietary regimens. Significantly more emulsion triglyceride was recovered in the liver of rats eating no fat than those on regular chow or fat-supplemented diets. The greater radiolabeled fatty acid than glycerol recovery in hepatic lipid is possibly a reflection of hepatic lipolysis of chylomicron and emulsion triglyceride. The mean ± SE plasma triglyceride was variable for each dietary group, but not significantly different for any of the groups (fat-free, 106 ± 25; regular, 85 ± 22; high-fat, 114 ± 34 ml/dl; n = 5). The endogenous dilution of the intravenous triglyceride bolus was no different for the three groups.

TABLE 1. Recovery of [^{14}C]glycerol-[^{3}H]triolein-labeled chylomicrons in the hepatic lipids of rats fed diets with differing triglyceride content[a]

Diet	^{14}C	^{3}H
	% injected activity/g liver	
High-fat (5)[b]	0.35 (0.07)[c]	0.55 (0.3)[c]
Regular (9)	0.39 (0.02)	0.58 (0.09)
Fat-free (9)	0.56 (0.05)[d]	0.89 (0.06)[e]

[a] The hepatic recoveries were obtained 30 min after the intravenous injection of 2 mg of the double-labeled chylomicrons. Animals were maintained on the diet for 2 weeks prior to the injection.
[b] Number of animals is in the parentheses.
[c] Standard error of the mean is in parentheses.
[d] Significantly different from high-fat and regular diets at $P < 0.05$ and $P < 0.01$, respectively.
[e] Significantly different from high-fat and regular diets at $P < 0.001$ and $P < 0.02$, respectively.

TABLE 2. Recovery of a [^{3}H]glycerol-[^{14}C]triolein-labeled emulsion in the hepatic lipids of rats fed diets with differing triglyceride content[a]

Diet	^{3}H	^{14}C
	% injected activity/g liver	
High-fat (5)[b]	0.46 (0.03)[c]	1.03 (0.06)[c]
Regular (5)	0.63 (0.07)[d]	2.02 (0.19)[e,f]
Fat-free (5)	1.51 (0.31)	3.33 (0.39)

[a] The hepatic recoveries were obtained 30 min after the intravenous injection of 2 mg of a double-labeled triolein emulsion. Animals were maintained on the diet for 2 weeks prior to the injection.
[b] Number of animals is in parentheses.
[c] Standard error of the mean is in parentheses.
[d] Significantly different from high-fat and fat-free at $P < 0.05$.
[e] Significantly different from fat-free at $P < 0.02$.
[f] Significantly different from high-fat at $P < 0.001$.

When littermates were begun on diets of high fat, regular chow, and no fat and evaluated after 2 weeks, significant differences in weight gains were appreciated. Despite eating more calories per hundred grams of weight (fat-free 38, regular 27) those rats on the fat-free diet demonstrated significantly ($P < 0.001$, n = 10) less weight gain over the 2-week period (regular, 134 ± 9.2; fat-free, 78 ± 5.5 g). The animals on the high-fat diet had weight gains comparable to those on regular chow.

The fat-free and high-fat diets resulted in hepatic triglyceride contents significantly greater than those observed for the regular chow animals (**Table 3**). The fatty acid patterns of triglyceride recovered in the livers of animals fed the various diets were similar to the lymph chylomicron triglyceride of these animals (Table 3), aside from the higher content of stearic acid in the lymph chylomicrons from animals fed the fat-free diet. Despite the

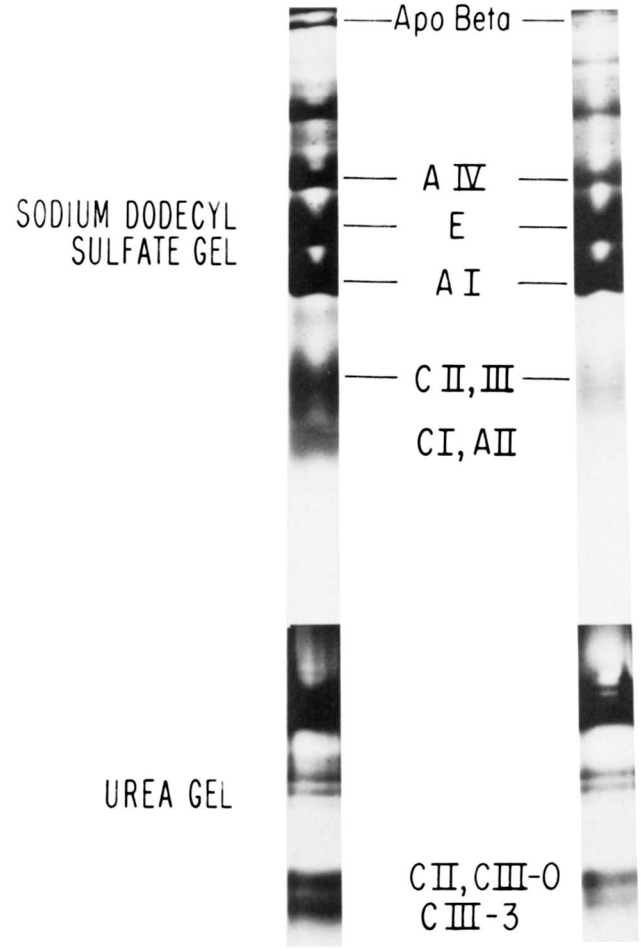

Fig. 1. The SDS-PAGE gel (top) and urea-PAGE (bottom) of delipidated d < 1.21 g/ml lipoproteins, reduced with mercaptoethanol, from a representative rat fed regular chow (left) or a fat-free diet (right), stained with Coomassie blue. The amount of apolipoprotein applied was from the same amount of plasma in each of the dietary states (equivalent to 0.1 ml). The characterized apolipoprotein bands are indicated.

TABLE 3. Hepatic triglyceride mass and fatty acid pattern in rats fed diets of differing triglyceride content[a]

Diet	Hepatic Triglyceride Content		Hepatic and Lymph Triglyceride Fatty Acid				
			16:0	16:1	18:0	18:1	18:2
	mg/g				*%*		
High-fat (5)[b]	26.4 (2.6)[c,d]	Liver	20.3	0.8	2.5	25.7	50.6
		Lymph chylomicron	12.2	1.1	2.8	29.0	52.9
Regular (5)	7.2 (0.8)	Liver	30.3	1.6	3.0	40.2	25.1
		Lymph chylomicron	24.0	1.2	3.6	45.0	26.0
Fat-free (5)	13.9 (2.4)[e]	Liver	35.9	8.7	3.1	41.9	10.4
		Lymph chylomicron	26.3	4.5	19.1	31.8	18.3

[a] The data are on littermates maintained for 2 weeks on the respective diets. Lymph collections were obtained after 10 days on the diet. The hepatic and lymph triglyceride fatty acid composition are the means of two determinations for each.
[b] Number of animals is in parentheses.
[c] Standard error of the mean is in parentheses.
[d] Significantly different from fat-free at $P < 0.01$.
[e] Significantly different from regular at $P < 0.05$.

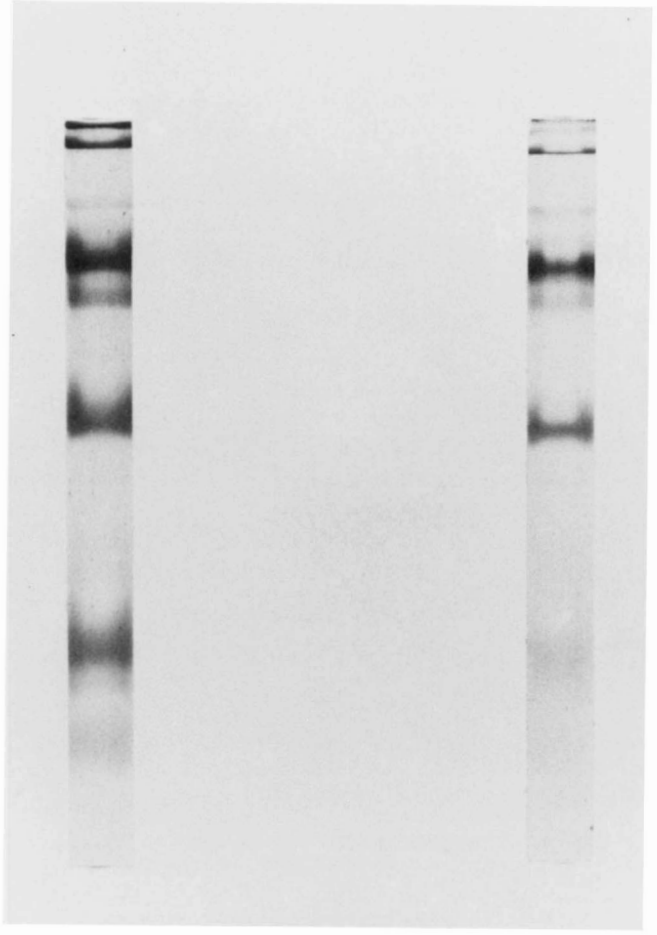

Fig. 2. The SDS-PAGE of the delipidated triglyceride-rich lipoproteins from rats fed fat-free or regular diets, run under the same conditions as described for Fig. 1. The major proteins observed (beginning at the largest) are apoB, an uncharacterized 65,000 dalton protein, apoE, apoC-III and C-II, and apoC-I. Right, fat-free diet; left, regular chow.

absence of triglyceride in the fat-free diet, intestinal lymph triglyceride output varied from 1.7 to 2.6 mg/hr.

The apolipoprotein composition of the plasma from rats fed a fat-free diet was appreciably different from that in rats on a regular or fat-supplemented diet, particularly with respect to C protein content (**Fig. 1**). The animals fed the high carbohydrate fat-free diet had considerably less apoC-II, C-III-0, and C-III-3 on plasma lipoproteins than did animals fed the regular chow. The triglyceride-rich lipoproteins of fat-free rats had less C protein (**Fig. 2**) than rats on regular chow. These qualitative impressions were supported by molecular sieve chromatography to isolate the total C and A-II apoproteins from equivalent plasma volumes of rats fed the fat-free and fat-supplemented diets (**Fig. 3**). The rats fed no fat had 9 μg of C and A-II protein/ml plasma by protein assay (16), whereas the fat-supplemented rats had 26 μg/ml. Laser densitometer scans of a urea gel of the isolated apoC, apoA-II protein fraction (Fig. 3) demonstrated 1.1×10^7 area

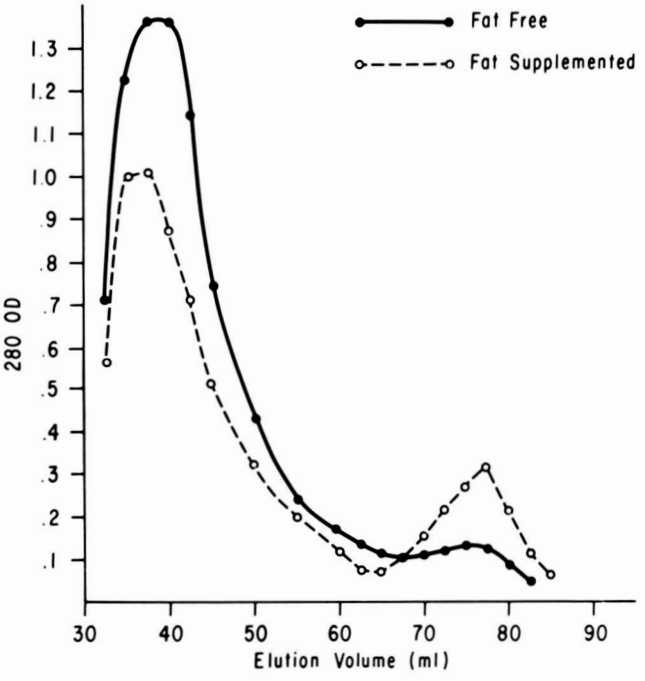

Fig. 3. The apoprotein elution of d < 1.21 g/ml lipoproteins from 20 ml of plasma obtained from three rats fed a fat-free diet and the same volume of plasma from three rats fed regular chow. The second peak in each contains C protein and A-II. The apoproteins were dissolved in 5 M urea, 0.1 M Tris, pH 8.2, and eluted from a 100 × 1 cm S200 Sephadex column with the same buffer.

units for C-III-3 from fat-free rats and 4.6×10^7 area units from fat-fed rats per ml of plasma. The A-II area unit was 0.9×10^7 for the fat-free and 1.2×10^7 for the fat-fed animal. The apolipoprotein pattern for rats fed fat-supplemented and regular chow diets did not differ significantly with respect to C apoprotein content.

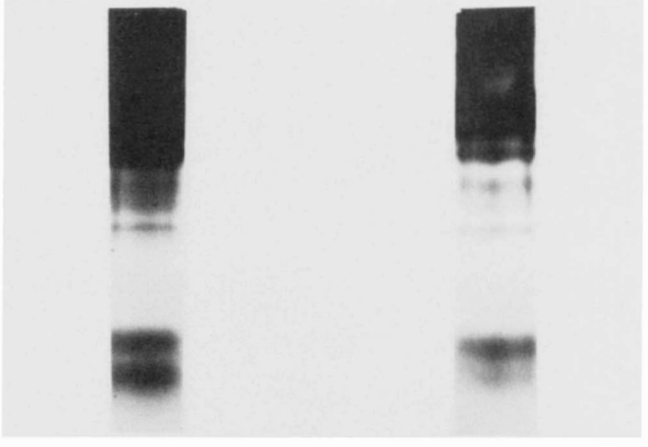

Fig. 4. The urea-PAGE gel of apoproteins recovered on the ultracentrifuged triglyceride emulsion after incubating 5 mg of Liposyn with 1 ml of plasma from a rat fed regular chow or a low-fat diet. The gels were overloaded with 120 μg of protein to easily identify the anionic C proteins. The two anionic bands are the same as those identified in the urea gel of Fig. 1.

TABLE 4. Plasma and hepatic recovery of a parenterally administered triglyceride emulsion in rats fed fat-free diets[a]

	% Injected Activity Recovered				
	Plasma		Liver		Hepatic Triglyceride Mass
	^{3}H	^{14}C	^{3}H	^{14}C	
					mg/g
Emulsion	47.5 (4.5)	50.5 (4.5)	10.7 (0.2)	18.7 (1.4)	9.6 (1.8)
Emulsion + C-III	85.3 (7.9)[b]	87.0 (8.5)[c]	6.2 (0.4)[d]	10.9 (1.3)[b]	4.5 (0.6)[e]

[a] Data are the means (±SE) of three rats in each group injected with 100 mg of [^{3}H]glycerol-[^{14}C]triolein-labeled emulsion. Recoveries were obtained 45 min after injection. Five mg of the C-III protein with the emulsion was injected at a time when the rat had been on the diet for 5 days.
[b] Significantly different from unsupplemented emulsion at $P < 0.01$.
[c] Significantly different from unsupplemented emulsion at $P < 0.02$.
[d] Significantly different from unsupplemented emulsion at $P < 0.001$.
[e] Significantly different from unsupplemented emulsion at $P < 0.05$.

When a synthetic triglyceride emulsion (Liposyn) was added to the same volume of plasma from rats fed fat-free or regular diets and subsequently recovered by centrifugation at 0.8×10^{6} *g*-min, the apoproteins on the emulsions were significantly different for the two dietary states (**Fig. 4**). Considerably less apoC-III-3 was present on the recovered emulsion from the plasma of rats fed no fat in comparison to rats on regular and high-fat diets. The apoprotein band containing the apoC-II and C-III-0 proteins (17) was also diminished.

The triglyceride emulsion was supplemented with human apoC-I, C-II, and C-III (200 μg of C protein per 2 mg of tri-[^{14}C]oleoylglycerol) prior to injection into rats fed a fat-free diet for 2 weeks and the hepatic ^{14}C-labeled triglyceride recovery after 30 min was compared to that of unsupplemented emulsions (control). The control had 2.73 ± 0.41%/g of liver; for apoC-I, recovery was 1.92 ± 0.46%; for C-II, 2.10 ± 0.31%; and for C-III, 1.44 ± 0.20% (n = 4 for each; control > C-III, $P < 0.05$).

Rat littermates fed a fat-free diet for 5 days were administered intravenously 5 mg of human apoC-III-1 along with a large bolus (100 mg) of double-labeled triolein (**Table 4**), and hepatic triglyceride mass and radioactivity recoveries were compared to rats receiving the emulsion alone. The animals supplemented with the heterologous apoprotein had less of both the [^{3}H]glycerol and the [^{14}C]-labeled fatty acid of the triglyceride in the liver and a lower hepatic triglyceride mass. The 50% of injected triglyceride ^{14}C recovered in control plasma after 45 min was probably due to the large mass (100 mg) injected. The plasma and total radiolabel recoveries were appreciably greater for the rats injected with human apoC-III-1. When the lipid was administered as an enteral bolus of [^{3}H]oleic acid in triolein, differences in hepatic recovery of the radiolabeled lipid in liver similar to those seen for parenteral emulsion injections (Table 2) were noted for the rats on regular chow and fat-free diets (**Table 5**). The rats on the fat-free diet absorbed less [^{3}H]oleic acid than the rats on regular chow and more of the absorbed lipid was recovered in the liver. Rats infused with apoC-III-1 had appreciably less radiolabel in the liver when compared to their respective controls in both dietary regimens.

TABLE 5. Hepatic recovery after the enteral administration of triolein containing [^{3}H]oleic acid in rats fed fat-free diets and regular chow[a]

	Regular Chow		Low-Fat Chow	
	Without ApoC-III (5)[b]	With ApoC-III (3)[b]	Without ApoC-III (5)[b]	With ApoC-III (3)[b]
			%	
[^{3}H]Oleic acid absorbed	91.7 (4.4)	94.2 (2.2)	68.5 (13.5)	70.5 (1.4)[c]
			%/g	
Absorbed [^{3}H]oleate recovered in liver	0.11 (0.02)	0.07 (0.02)	0.29 (0.06)[d]	0.15 (0.04)

[a] Each rat was given 1,200 mg of triolein containing [^{3}H]oleic acid by means of gastric intubation. The animals receiving apoC-III-1 were intravenously infused with 10 mg of human apoprotein over the 12–14-hr time interval. The absorbed fatty acid and hepatic lipid ^{3}H recoveries were determined after 12–14 hr. Means ± SE.
[b] Number of animals in parentheses.
[c] Significantly different from the respective regular chow value at $P < 0.001$.
[d] Significantly different from the respective regular chow value at $P < 0.02$.

DISCUSSION

The in vivo hepatic uptake of both chylomicrons and triglyceride emulsions was reciprocally related to the fat content in the diet prior to the time of study. Animals fed a fat-free diet demonstrated the most active uptake while those on a triglyceride-supplemented diet the least active. Rats with no triglyceride intake had a considerable decrease in the content of total plasma apolipoprotein C as well as in the amount of C apolipoprotein that transferred to an emulsion of a chylomicron on entry into plasma. The addition of human apoC-III-1 apoprotein to the plasma in the fat-free diet state substantially decreased the hepatic uptake of a parenterally administered triglyceride bolus and enteral fatty acid to uptakes near those of chow-fed animals who had greater plasma contents of C apolipoproteins. These data suggest that decreased plasma apolipoprotein C content in the fat-free state may be an important reason for the enhanced hepatic distribution of triglyceride entering plasma.

Although plasma C apoprotein content may be one important reason for the enhanced hepatic triglyceride partition in the fat-free diet state, this explanation is not tenable for the significantly lower triglyceride emulsion uptake of the rats receiving the corn oil-supplemented chow. The plasma lipoprotein apoC content of these animals was quite similar to those eating regular chow, yet the hepatic uptake of emulsion triglyceride was significantly less. The uptake of apoE-supplemented triglyceride emulsions by monolayer cultures of hepatocytes from rats on fat-free diets was no different (data not shown) than hepatocytes from fat-supplemented rats. The reason for the decreased fractional hepatic triglyceride uptake of corn oil-supplemented rats is due neither to the C apoprotein content of the plasma of these rats nor to their hepatocyte receptors, and remains unknown.

The reduction in plasma apolipoprotein C content observed in rats fed only carbohydrate and proteins is at present unexplained. From recent work (18) that demonstrates enhanced hepatic triglyceride-rich lipoprotein secretion in monolayer cultures of hepatocytes from rats fed a high carbohydrate diet, it would appear that the secretion of the apoprotein would, if anything, be enhanced. The kinetics of each of the C apolipoproteins is quite complex for both the human (19) and the rat. Protein turnover data to determine if the steady state decrease in these apolipoproteins in rats on no dietary fat is the result of altered body distribution, anabolism or catabolism are difficult to interpret but are currently being acquired.

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