Formation of Cholesteryl Ester-Rich Particulate Lipid during Metabolism of Chylomicrons

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ABSTRACT The metabolism of chylomicrons doubly labeled with cholesterol-^{3}H and triglyceride-^{14}C was studied in unanesthetized rats which were absorbing a fatty test meal. 10 min after intravenous injection, 80% of chylomicron cholesteryl ester, but only 20% of chylomicron triglyceride, was found in the liver. Treatment of recipient rats with puromycin to block hepatic triglyceride release did not increase the proportion of chylomicron triglyceride found in the liver.

Rapid partition of chylomicron triglyceride from cholesterol ester also occurred in rats in which the liver had been excluded from the circulation. However, now the cholesteryl ester accumulated in the plasma, whereas triglyceride was cleared by peripheral tissues. Residual labeled cholesterol in the plasma of such rats was shown to be present in particulate form, together with some residual triglyceride. The remnant particles contained about 13% cholesteryl ester. When injected into other rats the remnant particles appeared in the liver more rapidly than did chylomicrons.

These observations were consistent with the hypothesis that the first step in chylomicron metabolism occurred in extrahepatic tissues where a large portion of the triglyceride was removed by the action of lipoprotein lipase. The remnant particles so produced contained the chylomicron cholesteryl ester and residual triglyceride, and they were removed from the plasma by the liver.

INTRODUCTION

Newly absorbed dietary triglyceride and esterified cholesterol appear in intestinal lymph in chylomicrons which enter the bloodstream via the thoracic duct. The subsequent fate of these chylomicron constituents has been the subject of considerable investigation (for reviews see references 1–3). It has been established that both triglyceride and cholesteryl ester are removed from the blood very rapidly. Chylomicron cholesteryl ester is cleared almost exclusively into the liver (4, 5) whereas the liver accounts for only about 20–30% of chylomicron triglyceride removal (6–8), the remainder being cleared by extrahepatic tissues.

The mechanism whereby chylomicron cholesteryl ester becomes separated from most of the triglyceride has not been elucidated. Obviously two main possibilities exist. On the one hand the observed distribution could be reached if the liver processed chylomicrons in such a way as to remove the cholesteryl ester and some of the triglyceride, and then released cholesteryl ester–depleted particles back into the blood to be carried to peripheral tissues for final removal. On the other hand, if chylomicrons were initially processed in extrahepatic tissues where most of their triglyceride was removed, a cholesteryl ester–rich particle could be returned to the liver for final removal. The second possibility received support from the experiments of Nestel, Havel, and Bezman (9) who showed that in functionally hepatectomized dogs chylomicron triglyceride was cleared but cholesteryl ester remained in the blood. Their experiments suggested therefore that partition of these chylomicron components was accomplished in the extrahepatic tissues.

In this investigation the clearance of chylomicron components has been reexamined in functionally hepatectomized rats. The observations of Nestel et al. (9) have been confirmed in rats and the nature of the cholesteryl ester–rich particles which accumulate in the plasma has been studied. The uptake by the liver of these cholesteryl ester–rich "remnant" particles has been examined and a scheme for metabolism of chylomicrons has been proposed.

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Previous investigations of chylomicron clearance have used animals which were either fasted or carbohydrate-fed. Under such conditions chylomicron handling might not be representative of metabolism during normal absorption of a fatty meal and therefore in the present experiments clearance was studied in animals absorbing fat. Relatively small doses of chylomicrons were injected so that they would behave as a tracer of general chylomicron metabolism. Finally anesthesia was avoided because it severely impaired clearance of cholesteryl ester.

METHODS

Preparation of chylomicrons. The thoracic ducts of female 200–250 g rats (Holtzman) were cannulated by the method of Bollman, Cain, and Grindlay (10) and the animals were placed in restraining cages (11). 24 hr or 48 hr later the rats were given a test meal of 1–2 ml corn oil or olive oil containing 2% cholesterol, cholesterol-1,2-^{3}H (25–50 μCi),[1] and triolein-1-^{14}C (5–10 μCi).[1] Lymph samples were collected in plastic vessels in ice from about 2–8 hr after the test meal. No anticoagulant was used. Lymph was filtered through gauze to remove clots. Aliquots were layered under a discontinuous NaCl gradient (12) and then centrifuged at 63,581 *g* (average) for 68 min at 10°C in the SW 25.1 rotor of the Spinco model L ultracentrifuge.[2] The top layer of the gradient was 0.9% NaCl. The packed chylomicrons were dispersed by shaking vigorously in 0.9% NaCl to a final triglyceride concentration of 20–30 mg/ml. Contact with glass surfaces was avoided throughout. The washed chylomicrons were stored at 4°C for up to 72 hr. Analysis of chylomicron lipid extracts by thin-layer chromatography on silica gel showed 95% of ^{14}C label traveled with triglyceride, and 60% of ^{3}H label traveled with cholesteryl ester and the remainder with free cholesterol in the solvent system Skellysolve B, diethyl ether, acetic acid 80 : 20 : 1.

Distribution of chylomicrons. Under ether anesthesia a plastic cannula was placed in the right jugular vein of female rats weighing 200–250 g. The animals were placed in restraining cages and 0.2 ml/hr of 0.9% NaCl was infused steadily into the cannula to prevent clotting at the tip. Tap water was available to drink. The next day animals received 1 ml of 2% cholesterol in corn oil by stomach tube, avoiding anesthesia. Chylomicrons or serum was injected 1½–2 hr later in a volume of 0.3–0.5 ml followed by sufficient saline to clear the cannula. From 2.5–12.2 mg of chylomicron triglyceride were injected, the mean dose being 7.2 mg. 10 min later 0.2 ml of 6% sodium pentobarbital was given via the cannula and then the animals were exsanguinated as completely as possible via the abdominal aorta. The liver was rapidly removed without perfusion and washed under running water. Perfusion was avoided but, as plasma clearance in most cases was very rapid any corrections due to plasma trapped in the liver would be very small. The whole liver was extracted in 20 vol of chloroform-methanol 2 : 1 to avoid sampling difficulties which have been reported for rat liver (13). Aliquots of the extracts were washed (14). Plasma samples were similarly extracted and washed. Lipids were separated into cholesteryl esters, other neutral lipids and phospholipids on silicic acid-Super Cel[3] columns (15). All fractions were checked by thin-layer chromatography. Aliquots were dried under nitrogen and radioactivity was determined by liquid scintillation counting. All counting was performed at discriminator settings to exclude all ^{3}H disintegrations from the ^{14}C channel. Overlap of ^{14}C into the ^{3}H channel was determined by using standard curves constructed from samples of known quenching. Quenching was monitored by the external standard ratio (Packard Model 3375 Tri-Carb scintillation spectrometer)[4] and the method was checked frequently by adding internal standards to samples and recounting.

Some rats were killed 1 min after injection of chylomicrons or serum; a guillotine was used to minimize manipulative delays. Animals receiving puromycin[5] were given 2.5 mg intravenously in 0.04 M phosphate buffer, 0.15 M NaCl (pH 7.4) each hour for 4 hr before receiving chylomicrons.

Functionally hepatectomized animals. Functionally hepatectomized rats were prepared by two different techniques. One group of unfasted rats (250–350 g) was anesthetized with sodium pentobarbital (30 mg/kg body weight) and then the celiac axis, the superior mesenteric artery, and the portal vein were ligated. A dose of chylomicrons was injected intravenously and the animal was maintained under anesthesia for 30 min before a blood sample was taken from the abdominal aorta. Another group of rats was operated under ether anesthesia and the whole intestinal tract including spleen and pancreas was removed and the portal vein was ligated before the abdominal wall was sutured. A cannula was inserted into the right jugular vein and the animals were allowed to regain consciousness in restraining cages before chylomicrons were injected via the cannula. A solution of 5% glucose was infused intravenously at 1.0 ml/hr throughout the experimental period.

Blood samples were allowed to clot and then incubated at 37°C for 30 min for clot retraction. Serum was recovered by centrifugation, stored at 4°C, and used for injection studies or analyses within 24 hr.

Analytical. Lipids were extracted from most samples using chloroform–methanol as described above. Cholesterol (16), triglyceride (17), and lipid phosphorus (18) were determined on extracts or column eluates. For protein determinations (19) delipidation was achieved with ethanol–diethyl ether 3 : 1, and the protein was then redissolved in 1 N sodium hydroxide.

RESULTS

Unanesthetized rats which were actively absorbing lipid were injected with chylomicrons via a chronic right jugular cannula. Within 10 min of injection about 20% of labeled triglyceride was recovered in the liver (Table I). Variation in this value was small over a large number of rats. Under the conditions of these experiments metabolism of ^{14}C-labeled lipid was not sufficient to account for slightly lower hepatic recoveries of triglyceride than those which had been reported previously (6, 7) by other workers. From two rats 10 min after receiving chylomicrons, a blood sample was

[1] New England Nuclear Corp., Boston, Mass.

[2] Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.

[3] Johns-Manville Products Corp., New York.

[4] Packard Instrument Co., Downers Grove, Ill.

[5] Nutritional Biochemicals Corporation, Cleveland, Ohio.

TABLE I

*Hepatic Content of Chylomicron Triglyceride, Cholesteryl Ester, and Total Cholesterol**

Treatment	Triglyceride		Cholesteryl ester		Total cholesterol	
	% of injected dose	% of cleared dose	% of injected dose	% of cleared dose	% of injected dose	% of cleared dose
A. Unanesthetized controls	19.6 ±0.66 (10)	21.0 ±0.66 (9)	82.8 ±4.15 (6)	97.0 ±6.74 (5)	64.2 ±2.88 (5)	79.7 ±6.27 (4)
B. Anesthetized						
(i) Ether	‡13.7 ±1.24 (3)	19.1 (2)	—	—	§22.7 ±1.52 (3)	
(ii) Barbiturate	24.0 (2)	24.9 (2)	‡44.0 ±4.31 (5)	73.0 ±4.89 (4)	—	—
C. Puromycin	§14.0 ±0.39 (4)	14.2 ±0.42 (4)	70.4 (2)	85.1 (2)	66.8 ±3.09 (4)	79.0 ±4.39 (4)

* All observations made 10 min after intravenous injection of chylomicrons labeled with triglyceride-^{14}C and cholesterol-^{3}H. A plasma volume of 4.0 ml/100 g body weight was assumed in calculating what proportion of the dose cleared from the plasma was found in the liver. Results are mean ±SE, or mean. Numbers in parenthesis indicate number of observations.
‡ $P < 0.01$, comparison by t test with control values.
§ $P < 0.001$.

taken and the liver removed; then the carcasses were ground up and extracted in chloroform–methanol 2:1. Total recoveries were 96.5 and 100.6% of the injected dose of radioactivity.

The distribution of chylomicron cholesterol was quite different (Table I). 10 min after injection more than 80% of the dose of cholesteryl ester was found in the liver. By calculation this accounted for 97% of the amount which had left the plasma compartment. If chylomicron total cholesterol radioactivity was followed, 64% of the injected dose which represented 80% of the dose cleared from plasma, was recovered in the liver after 10 min. These lower values were interpreted to be caused by exchange of labeled free cholesterol from the chylomicrons with unlabeled cholesterol of plasma lipoproteins and cell membranes (20).

Under the same conditions the clearance of ^{32}P-labeled chylomicron phospholipid was studied. In a group of five rats 36.9 ±2.39 (SE)% of the injected dose of ^{32}P was recovered in the liver lipids after 10 min. The mechanism of phospholipid clearance was not studied further but it, too, is subject to rapid exchange (12, 21).

Anesthesia inhibited the clearance of the cholesterol moiety of injected chylomicrons (Table I). After ether only 23% of chylomicron total cholesterol was found in the liver and barbiturate anesthesia reduced the hepatic recovery of injected chylomicron cholesterol ester by half. On the other hand, the effect of anesthesia on triglyceride clearance by the liver was much less marked, although statistically significant for ether (Table I).

All experiments were carried out using washed chylomicrons, but similar results were obtained in two rats that were injected with whole lymph collected less than 30 min before at room temperature. It was therefore unlikely that the chylomicrons had been altered by the washing procedure or by storage at 4°C. Another group of rats was given washed chylomicrons collected from a lymph-cannulated animal which was absorbing triolein-^{14}C–labeled olive oil and only a tracer dose of cholesterol-^{3}H. The mass ratio of cholesteryl ester to triglyceride in these chylomicrons was 0.53% compared to 1.54% in animals absorbing olive oil plus 2% cholesterol. Again, within 10 min, 70% of the injected dose of cholesteryl-^{3}H ester was found in the liver, together with 19% of the triglyceride.

Puromycin blocks the release of triglyceride from the liver (22) and triglyceride accumulated in the livers of rats given puromycin under conditions similar to the present experiments (23). It seemed useful therefore to examine the fate of chylomicrons in rats treated with puromycin to see if a larger fraction of injected triglyceride would accumulate in the liver. However, the amount of triglyceride label recovered in the liver was moderately reduced (Table I), whereas the amount of chylomicron esterified or total cholesterol found in the liver was similar to that found in controls.

Chylomicrons were injected into functionally eviscerated rats which were maintained either inactive under barbiturate anesthesia or awake in restraining cages. Animals under barbiturate anesthesia were given doses of chylomicrons containing between 12 and 30 mg triglyceride. After a period of 30 min a considerable amount of the injected triglyceride had been cleared, but much more of the cholesteryl ester remained in the plasma (Table II). The mean ratio of recovery of the injected dose of triglyceride to that of the injected dose of cholesteryl ester was 0.22. Animals which were allowed to recover from anesthesia before administration of chylomicrons showed a lower ratio of recovery after 90 min, vis. 0.07 (Table II). Triglyceride was cleared very efficiently in these rats, although higher values seen in anesthetized rats might have been due in part to hemoconcentration. In addition, unanesthetized rats were able to clear a much

TABLE II

*Plasma Clearance of Chylomicrons in Functionally Eviscerated Rats**

Group	Plasma recovery % Injected dose/ml		Recovery ratio (triglyceride: cholesteryl ester)
	Cholesteryl ester	Triglyceride	
Anesthetized (9) (dose 12–30 mg)	10.7 ±1.30	2.6 ±0.81	0.22 ±0.06
Unanesthetized (4) (dose 48–145 mg)	6.6 ±0.17	0.5 ±0.1	0.07 ±0.01

* Chylomicrons were injected intravenously into animals in which the intestinal arteries and the portal vein had been ligated. Blood samples were taken after 30 min (anesthetized rats) or after 90 min (unanesthetized rats). The intestinal tract remained *in situ* in anesthetized rats, but was removed from unanesthetized animals.

larger dose of injected chylomicron lipid. The distribution of ^{14}C radioactivity in the plasma was examined by thin-layer chromatography in two samples and 69.3 and 74.0% were found in triglyceride, with most of the rest present in unesterified fatty acids. In two of the unanesthetized rats second doses of chylomicrons were given and then plasma was taken 60 or 90 min later. There was no evidence that the second dose was cleared less efficiently than the first. Thus removal of only 80–90% of the chylomicron triglyceride did not appear to be caused by saturation of the clearance mechanism under these conditions.

Chylomicron "remnants" in serums from functionally eviscerated rats were centrifuged in discontinuous sucrose density gradients by modifying slightly the method of Pinter and Zilversmit (24). Under conditions where 50% of chylomicrons reached the top of the gradient, less than 10% of remnant material had reached the top and more than 50% remained near the origin, thereby indicating a much smaller size of those particles (Fig. 1 A). When conditions were such that most of the remnant material was removed from the origin (Fig. 1 B), there was a fairly uniform distribution in the gradient, and no evidence of a discrete monodisperse particle was obtained. The median particle diameter was about 90 mμ for the remnants compared with about 200 mμ for chylomicrons. Confirmation of the flotation characteristics was obtained in discontinuous saline gradients, slightly modified from the method of Lossow, Lindgren, Murchio, Stevens, Jensen (25). Only about 5% of labeled remnant material floated at $S_f > 5000$, compared with 60% of chylomicron material (Table III). Most of the remnants which floated were recovered at $S_f > 400$. Analysis of this $S_f > 400$ material on two samples gave the following composition: triglyceride 79.0 and 78.7%; unesterified cholesterol 2.9 and 4.2%; cholesteryl ester (calculated as oleate) 12.3 and 13.4%; phospholipid 4.2 and 2.3%; protein 1.5 and 1.4%. On paper electrophoresis 55% of the cholesterol radioactivity was recovered at the origin and 20% with the β-globulin band with most of the remainder present as a streak between these areas.

When remnant-containing serum was injected into rats, in 10 min more than 90% of injected cholestryl ester and more than 50% of the injected labeled fatty acids were found in the liver (Table IV). 1 min after injection 40% of the injected remnant cholesteryl

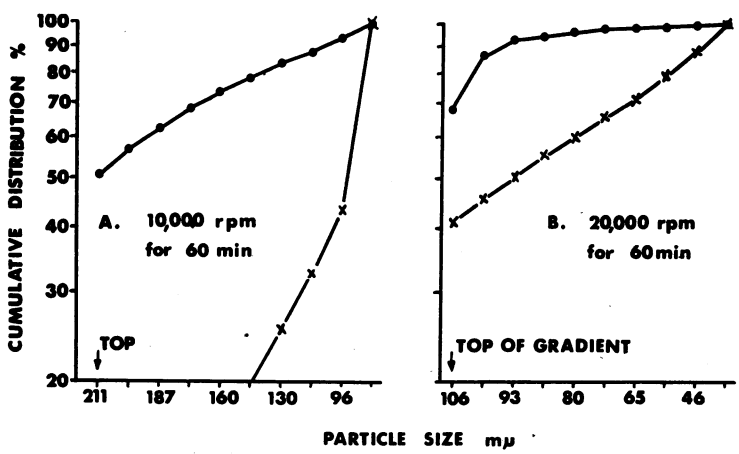

FIGURE 1 The size distribution of remnant particles compared with whole chylomicrons. Samples of washed chylomicrons or remnant-containing serum were centrifuged in sucrose gradients (24) in the SW 25.1 rotor of a Spinco preparative ultracentrifuge under the conditions indicated. Each point represents the mean of three to five observations. Total cholesterol-^3H activity was used to assess the distributions. ●——● chylomicrons, ×——× remnants.

ester and triglyceride was found in the liver, compared with only 17% of chylomicron cholesteryl ester at this time interval.

DISCUSSION

These experiments showed that chylomicron cholesteryl ester was rapidly partitioned from most of the chylomicron triglyceride. Within 10 min most of the cleared cholesteryl ester appeared in the liver, whereas only about 20% of chylomicron triglyceride was found in this organ. It must be stressed that these observations were made in rats that were actively absorbing cholesterol and fat, so the immediate fate of injected labeled chylomicrons might be representative of physiological conditions. Anesthesia was avoided, because it was shown that both ether and barbiturate impaired hepatic clearance of cholesteryl ester by the liver.

In functionally hepatectomized rats chylomicron cholesteryl ester was again rapidly partitioned from most of the chylomicron triglyceride. This partition caused accumulation of cholesteryl ester–rich remnant particles in the blood of these rats. Analysis of isolated remnant material showed that the particles contained about 79% triglyceride and about 13% cholesteryl ester which represent a mass ratio of cholesteryl ester to triglyceride of 16.5%. The mass ratio in injected chylomicrons was 1.54%. It can be calculated that removal of 90% of the triglyceride from the injected chylomicrons would result in the observed composition of the remnant particles. Because the particles were prepared in functionally hepatectomized animals, however, removal of triglyceride might have been more extensive than in the intact animal where partially degraded particles would be continuously removed by the liver. The median diameter of the remnant particles was about 90 mμ compared with about 200 mμ for chylomicrons. By calculation, such a reduction in median diameter would result from the removal of 90% of the chylomicron volume. This value is the same as that arrived at above from the change in chemical composition of the remnant particle.

TABLE III

*Flotation Characteristics of Remnant Particles**

	Recovery % of cholesterol-^{3}H—loaded			
	$S_f > 5000$	$S_f > 400$	$S_f > 100$	$S_f > 60$
Remnants (4)	4.9 ±1.08	49.3 ±5.16	7.7 ±0.37	7.7 ±2.72
Chylomicrons	61.3	36.4	0.7	1.3

* Serum from functionally eviscerated rats was centrifuged in salt gradients to float particles of the indicated S_f values to the tops of the tubes, essentially as described by Lossow et al. (25).

These observations were consistent with the hypothesis that the first step in chylomicron metabolism was lipolysis in the peripheral tissues. The remnant, from which a large portion of the triglyceride had been removed, consisted of triglyceride, free and ester cholesterol, phospholipid, and protein. Free cholesterol and phospholipid exchanged rapidly with plasma lipoproteins and cell membranes. The cholesteryl ester moiety, together with residual triglyceride, remained in the plasma until the remnants reached the liver, where the particles were removed.

It should be pointed out that the interpretation offered to explain the observations made in these experiments is not new. Nestel et al. (9) suggested that the liver might take up some intact chylomicrons and some which had been depleted of variable portion of their triglyceride. Goodman (2) proposed that shrunken chylomicrons containing cholesteryl ester might be re-

TABLE IV

Hepatic Clearance of Remnants and Chylomicrons*

Time after injection		Recovery in liver			
		Cholesteryl ester		Triglyceride	
		% of injected dose	% of cleared dose	% of injected dose	% of cleared dose
A. 1 min	Remnants	39.5 ±2.30 (3)	83.0 ±9.62 (3)	40.5 ±1.12 (3)	75.3 ±10.14 (3)
	Chylomicrons	‡16.8 ±3.78 (3)	39.5 ±13.22 (3)	‡16.0 ±3.70 (3)	36.7 ±13.68 (3)
B. 10 min	Remnants	91.5 ±2.29 (4)	—	53.3 ±1.73 (4)	—
	Chylomicrons	82.8 ±4.15 (10)	97.0 ±6.74 (9)	§19.6 ±0.66 (10)	21.0 ±0.66 (9)

* Serum obtained from donor functionally eviscerated rats was injected intravenously into rats which were actively absorbing a test meal of corn oil and cholesterol.
‡ $P < 0.01$.
§ $P < 0.001$.

turned to the liver after peripheral lipolysis. Also Schotz, Arnesjö, and Olivecrona (26) suggested that chylomicrons might be metabolized by the liver after a structural change induced by passage through extrahepatic tissues. By demonstrating the existence of a cholesteryl ester–rich remnant particle in the blood of functionally hepatectomized rats the present experiments provide strong evidence in favor of such a mechanism. Very rapid clearance of these remnant particles by the livers of other rats might indicate the reason why these particles have not been demonstrated previously under physiological conditions of fat absorption.

Further indirect support for the proposed mechanism was obtained in animals treated with puromycin, which blocks the release of hepatic triglyceride (22). When chylomicrons were injected into these rats cholesterol continued to accumulate in the liver but triglyceride label did not, showing that chylomicrons were not taken up by the liver intact, hydrolyzed and then depleted of cholesterol before resynthesis of triglyceride and reexcretion. In fact there was a little less triglyceride label in livers from these animals possibly because of other metabolic effects of puromycin.

Impairment of hepatic clearance of chylomicron cholesterol by anesthesia has previously obscured demonstration of rapid partition of cholesterol from most of the chylomicron triglyceride. For example Di Luzio and Riggi (27) found only 28% of chylomicron cholesterol in the livers of etherized rats 10 min after injection of chylomicrons. This compares with 23% found in the present experiments, which was much less than in unanesthetized animals (Table I).

The mechanism by which the remnant particles were taken up by the liver was not studied in these experiments. However as shown in Table IV after 1 min a large portion of the remnants was taken up by the liver with the same ratio of cholesteryl ester to triglyceride as the injected material. Because these animals were actively absorbing lipid the injected material behaved as a tracer and therefore it is unlikely that "trapping" of these particles in hepatic sinusoids could account fully for the observed distribution. However the presence of some labeled unesterified fatty acids in the injected material makes interpretation difficult. Further experiments will be necessary to establish whether the distribution indicates uptake of whole remnant particles, which would be compatible with the results of Olivecrona and Belfrage (8, 28) who found no change in the ratio of glycerol to fatty acid labels in chylomicron triglyceride taken up by the liver at short time intervals.

Friedman, Byers, and St. George (29) suggested that the Kupffer cells might be responsible for removal of chylomicron cholesterol. In view of the rapid appearance of remnants in the liver demonstrated by this study four rats were injected intravenously with labeled chylomicrons and 10 min later colloidal iron was also injected. 15 min later the livers were perfused and then Kupffer cells were separated from parenchymal cells as described by Ontko (30). The distribution of total cholesterol label in Kupffer cells compared with whole liver, compared per unit weight of protein, was 1.4 ± 0.11 (SE). Because the Kupffer cells comprise only about 10% of intracellular liver volume (31) it is clear that they were responsible for a relatively small proportion of hepatic cholesterol uptake, which supports the conclusions of Di Luzio and Riggi (27).

The in vitro perfused liver is able to take up triglyceride-labeled chylomicrons to the extent of 20–30% (32, 33). Chylomicron cholesteryl ester is taken up by the perfused liver to about the same extent (34, 35), i.e., much less than has been observed in vivo in the present experiments and by others (4, 5). While uptake of whole chylomicrons might account for the 20% triglyceride uptake seen in vivo, the present experiments suggest an alternative explanation. If the remnant particle containing cholesteryl ester contains also 20% residual triglyceride after peripheral lipolysis, then uptake of the remnants by the liver might account for the observed distribution. However, either uptake or trapping of some intact chylomicrons by the liver cannot be excluded completely.

Further experiments are needed to characterize more fully the short-lived cholesteryl ester–rich particles demonstrated in these experiments and to establish the mechanism of their uptake into the liver. The physiological significance of the process appears to involve not only chylomicrons produced during absorption of a cholesterol supplemented dietary fat load, but also chylomicrons from absorption of a test meal containing only a tracer dose of cholesterol.

The relevance of the process demonstrated by these experiments to the over all metabolism of cholesterol remains to be elucidated. However any deficiency in such a rapid removal process would be expected to lead very quickly to increased plasma cholesterol and might therefore be related to deposition of cholesterol in abnormal situations.

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