Effect of Apoproteins on Hepatic Uptake of Triglyceride Emulsions in the Rat

FRANK SHELBURNE, JOHN HANKS, WILLIAM MEYERS, and STEVEN QUARFORDT,
Cooperative Lipid Laboratory, Durham Veterans Administration Medical Center; and Department of Surgery, Duke University Medical Center, Durham, North Carolina 27705

ABSTRACT The addition of apoprotein E isolated from human very low density lipoproteins to both rat lymph chylomicrons and a triglyceride emulsion significantly increased the hepatic uptake of these particles in a nonrecycling isolated rat liver perfusion system. The cleared triglyceride was removed without apparent hydrolysis by the hepatocyte. When lymph chylomicrons were loaded with both Apo E and Apo C proteins by exposure to rat plasma, no increment in hepatic clearance was observed. Sequential evaluations of the influence of the C apoproteins on the hepatic clearance of both emulsions and chylomicrons revealed that the CIII (CIII-1) protein had a pronounced inhibitory effect on hepatic removal. The inhibition was observed for both Apo E-enriched chylomicrons and those containing little of this apoprotein.

INTRODUCTION

Unmetabolized triglyceride-rich chylomicrons appear to be poorly removed by the liver (1). The bulk of the triglyceride in the particle is distributed to peripheral tissues and the cholesterol that remains after this metabolism returns to the liver. When the liver is directly perfused with either lymph- or plasma-exposed chylomicrons, some observers (1) have noted virtually no removal of the particles whereas others have seen some uptake (2, 3). However, after the lymph chylomicrons are metabolized by peripheral tissues, the liver appears to actively clear the remnants (4). It has been speculated (5) that the metabolized chylomicron contains some lipoprotein lipase which, in turn, changes its affinity for the hepatocyte. However, there is currently no mechanism to explain why the lymph- or plasma-exposed chylomicrons enter the liver, whereas the remnant chylomicron is briskly removed (6). The remnant differs from the native chylomicron not only in its relative enrichment in sterol ester and depletion in triglyceride, but its apoprotein content is also quite different (7). There appears to be relatively more apoprotein E in remnants. The data presented here describe the influence of Apo E[1] and C proteins obtained from human triglyceride-rich lipoproteins on the hepatic uptake of both a synthetic triglyceride emulsion and the rat lymph chylomicron.

METHODS

Preparation of the chylomicron and synthetic emulsion: chylomicrons were obtained from a mesenteric lymph-fistulized Sprague-Dawley rat by a modification of the technique of Bollman et al. (8). Animals were fed a corn oil diet containing [2(*N*)-^{3}H]glyceryl trioleate and [1-^{14}C]oleic acid (Amersham/Searle Corp./Arlington Heights, Ill.) in a ratio of 5:1. The chyle was collected under EDTA (1 mg/ml) and the chylomicrons isolated by centrifugation (6×10^{5} g-min). A synthetic emulsion was prepared from a mixture containing 200 parts of triolein, 50 parts of egg lecithin (Grand Island Biological Co., Grand Island, N. Y.) and 10 parts of free cholesterol. All the lipid components were chromatographically pure. [2(*N*)-^{3}H]glycerol trioleate and glycerol-tri[1-^{14}C]oleate (Amersham/Searle Corp.) in a ^{3}H:^{14}C ratio of 5:1 were incorporated into the lipid mixture. The lipids were dried down from anhydrous chloroform methanol 1:1 and hydrated with a Krebs-Ringer bicarbonate buffer (9) containing 3 g/dl of bovine albumin (Reheis Co., Inc., Phoenix, Ariz.) and 2% glycerin. The albumin was found to be free of apoproteins by immunochemical assay using antisera to the CI, CII, CIII, AI, and E obtained from rabbits in this laboratory. Sonication at 50 W was performed using a Branson sonifier (Branson Sonic Power Co., Danbury, Conn.) for 1.5 min under nitrogen at 4°C. The resulting emulsion was maintained at 4°C for 2 h and the unemulsified lipid floated up and decanted. The infranate or stable emulsion was applied to a 1×40-cm Bio-Rad 1% Agarose column (Bio-Rad Laboratories, Richmond, Calif.) and eluted overnight with a 2-mM phosphate (pH 7.4), 2% glycerin buffer. The lipid dispersions recovered at the void volume and penetrating the gel were com-

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[1] *Abbreviations used in this paper*: Apo C and E, apoprotein C, E; SDS, sodium dodecyl sulfate.

bined. Intralipid that had been freed of the infranatant lecithin mesophase (10) was added to the resulting column fractions to obtain a stock emulsion of 2 mg of triglyceride/ml of buffer. Both the chylomicrons and the synthetic emulsions were centrifuged at 6×10^5 *g*-min before incubations with various apoproteins.

Apoprotein preparation. Human very low density lipoprotein was obtained from fasting humans and patients with hyperprebetalipoproteinemia (Type IV) and isolated from plasma by a centrifugation at 1×10^8 *g*-min in a Beckman model L5-65 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) using a 50 rotor. The lipoproteins were repurified by a second centrifugation, lyophilized and delipidated with 50 vol of 3/1 ethanol ether/vol of lipoprotein. The resulting apoproteins were dissolved in a 5-M urea, 2-mM phosphate buffer at pH 7.4 and applied to a heparin affinity column as previously described (11) to isolate Apo E protein. The unbound apoproteins were separated from the Apo E protein and put on a S-200 Sephacryl (Pharmacia Fine Chemicals, Div. Pharmacia, Inc., Piscataway, N. J.) column, and subsequently the C proteins were put on DEAE Sephadex (Pharmacia Fine Chemicals, Inc.) as described by Shore and Shore (12) to isolate the individual apoproteins. The isolated apoproteins were assayed for purity by sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis using a 10% gel exactly as previously described (11, 13). Urea polyacrylamide gel electrophoresis was used to assure purity of the C proteins (14). Only those proteins having <5% contaminant, when evaluated by densitometry of the stained gel, were used in these studies. The isolated Apo E protein and the CIII-1 protein were iodinated by the iodine monochloride method previously described (11). The specific activity of these purified apoproteins was established by a SDS polyacrylamide electrophoresis, a Lowry protein determination (15) and radioactivity assay (Beckman Biogamma, Beckman Instruments, Inc.). These two labeled apoproteins were used to more sensitively quantitate the apoprotein transfers onto chylomicrons or emulsion after incubation.

Incubations of apoproteins with triglyceride emulsions. The isolated Apo E, CI, CII, and CIII proteins were incubated either singly or in pairs with the synthetic emulsions and chylomicrons. The incubations were performed in either 5 ml of 2 mM (pH 7.4) phosphate buffer containing 0.12 M sodium chloride or in the Krebs-Ringer bicarbonate buffer used for the liver perfusions. To incorporate ~15 μg of Apo E protein on 1 mg of emulsion triglyceride, 50 μg was added to the incubation. For a similar incorporation onto chylomicrons, 100 μg was necessary. The C proteins were incubated at twice the concentration of the E protein except in instances where other proportions were used. Incubations were performed in a metabolic shaker at 37° for 30 min. The incubation mixture was then centrifuged under the conditions described previously to remove unincorporated-protein. The supernate was removed from the unincorporated protein and made to a volume of 12 ml in either Krebs-Ringer bicarbonate buffer containing 3 g bovine albumin/100 ml or saline. 10 ml of this solution was perfused.

The influence of the apoprotein incubations on the emulsion characteristics was evaluated by sucrose density gradient centrifugation (16). The lipid dispersions were also subjected to agarose electrophoresis by a standard method (17). The phospholipid and triglyceride contents of the pre- and postincubation emulsion and chylomicrons were determined by standard methods (18, 19). Heparin affinity was evaluated with heparin Sepharose (11) by a batch approach. This was done by incubating an aliquot of the radioactive triglyceride dispersion with heparin Sepharose for 15 min and assaying samples before and after the heparin Sepharose had been spun down at 2,000 rpm for 10 min. Radioactivity assays on the two samples enabled a determination of the amount of emulsion or chylomicrons bound to heparin.

Liver perfusion. The animals used in these studies were male Sprague Dawley rats weighing between 350 and 450 g. The animals were permitted access to normal rat chow on the evening before the experiment and the perfusions were usually performed at noon. Perfusions were performed in a nonrecycling fashion essentially by the method of Mortimore (20). The perfusate was Krebs-Ringer bicarbonate buffer prepared so that the calcium concentration varied between 8.5 and 10 mg/dl, and contained 3 g of bovine albumin/100 ml free of all C and E apoproteins when assayed by Ochterlony immunodiffusion, using antisera prepared in rabbits to each apoprotein, and by SDS polyacrylamide gel electrophoresis (13). Oxygenation of the rotating reservoir containing 250 ml buffer was produced with 95% O_2, 5% CO_2. The rat liver was perfused by initially cannulating the portal vein, ligating the hepatic artery and promptly cannulating the vena cava. Approximately 100 ml of washout was put through the liver before the emulsion was infused. It was determined that <50 cm^3 was actually necessary to free the effluent perfusate of any residual apoprotein. After the 100-ml washout was completed, the triglyceride emulsion (2 mg/10 ml) was infused at a constant rate for 5 min. The flow rates averaged between 1.0 and 1.2 ml/g per min. Bile volumes during the 20 min of these perfusions averaged 0.3 ml. The serum glutamate pyruvate transaminase contents of the effluent were low and the histology was normal. The nonrecycling perfusate was collected at 1-min intervals and after the infusion was terminated a 50-ml washout was performed. No further activity was eluted from the hepatic bed after this volume of washout. The effluent perfusate was immersed in ice and the liver was weighed and also placed in ice within 1 min after the discontinuation of the perfusion. An aliquot of some of the livers from these studies was frozen or formalin-fixed for radioautography. The radioautography was performed by a standard approach (21).

Chemical isolation. 4 ml of the effluent perfusate and 1ml of the injection solution were extracted by the Dole method (22). An aliquot of these lipid extracts was counted in a liquid scintillation spectrometer (Intertechnique model SL-4000) under double labeled conditions. Another aliquot of the heptane phase was chromatographed on a neutral lipid thin layer system and each lipid band assayed for radioactivity. After it was established that >85% of both labels was in the triglyceride band, the extract was used without chromatography. After the perfusion washout, the livers were weighed and homogenized and extracted by either a Folch (23) or a Dole (22) method. Aliquots of the lipid phase were chromatographed on a neutral lipid thin layer system to determine the distribution of lipid radioactivity. Because >90% of the hepatic radioactivity was triglyceride, the lipid phase was counted directly in the later experiments. Radioactivity was assayed under double labeled conditions and both ^3H and ^{14}C internal standards were added to the hepatic samples to correct for quenching.

RESULTS

The incubation of the ^{125}I-labeled E apoprotein with synthetic emulsions at concentrations <5% of the total lipid weight produced an E protein content of the emulsion ranging from 7 to 20 μg/mg total lipid. Chylomicron incubations with pure apo E protein at 10% of the total lipid content produced a similar mean increment in E

protein (12 μg/mg chylo lipid). This increment in protein after E apoprotein incubations produced no change in the chylomicron radioactivity distribution on a sucrose density gradient. The lipid content, electrophoretic mobility, and electron microscopic appearance of the recovered chylomicrons were no different for buffer and E protein incubations. Incubations of the synthetic emulsions with Apo E ratios higher than those used here (>7.5%) produced dramatic changes in emulsion appearance and properties,[2] but this was not seen at these lower concentrations.

When the E apoprotein was incubated with these emulsions together with individual C proteins at double the E concentration, a decrease in the amount of E protein on the recovered emulsion was noted. The CI protein incubations produced an E-protein content that was 83% of those incubations without C proteins. A decrease to 70% was noted for the CII protein and to 81% for the CIII-1. The estimated mean incorporations of these proteins on the emulsion when incubated at twice the E concentration were: CI, 40 μg/mg; CII, 26 μg/mg; CIII-1, 28 μg/mg total emulsion lipid. The same data for chylomicrons were: CI, 48 μ/mg; CII, 34 μg/mg; CIII-1, 38 μg/mg total chylomicron lipid.

The SDS polyacrylamide pattern for the synthetic emulsion showed selective increments of the Apo E and C proteins after incubation with these apoproteins (Fig. 1). The rat chylomicron apoprotein pattern similarly changed with these incubations. The added proteins were in much greater amounts than the endogenous AI and AIV proteins of the lymph chylomicron (3 μg/mg chylolipid). The amounts of chylomicron AI and AIV proteins did not appear to substantially differ after the protein additions but a definite statement on the contents of these proteins would require immunoassay. It is possible that significant changes in these proteins were produced by the incubations and were not detected by the SDS polyacrylamide method.

The incorporation of human Apo E protein on a synthetic emulsion produced a pronounced change in the clearance of the emulsion by a single pass through a rat liver. Both the triglyceride [^{3}H]glycerol and ^{14}C fatty-acid label recoveries in the lipid extract of the effluent perfusate were significantly less in the emulsions containing Apo E (Table I) than the controls. The recovery of the glyceride labels in the liver was threefold greater for the Apo E-exposed emulsion than the controls, both when expressed per gram or per total liver. The hepatic uptake of the glyceride labels was identical to the ratio of the injection mixture for

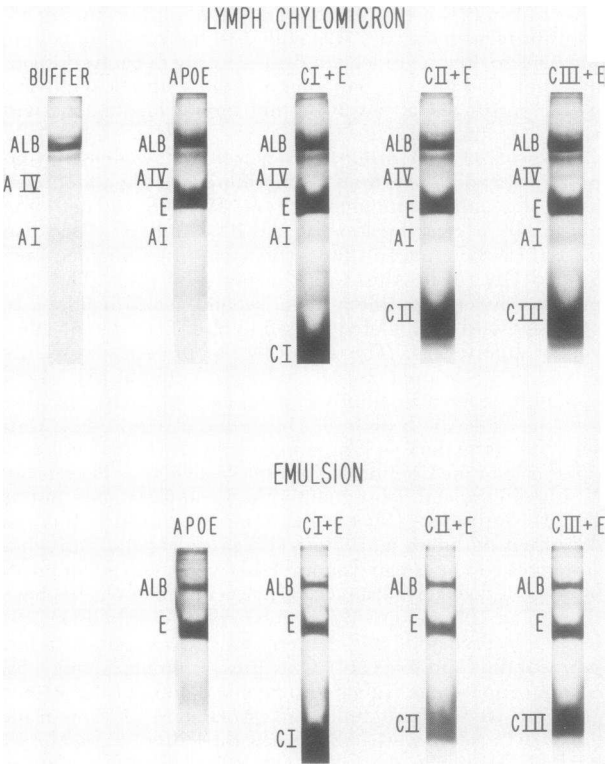

FIGURE 1 An SDS-10% polyacrylamide gel electrophoretic pattern of delipidated chylomicrons and emulsion after Krebs-Ringer bicarbonate buffer incubation; Apo E; CI and E; CII and E; CIII and E incubations. The protein applied to the gel in each case was that amount associated with 3 mg of either chylomicron or emulsion lipid. The respective protein bands are labeled including the bovine albumin from the buffer.

both the control and Apo E emulsions. Radioautography of a liver that had been perfused with an Apo E emulsion containing [^{3}H]glyceride demonstrated the activity to be predominantly in the hepatocytes (Fig. 2).

We have noted[2] that Apo E can change the sucrose density distribution of synthetic emulsions when high concentrations of apoprotein (>7.5% of the emulsion mass) are employed. To normalize the emulsions for any potential size effect, we harvested similar sized particles from the sucrose density gradients (24) of control and Apo E emulsions and investigated their hepatic uptake. The same differences were observed for the more homogeneously sized emulsions as were seen for the entire preparation.

When rat mesenteric lymph chylomicrons were incubated with the purified human Apo E protein, results quite similar to that observed for the in vitro prepared emulsion were observed (Table I). There was significantly less of the double-labeled triglyceride recovered in the lipid extracts of the effluent perfusate in the Apo E-exposed chylomicrons when com-

[2] Shelburne, F., and S. H. Quarfordt. The effect of Apoprotein E on the emulsion properties of triglyceride rich emulsions. Submitted for publication.

TABLE I

*Effect of E Protein on the Hepatic Uptake of Both Triglyceride Emulsion and Lymph Chylomicrons**

	Recovery of injected activity in the effluent perfusate		Recovery of injected activity in the liver			
			Per gram		Total	
	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
	%		%			
Triglyceride emulsion						
Control	71.7 (5.1)	81.7 (3.5)	0.69 (0.11)	0.79 (0.13)	7.1 (0.8)	7.1 (0.8)
Apo E incubated	56.2 (4.5)‡	57.8 (4.1)§	2.07 (0.21)‖	1.93 (0.17)‖	22.8 (2.7)‖	22.8 (2.7)‖
Chylomicrons						
Lymph chylomicron	92.1 (2.4)	95.5 (1.8)	0.52 (0.11)	0.57 (0.15)	5.1 (1.1)	5.4 (1.0)
Apo E-rich lymph chylomicron	54.5 (4.2)‖	61.5 (5.4)‖	2.52 (0.48)§	2.68 (0.46)§	29.6 (5.1)‖	30.2 (4.2)‖
Plasma-exposed chylomicrons	93.0 (2.0)	94.0 (2.0)	0.39 (0.10)	0.40 (0.10)	3.1 (0.3)	3.2 (0.3)

* The infused emulsion and chylomicrons contained a ^3H in the glycerol moiety and a ^{14}C in the fatty acid of the glyceride. Each datum represents the mean of nine determinations except for the plasma-exposed chylomicrons which represent five, with the standard error within parentheses. Each study was done at a 400-μg/min glyceride inflow as described in the text.

‡ Significant at the $P < 0.5$ level.

§ Significant at the $P < 0.01$ level.

‖ Significant at the $P < 0.001$ level.

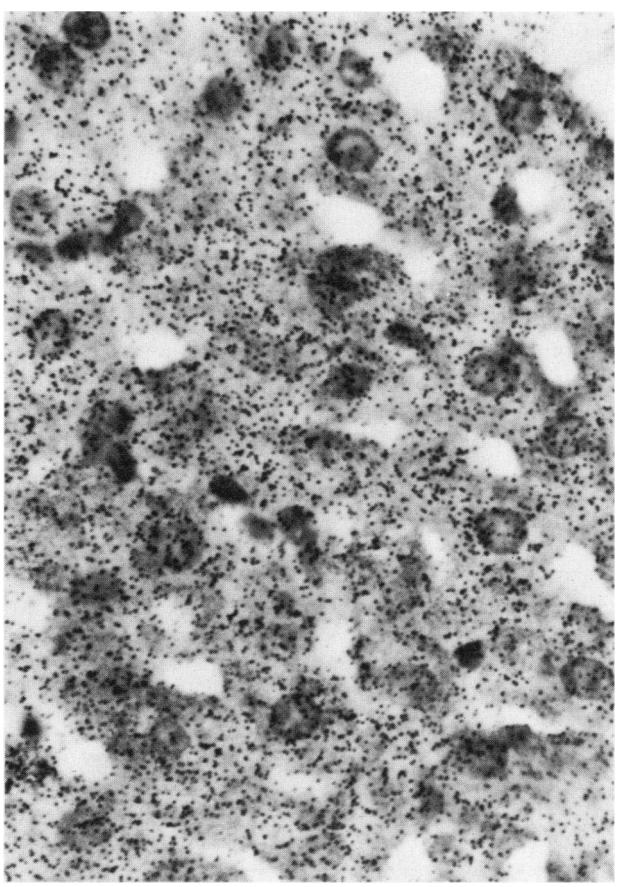

FIGURE 2 A radio-autograph of the frozen section of a liver perfused with a synthetic [^3H]triglyceride emulsion containing Apo E. The liver was processed by the method of Stein and Stein (21) are developed at a magnification of 400. The dark granules are the developed radioactivity.

pared with the control. A four- to fivefold greater recovery of the triglyceride labels was observed in those livers perfused with the Apo E enriched chylomicrons. Again, the labels were recovered in the same ratio as they had been injected, and >90% of the activity was in hepatic triglyceride, similar to the radioactivity distribution on the infused chylomicron.

When the chylomicrons enter plasma, it has been noted that they are enriched in Apo E as well as Apo C (25, 26). Plasma-exposed rat mesenteric lymph chylomicrons were also evaluated in terms of their clearance by the liver on a single pass perfusion at similar inflow rates (400 μg triglyceride/min). Rat plasma-incubated chylomicrons that demonstrated the previously described apoprotein alterations (25) revealed no significant differences (Table I) when compared with the control lymph chylomicron, despite a considerable enrichment in Apo E. These Apo E-enriched chylomicrons were also enriched in the C proteins, suggesting the possibility that one or a combination of the proteins modified the Apo E effect.

The three human C proteins CI, CII, and CIII were tested as to their influence on the hepatic removal of lipid emulsions. These emulsions and chylomicrons were exposed to 2:1 ratios of C:E in an attempt to approximate their relative contents in human very low density lipoprotein (27). The CI and CII proteins did not appear to affect the increased hepatic uptake of either the synthetic emulsion or chylomicron produced by the Apo E protein (Table II). The CIII protein that was pure CIII-1 by urea polyacrylamide gel electrophoresis (14) had a pronounced inhibitory effect on the hepatic uptake of both the synthetic emulsion and the lymph chylomicrons that had been loaded with Apo

TABLE II
*Hepatic Uptake of Chylomicrons and Triglyceride Emulsions Exposed to Apo E and C Proteins**

Apoprotein additions	Recovery of injected activity in the effluent perfusate		Recovery of injected activity in the liver	
	^3H	^{14}C	^3H	^{14}C
	%		%	
Apo E	58 (9)	62 (11)	24 (2)	27 (4)
Apo E and CI	55 (9)	59 (7)	27 (7)	28 (8)
Apo E and CII	64 (5)	69 (5)	22 (5)	21 (4)
Apo E and CIII	88 (4)	89 (5)	6 (3)	7 (3)

* The infused emulsions and chylomicrons contained a ^3H in the glycerol moiety and a ^{14}C in the fatty acid of the glyceride. Each datum represents the mean of four determinations; two with the emulsions and two with the chylomicrons. The standard errors are within the parentheses.

E protein (Table II). Further studies of the addition of CIII protein to lymph chylomicrons (Table III) revealed a marked inhibition of hepatic uptake of chylomicron glyceride. This was observed both in comparisons of Apo E-loaded chylomicrons with and without CIII, and in comparisons of the control chylomicron with the CIII-loaded particle (Table III). Although the CIII protein decreased the amount of Apo E on the emulsion when compared with the E-incubated dispersion alone, both CI and CII had similar if not greater effects on the emulsion content of Apo E, but neither significantly influenced hepatic uptake. None of the C apoproteins altered the lipid composition or gross physical characteristics of the E-loaded chylomicron or emulsion. Incubations of chylomicrons with CIII concentrations one half that of the Apo E still inhibited the hepatic removal of chylomicrons (Table IV) when compared with the Apo E-loaded particle, but not to the same extent as the higher concentrations.

DISCUSSION

After the in vivo injection of lymph chylomicrons in rats appreciable amounts of chylomicron triglyceride (~20%) and virtually all of the sterol was recovered in the liver (28). Most of the chylomicron triglyceride was distributed to peripheral tissues. Studies with double-labeled chylomicron triglyceride showed initial hepatic uptake of unhydrolyzed triglyceride whereas uptake in the periphery required hydrolysis (28). Several investigators (1) have suggested that no chylomicron triglyceride has direct access to the liver and only chylomicron triglyceride that has previously been metabolized at the periphery is subsequently recovered in the liver. Even though controversy surrounds the quantitative significance of the hepatic clearance of lymph chylomicrons, a consensus appears to exist on the ready hepatic uptake of chylomicron remnants (6, 29, 30). The remnant differs from the lymph chylomicron not only in its lesser content of triglyceride and increment in cholesterol ester, but its apoprotein pattern as well. The remnant is enriched in the Apo E protein (7) and probably has less C protein than does the lymph chylomicron that gains these proteins upon entry into plasma (26). The molecular basis for the apparently more avid hepatic uptake of the remnant than the lymph chylomicron is unknown.

It has been suggested that an interaction of a hepatic receptor with possibly the remnant E protein may facilitate the uptake of this particle (29). However, the lymph chylomicron entering the plasma becomes appreciably enriched in Apo E and if this were the major mechanism responsible for uptake, the triglyceride of these particles should be predominantly

TABLE III
*Hepatic Uptake of Apo E and CIII Containing Chylomicrons**

Chylomicrons	Recovery of injected activity in the effluent perfusate		Recovery of injected activity in the liver			
			Per gram		Total	
	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
	%		%			
Control lymph	87.8 (2.7)	95.2 (2.0)	0.76 (0.1)	0.89 (0.2)	8.5 (1.1)	9.7 (0.5)
Apo E	54.9 (2.8)‡	65 (5.4)‡	2.81 (0.5)§	3.11 (0.5)§	30.4 (5.3)§	33.6 (4.4)‡
Apo E and CIII	94.5 (2.8)	96.5 (1.7)	0.49 (0.06)	0.56 (0.06)	5.4 (0.9)	6.4 (1.0)
CIII	97.6 (1.5)	98.5 (0.9)	0.32 (0.09)‖	0.38 (0.12)	2.35 (0.5)‡	2.9 (0.6)‡

* The infused chylomicrons contained a ^3H in the glycerol moiety and a ^{14}C in the fatty acid of the glyceride injected at 400 μg/min. Each datum represents the mean of five determinations with the standard error within parentheses.
‡ Significantly different from control at $P < 0.001$.
§ Significantly different from control at $P < 0.01$.
‖ Significantly different from control at $P < 0.02$.

TABLE IV
*Effect of Differing CIII Contents on the Hepatic Uptake of Apo E Containing Chylomicrons**

Chylomicrons	Recovery of injected activity in the liver			
	Per gram		Total	
	^3H	^{14}C	^3H	^{14}C
	%			
Control lymph	0.59	0.74	7.4	9.2
Apo E	1.29	1.61	15.6	19.5
Apo E/CIII (2:1)‡	0.98	1.14	11.5	13.3
Apo E/CIII (1:1)	0.46	0.77	5.3	8.8
Apo E/CIII (1:2)	0.21	0.23	2.8	3.0

* The infused chylomicrons contained a ^3H in the glycerol moiety and a ^{14}C in the fatty acid in the glyceride injected at 400 μg/min.
‡ The ratio within the parenthesis is the relative amount of Apo E and CIII incubated with the lymph chylomicrons as described in the text.

distributed to the liver. The inhibition of hepatic uptake observed when CIII is added to either synthetic emulsions or lymph chylomicrons may explain the apparently more active hepatic uptake of remnant than unmetabolized plasma chylomicrons. The CIII protein that is added to the plasma-exposed lymph chylomicron along with the Apo E protein would act to prevent access of the particle to the liver. If the peripheral tissue (i.e., muscle, adipose, etc.) metabolism of chylomicrons reduced the CIII content along with the triglyceride, the resultant remnant would be more readily cleared by the liver. Although this sequence has no current experimental confirmation, the change in the apoprotein pattern of the chylomicron remnant (7) suggests that this event might occur.

The rat high density lipoprotein reservoir for Apo E protein appears to be a separate lipoprotein than for the C apoproteins (31). If the hepatic uptake of chylomicron lipid is importantly influenced by the content of these two apoproteins, the amount of the two high density lipoproteins may importantly influence the partition of the lipid between the liver and periphery. The triglyceride rich very low density lipoproteins emerging from the liver contain both E and C protein (32) with probable increments of both upon entering plasma. If CIII were not present on these E containing triglyceride-rich lipoproteins, an ineffectual recycling to the liver might result. The observation that at least two apoproteins control hepatic uptake provides a mechanism for denying these lipoproteins hepatic access until appropriate peripheral metabolism has occurred at which point retrieval occurs. These issues are presently speculative, and need further experimental development. The lack of accurate measurements of the lymph chylomicron apoproteins (AI and AIV) in the present study cloud the interpretation of the chylomicron data. A more sensitive assay system needs to be employed to evaluate the influence of these apoproteins on hepatic retrieval.

The ability of the E protein to increase the hepatic uptake of both the chylomicron and synthetic emulsion may be on the basis of the apoproteins' known affinity for glycosaminoglycans. Previous studies (11) of very low density lipoproteins containing considerable quantities of both Apo E and CIII reveal an intact heparin affinity. Preliminary data[2] have defined an influence of E protein on emulsion properties other than heparin affinity which CIII appears to counteract. However, despite the possibility of considerable glycosaminoglycan contents in the space of Disse (33) other mechanisms may determine the hepatic uptake of these lipoproteins. The previous work of Higgins (34) and these data also make it doubtful that an Apo E hepatocyte receptor similar to that on the fibroblast (35) mediates this enhancement.

Reasons for the inhibitory influence of the CIII protein (virtually all CIII-1) on the uptake of chylomicron lipid by the liver are under investigation. The state of sialylation of this protein may importantly govern its inhibitory potential. It is possible that this glycoprotein is similar to other sialylated plasma glycoproteins that demonstrate a negative correlation between neuraminic acid content and hepatic receptor affinity (36). It is possible that this inhibition of hepatic lipid uptake shown by Apo CIII is of clinical importance with regard to the partition of lipid into the liver. Many toxins, ethanol in particular, are associated with dramatic increments in hepatic triglyceride (37). If insufficient CIII were available in plasma or improperly distributed on lipoproteins, the inhibitory effect may be blunted and enhanced hepatic lipid deposits anticipated.

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