Differing Uptake of Emulsion Triglyceride by the Fed and Fasted Rat Liver

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ABSTRACT The recycling perfusion of a fasted rat liver with an apoprotein E-enriched synthetic triglyceride emulsion revealed a significantly greater hepatic uptake of both the apoprotein and the triglyceride than did the liver of a chow-fed animal. This greater hepatic triglyceride uptake by the perfused fasted liver in comparison to the fed was also noted for emulsions containing no added apoprotein or supplemented with both the E and CIII-1 proteins. However, no difference in the uptake of the triglyceride emulsion was seen for the fed and fasted livers when evaluated by a non-recycling single pass perfusion. The isolated hepatocyte plasma membranes from fasted rats failed to demonstrate enhanced binding of apoprotein or lipid when compared to those from fed animals. If the residual E loaded triglyceride emulsion was recovered from the recycling perfusates of fed and fasted livers and evaluated in a non-recycling single-pass system, the emulsion from the fasted perfusion was cleared as facilely as previously, whereas that from the fed was less actively cleared. The emulsions retrieved from the perfusion of the fed liver contained significantly more protein than did the fasted; in particular apo C. This apparent alteration of emulsion apoproteins by the fed liver possibly results in a less active hepatic retrieval and may be important in downregulating the entry of lipoprotein triglyceride in the postabsorptive liver.

INTRODUCTION

The fact that the dietary status of the rat profoundly influences the tissue distribution and metabolism of chylomicron triglyceride has been realized for many years (1). Carbohydrate fed animals appear to retrieve relatively less chylomicron triglyceride in their livers in comparison to fasted animals. This observation was originally suggested to be due to the less active peripheral tissue lipolytic removal of chylomicron triglyceride in the fasted state with the residual lipid subsequently retrieved by the liver. Although the in vivo quantitation of hepatic unhydrolyzed triglyceride uptake suggests that in some species (2) this is a minor pathway for the entry of chylomicron triglyceride fatty acid into the liver, this is probably not the case in the rat. The double-labeled chylomicron tryglyceride distribution data (3) and the relative triglyceride content of the chylomicron remnant of a rat (4) indicate that in this specie the hepatic uptake of unhydrolyzed triglyceride is of quantitative importance.

A recent study (5) of isolated in vitro perfused rat livers has noted that a greater partition of added emulsion triglyceride into the fasted liver was seen when compared to the isolated fed liver. This suggests a primary hepatic contribution for the less active hepatic uptake of chylomicron triglyceride fatty acid previously noted (1) for the postprandial rat. The current studies were performed to elucidate the hepatic mechanism for the differing partition of emulsion triglyceride into the liver in the fasted and fed state.

METHODS

Apoprotein preparation. Human very low density lipoprotein (VLDL)[1] was obtained from fasted normal humans and patients with hyper pre-β-lipoproteinemia (type IV) and isolated from plasma by an ultracentrifugation at 1×10^8 g-min in a Beckman model L5-65 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) using a 50 rotor. The VLDL were repurified by two more centrifugations, then lyophilized and delipidated with 50 vol of 3:1 ethanol ether per volume of lipoprotein. The thoroughly nitrogen-dried apoproteins were dissolved in 5 M urea, 2 mM phosphate buffer and applied to a heparin affinity colunm as previously described (6) for the isolation and purification of apo E protein. The unbound proteins were dialyzed and

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[1] *Abbreviations used in this paper:* VLDL, very low density lipoprotein.

sequentially applied to G-200 Sephadex and DEAE Sephadex (Pharmacia Fine Chemicals, Div. Pharmacia, Inc., Piscataway, NJ) as previously described (7). The purified apoproteins were evaluated by sodium dodecyl sulfate (8) and urea (9) polyacrylamide electrophoresis for purity. If any significant contaminant was observed, the proteins were reprocessed as described above for subsequent purification.

Preparation of synthetic emulsion. A mixture containing 200 parts of chromatographically pure Triolein (by weight), 50 parts of egg lecithin (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), and 10 parts of free cholesterol were used to prepare the synthetic emulsion. Additions of [2(*N*)-^{3}H]glycerol trioleate and glycerol-tri[1-^{14}C]oleate (Amersham Searle Corp., Arlington Heights, IL) in a $^3H/^{14}C$ ratio of 5:1 were made to the lipid mixture, and the mixture dried down from anhydrous chloroform methanol 1:1 to constant weight. The mixture was rehydrated with a Krebs-Ringer bicarbonate buffer containing 3 g/dl of bovine albumin (Reheis Co., Inc., Phoenix, AZ) and 2% glycerin. The albumin lot used was found to be free of CI, CII, CIII, and E apoproteins by urea and sodium dodecyl sulfate polyacrylamide gel electrophoresis, along with chromatography in 6 M guanidine hydrochloride. In those perfusions where the apoprotein content of the emulsion was evaluated, albumin was omitted. Sonication of the hydrated lipid mixture was performed at 50 W, using a Branson sonifier (Branson Sonic Power, Danbury, CT) for 10 min at 1-min intervals. The sonicated emulsion was maintained at 4°C for 2 h, so that the unemulsified lipid floated up and was decanted. The infranate or stable emulsion was centrifuged at 6×10^5 g-min after it had been added to 1 ml of Intralipid (Cutter Laboratories, Inc., Berkeley, CA) (10).

Incubations of apoprotein with the synthetic triglyceride emulsion. The isolated apo E and CIII-1 proteins were incubated either singly or as a pair with the synthetic emulsion. The incubations were performed in Krebs-Ringer bicarbonate buffer with or without albumin depending on whether residual emulsion apoprotein content was being determined. The same ratios of apo E and CIII protein and lipid were used as previously described (11). Incubation was performed in a metabolic shaker at 37°C for 15 min, and the mixture centrifuged at 6×10^5 g-min. The supernatant was removed from the unincorporated protein and subsequently used for perfusions.

Liver perfusion. Male Sprague-Dawley rats weighing between 400 and 600 g were used in these studies. The fed animals were maintained on a standard chow regimen and were permitted unlimited access to the chow until 30 min before the perfusion. Food was withdrawn from the fasted rats 36 h before the study. Perfusions were performed in the early or mid-mornings using an approach essentially as described by Mortimore (12). The perfusions were performed in either a recycling or a non-recycling fashion. The usual recycling perfusion lasted either 30 min or 1 h and used a Krebs-Ringer bicarbonate albumin buffer containing canine erythrocytes at a hematocrit of 10%. The erythrocytes were omitted from non-recycling perfusions and albumin from recycling perfusions where qualitative analysis of residual emulsion apoprotein was done. Oxygenation of the rotating reservoir that initially contained ~175 ml of perfusate was produced with 95% O_2, 5% CO_2. After the portal vein had been cannulated and the hepatic vein was also cannulated, the perfusate was run through in a non-recycling fashion so as to rid the liver of associated endogenous apolipoproteins. This was evaluated by radial immunodiffusion using antisera to rat E and AI apoproteins and was found to usually be completed with 75 ml of perfusate. After the washout phase, the perfusion was then established in a recycling mode, and the triglyceride emulsion (250–300 mg triglyceride) with appropriate apoproteins was added to the ~100 ml of remaining perfusate. The non-recycling hepatic perfusions were performed exactly as previously described (11). At the conclusion of each perfusion, the livers were flushed with 100–200 ml of 0.9% saline at 4°C.

Characteristics of residual perfusate emulsions. In experiments where the triglyceride emulsion was isolated after a 30-min perfusion, the entire perfusate was intially centrifuged to remove the erythrocytes and subsequently ultracentrifuged as described above, to recover the triglyceride emulsion. An aliquot of the isolated emulsion was extracted and assayed for radioactivity so as to reinject the same triglyceride loads for the single pass studies.

To characterize the apoprotein and lipid contents of the residual triglyceride emulsion after a 30-min hepatic perfusion, albumin was omitted from the perfusate and the emulsion was ultracentrifuged three times to minimize infranatant contamination. Total lipid mass was determined gravimetrically from the N_2 dried alcohol ether extracts and the individual lipids determined as described below; the protein content by either of two (13, 14) standard methods using a bovine albumin standard. The proteins were run in both urea and SDS polyacrylamide gels by standard methods (8, 9). To quantitate the C protein content of the residual emulsion ^{125}I human apo CIII-1 (<1 μg; >10,000 counts) was added to the emulsion apoproteins dissolved in 0.1% SDS. Conventional SDS-10% PAGE was run in tube gels and the C protein region defined by gamma counting a tube to which only labeled protein was added. This area was cut from the gels and electrodialyzed into treated cellulose dialysis tubing that retains proteins of >3,500 daltons. The resulting solution was depleted of residual SDS by cooling to 4°C or sequential addition of guanidine, then gamma counted and the protein assayed by the methods indicated above. Human apo CIII-1 was used as a standard with standard curves ranging from 2 to 30 μg. The determined protein was corrected for the recovery of radiolabel and expressed as micrograms per milligram emulsion lipid mass.

Membrane binding assay. Rat hepatocyte plasma membranes were prepared from fed and fasting animals by methods previously published (15, 16). The final pellets were frozen in liquid nitrogen and stored at −80°C. The membranes were assayed within one week by suspending in 50 mM NaCl, 1 mM $CaCl_2$, and 20 mM Tris-HCl, pH 7.5 using a 22 needle and sonication (Branson Instruments Co.). The double-labeled triglyceride emulsions supplemented with ^{125}I-apo E (~0.2–0.5% emulsion mass) that were used for the perfusions, were used for these binding assays. The assay was performed as previously described (15, 16) using ~100 μg of membrane protein and varying concentrations of apoprotein supplemented emulsion. The Ca^{++} dependent binding was calculated for both the ^{125}I and ^{14}C data by subtracting the binding in the presence of EDTA from the total binding. The only difference from the assays described (16, 17) was that the calf serum used to wash the pellet was supplemented with an unlabeled apo E-enriched triglyceride emulsion.

Chemical and radiochemical assay. Aliquots of the perfusate and the injected substrate were extracted by the Dole (17) procedure and fractions assayed for double label radioactivity and other fractions chromatographed on a neutral lipid thin-layer system (18). Triglyceride mass determinations were performed by a standard fluorometric method (19); cholesterol was determined by the Abell method (20) and phosphate as previously described (21). After the termination of either the recycling or non-recycling perfusate,

TABLE I

*Recovery of Double-labeled Triglyceride and ^{125}I Apoprotein E in the Liver and Perfusate of a Recycling Perfusion of Isolated Fasted and Fed Rat Livers**

	Percent administered activity in triglyceride			
	Perfusate		Liver	
	^{3}H	^{14}C	^{3}H	^{14}C
Fasted livers	27.8 (4.7)	33.7 (3.4)	40.5 (4.6)	44.5 (4.7)
Fed livers	53.0 (5.2)‡	55.5 (6.0)‡	21.6 (5.1)§	27.3 (5.6)§
	Percent administered ^{125}I activity			
Fasted livers	64.0 (2.7)		26.2 (1.9)	
Fed livers	79.2 (2.8)‡		13.8 (2.4)‡	

* The data represented the mean (SE) of seven studies of fasted and seven fed rats after a 30-min isolated hepatic perfusion with a [^{3}H]glycerol: [^{14}C]triolein triglyceride emulsion to which ^{125}I apo E protein was added. The mean weight of the fasted livers was 10.7 g and the fed 11.8 g.

‡ Significantly different from the fasted values at $P < 0.01$.

§ Significantly different from the fasted values at $P < 0.05$.

the livers were saline flushed and subsequently homogenized in perfusion buffer and extracted by the Dole procedure. Aliquots of the hepatic extract were directly radioassayed under double label conditions in an Intertechnique model SL4000 liquid scintillation counter (ICN Pharmaceuticals, Inc., Irvine, CA). Other aliquots were chromatographed on a neutral lipid thin-layer system. The perfusates and livers from studies using ^{125}I-labeled apoprotein E were assayed in a Biogamma counter (Beckman Instruments, Inc.). The apo E protein was iodinated with ^{125}I by the Bolton-Hunter technique (22) as described for this apoprotein (23). The hepatic lipid extracts were evaluated for quenching by the sequential additions of ^{3}H and ^{14}C internal standards and the appropriate corrections made.

RESULTS

The recycling perfusion of isolated fasted and fed rat livers with triglyceride emulsions containing the E apoprotein resulted in significantly different hepatic uptakes in the two dietary states (Table I). Significantly less double labeled triglyceride was recovered in the perfusates of the recycling fasted livers after 30 min, when compared to the fed. Greater hepatic recoveries of the radioactive triglyceride were reciprocally noted for the fasted livers. An even more significant difference was observed if the data was expressed per gram wet weight since the mass of the fasted livers were uniformly somewhat less than the fed. The mean triglyceride mass cleared in a net sense from the perfusate during the 30 min perfusion paralleled the radioactivity data (fasted: 132 mg of 210 mg of triglyceride added; fed: 72 of 206 mg added). It was not possible to confirm a similar mass radioactivity parallel for the liver. The preinfusion hepatic triglyceride content was significantly different between animals in the same dietary state and even between lobes of the same liver. This precluded a preinfusion estimate of hepatic triglyceride mass.

A small enrichment of the ^{14}C-fatty acid was appreciated in the triglyceride of both the liver and perfusate in each dietary state, indicating some reesterification. However, this process did not appear to significantly differ for the fasted and fed isolated perfused livers. Although somewhat more total triglyceride radioactivity was recovered in the fed state after 30 min this was not significantly different from the fasted. Thin-layer chromatography of the perfusate and hepatic lipid extracts revealed that in both dietary states, radioactivity in the free fatty acids was a relatively constant low amount (<5%) in the perfusate and at an even lower level (<3%) in the liver. Even less radioactivity was recovered in lipids other than triglyceride after the 30-min perfusion.

A similar dietary effect was noted on the partition between liver and perfusate of ^{125}I-apoprotein E, which had been added to the emulsion (Table I). Significantly less apoprotein was recovered in the perfusate and more in the liver of the fasted rat than of the fed. However, the relative hepatic clearance of the apoprotein from the perfusate was less than the lipid in both dietary states.

The dietary difference in hepatic lipid uptake was observed as well, using triglyceride emulsions with apoprotein supplements other than simply apoprotein E (Table II). These recycling perfusions were performed for 1 h and again demonstrated less hepatic

TABLE II

Recovery of a Double-labeled Triglyceride in Liver and perfusate in a 60-min Recycling Perfusion of Isolated Fed and Fasted Rat Livers

	Percent recovery in perfusate triglyceride		Percent recovery in hepatic triglyceride	
Apoproteins added	^{3}H	^{14}C	^{3}H	^{14}C
Fasted				
E (3)*	8.7	12.9	55.7	55.1
E + CIII-1 (2)	28.2	49.8	28.0	30.5
No apoproteins (2)	63.4	63.8	15.1	21.3
Fed				
E (3)	28.2	32.3	34.6	38.4
E + CIII-1 (2)	46.2	72.8	10.7	16.6
No apoprotein (2)	80.2	76.1	11.2	15.4

* The figure within the parenthesis represents the number of studies done using the respective apoprotein and dietary state with the same conditions as Table I except that the perfusion was continued for 60 min.

uptake in the fed state employing emulsions supplemented with both apo E and CIII-1 proteins in the proportions previously studied (11) as well as emulsions containing no added apoprotein. The emulsion supplemented with both E and CIII-1 proteins revealed a considerable difference between the ^{14}C and

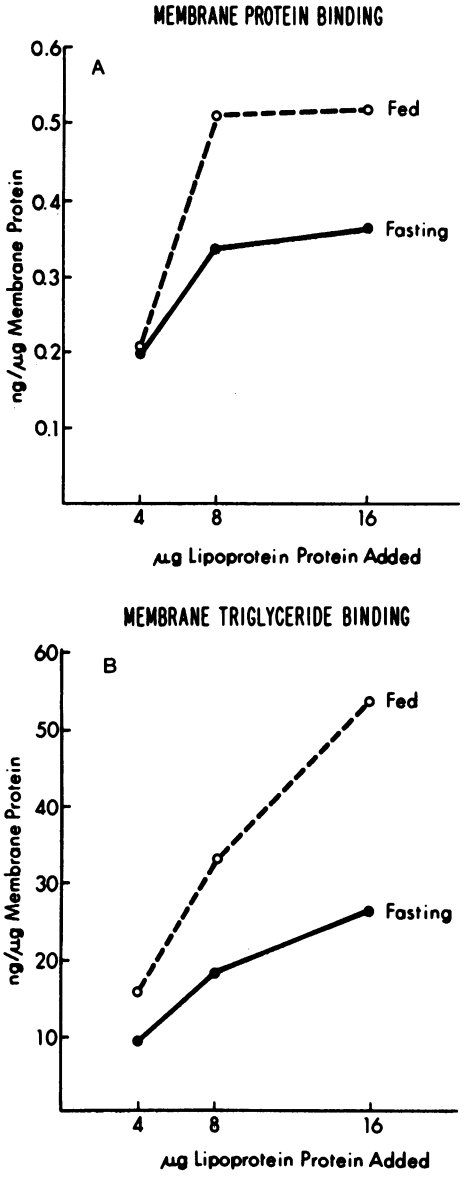

FIGURE 1 The plot represents the binding of the ^{125}I-apo E-enriched triglyceride emulsion to hepatic plasma membranes. (A) The ordinate is the emulsion protein (nanograms per microgram membrane protein) bound as a function of emulsion amount expressed as protein added to 0.8 ml of incubation mixture. (B) The ordinate is the emulsion [^{14}C]triglyceride (nanograms per microgram membrane protein) bound as a function of increasing emulsion concentration expressed as protein.

TABLE III
*Single-Pass Hepatic Uptake of Triglyceride Emulsions Recovered from Recycling Perfusions**

	Percent administered triglyceride activity recovered activity in liver	
	^{3}H	^{14}C
Emulsion from a recycling fasted hepatic perfusion	20.0 (2.0)	22.4 (3.3)
Emulsion from a recycling fed hepatic perfusion	9.7 (.8)‡	11.6 (1.8)§

* Data represent the mean (SE) of four different fasted perfusion emulsions and four different fed emulsions infused in a non-recycling fashion into isolated fasted rat livers.
‡ Significantly different from the fasted emulsion $P < 0.01$.
§ Significantly different from the fasted emulsion $P < 0.05$.

^{3}H recoveries in the perfusate. This was not observed when only the E apoprotein was added and suggests the possibility of an enhanced perfusate partition of resynthesized triglyceride as a consequence of the CIII-1 addition. The triglyceride ^{3}H activity in the perfusate and liver of the recycling perfusions to which CIII-1 was added confirmed (11) a diminished clearance of emulsion triglyceride when compared with the emulsions to which only E protein was added.

Single-pass non-recycling perfusions of fed and fasted livers with a synthetic triglyceride emulsion enriched with E apoprotein differed from the recycling data. No significant difference was observed for the single-pass percent hepatic uptake of either the ^{14}C labeled emulsion triglyceride (fed, 22.4 [1.9]; fasted, 21.8 [1.6]) or ^{125}I-E apoprotein (fed, 21.4 [1.5], fasted, 19.4 [1.4]) when the means (SE) of 10 studies of fasted and fed animals were compared. These data were obtained with single-pass hepatic perfusions of only 2 mg of triglyceride per minute but no difference was noted for triglyceride loads of up to 25 mg per minute. The calcium dependent binding (15, 16) of the apo E supplemented triglyceride emulsions to hepatic plasma membranes did not indicate enhanced binding by the fasted membranes (Fig. 1A and B). If anything, there appeared to be somewhat greater apo E emulsion binding to the fed hepatic plasma membrane preparations.

The perfusates from 30-min recycling fed and fasted hepatic perfusions were centrifuged as described to isolate the triglyceride emulsion containing the E apoprotein. Similar triglyceride loads (1.7 mg/min) of the recovered fasting and fed emulsions were infused into non-recycling isolated hepatic perfusions of fasted rats (Table III). Significantly less hepatic uptake was noted for the emulsion recovered from the fed perfusions when compared with the fasted or with the single-pass uptake of the original E-enriched triglyc-

TABLE IV
*Protein Content of Triglyceride Emulsions Recovered from Perfusion of Isolated Fed and Fasted Livers**

Emulsion	Total emulsion protein	Apo C comigrating emulsion protein‡
	μg/mg total lipid	
No added proteins		
Fasted	56 (12)	4 (3)
Fed	104 (17)§	12 (4)
Apo E-enriched		
Fasted	78 (8)	5 (3)
Fed	134 (22)§	29 (5)‖

* The data represent the mean (SE) of five albumin-free perfusions of fasted and five of fed rats using an emulsion that was not supplemented with apoprotein and similar studies with an emulsion to which apo E was added as described in the text.
‡ Protein electrodialyzed from the apo C region of an SDS-10% PAGE run and assayed as described in the text.
§ Significantly different from the fasted at $P < 0.05$.
‖ Significantly different from the fasted at $P < 0.01$.

eride emulsion described above. This decrease in hepatic clearance of the recovered fed emulsion was also seen when the single pass perfusions were done in fed rats.

To minimize infranatant protein contamination of the residual emulsion during perfusions of fed and fasted livers, albumin was omitted. The fasted liver demonstrated the same greater hepatic uptake of emulsion than the fed in the albumin-free system. The relative lipid composition of the ultracentrifugally isolated residual emulsion from the fasted and fed perfusions was not significantly different (phospholipid: fasted 4.7%, fed 4.9%; triglyceride: fasted 93%, fed 94%).

The relative protein contents were significantly different for the fasted and fed perfusions not only for these emulsions containing E, but also when no apoproteins had been added to the emulsion (Table IV). Not only did the residual emulsion obtained from the perfusates of the fed liver contain relatively more protein than the fasted, but the distribution appeared to differ (Fig. 2). Relatively more protein was qualitatively appreciated in the lower molecular weight region (Fig. 2, band 6) of the emulsions isolated from the fed liver perfusions. When the apo C comigrating (on urea and SDS) band 6 was recovered by electrodialysis, it was observed to be significantly greater for the fed perfusions (Table IV). The content of E protein determined by the ratio of ^{125}I activity to triglyceride radioactivity was similar for the emulsions recovered from fed and fasted hepatic perfusions. Despite the relatively greater protein content of the emulsions recovered from the perfusions of fed livers, the sucrose gradient distribution (24) of the emulsions did not differ.

DISCUSSION

The data presented indicates that the fed liver less actively removes both triglyceride and E protein from a recycling perfusion media containing an E protein-enriched triglyceride emulsion than does the fasted liver. The lower recovery of labeled triglyceride in the fed liver appears to be on the basis of a lesser net uptake rather than an enhanced secretion of cleared and resynthesized triglyceride. The lack of a significant difference in the single pass studies that estimate initial uptake and the plasma membrane binding data between fed and fasted livers suggests that the dietary effect was not due to a change in the hepatocyte plasma membrane receptor system (15, 16). These single pass studies have previously demonstrated (11, 25) dramatic differences in initial uptake of lipid depending on the apoprotein character of either the native lipoprotein or synthetic emulsion and are probably a good index of early, possibly receptor-mediated, hepatic lipid removal. The increased hepatic lipoprotein uptake suggested for the fasted canine has been observed to be mediated by the plasma membrane receptor that binds apo E-containing lipoproteins (26). This difference with the rat data presented here may either be a species difference or on the basis of the lipoproteins being studied.

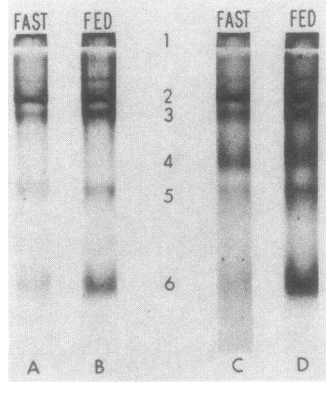

FIGURE 2 The Coomassie Blue-stained SDS 10% polyacrylamide gel electrophoresis of apoproteins on the recovered emulsion from a 30-min fasted (A) and fed (B) liver in studies where the original emulsion was not enriched in E apoproteins. The apoprotein pattern of the recovered emulsion from studies where the initial emulsion was supplemented with apoprotein E; lane C from a fasted hepatic perfusion and lane D from a fed. Band 1 represents undefined protein aggregates at the site of gel entry; 2 is serum albumin; 3 a partially characterized basic protein; 4 is apo E; 5 is an uncharacterized apoprotein; 6 is apo CII and CIII proteins.

The lack of any difference in the single pass and plasma membrane binding data, as well as the absence of any difference in the secretion of resynthesized triglyceride by the fed and fasted livers provoked investigations into other potential explanations for these observations. This decreased partition of triglyceride into a fed liver could be due to an enhanced rate of hepatic reflux of unmetabolized triglyceride (with the same [^{3}H]glycerol:[^{14}C]triolein ratio) that had been removed from the perfusate at normal rates. The possibility of enhanced hepatic-to-perfusate reflux of unmetabolized triglyceride was not tested in these studies. This would require a more complete kinetic appraisal with multiple sequential hepatic and perfusate samplings and would be technically difficult. Rapid sequence sampling of the hepatic single-pass effluent with analysis by indicator dilution kinetics (27) might also answer this issue and is being pursued.

Another possible explanation for the observation that the fed liver differs in perfusate lipid clearance from the fasted in a recycling, but not a single-pass perfusion is that the emulsion was being altered during recycling so as to be less readily cleared by the liver. This possibility was readily tested by recovering the emulsions from the fed and fasted recycled perfusates and determining their initial uptakes in a single-pass perfusates and determining their intial uptakes in a single-pass perfusion. With similar triglyceride loads infused, the fasted emulsions have hepatic extractions comparable to the initial E load emulsion, whereas the fed was appreciably less. This suggests that during the 30-min recycling interval, a change had occurred in the emulsion of the fed system so as to decrease its retrieval by the liver when compared to the original emulsion introduced into the recycling perfusate. It might be argued that after a period of perfusion, an unchanged remnant of the original triglyceride emulsion that is poorly cleared by the fed liver remains in the perfusate. However, the single pass studies were done in fasted rats, where from the recycling data, such an inability to clear a fraction of the emulsion should not be present, yet the difference between the recovered emulsions is pronounced.

The only significant compostional difference noted for the synthetic emulsion perfused through a fed liver when compared with the fasted, was its greater protein content. This was difficult to appreciate in the perfusion studies using albumin because of contamination, despite gel filtration or repeated ultracentrifugal isolation. In the albumin-free system, this was readily apparent. It was also clear that an appreciable amount of this protein increment was due to proteins comigrating with CII and CIII on both urea and SDS polyacrylamide gels. We have observed (11) the latter to be an important inhibitor of hepatic triglyceride emulsion uptake and others (28) have indicated that both CII and CIII proteins act in this manner. It is currently unknown whether the small amounts of these proteins (<0.3%) observed on the emulsion would be sufficient to inhibit hepatic uptake. It is possible that the fed liver in the course of secreting more triglyceride-rich lipoprotein than the fasted, makes a greater complement of C protein accessible to the perfusate, and therefore a greater potential for the inhibition of triglyceride-rich lipoprotein uptake by the liver.

The observation that the liver of the fed rat is less able to retrieve an emulsion similar to a chylomicron dispersion has important implications with respect to the in vivo distribution of lipid. Such a property would permit a higher fraction of the chylomicron influx to be distributed to peripheral lipid storage sites in the fed state. This is, in fact, what has been seen (1) for the in vivo effects of fasting and feeding on chylomicron triglyceride distribution. The time interval of these isolated hepatic perfusion studies (<30 min) is compatible with the time frame of in vivo chylomicron clearance (29) again suggesting that this intrinsic hepatic down-regulation of lipid entry into the fed liver is of physiologic significance. The mechanisms of this down-regulation in the rat appears to be quite different from that suggested for the canine, where plasma membrane binding for high density lipoprotein-C appears to be significantly different in the fasted and fed state (26).

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