Secretion and uptake of nascent hepatic very low density lipoprotein by perfused livers from fed and fasted rats

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Abstract Livers from fed or 24-hr fasted male rats were perfused in a recycling system. VLDL labeled with [1-^{14}C]oleate (95% in triglyceride), produced in separate perfusions of livers from fed rats, was added to the medium as a pulse. Uptake of VLDL ^{14}C-labeled triglyceride by livers from fasted rats was less than that from fed rats regardless of addition of oleate. During the interval in which radioactive triglyceride was taken up, the mass of triglyceride in the medium increased, indicative of the synthesis and net secretion of triglycerides. The rates of secretion of VLDL and uptake of VLDL were both more rapid in livers from fed rats in comparison to those from fasted animals. It was calculated that about 50% of the triglyceride synthesized and secreted by the liver was taken back by livers from fed rats. The VLDL from livers of fasted rats did not contain any apoE detectable by SDS gel electrophoresis or by radioimmunoassay when no fatty acid or 166 μmol of oleic acid was infused. In contrast, apoE comprised 6% of the VLDL apoprotein derived from perfusion of livers from fed animals in the absence of added fatty acid, and 20% when the fed livers were infused with 166 μmol of oleic acid. However, the net output (accumulation) of apoE by fasted liver was only two-thirds that from fed livers. When lipoprotein-free rat plasma containing apoE (4 mg/dl) was used in place of bovine serum albumin, the VLDL secreted by livers from either fed or fasted rats contained apoE and was taken up to a similar extent by such livers. These data suggested that the apoE of the d > 1.21 g/ml fraction was transferred to newly secreted VLDL which then stimulated uptake of the VLDL by livers from fasted rats. With further stimulation of secretion of VLDL triglyceride by infusion of 332 μmol of oleic acid/hr, the percent of apoE in the VLDL secreted by livers from fasted rats increased to 20%, which was similar to that of the VLDL produced by livers from fed rats when either 166 or 332 μmol/hr was infused.■ These data suggest a relationship between rates of hepatic secretion of VLDL (TG) and apoE, and the association of apoE with the secreted VLDL. During fasting, reduced secretion of both VLDL and apoE resulted in a VLDL particle that was considerably diminished in content of apoE and, therefore, that would be taken up by the liver at a reduced rate, in comparison to that observed in the fed animal.—**Wilcox, H. G., and M. Heimberg.** Secretion and uptake of nascent hepatic very low density lipoprotein by perfused livers from fed and fasted rats. *J. Lipid Res.* 1987. **28:** 351–360.

Supplementary key words apolipoprotein E • nascent VLDL • liver perfusion

It is now well established that the hepatocyte can bind and take up triglyceride-rich lipoprotein particles by means of two receptor systems (1). These receptor systems recognize TG-rich particles including *1*) chylomicron remnants (the apoB-48- and apoE-containing degradation products of chylomicron metabolism) and *2*) VLDL remnants (the apoB-100- and apoE-containing intermediate density lipoproteins derived from the metabolism of hepatic VLDL) (2, 3). The VLDL formed and secreted by the liver (nascent VLDL) also appears to be taken up and degraded by the liver (4). Much earlier data from our laboratory suggested hepatic uptake of the VLDL in studies of hepatic TG metabolism in the perfused rat liver (5). It has been observed that the apolipoprotein composition of the TG-rich particles plays a very important role in the rate at which such particles are removed by the liver. ApoE and apoC present in the lipoprotein particle seem to have opposing actions on hepatic uptake of the TG-rich particle; apoE has been reported to stimulate uptake, while the C apoproteins may have an inhibitory effect on uptake (6, 7). A similar relationship exists for the interaction of chylomicrons with rat liver parenchymal cells in vitro (8). The apolipoprotein composition of TG-rich particles can be altered by exchange and transfer reactions with free apolipoproteins or with those present in other lipoprotein classes (6, 7), and hepatic uptake of these TG-rich particles may be affected accordingly.

Most of the work on hepatic uptake of TG-rich particles has compared uptake of remnants with that of the native particles. Remnant uptake usually was faster (4, 9–11). Uptake of TG-rich particles (chylomicrons and TG-rich emulsions) by livers from fasted rats had been reported to exceed that of fed animals, a conclusion reached by Heimberg et al. (5) in 1962 and by Quarfordt et al. (12)

Abbreviations: TG, triglyceride; VLDL, very low density lipoprotein; apo, apolipoprotein; PL, phospholipids; C, cholesterol; CE, cholesteryl esters; FFA, free fatty acid.

more recently. It was of interest, therefore, to determine whether these differences in rate of uptake might be attributable to the nutritional state of the animal, and possibly, to differences in the protein composition of the VLDL or in the composition of the medium perfusing the liver in vitro.

To evaluate these possibilities, uptake of nascent VLDL, as measured by disappearance of radioactivity VLDL-TG, was compared in perfused livers isolated from fed and fasted rats. Conceivably, VLDL-TG metabolism may occur without uptake of the intact particle, but rather by extracellular hydrolysis to free fatty acid, which is then taken up. A residual "remnant" particle may then be processed by a separate mechanism. In this study we have equated uptake of VLDL with uptake of VLDL-TG. It was observed that nascent hepatic VLDL-TG was taken up extensively by livers from fed rats, but not by livers from fasted rats, when the livers were perfused with a medium devoid of rat plasma. Our data, moreover, suggested that the nascent VLDL was not taken up by livers from fasted animals, in part, because apoE, which is deficient in the nascent hepatic VLDL produced by livers from fasted rats, may be required for uptake of the VLDL. A preliminary report of this work has appeared (13).

METHODS

Preparation of radioactive VLDL

VLDL, labeled with [1-^{14}C]oleate, was prepared in vitro by perfusion of livers isolated from normal, fed Sprague-Dawley male rats (250–300 g) obtained from Harlan, Inc. (Indianapolis, IN). The livers were removed surgically and placed in a perfusion apparatus as described previously (14). The livers were perfused in a recycling system with an initial volume of 70 ml of a medium (14) consisting of 30% washed bovine erythrocytes, 6 g of delipidated (15) bovine serum albumin/dl, 100 mg of glucose/dl, all in Krebs-Henseleit bicarbonate buffer, pH 7.4. During a 2-hr perfusion, a complex of [1-^{14}C]oleic acid with bovine albumin (16) was infused at a rate of 11.7 ml/hr (166 μmol of oleic acid; 9 μCi of ^{14}C/hr). A portion of the fatty acid complex (37 μmol of oleic acid; 2 μCi of ^{14}C) was added as a pulse dose at the start of the perfusion. After 2 hr, the infusion of the fatty acid complex was discontinued, but the perfusion was continued for an additional 30 min to allow much of the oleic acid remaining in the medium to be taken by the livers and to be metabolized.

At the end of the experiment, the perfusate was centrifuged at low speed to sediment the erythrocytes. The perfusate plasma was then ultracentrifuged without further density adjustment in a Beckman Model L8 ultracentrifuge using a type 50.2 rotor at 39,000 rpm (138,000 g) for 18 hr at 15°C (16). The floating VLDL was harvested carefully from the top 10-15% of the tube volume (2–3 ml per tube) and dialyzed overnight against 0.15 M NaCl. The VLDL was used as the substrate for subsequent studies of hepatic uptake, 48 hr following the preparative perfusion. The distribution of radioactivity within the lipids of the substrate VLDL particle is shown in **Table 1.** In each experiment, radioactive VLDL (2–12.5 μmol, with a range of specific activities of 40,000–200,000 dpm μmol TG) was added to the perfusion medium.

Uptake of the nascent VLDL

For these studies, the livers were perfused as described in the previous section, except that [1-^{14}C]oleic acid was omitted from the medium, and perfusions were carried out for 3 hr. Bovine serum albumin (6 g/dl), with or without nonradioactive oleic acid, was infused continuously (0 or 166 μmol of oleic acid/hr; 11.7 ml/hr). Experiments were initiated by adding a pulse dose of the radioactive nascent VLDL to the perfusion medium (2–12.5 μmol of TG), and immediately thereafter the infusion was started. In certain studies no VLDL was added to the initial perfusion medium. Livers for perfusion were removed from male rats fed ad libitum (Purina Laboratory Chow), or from rats fasted for approximately 24 hr (deprived of food at 8 AM 1 day before the experiment). Samples of perfusate were removed hourly, and the final perfusate remaining at the end of the experiment was collected for analysis. At the termination of the experiment, the livers were flushed with ice-cold 0.9% NaCl solution, and 1.0-g samples of liver were removed for analysis of lipid and radioactivity.

Analytical procedures

Lipids were extracted from samples of perfusate plasma, VLDL, and liver with chloroform–methanol 2:1 (v/v) (17). Lipid classes were separated by thin-layer chromatography on silica gel G plates, and appropriate bands were analyzed for triglyceride, fatty acid, phospholipid, cholesterol, and cholesteryl esters (15). Aliquots of the CHCl$_3$ extracts of the triglyceride and cholesteryl ester bands were dried in a liquid scintillation vial, and radioactivity was measured in a Beckman LS-7500 liquid scintillation counter, after addition of 10 ml of scintillation fluid. An aliquot of the phospholipid band was counted directly in Biocount without extraction into chloroform. Radioactivity is reported as dpm, calculated from the H-number quench curve efficiency correction program of the instrument.

Uptake of triglyceride mass, which represented the major fraction of the radioactivity in the VLDL (Table 1), was calculated from the measured mass of TG and radioactivity in TG at each hour. It is reasonable to assume that the VLDL particles that were added as substrate (labeled with ^{14}C oleate) and those newly

TABLE 1. Distribution of radioactivity in lipids of the VLDL and liver

	% Total Radioactivity		
	TG	PL	CE
VLDL			
Before perfusion (3)	93.9 ± 1.6	2.5 ± 0.9	3.6 ± 0.9
After perfusion, fed (9)[a]	95.4 ± 0.4	1.3 ± 0.2	3.2 ± 0.4
After perfusion, fasting (9)[a]	95.4 ± 0.3	1.1 ± 0.2	3.5 ± 0.2
Liver			
Oleic acid infused, fed (3)	61.7 ± 3.2	32.9 ± 3.1	5.4 ± 0.7
Oleic acid infused, fasting (3)	49.3 ± 3.9	42.9 ± 3.8	7.8 ± 0.4[b]
Oleic acid not infused, fed (3)	66.6 ± 1.3	32.5 ± 2.8	2.1 ± 0.3
Oleic acid not infused, fasting (3)	47.8 ± 1.7[b]	48.8 ± 2.6[b]	5.6 ± 1.5[c]

Data are percent of radioactivity in VLDL and liver (means ± SEM) and were derived from the sum of the values for TG, PL, and CE. Numbers of observations are in parentheses.
[a]Since no differences were observed among the individual perfusion conditions (see legend to Fig. 1 for defini-tion), the values in the three groups (A, B, and C) were combined. The percent values presented for distribution of radioactivity among hepatic lipids are those for group A in Fig. 1 (oleic acid infused; 12.5 μmol of TG as initial pulse). When fatty acid was not infused, 7.8 μmol of TG was the initial pulse (group C).
[b]$2P < 0.05$ compared to fed values.
[c]$2P < 0.10$ compared to fed values.

secreted VLDL particles produced during perfusion of the liver equilibrated completely to form a normally dis-tributed population, and that, therefore, VLDL from both sources will be taken up at the same rate. Since a large load of fatty acid was infused to maintain physiolo-gical concentrations, contribution to the perfusate pool of radioactive VLDL-TG by recycling of the ^{14}C-labeled triglyceride fatty acid which had been taken up by the liver is negligible. When fatty acid is not infused, recy-cling of labeled TG may become important. The total mass of TG taken up from the perfusion medium can therefore be calculated by the following equation, where $\triangle$ dpm is the total TG radioactivity taken up (change during the hourly sampling period) and the denominator is the average TG specific activity ($SA = dpm/\mu mol$) of perfusate TG at the start (SA_1) and end (SA_2) of the sampling period. (Data presented represent the sum of calculations for the three hourly sampling periods.)

Total mass uptake

$$(\mu mol\ TG/g\ liver) = \frac{\triangle\ dpm}{0.5\ (SA_1 + SA_2)\ \ (liver\ weight,\ g)}$$

(Eq. 1)

The total mass of TG taken up includes TG originally present (the radiolabeled TG pulse), as well as the TG newly formed and secreted by the liver. The amount of the original radioactive pulse of TG taken up is simply calculated as the product of the proportion of label (in TG) remaining at the end of the experiment and the ori-ginal mass of the pulse of TG. The difference between this value and the total mass taken up (from equation 1) there-fore represents the additional TG mass that was synthe-sized and secreted by the liver and taken back up.

Radioimmunoassay for rat apoE

Rat VLDL (d < 1.006 g/ml) was isolated by ultracentri-fugation of pooled plasma from fed male rats, and reisolated at the same density. Blood was obtained at 9–10 AM. The VLDL was dialyzed against 1 mM EDTA and delipidated with ethanol–ether 3:1 (18). The apolipopro-teins were solubilized in 5 M urea (2 mM sodium phosphate–0.05 M NaCl, pH 7.4) and applied to a heparin-Sepharose column (18). ApoE was eluted with 0.3–0.5 M NaCl. This fraction proved to be contaminant-free, as indicated by SDS-polyacrylamide gel elec-trophoresis, and by its lack of reaction with antisera to rat apo-AI and B and to rat albumin (Cappel, Inc.). Rat apoE prepared in this manner was used as a standard in the radioimmunossay, for iodination (19), and for produc-tion of antisera. ApoE was also prepared by preparative SDS polyacrylamide (10%) gel electrophoresis in similar purity, and was used for preparation of antisera. Anti-serum to rat apoE was prepared by multiple intradermal injections of antigen preparations, diluted (1:1, v/v) with complete Freund's adjuvant, on the backs of male rabbits. This antiserum formed arcs of identity between rat serum and apoE, and did not react with preparations of apo-AI, apoB, or C apoproteins, or rat albumin when examined by double immunodiffusion.

The radioimmunoassay was carried out in a buffer con-sisting of 1% Triton-100 and 3% bovine serum albumin (Cohn Fraction V, Miles Laboratories) in 0.05 M barbital buffer, pH 8.6 (0.01% NaN₃). All dilutions were made in this buffer. Assay conditions were such that the linear (log-logit) standard curve (n = 35 assays) ranged from 6.25 to 200 ng with a B_0 (50% precipitation) obtained in a 1:20,000 dilution of antibody. After setting at 4°C over-

night with the primary antibody, the secondary antibody (goat anti-rabbit γ globulin, Cappel, Inc.) was added and mixed, and then 6% polyethylene glycol was added (20). After incubation at room temperature for 1 hr, pellets were obtained by centrifugation and were counted with a Micromedic 4/600 gamma counter. Data were obtained by a data reduction program (log-logit transformation, Hewlett-Packard 9815 A). Characteristics of the linear transformation of the standard curve were as follows (n = 35); slope = − 2.35 ± 0.07, y intercept = 3.72 ± 0.20 and $r^2 = 0.986 \pm 0.002$ (mean ± SEM). The assay conditions were similar to those which we have used to quantitate rat apoA-I (21). Intraassay and interassay coefficients of variation (CV) were 3% and 12%, respectively.

Apolipoprotein characterization

The apolipoprotein patterns of the VLDL were examined by SDS-gradient polyacrylamide gel (3.5–20%) electrophoresis, similar to the procedure described by Laemmli (22). Gels were stained with Coomassie Brilliant Blue R 250 and were scanned, and the peak areas were quantitated with an LKB laser gel scanner and integrator. Any differences in chromogenicity among the bands were not considered in calculation of areas.

Materials

Nonradioactive oleic acid was obtained from Nu-Chek-Prep, Elysian, MN and [1-^{14}C]oleic acid was from New England Nuclear. Bovine serum albumin (fraction V) was obtained from Miles Laboratories and was delipidated as described previously (15). Biocount (Research Products International) was used as the scintillation fluid. All other chemicals were reagent grade, obtained from Fisher and from Curtis Matheson Scientific.

Statistics

Values in all tables are reported as means ± standard error (SEM). Statistical comparisons were made using Student's unpaired t-test. Statistical significance was taken as $2P < 0.05$.

RESULTS

The uptake of radioactive TG (measured by disappearance of ^{14}C-labeled TG from the medium) by perfused livers from fed rats was 5–9 times greater than that from fasted rats when ^{14}C-labeled nascent VLDL was supplied as a substrate (Fig. 1). Proportionality between the initial pulse mass of VLDL (either 2.0 or 12.5 μmol of TG) and rate of uptake of VLDL was not apparent (groups A and B) but was similar for both pulses. When exogenous oleate was not infused (group C), uptake of
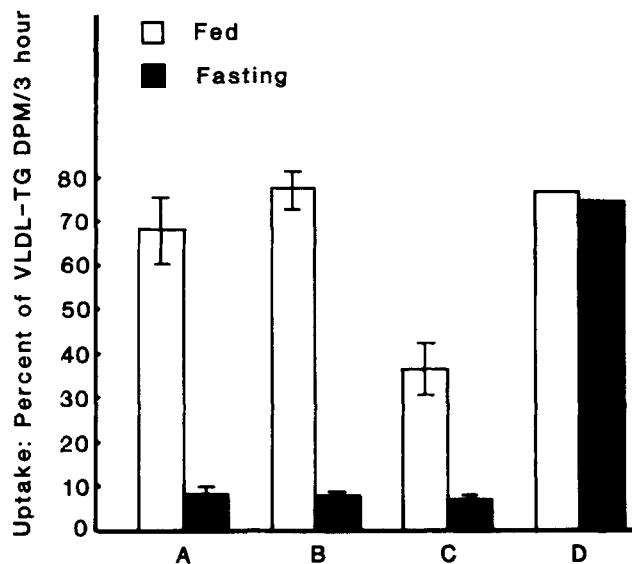

Fig. 1. Uptake of ^{14}C-labeled VLDL-triglyceride by the isolated perfused rat liver. Livers were perfused with a medium devoid of rat plasma (A, B, and C) as described in the text. In groups A (n = 3) and B (n = 3), 166 μmol of oleic acid was infused per hr for 3 hr. In groups C (n = 3) and D (n = 2), oleic acid was not infused. The initial pulse of ^{14}C-labeled VLDL-TG was: group A, 12.5 μmol; group B, 2.0 μmol; group C, 7.8 μmol; and group D, 9.6 μmol. Lipoprotein-free rat plasma (d > 1.210 g/ml) obtained from fed animals was added to the perfusion medium for group D (4 g of protein/dl), to replace the bovine serum albumin present in the medium used for the group A, B, and C perfusions. When uptake was normalized for liver weight and expressed as percent of initial pulse dose (% ^{14}C-labeled TG taken up per g liver), then the uptake for the combined groups A and B (n = 6) (oleic acid provided) in the fed state was 6.26 ± 0.30%, and in the fasted state was 1.00 ± 0.20% for the 3-hr period; uptake for group C (n = 3)(oleic acid not provided) in the fed state was 4.23 ± 0.68%, and in the fasted state was 1.00 ± 0.46%. Values for groups A, B, and C are statistically different for the fed state compared to the fasting state (2P<0.05). The values in groups A and B (fed state) are statistically higher than those for group C (2P<0.05).

radioactive TG was less than when fatty acid was supplied. In the absence of a liver, no change in TG radioactivity or mass was observed in the medium recycling through the perfusion apparatus for a 3-hr period (data not shown); therefore, nonspecific disappearance of TG during liver perfusion was negligible. Simultaneously with the disappearance of VLDL-TG radioactivity from the medium during perfusion of the liver, a net increase of VLDL-TG mass was observed, indicative of secretion of TG (**Fig. 2**). Infusion of oleic acid stimulated hepatic secretion of TG by livers from fed and fasted rats, as had been reported previously by us (5); net output of TG by livers from fed rats, as expected, exceeded that of livers from fasted rats (Fig. 2). Uptake of oleic acid by the perfused livers from fed or fasted animals was identical (the oleic acid concentration was maintained at 0.67 ± 0.03 μmol/ml, or 0.62 ± 0.05 μmol/ml in the medium perfusing livers from fed or fasted rats, respectively) in agreement with earlier data in female rats (23, 24) and male rats (5). The rapid decrease in specific activity of TG in the per-
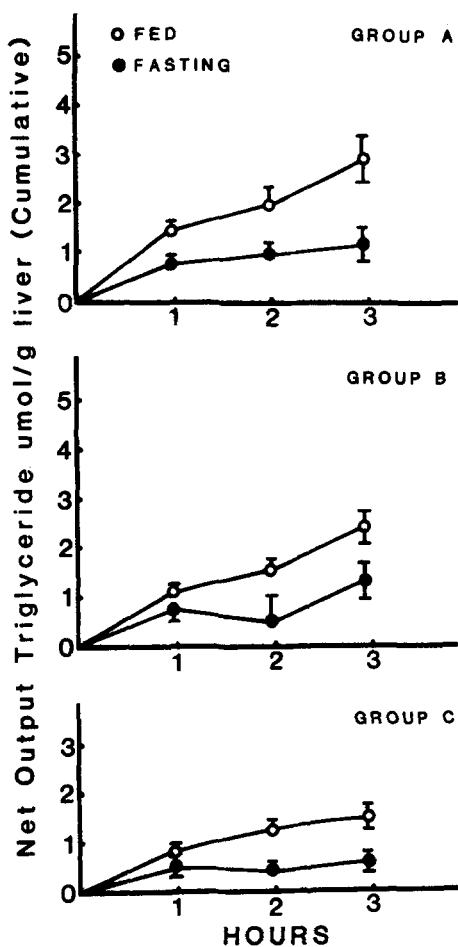
Fig. 2. Net output of triglyceride by the liver. Perfusion conditions for the data presented in the three panels (groups A, B, and C) are presented in Fig. 1. Data presented are means ± SEM for the cumulative TG output (measured chemically). Each plot is derived from three perfusions. Standard errors are given as vertical bars. Values at all time points for group A are significantly higher ($2P < 0.05$) in the fed state than in the fasting state. The values for the fed state are significantly higher ($2P < 0.05$) than for the fasting state for groups B and C at the second and third hour.

take of TG for fasted livers was very small, as indicated in Fig. 1, reflected by the small percent of the radioactive TG taken up.

During the 3-hr experiment, the VLDL-TG that had been taken up by the liver was metabolized extensively. In support of this, it may be seen that the distribution of radioactivity in hepatic lipid differed considerably from that of the substrate VLDL lipid (Table 1). Certainly, the VLDL taken up was not simply trapped or bound within the liver. The distribution of radioactivity in the VLDL at the termination of perfusion was almost exclusively in TG, regardless of the experimental condition, and was not different from the initial distribution in the VLDL substrate. In contrast, approximately 50–60% of the hepatic lipid radioactivity was in TG, with the remainder essentially in PL. When fatty acid was infused, the distribution of radioactivity among the hepatic lipids did not differ significantly, regardless of the nutritional state. In separate experiments, livers from fed rats were perfused without added radioactive VLDL but with [1-^{14}C]oleate. In those experiments, radioactivity distributed in hepatic lipids was similar to that observed when ^{14}C-labeled VLDL-TG was the substrate, and is in agreement with
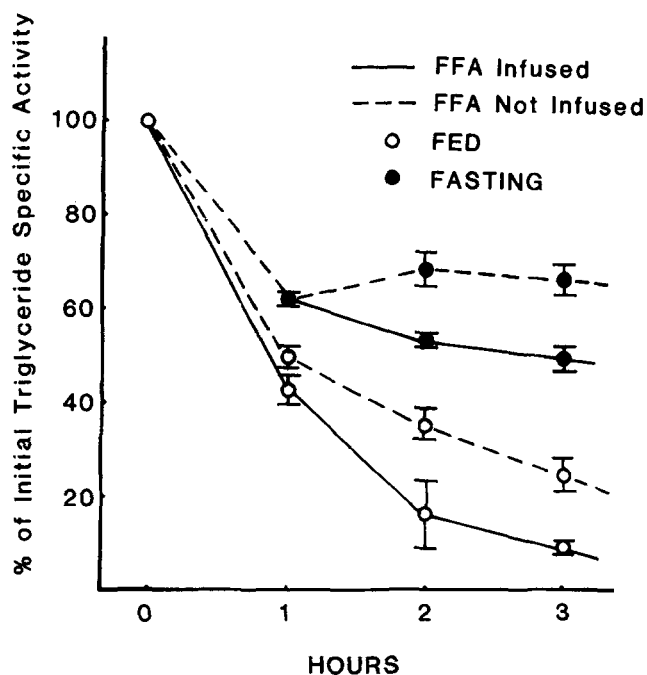
fusate is illustrated for groups A and C in **Fig. 3.** Clearly, both hepatic secretion of VLDL-TG and hepatic uptake of VLDL-TG were greater in the fed than in the fasted state as indicated by the more rapid decrease in TG specific activity with livers from fed rats. Furthermore, the addition of oleic acid to the medium resulted in a more rapid decrease in the specific activity, as would result from dilution of radioactivity by increased rate of secretion of unlabeled TG.

Reuptake (calculated with equation 1) of newly secreted VLDL-TG is presented in **Table 2.** In both groups of livers from fed rats in which exogenous fatty acid was provided, about 50% of the VLDL was taken back up, whereas similar calculations indicated that, in the absence of an exogenous supply of fatty acid, 27% was taken back up by livers from fed animals. Calculations of the reup-

Fig. 3. Changes in specific activity of ^{14}C-labeled VLDL-triglyceride during perfusion of the liver. Data are presented as means ± SEM (n = 3 for each point). When indicated (group A), 166 μmol of oleic acid was infused into the perfusion medium each hour; 12.5 μmol of VLDL-TG, with a specific activity of 129,400 dpm/μmol TG, was added at the beginning of the experiment. For group C, no fatty acid was infused; 7.0 μmol of VLDL-TG, with a specific activity of 213,600 dpm/μmol TG, was present at the start. The specific activity is statistically different between fed and fasted groups, and for presence or absence of oleic acid, at 2 and 3 hr ($2P < 0.05$).

TABLE 2. Effect of nutritional status on hepatic uptake and secretion of VLDL triglyceride

	TG Uptake (Total)	TG Reuptake	TG Net Output (Cumulative)	TG Secreted (Total)
	μmol/g liver per 3 hr			
Oleic acid infused (166 μmol/hr)				
Fed (group A) (3)	3.66 ± 0.48	2.92 ± 0.44	2.93 ± 0.56	5.85 ± 0.40
Fed (group B) (3)	1.97 ± 0.09	1.84 ± 0.09	2.25 ± 0.27	4.09 ± 0.34
Fasting (group A) (3)	0.38 ± 0.16	0.27 ± 0.18[a]	1.13 ± 0.24[a]	1.40 ± 0.42[a]
Fasting (group B) (3)	0.00 ± 0.07	0.05 ± 0.07[a]	1.03 ± 0.32[a]	1.08 ± 0.31[a]
Oleic acid not infused				
Fed (group C) (3)	0.90 ± 0.24	0.55 ± 0.19	1.46 ± 0.15	2.01 ± 0.31
Fasting (group C) (3)	0.12 ± 0.06	0.04 ± 0.02[a]	0.46 ± 0.03[a]	0.50 ± 0.04[a]

Data presented are means ± SEM. Numbers of observations are given in parentheses. In group A, 12.5 μmol of VLDL-TG was added as a pulse; in group B, 2.0 μmol of VLDL-TG was added; and in group C, 7.8 μmol of VLDL-TG was added. "TG uptake (total)" was calculated from equation 1, given in the Methods section; these data represent the sum of the uptake during the three individual consecutive sampling periods and were derived from the specific activity at zero time and at each of the three hourly sampling periods. This is necessary since uptake is nonlinear over the 3-hr period. "TG reuptake" is the total mass uptake minus the mass of added radioactive VLDL-TG taken up from the medium. "TG net output" represents the net mass of TG accumulating in the perfusion medium during the 3-hr experiment. "TG secreted (total)" is the sum of the calculated TG reuptake and the net output. Whether fasted or fed, between groups (12.5 μmol vs. 2 μmol TG originally present) neither the "TG reuptake" nor the "TG net output" is different; however, a comparison of fed versus fasted within a group shows that the above comparisons are statistically different, including group C in which no exogenous fatty acid was supplied.

[a]$2P < 0.05$ compared with appropriate fed groups (A, B, or C).

our previously published data of distribution of radioactivity from labeled oleate (16). The data suggest that VLDL-TG was hydrolyzed to fatty acids, which then entered a metabolic pool in the liver in common with that derived from exogenous free fatty acids.

The concentration of hepatic TG at the termination of the experiment reflects the metabolic state of the animal and the provision of exogenous fatty acid (Table 3). Hepatic TG concentration was depressed in the fasted state compared to the fed state. When oleate was infused, the concentrations of TG were higher in livers from fed or fasted rats, than when fatty acid was not infused. The cholesteryl ester content of livers from fed rats did not differ significantly from that of fasted rats when oleate was added, but was lower when fatty acid was omitted.

The apolipoprotein patterns of the VLDL isolated from the medium at the end of the perfusion suggested important differences between the VLDL particles secreted by livers from fed and fasted rats (Fig. 4). The VLDL substrate, as discussed previously, had been prepared by perfusion of livers from fed animals. The VLDL isolated after perfusion of livers from fed rats contained discernible amounts of apoE, while the VLDL isolated from the medium following perfusion with the radioactive substrate VLDL of livers from fasted animals was essentially devoid of apoE. No apoE was detectable by densitometric scanning of the stained gels of VLDL apolipoproteins isolated after perfusion of livers from fasted rats, whereas about 6% and 20% of the total stained material was apoE in the VLDL after perfusion of livers from fed rats in the absence and presence of FFA, respectively (Table 4). Densitometric scanning indicated that about 50% of the total stained material was in the apoC area of the apoprotein patterns of VLDL produced by both fed and fasted

TABLE 3. Hepatic lipid composition

Group	TG	PL	C	CE
	μmol/g liver			
Oleic acid infused (166 μmol/hr)				
Fed (group A) (3)	4.9 ± 0.2[a]	31.0 ± 2.3	3.7 ± 0.3	0.8 ± 0.2[a]
Fasting (group A) (3)	2.7 ± 0.6[b]	29.0 ± 2.5	4.2 ± 0.3	0.7 ± 0.4[a]
Oleic acid not infused				
Fed (group C) (3)	3.2 ± 0.1[b]	30.3 ± 1.2	4.5 ± 0.2	0.4 ± 0.1[b]
Fasting (group C) (3)	1.2 ± 0.3[c]	23.3 ± 2.7	4.2 ± 0.1	0.6 ± 0.1[a]

Data are means ± SEM. Numbers of observations are given in parentheses. Values with different superscripts in each column are significantly ($2P < 0.05$) different from each other. In group A, 12.5 μmol of labeled VLDL-TG was added, while in group C, 7.8 μmol of TG was added.
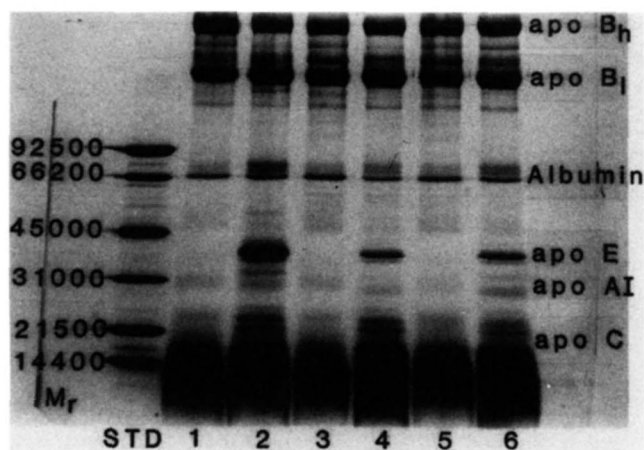
Fig. 4. Gradient gel electrophoresis of perfusate VLDL apolipoproteins secreted by the liver. The lane on the left presents molecular weight standards (Bio-Rad) with M_r given in daltons. One hundred μg of protein was applied to each sample lane. Odd-numbered lanes: VLDL isolated from perfusions of livers from fasted rats; even-numbered lanes: VLDL isolated from liver perfusions from fed rats. VLDL samples for these gels were obtained from group A (166 μmol of oleic acid infused/hr, plus an initial pulse of 12.5 μmol of TG). Essentially identical patterns were obtained in separate experiments in which VLDL had not been added to the perfusion medium.

livers. In recent unpublished experiments, the percentage of the apoCs ranged from 32 to 40% and no differences were apparent between the fed and fasting experiments with or without exogenous oleic acid supplied to the liver.

Separate additional experiments were carried out, except that substrate VLDL was not added to the perfusion medium. In those experiments, the presence of apoE in the VLDL secreted by livers from fasted rats perfused with or without exogenous oleic acid (166 μmol/hr) was essentially undetectable by radioimmunoassay, whereas the VLDL secreted by livers from fed rats had a significant content of apoE (Table 4).

The increase in percentage (6 to 20%) of VLDL-apoE when 166 μmol of oleate/hr was infused suggests stimulation of output of VLDL-apoE coincident with increased secretion of VLDL-TG by livers from fed rats. Total apoE, as measured by radioimmunoassay, was not statistically different between perfused livers from fed and fasted rats (Table 4), although the mean value in the absence of fatty acid was lower in the fasting group (88 ± 5 and 62 ± 8 μg of apoE/g of liver/4 hr, n = 3, respectively). Similarly, hepatic secretion of total perfusate apoE was not influenced by the infusion of oleic acid with livers from fed or fasting rats (92 ± 11 and 63 ± 4 μg of apoE/g liver/4 hr, n = 3, respectively). When the data are combined, the net output of apoE by livers from fasted rats was significantly lower than that from fed rats (62 ± 4 vs. 90 ± 6 μg/g of liver/4 hr, n = 6 each) without regard to whether fatty acid was supplied or not. Decreased incorporation of [^{3}H]leucine into apoE by livers from fasted rats compared to fed rats also has suggested depressed hepatic synthesis of this apoprotein in fasting (Salam, W., H. G. Wilcox, and M. Heimberg, unpublished results).

The apoE percentage of the VLDL secreted by livers from fasted rats was, however, increased (19.7%) by stimulation of the secretion of VLDL (TG) by additional fatty acid (332 μmol of oleic acid/hr). Under these same conditions, however, the percent of apoE in VLDL produced by livers from fed rats was similar to that observed when 166 μmol of oleic acid/hr was infused (Table 4).

DISCUSSION

The severely reduced uptake of radioactive VLDL-TG by perfused livers from fasting rats that was observed in these experiments was surprising, in view of implications from previous experiments. Heimberg et al. (5), using recycling liver perfusions with a medium containing

TABLE 4. Secretion of apolipoprotein E by livers from fed or fasted rats

Group	Perfusate ApoE	VLDL ApoE	ApoE
	μg/g liver/3 hr		*% of protein[a]*
Fed			
No oleate	82 ± 5 (3)	34 ± 4 (11)	6.2 ± 0.6 (6)
Oleate infused (166 μmol/hr)	92 ± 11 (3)	90 ± 10 (12)[b]	19.9 ± 2.0 (3)[b]
Oleate infused (332 μmol/hr)		134 ± 20 (3)	20.6 ± 3.2 (3)[b]
Fasted (24 hr)			
No oleate	62 ± 8 (3)	< 0.1 (6)	ND
Oleate infused (166 μmol/hr)	63 ± 4 (3)	0.3 ± 0.1 (4)	ND
Oleate infused (332 μmol/hr)		97 ± 3 (3)	19.7 ± 0.4

Values are means ± SEM. Figures in parentheses indicated number of independent observations. Analyses were performed in experiments in which ^{14}C-labeled VLDL was not added to the perfusion medium. Perfusate and VLDL apoE quantitated by radioimmunoassay. ND, none detected.
[a]Data were calculated from the area of Coomassie blue-stained gels of VLDL apolipoproteins.
[b]$2P < 0.05$ compared to "no oleate."

defibrinated rat blood, and Quarfordt et al. (12), using a medium containing erythrocytes and buffer, showed that livers from fasted rats removed TG-rich particles (chylomicrons and synthetic neutral fat emulsions) from the medium at a faster rate than livers from fed rats. Quarfordt et al. (12), however, when examining uptake with a non-recycling system, found no differences and attributed this to the association of apoC with the particle in recycling but not in single-pass perfusions. Our observation that apoE was deficient in the VLDL secreted by livers from fasted rats was unexpected and suggested a possible explanation for the very minimal uptake of VLDL by livers from fasted rats. Bar-On et al. (25) reported recently that the plasma half-life in normal rats for VLDL derived from the plasma of diabetic rats was about twice that of VLDL derived from plasma of normal rats; this observation was related to the lower relative content of apoE in the VLDL derived from diabetic rats, which presumably reduced the potential for receptor recognition. Similar findings and conclusions were reported by O'Looney et at. (26).

We observed that livers from fasted rats produced VLDL with reduced amounts of apoE or devoid of apoE (Table 4, Fig. 4). However, the radioactive VLDL used as the substrate in our experiments contained apoE, since it was produced by perfusion of livers from fed rats. ApoE, which was synthesized and secreted in the VLDL by the livers from fed animals, represented 39–100% (Table 4) of the total perfusate apoE produced by the liver, depending on the experiment, whereas the apoE content of VLDL produced by livers from fasted rats was negligible. Felker et al. (27) reported that about 50% of the apoE secreted by perfused livers from fed rats into an erythrocyte–buffer medium (albumin, free fatty acid, or plasma were not present) was associated with the VLDL, in good agreement with our data (39%, Table 4).

The apolipoprotein composition of the newly secreted VLDL must be influenced by transfer and/or exchange with other circulating lipoproteins or with free apolipoproteins present in the perfusion medium. Since the medium used in these perfusion studies did not contain plasma or plasma lipoproteins, the apolipoprotein content of the VLDL must reflect the direct contribution by the liver. Since apoE was secreted by livers from fasted animals, although in reduced amounts, it is surprising that little of it was found associated with the VLDL. The apoE secreted by livers from fasted rats may have differences in VLDL binding properties in comparison to the apoE produced by livers from fed animals. Alternatively, an apoE isoform required for VLDL uptake (28) may not be produced by the livers from fasted rats, but may be present in serum and produced by livers from fed animals. Dolphin, Forsyth and Krul (29) reported recently that the d > 1.210 g/ml fraction of rat serum contains an apoE spe-

cies that will actively transfer to VLDL, an observation that supports our observations. An attractive hypothesis is that differences in size of the VLDL influence its association with apoE. In general, we have observed that larger VLDL particles are produced by the liver as synthesis and secretion of triglyceride is stimulated. This is so, for example, for the VLDL secreted by livers from fed rats with the greater output of VLDL in comparison to the VLDL secreted by livers from fasted rats; similarly, an increase in size of the VLDL results from the infusion of free fatty acid into the medium perfusing the isolated liver (15). This concept of the association of apoE with VLDL is in agreement with the recently published data of Salam, Wilcox, and Heimberg (30). It was of particular interest that dialyzed lipoprotein-free plasma (d > 1.210 g/ml) obtained from fed rats, containing about 4 mg of apoE/dl (measured by radioimmunoassay), when added to the perfusion medium, stimulated the uptake of VLDL by livers from fasted rats. A total of 75% (n = 2) of the initial ^{14}C-labeled VLDL-TG radioactivity was taken up in 3 hr, a value similar to that obtained with livers from fed rats in the presence of the lipoprotein-free plasma (group D, Fig. 1) or in the presence of bovine serum albumin. Moreover, the VLDL isolated at the termination of perfusions of livers from fasted rats with lipoprotein-free plasma from fed rats contained apoE, indicating that this apoE will associate with the VLDL produced by livers from fasted rats. Support for this contention is also evident in our observation of the association of apoE with the VLDL secreted by livers from fasted rats provided with 332 μmol of oleic acid/hr. Interestingly, Blum (31) reported that, during alimentary lipemia, increased amounts of apoE became associated with circulating TG-rich particles, without coincident increases in total plasma apoE, and that this resulted from transfer of apoE from HDL. Perhaps a similar phenomenon occurred under our experimental conditions, except that the available apoE was either synthesized and secreted by the liver as a component of the VLDL as required for VLDL formation or was secreted independently in free form, which then later associated with the VLDL. It may be surmised, therefore, that both the rate of synthesis and secretion of VLDL-TG and of apoE represent important determinants of the amount of apoE associated with the circulating VLDL particles.

The extent to which nascent VLDL particles per se can be taken up by the liver is unclear in the literature. We reported that VLDL particles had a very short life in the liver perfusion system, a conclusion derived from our data on the incorporation of radioactive amino acids into VLDL protein (32); this suggested that newly synthesized VLDL must be taken up rapidly by the liver. A similar conclusion was derived recently by Windmueller and Spaeth (33) from the temporal incorporation of radioac-

tive amino acid into VLDL. Several reports have appeared which indicate that nascent VLDL may be taken up by the liver, but the major emphasis has been on the uptake of TG-rich remnant particles. Surprisingly, the calculated uptake of TG mass in the experiments reported here (Table 2), was about 50% of the nascent VLDL-TG synthesized and secreted by livers from fed rats during the 3-hr perfusion with added oleic acid. When oleate was not added, only about 27% (Table 2) of the nascent VLDL-TG was calculated to be taken up. This latter calculated value may be inaccurate if significant secretion of labeled TG, derived from hydrolysis of the pulse radioactive TG, occurred. Since fatty acid was not added, the smaller hepatic fatty acid metabolic pool may have become sufficiently radioactive that newly synthesized TG became detectable in the perfusion medium; thus, 27% may be a minimal value while the actual value may be equal to or greater than the 50% calculated for experiments with livers from fed rats perfused with fatty acid. One should also consider the likely possibility that the VLDL secreted by livers from fed rats, when fatty acid was not infused, contained a lower content of apoE than did the VLDL produced in the presence of oleic acid (6 vs. 20%, Table 4); because of this decreased apoE content, a lower percentage of the VLDL may have been taken up.

According to a previous interpretation, apo C-III may play an important role in VLDL uptake. ApoC-III in the serum is transferred to nascent VLDL, enriching it in that component (34). Presumably, apoC-III opposes stimulation of uptake by apoE, diminishing uptake of the VLDL (4, 6, 7, 34, 35). In our study, no attempt was made to correlate uptake with the ratio apoE/apoC. Clearly, however, in these experiments, the apoE content of the VLDL produced by livers from fasted rats was near zero, regardless of the amount of apoC-III present. The high affinity receptor (B, E) which is involved in remnant uptake is probably also involved in VLDL uptake. Our data strongly support this, particularly since it appears that VLDL devoid of apoE, as produced by livers from fasted rats, is not taken up by the liver. This suggests the importance of the E receptor in the VLDL uptake process, whether the particle is nascent, mature, or remnant, and the potential relation between association of apoE with VLDL after secretion and its effect on the subsequent metabolism of the VLDL. ■

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