Hepatocellular triglyceride synthesis and transfer to lipid droplets and nascent very low density lipoproteins

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Abstract The transfer of triglyceride from sites of synthesis in the endoplasmic reticulum to cytoplasmic lipid droplets and nascent VLDL (very low density lipoproteins) in rat liver in vivo has been examined with [^{3}H]glycerol, cell fractionation, and electron microscopy. Rates of mass transfer of newly synthesized triglyceride were estimated from the specific radioactivity of triglyceride present in microsomal membranes and the radioactivity observed in recipient triglyceride pools. Fasting decreased the transfer of triglyceride to nascent VLDL without affecting transfer to lipid droplets. Stimulation of triglyceride synthesis with 2-tetradecylglycidic acid (TDGA) increased transfer of triglyceride to nascent VLDL 5-fold, and to lipid droplets 14-fold, 1 hr after TDGA administration. Triglyceride transfer to nascent VLDL was increased 6-fold, and to lipid droplets 37-fold, above control rates 6 hr following TDGA treatment, indicative of saturation of triglyceride assembly into nascent VLDL and storage of excess triglyceride in lipid droplet reservoirs. These liver triglyceride pools were concurrently expanded and electron microscopy demonstrated more abundant VLDL particles in the endoplasmic reticulum together with a proliferation of lipid droplets in hepatocytes. TDGA progressively decreased hepatic *sn*-glycerol-3-phosphate in fasting rats while triglyceride synthesis increased, indicating that *sn*-glycerol-3-phosphate does not limit the rate of triglyceride synthesis in this metabolic state. Results implicate triglyceride transfer from endoplasmic reticulum membranes to nascent VLDL as a regulated determinant of hepatic VLDL assembly and VLDL triglyceride secretion in vivo.—**Chao, F-F., D. L. Stiers, and J. A. Ontko.** Hepatocellular triglyceride synthesis and transfer to lipid droplets and nascent very low density lipoproteins. *J. Lipid Res.* 1986. **27:** 1174–1181.

Supplementary key words endoplasmic reticulum • fasting • fatty acid oxidation • glycerol • glycerol-3-phosphate • hepatocyte • microsomes • *p*-phenylenediamine • ultrastructure

Triglyceride is synthesized in liver on the cytosolic surface of the endoplasmic reticulum (1). It then undergoes rapid transfer to sites of storage in cytoplasmic lipid droplets and to sites of incorporation into nascent very low density lipoproteins in the cisternae of the endoplasmic reticulum (2–9). Little is known about the mechanisms of these transfer processes and the factors that govern their rates.

The incorporation of newly synthesized triglyceride into cellular lipid droplets has been investigated with radioactive (4, 6–9) and fluorescent (10, 11) precursors. The synthesis of triglyceride and its appearance in nascent VLDL particles (4, 6, 12), isolated from hepatic subcellular compartments of lipoprotein assembly (13–15), have also been examined. However, the rates of mass transfer of newly synthesized triglyceride from the endoplasmic reticulum to lipid droplets and developing VLDL in liver in intact animals have not been determined. In the present study, an approach to the measurement of these rates is described, together with analysis of the effects of fasting and accelerated triglyceride synthesis on intracellular triglyceride transport and deposition.

MATERIALS AND METHODS

Materials

Glycerol-3-phosphate dehydrogenase and glycerokinase were obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. [1,2,3-^{3}H]Glycerol (200 mCi/mmol) was supplied by New England Nuclear, Boston, MA. TDGA (McN-3802) was provided by McNeil Laboratories, Fort Washington, PA.

Liver subcellular fractionation

Male Holtzman rats (250–320 g) were supplied by Charles River Breeding Laboratories, Wilmington, MA, and maintained on water and Purina Laboratory Chow ad libitum. Rats were housed at 22°C with lights on at 6 AM and off at 6 PM. Food was removed from fasted rats

Abbreviations: VLDL, very low density lipoproteins; TDGA, 2-tetradecylglycidic acid; apoB and apoE, apolipoproteins B and E; PAGE, polyacrylamide gel electrophoresis.

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at 4 PM on the day prior to experiment. [1,2,3-^{3}H]Glycerol was injected intraperitoneally (20 μCi/100 g body wt) at 8–9 AM in control rats and as indicated in TDGA-treated rats. TDGA was dissolved in 9% fatty acid-free bovine serum albumin in 0.15 M NaCl, pH 7.4, and injected intraperitoneally (10 mg/kg body wt) at 8 AM. Rats were decapitated and blood was collected. Livers were immediately removed and homogenized (10% w/v) in 0.25 M sucrose–3 mM Tris–1 mM EGTA (pH 7.4) and centrifuged at 500 *g* for 10 min at 0–4°C (16). The supernatant was centrifuged at 9,000 *g* for 10 min. The pellets were combined, rehomogenized in the original volume of sucrose–Tris–EGTA, and centrifuged at 9,000 *g* for 10 min. This sediment was designated the low speed pellet. The combined supernatants were centrifuged at 4°C for 2.5 hr at 105,000 *g*. The top fraction (lipid droplets) was removed by tube-slicing. The microsomal pellet was washed with 0.15 M Tris buffer at pH 8.0 (17) and centrifuged at 105,000 *g* for 2 hr. As measured by glucose-6-phosphatase activity in six different experiments, an average of 81% of the membranes of the endoplasmic reticulum was recovered in this fraction. Recoveries from liver homogenates of fed and fasted rats were similar. The absence of lipid droplets in these sedimented microsomes was verified by electron microscopy. The washed pellet was resuspended in ice-cold distilled water. Intravesicular contents were released by sonication (Branson W 140 Sonifier, Plainview, NY) at setting 5.0 for 3 min in an ice bath. The membranes were sedimented at 105,000 *g* for 2 hr. In six different experiments, an average of 85% of the microsomal glucose-6-phosphatase activity was recovered in this membrane fraction. Recoveries were similar in membranes isolated from fed and fasted rats. The supernatant (contents fraction) was removed, adjusted to 1.10 g/ml with solid NaCl and NaBr, overlayered with a solution of NaCl at a density of 1.03 g/ml [as recommended by Glaumann, Bergstrand, and Ericsson (6)] containing 0.05% EDTA and 0.01% Thimerosal, and centrifuged at 105,000 *g* at 4°C for 22 hr. The top fraction was isolated by tube-slicing and designated as microsomal VLDL (6). Only 9% of both triglyceride and triglyceride radioactivity of the contents fraction remained in the subnatant following flotation of the microsomal VLDL. An alternative method for the separation of microsomal membranes and VLDL (18) yielded similar results. Complete removal of nascent VLDL particles from the microsomes was established by electron microscopy.

Lipid analyses

Serum and liver fractions were extracted and washed (19). The lipid extracts were evaporated to dryness and dissolved in chloroform, an aliquot of which was assayed for triglyceride (20). The radioactivity in lipid fractions, separated by thin-layer chromatography on silica gel 60 G (21), was counted by liquid scintillation with correction for quenching by external standard.

Electron microscopy

Rats were anesthetized with Nembutal (50 mg/kg body wt, i.p.). Livers were perfused for 5 min via the hepatic portal vein with 1.5% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.4 (22). After perfusion, the livers were removed and portions were cut in 1-mm^3 blocks. These were immediately placed in 2% OsO_4 in 0.02 M sodium phosphate at pH 7.4 for subsequent immersion fixation for 2 hr at room temperature. The tissue blocks were then incubated in 1% *p*-phenylenediamine (free base) in 70% ethanol for 1 hr (23). The tissues were then en bloc stained in 2% uranyl acetate in 70% ethanol, dehydrated, and embedded in epoxy resin (24). Pelleted microsomes and microsomal membranes were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde (25) in 0.1 M cacodylate at pH 7.2 for 1 hr at room temperature, post-fixed in 2% OsO_4 in 0.1 M cacodylate at pH 7.2 for 2 hr, dehydrated in ethanol, and embedded as above. Ultrathin sections were cut on a Sorvall MT 6000 ultramicrotome and photographs were taken with a JEOL JEM-1200EX electron microscope.

Other analyses

Serum glycerol (26), *sn*-glycerol-3-phosphate from freeze-clamped liver (27) and glucose-6-phosphatase (28) were also measured. The significance of differences was determined by Student's *t* test with a two-tailed measurement of *P* values. Apolipoproteins of microsomal VLDL were analyzed by electrophoresis in sodium dodecylsulfate–3.5% polyacrylamide gel as previously described (29, 30). For comparison, hepatic VLDL was also isolated from the medium of rat liver perfused according to procedures described earlier (31, 32). The microsomal VLDL and liver perfusate VLDL were dialyzed against 5 mM NH_4HCO_3 (33), 1 mM EDTA, and 1.5 mM NaN_3 overnight. Prior to dialysis, the liver perfusate VLDL was washed with aqueous NaCl at a density of 1.006 g/ml and recentrifuged at 105,000 *g* for 22 hr at 4°C. Following dialysis, the VLDL preparations were concentrated by ultrafiltration with Centriflo membrane cones (CF-25, Amicon, Danvers, MA) and delipidated according to Herbert et al. (33) prior to loading on the SDS-polyacrylamide gels.

RESULTS AND DISCUSSION

Effects of fasting on the amounts of triglyceride in subcellular compartments of liver

Rat liver was fractionated into lipid droplets, microsomal membranes, microsomal VLDL, and a low speed

pellet that contained nuclei, mitochondria, and lysosomes. The yield of microsomes was 81% in both fed and fasted rats (see Materials and Methods). The low speed pellet contained about 18% of the total hepatic triglyceride in both fed and fasted rats, owing to the presence of adherent lipid droplets and microsomes. In fed rats the amounts of triglyceride in whole liver, lipid droplets, microsomal membranes, and microsomal VLDL were: 6.03 ± 0.34, 3.65 ± 0.21, 0.34 ± 0.02, and 0.42 ± 0.02 μmol/g liver, respectively. The corresponding values in liver after fasting for 16 hr were: 5.72 ± 0.38, 3.39 ± 0.12, 0.53 ± 0.05, and 0.26 ± 0.02 μmol/g liver, respectively. The triglyceride associated with the microsomal membrane was increased ($P < 0.005$) and that present in newly assembled VLDL was decreased ($P < 0.001$) by fasting. These alterations are consistent with the possibility that fasting decreased the transfer of newly synthesized triglyceride to developing VLDL particles and thereby caused accumulation in the precursor pool of triglyceride associated with the microsomal membrane.

Measurement of synthesis and transfer of triglyceride to specific compartments

[1,2,3-^{3}H]Glycerol was employed to trace the movement of newly synthesized triglyceride. Early after glycerol administration, the greatest amount of radioactivity was recovered in the microsomal membrane fraction (**Fig. 1**), in agreement with other observations (6, 12). [^{3}H]Triglyceride subsequently appeared in VLDL and lipid droplets with similar lag periods, consistent with the involvement of similar transport mechanisms. Triglyceride movement may be mediated by a transfer protein (34). Similar amounts of newly formed triglyceride were transferred to lipid droplets and to VLDL particles during the first 10–15 min, after which the amounts sequestered in lipid droplets increased. During this phase the radioactivity in the VLDL in the endoplasmic reticulum declined as the nascent VLDL were secreted into the circulation (Fig. 1). From this experiment, a 10-min period of labeling was selected to trace the initial synthesis and transfer of triglyceride to the droplet and VLDL targets.

The VLDL isolated from liver microsomes was characterized by analysis of the radioactivity present in lipid classes 10 min after [^{3}H]glycerol injection and by apolipoprotein composition. The distribution of radioactivity in total VLDL lipids was 94.5%, 1.6%, and 3.9% in the triglyceride, diglyceride, and phospholipid fractions, respectively. Apolipoproteins of the isolated microsomal VLDL were examined by SDS-3.5% PAGE (29, 30). As shown in gel A of **Fig. 2,** distinct apoB-100, apoB-48, and apoE bands (35–38) were observed. These are labeled 1, 2, and 4, respectively. Since the triglyceride-rich lipopro-

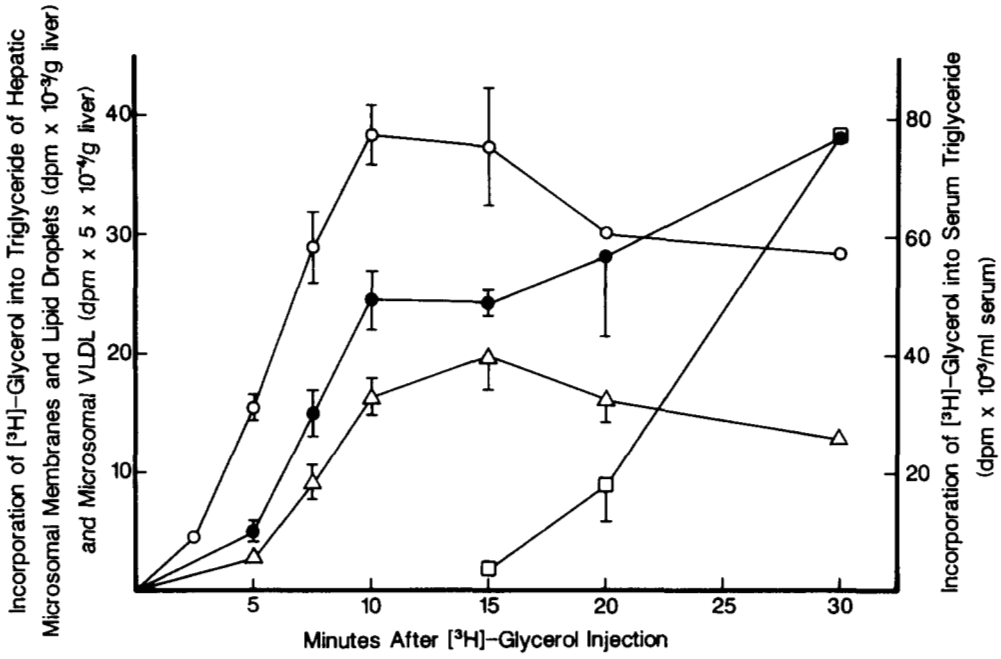

Fig. 1. Radioactivity in liver and serum triglyceride pools following [1,2,3-^{3}H]glycerol administration. Fed rats were injected intraperitoneally with [1,2,3-^{3}H]glycerol. At the indicated times thereafter, blood and livers were removed for the measurement of triglyceride radioactivity in liver microsomal membranes (○), liver microsomal VLDL (△), liver lipid droplets (●), and in serum (□) with n = 3 at 2.5, 7.5, 15, and 20 min, n = 6 at 5 min, n = 7 at 10 min and n = 1 at 30 min. The tritium in lipid droplets and microsomal VLDL at 2.5 min and in serum triglyceride prior to 15 min was very low and these data points were therefore not included. One of the microsomal membrane samples at 20 min was lost. The bars indicate SEM. The SEM not shown at 2.5, 5, 15, and 20 min were smaller than the symbols.

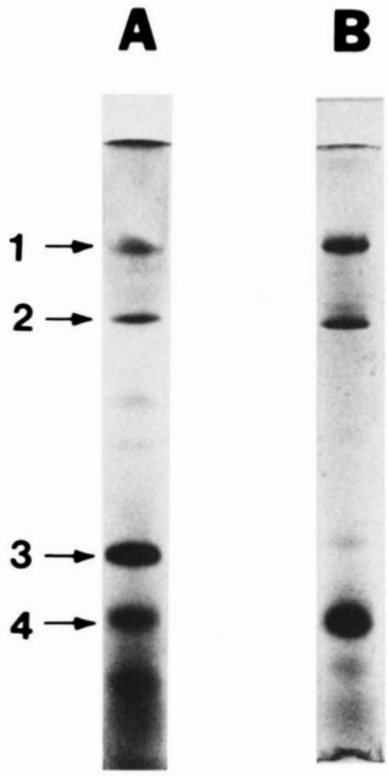

Fig. 2. Resolution of apolipoproteins in liver microsomal and liver perfusate VLDL by SDS-polyacrylamide gel electrophoresis. The polyacrylamide concentration was 3.5%. Gel A. Triglyceride-rich lipoprotein particles were isolated from sonicated rat liver microsomes. The livers from two rats, which were fasted overnight, were pooled for this isolation and analysis. Gel B. VLDL secreted by the liver was obtained by perfusion of the isolated intact organ with a serum-free medium by recirculation for 225 min. The donor was a fed rat in the gel shown. Blood was completely removed from the liver by single-pass perfusion prior to its initial connection to the recirculating perfusion circuit. ApoB-100, apoB-48, albumin, and apoE are labeled 1, 2, 3, and 4, respectively.

teins isolated from the liver microsomes were not washed by reflotation, a prominent albumin band (position 3 of gel A in Fig. 2) was also present. These bands were compared with those exhibited by VLDL isolated from a recirculating rat liver perfusion medium. In the gel shown (gel B in Fig. 2), the liver from a fed Holtzman rat was perfused with oleic acid as previously described (31, 32) and the secreted VLDL were harvested. These VLDL were washed by dilution and recentrifugation. Accordingly, the albumin band was relatively faint. Identical apoB-100, apoB-48, and apoE bands in both gels A and B in Fig. 2 established the identity of the isolated microsomal lipoprotein particles as nascent VLDL.

If the sonication procedure, employed to release nascent VLDL, promoted exchange of triglyceride between microsomal membranes and VLDL particles, triglyceride radioactivity data could not be used to calculate triglyceride transfer. However, the sonication treatment did not induce triglyceride exchange, since *1)* the microsomal membrane triglyceride specific radioactivity always exceeded that of microsomal VLDL; *2)* at 2.5 min, when the microsomal membrane triglyceride was labeled (Fig. 1), microsomal VLDL triglyceride did not contain detectable radioactivity; and *3)* similar results were obtained when we used the alkaline Na_2CO_3 treatment (18), instead of sonication, to separate microsomal membranes and content VLDL.

Alterations in triglyceride transfer to lipid droplets and VLDL following accelerated synthesis

The rates of transfer of triglyceride to these pools in the fed and fasting states were then compared. In addition, the rates of transfer in fasting animals were compared under conditions of accelerated triglyceride synthesis. TDGA, an inhibitor of long chain fatty acid oxidation (39), was employed to increase triglyceride synthesis.

All liver triglyceride pools were expanded by TDGA treatment (**Table 1**). Total liver triglyceride increased at a constant rate. One hr after TDGA, the amounts of triglyceride recovered in the lipid droplet, microsomal membrane, and secretory VLDL pools, were two to three times greater. At 6 hr, triglyceride deposition in all pools increased further, with the predominant elevation in the reservoir of lipid droplets. Serum glycerol was unaffected by TDGA treatment (not shown).

Sn-glycerol-3-phosphate is a key substrate in triglyceride synthesis (40, 41). The potential involvement of *sn*-glycerol-3-phosphate in the regulation of triglyceride synthesis has been discussed (41–46). The possibility that the *sn*-glycerol-3-phosphate concentration influences the rate of triglyceride synthesis in liver in the fasting state, when *sn*-glycerol-3-phosphate production from carbohydrate is diminished, was examined in the present study by the measurement of *sn*-glycerol-3-phosphate in freeze-clamped livers from fasted control rats and from rats treated with TDGA, as described in Table 1. The hepatic

TABLE 1. Effects of increased triglyceride synthesis on accumulation of triglyceride in subcellular compartments of rat liver

	Triglyceride (μmol/g wet wt liver)		
Fraction	Control (13)	TDGA, 1 hr (6)	TDGA, 6 hr (4)
Whole liver	5.72 ± 0.38	12.60 ± 1.14[a]	48.43 ± 2.51[a,b]
Lipid droplet	3.39 ± 0.12	6.50 ± 0.52[a]	33.31 ± 1.20[a,b]
Microsomal membrane	0.53 ± 0.05	1.10 ± 0.05[a]	1.35 ± 0.32[c]
Microsomal VLDL	0.26 ± 0.02	0.86 ± 0.18[a]	1.32 ± 0.22[a]

All rats were fasted from 4 PM to 8 AM. Triglyceride synthesis was elevated by TDGA (10 mg/kg body wt) and livers were removed 1 hr and 6 hr later. Livers were removed from control rats at 8 AM. Values are means ± SEM with n in parentheses.

[a] Difference between control and TDGA, $P < 0.001$.

[b] Difference between TDGA 1 hr and TDGA 6 hr, $P < 0.001$.

[c] Difference between control and TDGA, $P < 0.005$.

sn-glycerol-3-phosphate contents in the control-fasted, 1 hr after TDGA, and 6 hr after TDGA groups were: 271 ± 19 nmol/g wet wt, 161 ± 12 nmol/g wet wt ($P < 0.005$), and 117 ± 4 nmol/g wet wt ($P < 0.001$) with n = 4 in each group. Thus, the liver *sn*-glycerol-3-phosphate content decreased under conditions of increased triglyceride synthesis (Table 1). It therefore appears that the concentration of *sn*-glycerol-3-phosphate does not limit the rate of triglyceride synthesis in the fasting state under these conditions.

Deposition of triglyceride in lipid droplets within hepatocytes of liver following TDGA treatment was established by electron microscopy (**Fig. 3**). At 6 hr, numerous lipid droplets of variable size were visible, together with an increase in VLDL within membrane-bound vesicles. These vesicular profiles are shown at higher magnification in **Fig. 4a** and may be derived from VLDL particle assembly in the cisternae of the endoplasmic reticulum (Fig. 4b). Marked electron density of lipid droplets and VLDL particles results from the mordant effect of *p*-phenylenediamine with osmium (23). For reasons that are not yet clear, the intensity of this reaction was variable. Accordingly, a range of electron densities was exhibited. In both control and TDGA-treated livers (Fig. 3), lipid droplets exhibited no limiting membranes, the absence of which was also clear when *p*-phenylenediamine exposure was omitted (not shown). Abnormal triglyceride-rich structures were not evident. Therefore, the drug appears to accelerate normal processes of triglyceride synthesis and transfer to the storage droplet and secretory VLDL pools.

The rates of transfer of triglyceride from sites of synthesis in the membranes of the endoplasmic reticulum to cytoplasmic lipid droplets and to nascent VLDL particles in the secretory pathway were estimated from the specific radioactivity of the precursor pool of microsomal membrane triglyceride (**Table 2**). The lower specific radioactivity of this pool in fasted rats (Table 2) may result from the elevated plasma glycerol in these animals (47). Radioactivity accumulated in this triglyceride pool at a nearly constant rate during the first 10 min after [1,2,3-^{3}H]glycerol injection (Fig. 1). A lag period of 1.5 min, based on extrapolation of the microsomal membrane data points (Fig. 1), was exhibited. Tritium appeared in lipid droplet and microsomal VLDL triglyceride after a lag period of approximately 4 min (Fig. 1). Therefore, the transfer of triglyceride to these two pools, measured at 10 min, represents the transfer of triglyceride synthesized between 1.5 and 7.5 min following [1,2,3-^{3}H]glycerol injection. The average triglyceride specific radioactivity in the microsomal membrane precursor pool during this 6-min period was estimated to be the calculated value at 4.5 min. The rates of transfer of triglyceride from the membranes of the endoplasmic reticulum to lipid droplets and nascent VLDL were estimated from the tritium incorporation into these pools at 10 min divided by the average specific radioactivity of the precursor pool and then divided by 6. The kinetics of [1,2,3-^{3}H]glycerol incorporation (Fig. 1) were also examined in fasted and fasted TDGA-treated rats. Similar lag periods of tritium incorporation were observed under all conditions. In ad-

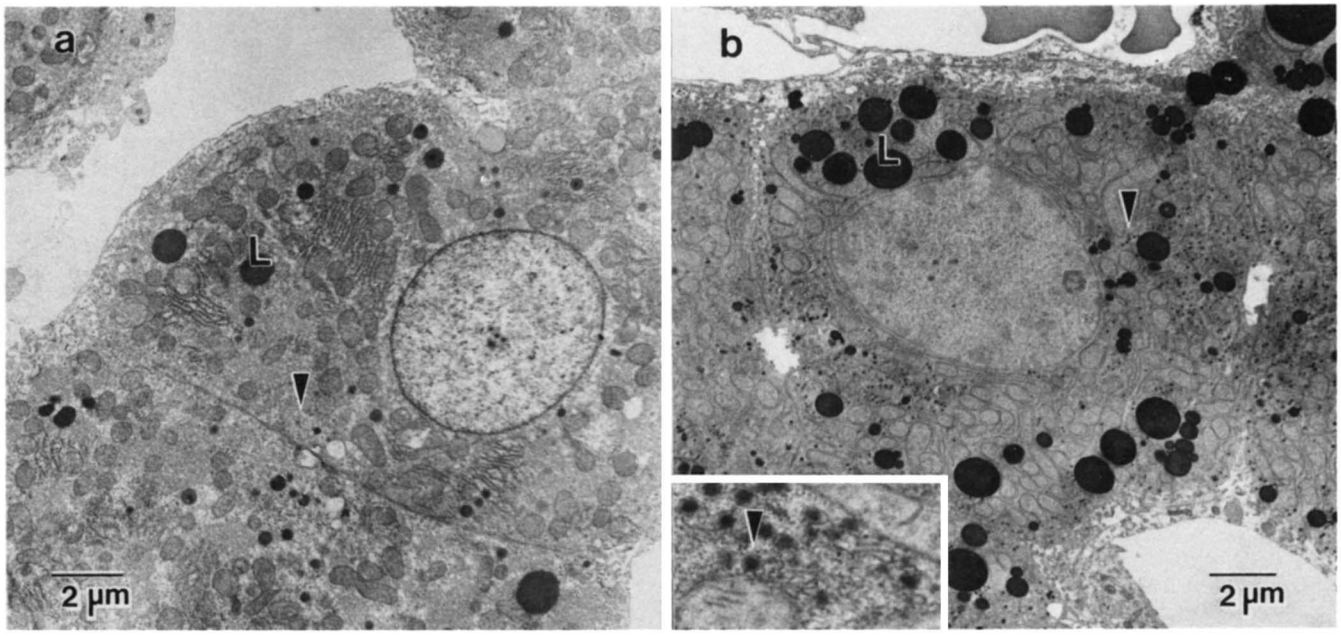

Fig. 3. Electron micrographs of ultrathin sections post-fixed with OsO_4 and reduced with *p*-phenylenediamine. (a) Liver of fasted control rat; (b) liver of fasted rat 6 hr after TDGA treatment. Magnification of (a) and (b) are both ca. 4,000; the insert is ca. 30,000. Arrows indicate individual VLDL particles. Lipid droplets and VLDL particles of normal appearance and much greater abundance were found in hepatocytes following TDGA treatment. Abbreviation: L, lipid droplet.

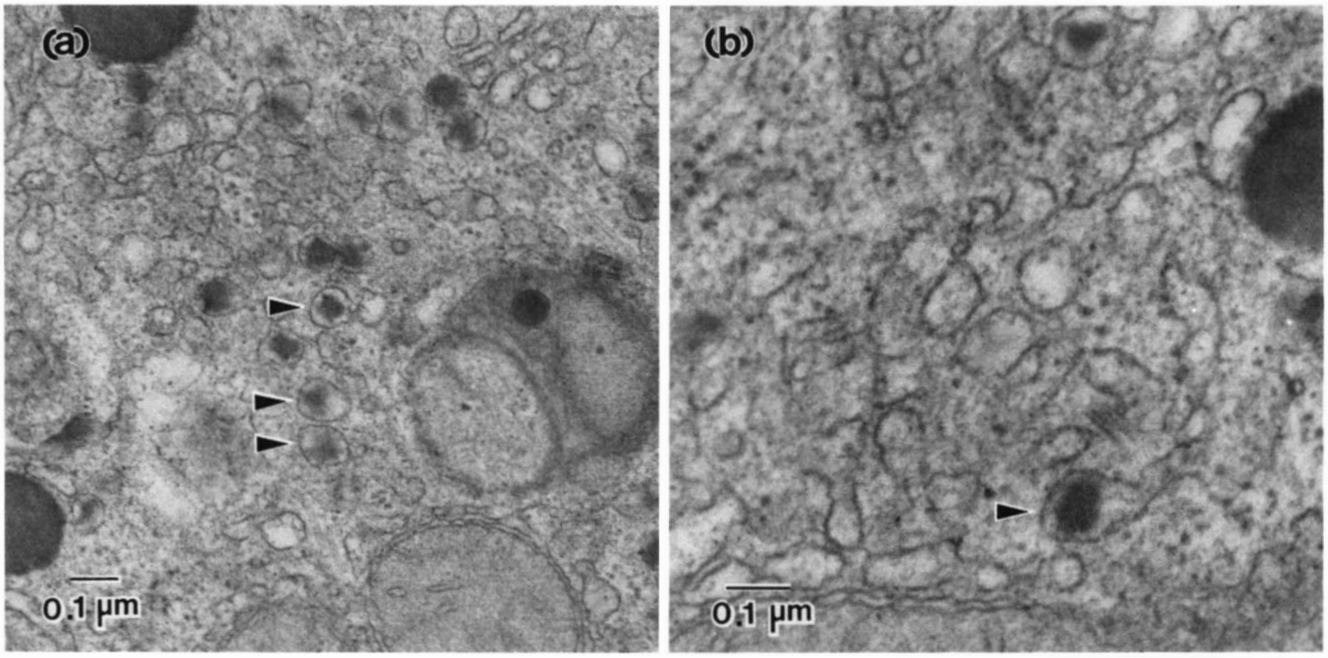

Fig. 4. Electron micrographs prepared as described in Fig. 3 from the liver of a fasted rat 6 hr after TDGA treatment. The arrows in panel (a) point to vesicular profiles which contain lipid particles with diameters of VLDL [300–700 Å (13–15)]. The upper arrow points to a vesicle, the right side of which appears to be budding from, or fusing with, an adjacent membrane structure. A VLDL particle in the cisternae of the endoplasmic reticulum is shown in panel (b). Magnification of (a) ca. 60,000 and (b) ca. 85,000.

dition, the rates of tritium accumulation in the liver triglyceride pools, following the initial delay, remained constant in all animal groups for at least 10 min after [1,2,3-^{3}H]glycerol administration. The rate of assembly of newly synthesized triglyceride into VLDL was depressed by fasting whereas transfer to lipid droplets was unaffected (Table 2). These results suggest that decreased hepatic triglyceride secretion in the fasting state (48) occurs not as a result of decreased triglyceride synthesis but as a consequence of decreased transfer of newly synthesized triglyceride to developing VLDL particles. Considering the losses of hepatic VLDL inherent in the isolation procedure, the observed rate of triglyceride transfer to VLDL in fasted rat liver (75 nmol/min per g; Table 2) agrees well with the rate of release of hepatic triglyceride into serum calculated by Palmer, Cooper, and Shipley (7). When triglyceride synthesis was elevated by TDGA, the rates of transfer to lipid droplets were increased 14-fold and 37-fold at 1 hr and 6 hr following TDGA treatment (Table 2). The amounts of triglyceride that migrated to the developing VLDL particles were increased 5-fold at 1 hr, with no further increase at 6 hr, indicating attainment of a maximum rate of VLDL assembly. In the liver perfusion study of Kondrup (8), when triglyceride synthesis was elevated by an increased concentration of [1-^{14}C]palmitate, it was also found that labeled triglyceride in lipid droplets increased to a greater degree than did radioactive triglyceride in the microsomal fraction.

In the present study, the specific radioactivity of triglyceride in microsomal VLDL approached 90% of that found in newly synthesized triglyceride in the microsomal membrane 15 min after [^{3}H]glycerol injection. The tri-

TABLE 2. Rates of transfer of newly synthesized triglyceride from microsomal membranes to lipid droplets and nascent VLDL in livers of fed, fasted, and fasted TDGA-treated rats

Rats	Triglyceride Transferred (nmol/min per g wet wt liver)	
	Lipid Droplets	Microsomal VLDL
Fed (7)	93 ± 7	125 ± 13
Fasted (5)	94 ± 7	75 ± 4[a]
Fasted + TDGA, 1 hr (4)	1253 ± 33[b]	342 ± 55[c]
Fasted + TDGA, 6 hr (4)	3260 ± 166[b,d]	415 ± 61[b]

Rats were injected with [1,2,3-^{3}H]glycerol and 10 min later livers were removed for isolation of lipid droplets, microsomal membranes, and microsomal VLDL. Livers were removed from TDGA-treated rats 1 hr and 6 hr after injection of 10 mg TDGA/kg body wt. The mean microsomal membrane triglyceride specific radioactivities at 10 min were: fed, 123,230 ± 6520; fasted, 68,140 ± 5350; fasted + TDGA, 1 hr, 82,250 ± 4450; fasted + TDGA, 6 hr, 76,470 ± 7290 dpm/μmol (fed versus each fasted group, $P < 0.001$). Triglyceride transfer rates were calculated as described in the text. Values are means ± SEM with n in parentheses.

[a] Difference between fed and fasted, $P < 0.001$.
[b] Difference between fasted and fasted + TDGA, $P < 0.001$.
[c] Difference between fasted and fasted + TDGA, $P < 0.005$.
[d] Difference between TDGA 1 hr and TDGA 6 hr, $P < 0.001$.

glyceride specific radioactivity in lipid droplets was, in contrast, comparatively very low (6% of that found in the microsomal membrane). Similar observations were reported by others (7, 12). These data indicate that en bloc transfer of intact triglyceride molecules from lipid droplets to developing VLDL does not contribute appreciably to the process of VLDL assembly.

It is clear from several lines of evidence that fasting decreases the hepatic production of VLDL. The earlier literature on the subject has been summarized (48–51). More recent data have been provided by studies of VLDL secretion by perfused rat livers (52, 53) and by rat hepatocytes in primary culture (54), in which fasting diminished the synthesis and secretion of apolipoprotein B. This decrease may be related to the decreased transfer of triglyceride from intracellular membrane sites of synthesis to developing VLDL particles reported here (Table 2). High concentrations of plasma free fatty acids provide the principal substrates for liver triglyceride synthesis in the fasting animal. Decreased incorporation of this triglyceride into VLDL may cause a reduction in both the size and the number of nascent VLDL particles. The present results suggest that the transfer of triglyceride to developing VLDL in the liver is regulated and that the rate of transfer is a determinant of the overall rate of VLDL triglyceride secretion. The mechanism of this transfer process and the factors that govern its rate remain to be elucidated.

The present studies define and contrast the limited capacity of the process of triglyceride transport from sites of synthesis to developing VLDL particles and the markedly greater potential for the net transfer of triglyceride to lipid droplets. Lipid droplets are much larger than hepatic VLDL, suggesting that restrictions in the maximum rate of transfer of triglyceride to the developing VLDL particle are imposed by the factors that determine the maximum size of these lipoproteins and the maximum number of VLDL particles produced per unit time. Although the appearance of ^{3}H-labeled triglyceride in hepatic VLDL and lipid droplets after similar lag periods following [^{3}H]glycerol administration is indicative of similar triglyceride transport mechanisms, factors that control the size of VLDL, possibly apolipoprotein B, are apparently absent in lipid droplets, thereby permitting their unrestricted growth.

The technical assistance of Latricia S. Horne and Lynda W. Perrin is gratefully acknowledged. We thank Dr. Munehiko Yamamoto for the rat liver perfusion, which provided VLDL secreted by the isolated intact liver. We also thank Dr. G. F. Tutwiler of McNeil Laboratories, Fort Washington, PA, for the sample of TDGA. This work was supported by United States National Institutes of Health Grant HL 32609.

Manuscript received 12 March 1986.

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