Inhibition of fatty acid synthesis decreases very low density lipoprotein secretion in the hamster

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Abstract The hamster was developed as a model to study very low density lipoprotein (VLDL) metabolism, since, as is the case in humans, the hamster liver was found to synthesize apoB-100 and not apoB-48. The effect of inhibiting fatty acid synthesis on the hepatic secretion of VLDL triglyceride (TG) and apolipoprotein (apo) B-100 in this model was then investigated. In an in vivo study, hamsters were fed a chow diet containing 0.15% TOFA (5-tetradecyloxy-2-furancarboxylic acid), an inhibitor of acetyl-CoA carboxylase. After 6 days of treatment, plasma triglyceride and cholesterol levels were decreased by 30.2% and 11.6%, respectively. When the secretion of VLDL-TG by the liver was measured in vivo after injection of Triton WR 1339, TOFA treatment was found to decrease VLDL-TG secretion by 40%. In subsequent in vitro studies utilizing cultured primary hamster hepatocytes, incubation with 20 μM TOFA for 4 h resulted in 98% and 76% inhibition in fatty acid and triglyceride synthesis, respectively; VLDL-TG secretion was decreased by 90%. When hepatocytes were pulsed with [^{3}H]leucine, incubation with TOFA resulted in a 50% decrease in the incorporation of radiolabel into secreted VLDL apoB-100. The results of this study indicate that inhibition of intracellular triglyceride synthesis decreases the secretion of VLDL-TG and apoB-100, and does not result in the secretion of a dense, triglyceride-depleted lipoprotein.—**Arbeeny, C. M., D. S. Meyers, K. E. Bergquist**, and **R. E. Gregg**. Inhibition of fatty acid synthesis decreases very low density lipoprotein secretion in the hamster. *J. Lipid Res.* 1992. **33:** 843–851.

Supplementary key words triglyceride production • lipogenesis • apolipoprotein B • acetyl-CoA carboxylase • fatty acid synthesis

Very low density lipoproteins (VLDL) have an important metabolic function in transporting triglycerides, which are synthesized by the liver, to the peripheral tissues for utilization. The hepatic synthesis of VLDL involves the biosynthesis of the lipid and apolipoprotein moieties, the assembly of the lipid and apolipoproteins to form the nascent VLDL particle, and the secretion of the mature VLDL into the circulation (1, 2). However, the processes regulating the assembly and secretion of VLDL-TG and apolipoproteins are not clearly understood.

Studies performed principally in the rat and in HepG2 cells have shown that fatty acid levels regulate the synthesis and secretion of VLDL triglyceride. In rat liver perfusions (3, 4) and in studies with primary rat hepatocytes (5, 6), incubation with oleic acid stimulated the synthesis of cellular triglyceride and the secretion of VLDL-TG. Studies with HepG2 cells (7, 8) have also shown that oleic acid increased the secretion of lipoprotein triglyceride. There is evidence that limiting fatty acid synthesis decreases VLDL-TG secretion. TOFA (5-(tetradecyloxy)-2-furancarboxylic acid) has been developed as a potent inhibitor of acetyl-CoA carboxylase, the rate-limiting step in de novo fatty acid synthesis (9). Previous studies in the rat have shown that chronic treatment with TOFA resulted in a significant decrease in plasma triglyceride and cholesterol levels (9). TOFA treatment also decreased triglyceride synthesis and VLDL-TG secretion by the perfused rat liver (4). However, the effect of inhibiting triglyceride synthesis on the synthesis and secretion of apoB-100 has not been reported.

Although a great deal of information on VLDL metabolism has been obtained in the rat and in HepG2 cells, there are limitations to using these systems as models to study the regulation of VLDL synthesis and secretion in humans. In contrast to human lipoprotein metabolism, the rat has very low levels of LDL in plasma (10) and does not express cholesteryl ester transfer protein (CETP) activity (11). Furthermore, rat hepatocytes synthesize VLDL particles that contain either apoB-48 or B-100 (12, 13), which is dissimilar to the VLDL secreted by the human liver which contains solely apoB-100 (14). The human hepatoma cell line, HepG2, also has its limitations for these studies as these cells secrete apoB-100 triglyceride-rich lipoproteins that are isolated in the LDL rather than VLDL density range (15).

Abbreviations: VLDL, very low density lipoprotein; apo, apolipoprotein; TG, triglyceride; TOFA, 5-(tetradecyloxy)-2-furancarboxylic acid; CETP, cholesteryl ester transfer protein; DMEM, Dulbecco's modified Eagle medium; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; LDL, low density lipoprotein; HDL, high density lipoprotein.

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In the present study we have developed the hamster as a small rodent model to study the regulation of VLDL-TG and apoB synthesis and secretion. The plasma lipoprotein profile in the hamster is more analogous to that found in human subjects in that there are appreciable levels of LDL as well as HDL (16). The hamster has been shown to express plasma CETP activity which is regulated by the level of cholesterol-containing lipoproteins in plasma (17, 18). We (19) and others (20) have recently found that the VLDL that is secreted by the perfused hamster liver contains only apoB-100 as is the case in humans. We have used the hamster model to study the effect of limiting triglyceride synthesis on the synthesis and secretion of VLDL-TG and apoB-100 by the liver.

EXPERIMENTAL PROCEDURES

Materials

Dulbecco's modified Eagle medium (DMEM, low glucose, leucine-free) was obtained from Gibco BRL (Grand Island, NY). Fatty acid-free bovine serum albumin (Fraction V), collagenase (Type IV), collagen (rat tail, Type I), insulin (from bovine pancreas), and oleic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Radiolabeled glycerol ([2-^{3}H]glycerol, 7.4 GBq/mmol) was obtained from New England Nuclear (Boston, MA), leucine ([4,5-^{3}H(N)]leucine, 4.4–7.0 TBq/mmol) was obtained from Amersham (Arlington Heights, IL) and acetate ([1-^{14}C]acetic acid, 2.03 GBq/mmol) was obtained from ICN Biomedicals, Irvine, CA. TOFA (5-(tetradecyloxy)-2-furancarboxylic acid) was a generous gift from Merrell Dow Research Institute of Marion Merrell Dow, Inc. (Cincinnati, OH).

Animals

Male Syrian Golden hamsters (Sasco Breeders, Omaha, NE) weighing 120–140 g were used in these studies. The animals were given Purina rodent chow (#5001) ad libitum. In one study, hamsters were fed the chow diet or chow supplemented with 0.15% TOFA for 6 days. For lipid and lipoprotein analyses, a blood sample was taken from nonfasted animals under ether anesthesia (at 11 am-noon) from the eye orbital venous plexis.

Measurement of the in vivo secretion of VLDL-TG

The effect of TOFA treatment on the in vivo secretion rate of VLDL by the liver was determined using Triton WR 1339 (21). After a 30-h fast, the hamsters were anesthetized with ether and a blood sample was taken from the eye orbital venous plexis. The animals were injected with Triton WR 1339 (250 mg/kg) via the jugular vein, and then allowed to recover from the anesthesia. At 2 h after the Triton injection, the animals were test-bled and the increase in plasma triglyceride relative to time 0 was determined.

Liver perfusion

In order to isolate nascent hepatic VLDL, the livers from chow-fed Sprague-Dawley rats (125 g, Taconic Breeders, Germantown, NY) or hamsters were perfused in a recirculating system. The isolated liver perfusion procedure was similar to that described by Miller (22), except that a membrane oxygenator was used. The perfusate consisted of Krebs-Henseleit buffer at pH 7.4 which contained 3% albumin, antibiotics, and essential amino acids. The liver was perfused in a recirculating system at a flow rate of 2–3 ml/min per g of liver with a pressure of 12 cm of water. Liver viability was assessed by maintenance of hepatic flow, bile flow, and the level of SGOT and SGPT in the perfusate. After 2.5 h, the perfusate was collected and concentrated. The VLDL was isolated from the perfusate by ultracentrifugation at a density of 1.006 g/ml.

Isolation and culture of primary hamster hepatocytes

Hepatocytes were isolated from the livers of male hamsters by collagenase perfusion, by a modification of the procedure used for the isolation of rat hepatocytes (23). After cannulation of the portal vein and vena cava, the liver was perfused for 10 min with oxygenated Hank's balanced salt solution (HBSS, Ca^{2+}- and Mg^{2+}-free) containing antibiotics, 25 mM Na-bicarbonate, and 10 mM HEPES buffer at pH 7.4. During this time interval, the liver was isolated and placed in a 37°C chamber. The flow rate was 12–18 ml/min with a hydrostatic pressure head of 12 cm. The liver was then perfused for 20 min with HBSS containing antibiotics, 25 mM Na-bicarbonate, 10 mM HEPES, 5 mM $CaCl_2$, and 50 mg/dl collagenase (Type IV), at pH 7.6. The liver cells were dissociated by gentle scraping and then were suspended in DMEM containing 1% BSA and antibiotics, and filtered. The cells were centrifuged at 100 *g* at 4°C for 3 min, and washed three times in this buffer by centrifugation. The cell yield was 1–3 × 10^{8} cells/liver with a viability of >85% (as determined by trypan blue exclusion).

The cells were plated on a collagen matrix at a density of 1.5 × 10^{6} cells/60-mm dish in DMEM containing 20% fetal calf serum, 1 μg/ml insulin, 5 μM $MgCl_2$, and antibiotics. After an 18-h culture, the hepatocyte cultures were incubated with 3.0 ml of serum-free media containing 15 μCi [^{3}H]glycerol, 30 μCi [^{14}C]acetate, or 15 μCi[^{3}H]leucine, which was supplemented with 0.25 mM fatty acid-free bovine serum albumin (BSA), 1.0 mM oleic acid or 20 μM TOFA (both complexed to BSA at a 4:1 molar ratio), for up to 4 h.

The incorporation of [^{3}H]glycerol into cellular and secreted triglyceride (24), and the incorporation of

[^{14}C]acetate into cellular total cholesterol (25), esterified cholesterol (26), and nonesterified fatty acids (25) were determined by analyses of the appropriate spots after thin-layer chromatography of the lipid extracts. The incorporation of labeled [^{3}H]leucine into intracellular apoB-100 was determined by an immunoprecipitation procedure (27) using a polyclonal rabbit anti-hamster apoB-100 IgG fraction. The immunoprecipitate was subjected to SDS gel electrophoresis using gradients of 4–20% acrylamide, and the radioactivity associated with the apoB band was then determined. In order to determine the effect of the various incubation conditions on the secretion of apoB-100, VLDL was isolated from the media by ultracentrifugation, followed by precipitation of the radiolabeled apoB-100 with isopropanol (28). The radioactivity in the gels or in the isopropanol precipitates was extracted using Solvable (NEN), prior to the addition of NEN Formula 989 scintillation cocktail.

Lipoprotein analyses

In the in vivo studies, the d<1.063 g/ml fraction (VLDL+LDL) and d>1.063 g/ml (HDL and other plasma proteins) were isolated from 100 μl of plasma using a Beckman TL100 ultracentrifuge with the TLA 100 rotor. The TL100.3 rotor was used to isolate VLDL (d<1.006 g/ml), LDL (d 1.006–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) from perfusate or cell culture media (29). The isolated lipoproteins were dialyzed against 0.01% EDTA and 0.01% sodium azide at pH 7.0. Plasma and lipoprotein cholesterol and triglyceride were determined using a Roche Cobas Blood Chemistry Analyzer. The apolipoprotein profile of the serum lipoprotein fractions was characterized by SDS gradient gel electrophoresis. Lipoprotein protein concentrations were quantitated using the Bradford method (Bio-Rad Laboratories, Richmond, CA). The apolipoprotein distribution was determined utilizing polyacrylamide gels with a gradient of 4–20% acrylamide in a Laemmli system (30).

Statistical analysis

All statistical comparisons were based on a two-tailed Student's *t*-test, and are relative to the control value.

RESULTS

The apolipoprotein distribution of the VLDL that was secreted by the perfused hamster liver was analyzed by SDS gradient gel electrophoresis. The results (**Fig. 1**) indicate that the VLDL that was secreted by the perfused hamster liver (lane 3) contained apoB-100 without any apoB-48, as is found with VLDL secreted by the human liver. In contrast, nascent rat VLDL (lane 2) contained apoB-100 and B-48 in approximately equal proportions. The VLDL from both species also contained apoE and albumin (possibly a contaminant of the perfusate). In studies with cultured hamster hepatocytes (see below, Fig. 2), incubation with [^{3}H]leucine resulted in incorporation of radiolabel into VLDL apoB-100 and apoE. Thus, the nascent VLDL that is secreted by the hamster liver has an apolipoprotein profile similar to that of humans and strikingly different than that in rats.

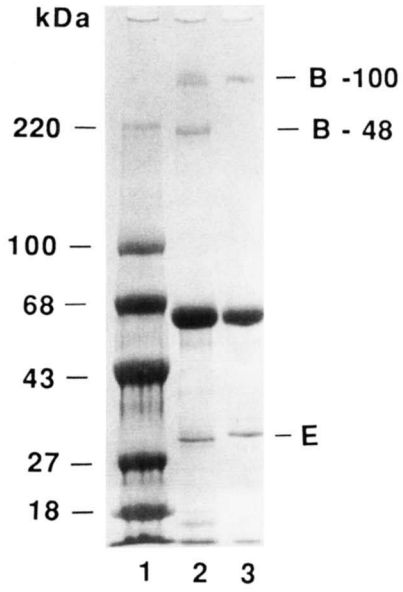

Fig. 1. SDS gel electrophoresis of nascent hepatic VLDL. Hamster and rat liver perfusions were performed, and the VLDL was isolated from the perfusate by ultracentrifugation. The apolipoprotein distribution of the VLDL fractions was then determined by SDS-PAGE. Five μg of protein was applied to each position. The molecular weight markers are shown in lane 1. The apolipoprotein profiles of the nascent VLDL that was secreted by the perfused rat and hamster liver are shown in lanes 2 and 3, respectively.

In vivo studies

The effect of inhibiting fatty acid synthesis on the in vivo secretion of VLDL-TG in the hamster was investigated. In chow-fed hamsters, plasma triglyceride concentrations were slightly above 200 mg/dl, while plasma cholesterol levels averaged 160 mg/dl (**Table 1**). The cholesterol concentration of the apoB-containing lipoproteins represented 54% of the plasma cholesterol levels. Treatment of hamsters with TOFA, an inhibitor of fatty acid synthesis, resulted in a significant 30.2% (67.1 mg/dl) decrease in plasma triglyceride concentrations relative to the control group. In addition, there was a significant 11.6% (18.7 mg/dl) decrease in plasma cholesterol levels in the TOFA group, relative to the control group. The decrease in plasma cholesterol levels was found to be due to a lowering in VLDL+LDL cholesterol concentrations, while HDL cholesterol levels were not significantly lowered.

TABLE 1. The effect of TOFA on plasma lipid and lipoprotein concentrations

Group	Day 0	Day 6	% Change
	mg/dl of plasma		
Triglyceride			
Placebo	230.6 ± 19.1	224.2 ± 29.7	− 3.9 ± 5.5
TOFA	204.5 ± 17.2	130.5 ± 15.1	− 34.1 ± 9.6[a]
Cholesterol			
Placebo	160.2 ± 6.9	155.6 ± 5.7	− 2.6 ± 2.9
TOFA	160.9 ± 4.9	137.6 ± 1.4	− 14.2 ± 2.9[a]
VLDL + LDL Chol			
Placebo	86.9 ± 2.8	85.2 ± 6.8	− 2.3 ± 5.3
TOFA	87.1 ± 2.9	70.5 ± 2.2	− 18.8 ± 2.9[a]
HDL Chol			
Placebo	79.1 ± 4.4	83.7 ± 5.7	+ 3.5 ± 3.9
TOFA	81.4 ± 2.0	79.2 ± 3.0	− 2.9 ± 1.6

A blood sample was obtained from chow-fed hamsters for plasma lipid analysis (representing day 0 of treatment). The animals were then fed the chow diet (n = 5) or the diet supplemented with 0.15% TOFA (n = 5). At day 6 of treatment, a blood sample was obtained for plasma lipid analysis.

[a]The statistical comparison is relative to the untreated group ($P < 0.05$).

In order to determine whether the lipid-lowering effect seen in the chow-fed animals was due to a decrease in hepatic VLDL-TG secretion, the in vivo secretion rate of VLDL-TG by the liver was determined using Triton WR 1339. This detergent inhibits lipolysis and blocks the uptake of VLDL particles. Thus, the accumulation of VLDL-TG over time is a measure of hepatic secretion. In an initial study, hamsters were injected with Triton WR 1339 and then test-bled at 2, 4, and 6 h after the injection. There was a linear rate of secretion of VLDL-TG over the 6-h time frame (data not shown). The 2-h time point was chosen for subsequent studies in which the VLDL-TG secretion rate was determined in controls and in hamsters treated with TOFA. The VLDL-TG secretion rate, expressed as mg/min per 100 g body weight (represented as the mean ± SEM, n = 5 animals/group) for the controls was 0.43 ± 0.03 versus 0.26 ± 0.04 in the hamsters treated with TOFA. Thus, after inhibition of de novo fatty acid synthesis by TOFA administration, there was a significant 40% decrease ($P < 0.01$) in the secretion of VLDL-TG by the liver.

Studies with cultured hepatocytes

The cultured hamster hepatocyte system was developed to study, in more detail, the regulation of VLDL-TG and apoB synthesis and secretion. In order to characterize the lipoproteins that were secreted by the hamster hepatocytes, the cells were incubated with radiolabeled glycerol or leucine, and the secreted lipoproteins were isolated from the media by sequential ultracentrifugation. Greater than 90% of the radiolabeled triglyceride and 80% of the radiolabeled apoB was isolated at a < 1.006 g/ml. The distribution of the radioactivity among the VLDL apolipoproteins is shown in **Fig. 2**, and indicates that the radiolabeled leucine was incorporated into apoB-100, E, and C. The incorporation of radiolabel was principally into VLDL apolipoproteins, with minimal incorporation into LDL and HDL apolipoproteins. Although this result was obtained using radiolabeled precursor and mass

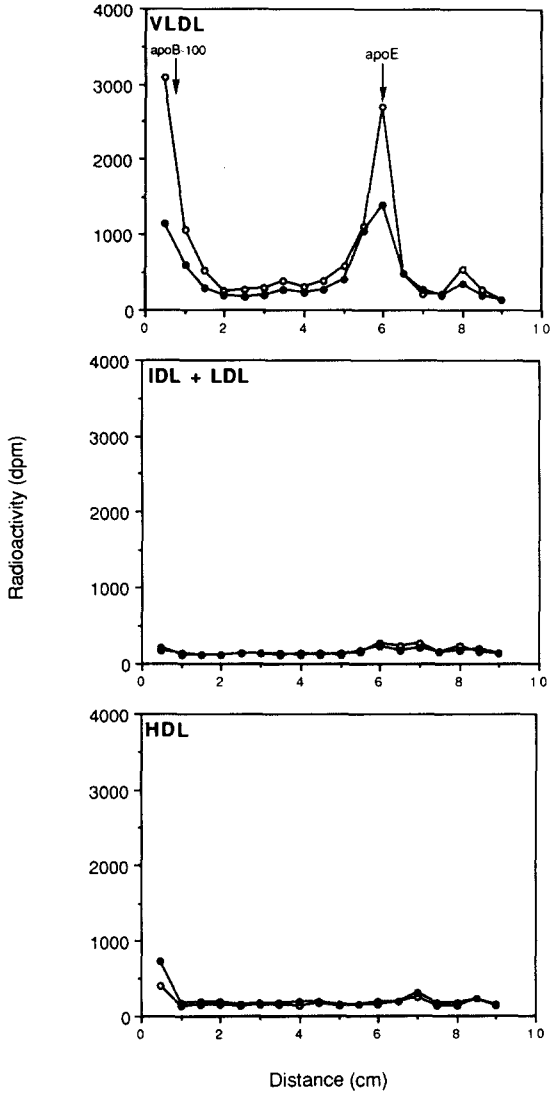

Fig. 2. Distribution of radioactivity among the apolipoproteins of the lipoproteins secreted by hamster hepatocytes. Hepatocytes were cultured overnight in DMEM containing 20% FCS, and then incubated in serum-free media or media containing 20 μM TOFA. The cells were pulsed with [^{3}H]leucine for 4 h. Radiolabeled VLDL was isolated from the pooled media from five plates of control (O--O) or TOFA-treated (●--●) hepatocytes by ultracentrifugation. The VLDL was then concentrated, dialyzed against 0.01% EDTA and 0.01% NaN_3, and subjected to SDS-PAGE. Plasma VLDL (5 μg of protein) and molecular weight standards were also loaded onto the gel. The apolipoprotein bands were visualized following Coomassie staining. The gel was then sliced and the radioactivity in the bands was determined.

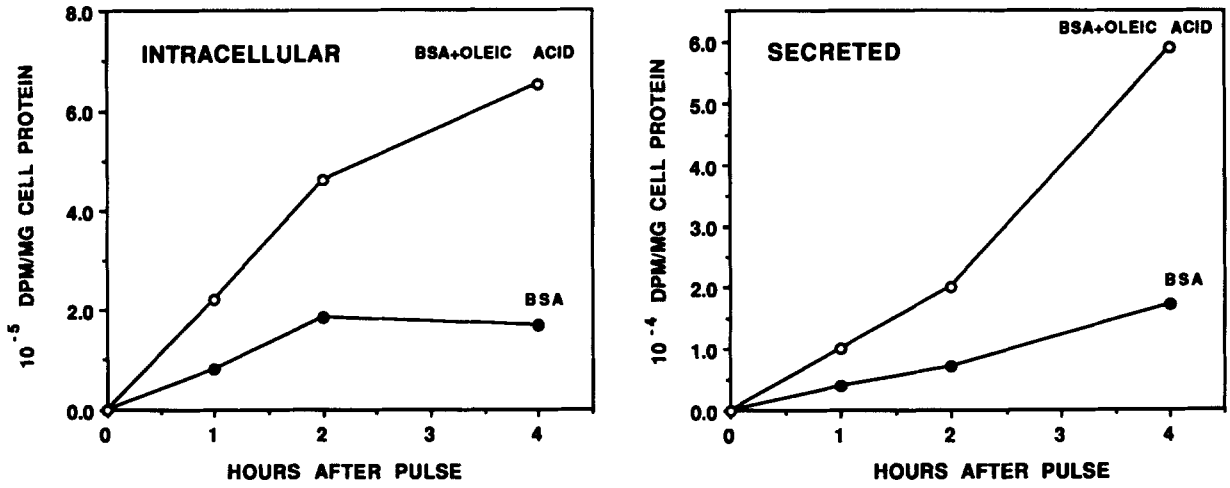

Fig. 3. The time course for the effect of oleic acid on the synthesis and secretion of ^{3}H-labeled triglyceride. After an overnight culture, hamster hepatocytes were incubated in serum-free DMEM containing 1.0 mM oleic acid complexed to BSA (4:1 molar ratio) or 0.25 mM BSA. The cells were pulsed with [^{3}H]glycerol for up to 4 h. At the specific time intervals, the amount of radiolabel that was incorporated into intracellular and secreted triglyceride was determined. Each time point represents the mean of four plates, and the data are from a representative experiment.

measurements were not performed, this finding suggests that VLDL is the principal lipoprotein that is secreted by primary hamster hepatocytes.

Since this was the first investigation using primary hamster hepatocytes to study VLDL metabolism, the effect of incubation with oleic acid on the synthesis and secretion of triglyceride and apoB was determined and compared to previous findings with rat hepatocytes or HepG2 cells. The time course for the effect of oleic acid on the incorporation of [^{3}H]glycerol into intracellular and secreted triglyceride is shown in **Fig. 3**. Note that the units are different on the Y-axis for the two graphs. In the BSA control, the incorporation of label into intracellular triglyceride was linear for up to 2 h. Incubation with oleic acid resulted in an increase in intracellular radiolabeled triglyceride that was observed within 1 h of incubation, and by 4 h there was a three-fold increase over the control in the level of intracellular triglyceride. There was a linear rate of secretion of labeled triglyceride into the medium over the time-frame of the study by the hamster hepatocytes. Similar to the intracellular triglycerides, the stimulation of triglyceride secretion by oleic acid was observed at 1 h of incubation, and by 4 h there was a marked increase in triglyceride accumulation in the media, over the control.

The results of a 4-h incubation with oleic acid on the incorporation of labeled precursors into intracellular and secreted triglyceride and apoB are shown in **Table 2**. Incubation with oleic acid resulted in a 352% increase in incorporation of label into intracellular triglyceride. The incorporation of radiolabel into intracellular apoB-100 was not significantly different from the control. Incubation with oleic acid also resulted in a 310% increase in secreted VLDL-TG, while secretion of VLDL apoB-100 was unchanged.

The effect of inhibiting fatty acid synthesis by TOFA on

TABLE 2. The effect of inhibiting fatty acid synthesis on the synthesis and secretion of VLDL-TG and apoB-100

Treatment	Intracellular		Secreted	
	TG	ApoB	TG	ApoB
	% of control			
Oleic acid, 1.0 mM (n = 5)	352.8 ± 24.7[b]	139.4 ± 27.8	309.6 ± 17.1[b]	89.8 ± 11.8
TOFA, 20 μM (n = 3)	22.3 ± 2.0[b]	113.0 ± 8.2	9.7 ± 0.7[b]	50.5 ± 12.9[a]
Oleic acid, 1.0 mM + TOFA, 20 μM (n = 3)	195.3 ± 71.6	151.7 ± 17.3	145.7 ± 15.6	78.8 ± 13.4

Hamster hepatocytes were incubated with 1.0 mM oleic acid, 20 μM TOFA or both treatments, and pulsed with either [^{3}H]glycerol or [^{3}H]leucine for 4 h (as described under Experimental Procedures). The incorporation of radiolabeled glycerol into intracellular and secreted triglyceride was determined after extraction and separation by thin-layer chromatography. The incorporation of radiolabeled leucine into intracellular apoB was determined by immunoprecipitation; the incorporation of radiolabel into secreted VLDL-apoB was determined by isopropanol precipitation. The results are expressed as the mean ± SEM for the number of experiments shown in parentheses.

The statistical analysis is relative to the control value: [a]$P < 0.05$; [b]$P < 0.001$.

cellular lipid synthesis and VLDL-TG and apoB secretion was also investigated. In an initial study, the magnitude of the inhibition of cellular fatty acid synthesis and also its effect on cholesterol synthesis were determined. When cells were incubated with 20 μM TOFA for 4 h, there was a 98% ± 0.55 (mean ± SEM, n ' 3) inhibition in the incorporation of [^{14}C]acetate into cellular fatty acids. However, total cholesterol biosynthesis was unchanged. Therefore, TOFA specifically inhibited fatty acid synthesis, without adversely affecting the synthesis of cholesterol.

A dose-response study was performed in which hepatocytes were incubated with 1-50 μM TOFA for 4 h. The ED_{50} for the inhibition of intracellular triglyceride synthesis and for the inhibition of VLDL-TG secretion was 10.2 μM and 15.1 μM, respectively. Therefore, in subsequent studies, cells were incubated with TOFA at a concentration of 20 μM. The time course for the effect of TOFA on the incorporation of [^{3}H]glycerol into intracellular triglyceride and secreted VLDL-TG is shown in **Fig. 4**. Inhibition of cellular and secreted triglyceride was observed within 1 h of incubation and was sustained over the 4-h time frame of the study.

At the 4-h time point, incubation with 20 μM TOFA resulted in a 78% decrease in intracellular radiolabeled triglyceride (Table 2). However, intracellular apoB-100 was unchanged. Incubation with TOFA also resulted in a 90% and 50% decrease in secreted VLDL-TG and apoB-100, respectively. In order to determine whether TOFA treatment resulted in the secretion of a more dense lipoprotein fraction, the distribution of radiolabeled apolipoproteins among the lipoprotein fractions was determined (Fig. 2). The results indicate that TOFA treatment decreased VLDL apoB-100 and apoE, and did not result in the secretion of more dense apoB-containing lipoproteins.

When hepatocytes were incubated in the presence of both 1 mM oleic acid and 20 μM TOFA, the fatty acid restored the synthesis of intracellular and secreted triglycerides to levels somewhat above that found in the control hepatocytes (Table 2). Intracellular apoB was 50% higher than that found in the control cells, but this difference was not statistically significant; while VLDL apoB-100 secretion was similar to controls.

DISCUSSION

In this study, we have used the hamster as a model to investigate the processes that mediate hepatic VLDL-TG and apoB synthesis and secretion. The similarities between the hamster and human in terms of VLDL composition and lipoprotein distribution and metabolism make this species very appropriate for these studies. The finding that the VLDL that was secreted by the hamster liver contained only apoB-100, rather than both apoB-48 and apoB-100 as is found in the rat, demonstrated that the hamster is a good model to study human VLDL metabolism. The protocol, using Triton WR 1339, made it possible to quantitate the in vivo hepatic secretory rate for VLDL-TG. The cultured hamster hepatocyte system that was developed for the in depth in vitro analysis of de novo synthesis and secretion of VLDL-TG and apoB had several advantages over previously utilized in vitro models.

In vivo studies

VLDL-TG secretion by the hamster liver was found to be decreased by inhibition of fatty acid synthesis and subsequent triglyceride synthesis. In the in vivo experiments, TOFA treatment resulted in a 25-30% decrease in plasma triglyceride levels in the hamster. This was found to be

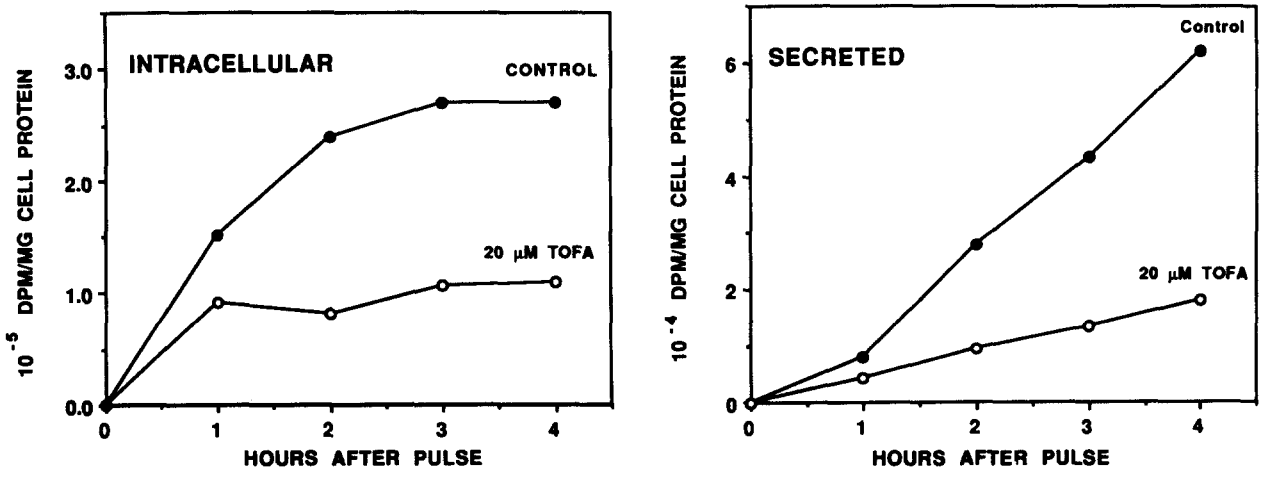

Fig. 4. The time course for the effect of TOFA on the synthesis and secretion of ^{3}H-labeled triglyceride. Hepatocytes were incubated with 20 μM TOFA complexed to BSA or BSA alone, and pulsed with [^{3}H]glycerol for up to 4 h. At the specific times, the amount of radiolabel that was incorporated into intracellular and secreted triglyceride was determined. Each time point represents the mean of four plates, and the data are from a representative experiment.

due to a 40% decrease in hepatic secretion of VLDL-TG. Although TOFA is not liver-specific and inhibits fatty acid synthesis throughout the body, the effect of TOFA on the secretion of VLDL-TG can be considered as liver-specific, as the liver is a major site of fatty acid synthesis and VLDL-TG production. Previous studies in the rat have shown that there was a 60–70% decrease in plasma triglyceride levels after inhibition of fatty acid synthesis (9). This greater decrease in plasma triglyceride levels in the rat after TOFA treatment may be due to the lower level of plasma free fatty acids in the rat (31) that can be used as precursors for triglyceride synthesis. In the present study, VLDL+LDL cholesterol levels were also decreased after TOFA treatment. As the hamster plasma contains appreciable levels of LDL cholesterol, this suggests that VLDL cholesterol is the precursor to LDL cholesterol in the hamster, as is the case in humans. Thus, a decrease in the secretion of VLDL particles by the liver after TOFA treatment appears to result in a decrease in LDL cholesterol in this animal model.

Studies with cultured hepatocytes

The studies with cultured hamster hepatocytes determined the effect of increasing or decreasing fatty acid levels on VLDL-TG and apoB secretion. In initial studies to evaluate this in vitro model, hamster hepatocytes were found to secrete triglyceride-rich apoB-containing lipoproteins having a buoyant density of < 1.006 g/ml, which is a characteristic of plasma VLDL. This system differs from HepG2 cells, which synthesize dense apoB-100 lipoproteins that are isolated within the LDL density range (15). When hamster hepatocytes were incubated with oleic acid, the secretion of VLDL-TG, but not apoB, was stimulated. Thus, there was secretion of a triglyceride-enriched VLDL particle rather than an increase in the number of VLDL particles. These results are similar to those obtained with cultured rat hepatocytes (6, 32). In studies in which HepG2 cells were incubated with oleic acid, the secretion of apoB has been reported to be stimulated 2- to 4-fold (7, 25, 33, 34) or to be unchanged (35). The difference between the HepG2 cells versus the rat and hamster hepatocytes may be due to loss of normal hepatocyte regulatory function or to a defect in VLDL assembly in the HepG2 cells. However, the McA-RH7777 rat hepatoma cell line differs from the HepG2 cells in that these cells secrete a VLDL particle that is similar to that found with primary rat hepatocytes (36).

When the hepatocytes were incubated with TOFA, inhibition of fatty acid synthesis markedly inhibited the secretion of VLDL-TG and apoB, resulting in the secretion of fewer VLDL particles. This is in contrast to the finding with oleic acid in which the increase in fatty acids resulted in the secretion of a triglyceride-rich VLDL, without increasing the secretion of apoB. The reason for this is not apparent, but indicates that changes in VLDL-TG secretion may or may not reflect changes in VLDL-apoB secretion.

It has been proposed that cholesteryl ester synthesis plays the key regulatory role in the assembly and subsequent secretion of the apoB-containing lipoproteins. Studies in HepG2 cells have shown that the secretion of apoB was more closely correlated with intracellular cholesteryl ester content than intracellular triglyceride (26). When HepG2 cells were incubated with oleic acid, intracellular triglyceride was increased 6-fold, while intracellular cholesteryl esters and apoB secretion was increased 3- and 2.5-fold, respectively. When the incorporation of [^{14}C]acetate into intracellular cholesteryl esters was determined in hamster hepatocytes in this present study, incubation with oleic acid resulted in a 56% increase in intracellular cholesteryl ester, while TOFA treatment resulted in a 38% decrease. As the secretion of apoB was decreased by 57% following TOFA treatment, this is somewhat consistent with the hypothesis derived from studies with HepG2 cells. However, VLDL-TG but not apoB-100 secretion by primary hamster hepatocytes was increased after incubation with oleic acid. Therefore, in hamster hepatocytes, an increase in intracellular cholesteryl ester does not result in an increase in apoB secretion.

The results using primary hamster hepatocytes indicate that when triglyceride synthesis was inhibited by inhibiting fatty acid synthesis, there was a decrease in the secretion of both VLDL-TG and apoB, while apoB synthesis was unchanged. Results from several studies have suggested that there is a large pool of intracellular apoB, and that only a small fraction of this pool is utilized for VLDL assembly (37–39). Thus, the hepatocyte may modulate triglyceride synthesis in response to increases or decreases in fatty acid availability, while keeping intracellular apoB-100 synthesis constant. It has been recently reported that incubation of HepG2 cells with oleic acid was protective against the early intracellular degradation of apoB (40). As intracellular apoB was unchanged after incubation with TOFA but secretion was markedly reduced, this suggests that inhibition of fatty acid synthesis may lead to an increase in the intracellular degradation of apoB. When hepatocytes were incubated with oleic acid in addition to TOFA, VLDL-apoB secretion was restored to control levels, suggesting that oleic acid was protective against apoB degradation. This is the subject of further investigation.

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