Bile Acids Suppress the Secretion of Very-Low-Density Lipoprotein by Human Hepatocytes in Primary Culture

YUGUANG LIN,[1] RICK HAVINGA,[1] HENKJAN J. VERKADE,[1] HAN MOSHAGE,[2] MAARTEN J. H. SLOOFF,[3] ROEL J. VONK,[1] AND FOLKERT KUIPERS[1]

The existence of a relationship between bile acid and triacylglycerol metabolism in humans has been established, but the underlying mechanism and its physiological relevance have remained unclear. We have studied the effects of bile acids on the secretion of very-low-density lipoprotein (VLDL)-associated triacylglycerol, using [^{3}H]glycerol labeling technique, and apolipoprotein B (apoB) in human hepatocytes in primary culture. Human hepatocytes secrete nascent VLDL with an average diameter of about 40 nm. Lipid composition of the particles resembles that reported for plasma VLDL, with the exception of a markedly lower cholesterylester content. In 24-hour cultured human hepatocytes, physiological (i.e., portal) concentrations of taurocholic acid (10 to 200 μmol/L) suppressed [^{3}H]triacylglycerol secretion dose dependently. The degree of inhibition highly correlated (r = .87, P < .01) with taurocholic acid content of the cells of different preparations (n = 7). ApoB secretion was inhibited by taurocholic acid to a similar extent as [^{3}H]triacylglycerol secretion (r = .93, P < .01). Lipid composition of secreted VLDL particles did not change during taurocholic acid–induced suppression. No effects on intracellular apoB, [^{3}H]triacylglycerol, triacylglycerol, and cholesterol mass were observed, nor did taurocholic acid affect protein synthesis, albumin secretion, or lactate dehydrogenase (LDH) release. Cellular cholesteryl ester (CHE) mass, however, was markedly reduced. Our results show that bile acids strongly interfere with the assembly or secretion of VLDL particles by human hepatocytes, suggesting a physiological function of the enterohepatic circulation of bile acids in the regulation of postprandial serum lipid levels. (HEPATOLOGY 1996; 23:218-228.)

Hepatocytes secrete substantial amounts of lipids at their apical (canalicular) pole into bile and at the basolateral (sinusoidal) side into the blood, the latter mainly in the form of very-low-density lipoprotein (VLDL). The apical route, i.e., the flux of free cholesterol (CH) and phospholipids (PL) into the bile, is thought to occur mainly through vesicle formation at the canalicular membrane in a process under control of intracanalicular bile acids and mdr2 P-glycoprotein.[1,2] VLDL secretion involves the packing of triacylglycerol (TG), cholesteryl ester (CHE), CH, and PL together with apolipoprotein (apo) B into nascent VLDL in the endoplasmic reticulum, processing of the particles in the Golgi complex, and secretion through an exocytotic pathway.[3,4] In spite of the different nature of both secretory processes, they appear to be connected in a functional sense. In animals, dietary and pharmacological manipulations that increase or decrease biliary CH secretion generally have an opposite effect on serum CH and TG levels and VLDL production.[5-7] Interactions of bile acids with both secretory processes have been reported. The stimulatory effects of bile acids on biliary lipid secretion in various experimental models as well as in humans are well established.[1] Likewise, a relationship between bile acid metabolism and VLDL production has been recognized for many years. Interruption of the enterohepatic circulation of bile acids by sequestrant therapy[8-11] or ileal resection[8,12] is often associated with increased serum levels of VLDL-TG, because of an increased hepatic VLDL production. Conversely, bile acid administration, which increases bile acid pool size and decreases hepatic bile acid synthesis, results in decreased levels of serum TG in hyperlipidemic patients.[10,13,14] Patients with cerebrotendinous xanthomatosis, caused by a defective bile acid synthesis, have high levels of serum VLDL-TG, which have been reported to normalize during chenodeoxycholic acid treatment.[15] Finally, synthesis and turnover of primary bile acids are increased in patients with familial hypertriglyceridemia, which has been attributed to an

Abbreviations: VLDL, very-low-density lipoprotein; CH, cholesterol; PL, phospholipids; TG, triacylglycerol; CHE, cholesterol ester; apoB, apolipoprotein B; FCS, fetal calf serum; BSA, bovine serum albumin; HBSS, Hank's balanced salt solution; OA, oleic acid; PBS, phosphate-buffered saline; LDH, lactate dehydrogenase; cAMP, cyclic adenosine monophosphate.

From the Departments of [1]Pediatrics, [2]Internal Medicine, and [3]Surgery, Groningen Institute for Drug Studies, Academic Hospital Groningen, Groningen, The Netherlands.

Received May 11, 1995; accepted July 21, 1995.

Supported by grants from the Royal Netherlands Academy of Arts and Sciences and from the "Alternatives to Animal Experiments Platform," Ministry of Health, Welfare and Sports, The Netherlands (Y.L.), and grant no. NWO-KWO (#900-716-028) (H.J.V.). Dr. Kuipers is an established investigator of the Netherlands Heart Foundation.

This work has been performed in collaboration with the "Human Liver Group Groningen" and has been presented in part at the Annual Meeting of the American Association for the Study of Liver Diseases, November 13-16, 1994, at Chicago, IL (HEPATOLOGY 1994;20:A673).

Address reprint requests to: Folkert Kuipers, Ph.D., Laboratory of Nutrition and Metabolism, CMC IV, Room Y3137, Academic Hospital Groningen, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

0270-9139/96/2302-0004$3.00/0

impaired intestinal bile acid absorption.[16,17] The suggestion has been made that bile acid malabsorption may be a primary defect in (some) patients with hypertriglyceridemia.[16,17]

Little is known about the mechanism(s) by which bile acids interfere with the process of hepatic VLDL production. Data from clinical and experimental *in vivo* studies have been interpreted to suggest the existence of a functional coupling between bile acid synthesis and TG synthesis,[18] the latter being a major determinant of hepatic VLDL production.[3,4] Thus, a high synthesis of TG available for VLDL assembly would occur in situations with high bile acid synthesis and, vice versa, a low TG synthesis when bile acid synthesis is downregulated. Conversely, incubation of freshly isolated rat hepatocytes with taurocholic acid leads to an instantaneous suppression of TG secretion by these cells without altering cellular TG synthesis,[19] suggesting the existence of another, probably more directly exerted effect of bile acids on VLDL production in rat cells. So far, effects of bile acids on VLDL production by the human hepatocyte have not been documented. Human hepatoma cells like HepG2 have been used extensively for VLDL secretion studies. However, it has been shown that HepG2 cells have lost their ability to actively take up bile acids from their incubation medium.[20] In addition, certain tumor-specific characteristics have to be taken into account when interpreting results obtained with these cells.[21,22] We have investigated the influence of bile acids on VLDL-lipid and apoB secretion by primary cultured human hepatocytes.[23]

MATERIALS AND METHODS

Materials. All bile acids, as sodium salts, and human albumin were obtained from Calbiochem (La Jolla, CA). Williams' E medium, glutamine, penicillin-streptomycin, fetal calf serum (FCS), and collagenase were purchased from Life Technologies Ltd., Paisley, Scotland. Insulin and dexamethasone came from Novo Nordisk Pharma B.V., Amsterdam, The Netherlands. Hydrated colloidal silica (Cab-O-Sil) (fumed silica, particle size 0.011 μm) was purchased from Sigma Chemical Co., St. Louis, MO. [2-^{3}H]Glycerol (1 Ci/mmol), [9,10(n)-^{3}H]oleic acid (OA) (10 Ci/mmol), L-[4,5-^{3}H]leucine (61 Ci/mmol), tauro[carbonyl-^{14}C]cholic acid (56 mCi/mmol), and Enhance chemiluminescence Western blotting reagent were obtained from Amersham International, Amersham, UK. Cell culture plates were supplied by Costar, Cambridge, MA. Thin-layer chromatography plates were obtained from E. Merck, Darmstadt, Germany. Triglycerides GPO-PAP kit and polyclonal sheep anti–human apoB antibodies were obtained from Boehringer Mannheim G.m.b.H., Mannheim, Germany. Peroxidase-conjugated anti-sheep-immunoglobulin G antibody developed in donkey, human-specific monoclonal antibody against human serum albumin and essentially fatty acid–free bovine serum albumin (BSA) were supplied by Sigma. Peroxidase-conjugated rabbit anti–human albumin antiserum was purchased from Dakopatts, Glostrup, Denmark. All other chemicals and solvents were high purity commercial materials.

Preparation and Culturing of Human Hepatocytes. Human liver tissue was obtained from healthy liver transplant donors and from patients with liver cancer undergoing partial hepatectomy. Consent from legal authorities, patients, or donor families was obtained. Hepatocytes were isolated from nontransplanted liver tissue when the donor livers were split to perform reduced-size liver transplantation in children. Livers were perfused with University of Washington (UW) organ preservation solution[24] at the time of harvesting and kept at 0 to 4°C until cell isolation (2-12 hours). In addition, hepatocytes were isolated from resected healthy liver parts obtained during partial hepatectomy for treatment of liver cancer. Donor characteristics are shown in Table 1. Wedge-shaped pieces of 30 to 200 g with a single cut surface were used for hepatocyte isolation as described in detail elsewhere.[25] Briefly, two to four major branches of the portal vein were cannulated with plastic cannulae for perfusion, and other major vessels were tied off. The tissue was first perfused with 1,000 mL calcium-free Hanks' balanced salt solution (HBSS) containing glucose (10 mmol/L) and HEPES (10 mmol/L) at a flow rate of 90 mL/min to wash out the preservation fluid or blood and to warm the liver to 37°C. This was followed by perfusion of 1,500 mL calcium-free HBSS containing $NaHCO_3$ (25 mmol/L) and glucose (10 mmol/L) and 200 mL calcium-containing HBSS, before perfusion with 400 mL of enzyme solution (50 mg of collagenase/100 mL calcium-containing [5 mmol/L] HBSS) in a recirculating fashion for 15 minutes. All solutions were pregassed with 95% O_2 and 5% CO_2 and adjusted to pH 7.42.

TABLE 1. Characteristics of Liver Tissue Source

Donor	Age (yr)	Sex	Source
a	20	Male	Transplant
b	12	Female	Transplant
c	33	Female	Transplant
d	31	Male	Transplant
e	46	Female	Hepatectomy
f	3	Male	Transplant
g	47	Male	Hepatectomy
h	68	Female	Hepatectomy
i	24	Female	Transplant

Nonviable hepatocytes were separated from viable ones by suspending cells in 36% Percoll, followed by centrifugation at 100g for 10 minutes. The viability of hepatocytes after isolation was 85% to 95% as examined by trypan blue exclusion (final concentration, 0.2%). Cells were routinely plated in 35-mm 6-well plastic dishes (precoated with collagen) at a density of 1.5×10^6 viable cells/well in 2 mL culture medium.

The culture medium consisted of Williams' E medium supplemented with 10% FCS, 2 mmol/L glutamine, 100 μg/mL penicillin, 100 μg/mL streptomycin, 4 mU/mL insulin, and 0.02 μg/mL dexamethasone as described by Princen et al.[26] After cells had attached to the plates, usually after 4 hours, medium was renewed. Cells were incubated at 37°C in an atmosphere of 95% air and 5% CO_2.

Experimental Protocol. Because pilot experiments had shown that the ability of the cells to concentrate bile acids rapidly declines during cell culture, experiments were conducted 24 hours after seeding. BSA–oleic acid (OA) complexes were freshly prepared on the day of an experiment. A sterile solution of 0.25 mmol/L BSA in Williams' E medium with penicillin and streptomycin but without FCS and hormones (FCS hormone-free medium) was adjusted to pH 10. The fatty acid was solubilized in chloroform in glass tubes and dried under nitrogen. The BSA-containing medium was

mixed with the dried fatty acid while sonicating vigorously to produce a solution of 1 mmol/L OA/0.25 mmol/L BSA. The pH was adjusted to 7.4, and media were warmed to 37°C before use.

FCS-containing medium was removed from the wells, and cells were washed two times with FCS hormone-free medium and incubated in this medium for 4 hours. Then the medium was replaced by the same lipid-free medium (1 mL/well) containing OA-BSA complexes with or without addition of bile acids at the concentrations indicated. Cells were incubated for 1 to 3 hours. To determine the glycerolipid synthesis and secretion rates at indicated times, [^{3}H]glycerol (4.4 μCi, 25 μmol/L) or [^{3}H]OA (10 μCi, in 10 μL ethanol) was added to each well according to different experimental protocols (see legends in Results). All incubations were performed in triplicate and terminated by placing the culture plates on ice. Media were collected and centrifuged at 14,000*g* for 2 minutes to remove suspended cells. Cells were washed twice with phosphate-buffered saline (PBS) and harvested by using a rubber policeman in 2 mL PBS. Samples were frozen at −20°C before analysis. Cells and media used for apoB determination were harvested, as described in Western blotting of apoB.

Assessment of Taurocholic Acid Uptake by Cultured Human Hepatocytes. In all individual preparations used, the capacity of the cells to take up [^{14}C]taurocholic acid was determined in separate experiments. For this purpose, 0.1 μCi of the radiolabeled compound, supplemented with unlabeled taurocholic acid to reach the desired concentrations, was added to each well. At the indicated time points, dishes were placed on ice, the medium was aspirated, and cells were washed five times with 0.5 mL cold HBBS. Cells were then scraped off with a rubber policeman and suspended in water. An aliquot was taken for protein measurement and another one for determination of radioactivity by liquid scintillation counting. Data were corrected for nonspecific binding, as determined from incubations at 0°C. Intracellular accumulation was expressed as nanomoles per milligram of cell protein.

Assessment of Cellular and VLDL Lipid Content. Cells were thawed and resuspended by passing through 26-gauge × 1″ (0.45 × 25 mm) needles five times. The cellular and VLDL lipids were extracted with chloroform/methanol (1/2, v/v).[27] TG, CH, and CHE and PL were separated by thin-layer chromatography with hexane/diethylether/acetic acid (80/20/1) as developing solvent. After iodine staining, the spots containing the lipids of interest were scraped into vials and assayed for radioactivity by scintillation counting. Cellular TG mass was assayed using a commercially available kit according to manufacturer's instruction with glycerol as standard. Cellular CH and CHE mass were determined according to the methods developed by Gamble et al.[28] Phospholipids were determined by measuring phosphorus content after perchloric acid treatment.[29]

Assessment of VLDL-Associated [^{3}H]Lipid Secretion. [^{3}H]TG in medium has been used extensively to estimate the VLDL-[^{3}H]TG secretion by primary cultured rat hepatocytes.[30] Because our pilot experiments showed that more than 90% of [^{3}H]TG secreted into the medium of cultured human hepatocytes was recovered in the VLDL fraction ($d < 1.006$ g/mL), we followed [^{3}H]TG secretion as a measure of hepatocytic VLDL formation. Before medium lipids were extracted, 30 μL of 2 mmol/L triacylglycerol solution in chloroform was added to each sample as a carrier. TG was separated from other lipids by thin-layer chromatography in a similar procedure as the assay of cellular lipids. Silica gel containing TG was scraped into vials and assayed for radioactivity by scintillation counting.

To further investigate the lipid composition of VLDL secreted into the medium, immunoaffinity isolation of medium apoB and its associated lipids was conducted in two separate experiments. The validity of this method for the determination of apoB-associated lipids has been described by Rusiñol et al.[31] To fresh medium samples, anti-apoB serum was added to a final dilution of 1:200, and phenylmethylsulfonylfluoride (0.02% final concentration) was added to inhibit the activity of proteases. The tubes were rotated overnight followed by the addition of 40 μL 12% (wt/vol) protein A beads and incubated for another 2 hours. Protein A–antibody-apoB complexes were collected by centrifugation. The beads were washed two times with PBS buffer. [^{3}H]-Lipids associated with the bound apoB were extracted, separated, and assayed as described.

Western Blotting of ApoB. The cell monolayer was solubilized by addition to each well of 0.5 mL of detergent buffer (25 mmol/L ethylenediaminetetraacetic acid, 1% Triton X-100, 1% deoxycholic acid, 125 mmol/L NaCl, 50 mmol/L Tris-HCl, 0.1% sodium dodecyl sulfate, and dithiothreitol and phenylmethylsulfonylfluoride at a final concentration of 1 mmol/L each. The cell lysates were pipetted into tubes and heated at 70°C for 30 minutes and diluted with an equal volume of a solution containing 0.01 mmol/L Tris/HCl (pH 7.4) and 0.15 mol/L NaCl. Cell lysates were centrifuged at 10,000 rpm for 2 minutes, and the supernatant of the cell lysates was used for apoB determination.

ApoB in cell lysates was precipitated by adding anti-apo B-serum at a final dilution 1:200. The tubes were rotated overnight, followed by adding 40 μL of 12% (weight per volume) protein A beads and incubated for another 2 hours. Protein A–antibody-apoB complexes were collected by centrifugation. ApoB in media were absorbed to Cab-O-Sil according to the methods described by Vance et al.[32] Control experiments showed that when the initial immunoprecipitation (cell lysate) or Cab-O-Sil adsorption (media) was followed by a second immunoprecipitation of the supernatant, no additional apoB was recovered. After washing two times with PBS, apoB was solubilized from the protein A beads or silica pellet with an extraction buffer that contained 2% sodium dodecyl sulfate, 0.05 mol/L Tris (pH 9.0), 6 mol/L urea, 0.1% ethylenediaminetetraacetic acid 0.1% dithiothreitol, 0.13% ϵ-aminocaproic acid, and 0.05% glutathione at 95°C for 10 minutes. ApoB was separated from other proteins by electrophoresis in 5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate. Subsequently, apolipoproteins were transferred to nitrocellulose membranes. For immunoblotting, the membrane was immersed in a solution of Tris-buffered saline (20 mmol/L Tris, pH 7.5, and 0.5 mol/L NaCl) containing 5% dried milk for 1 hour at room temperature to block nonspecific binding and incubated with the primary antibody (1:5,000) overnight. The membrane was washed four times with Tris-buffered 5% milk solution and subsequently incubated with secondary peroxidase-conjugated antibody (1:10,000) for 2 hours and then washed as before. ApoB-antibody complexes were visualized with Enhance Chemiluminescence Western blotting reagent by exposure to Hyperfilm according to the manufacturer's instruction. Quantification of the bands were performed with an UltroScan XL densitometer.

Assessment of Albumin Secretion. The amount of albumin secreted by human hepatocytes into medium was estimated by sandwich enzyme-linked immunosorbent assay. Microplates were precoated with monoclonal anti–human albumin antibody in PBS by incubating overnight at 4°C. The

buffer containing the unbound antibodies was drained from the plate, and the wells were washed four times with PBS containing 0.05% Tween-20. The unbound sites on the wells were blocked by adding to each well 200 μL of a block solution (1% milk-powder in PBS), and incubated for 2 hours at room temperature. The wells were then washed as described above. Human albumin standard was diluted with PBS buffer (buffer A) containing 0.05% Tween-20 and 0.1% milk-powder in concentrations ranging from 0 to 5 ng per 100 μL. One hundred microliters of these human albumin standards were added per well in duplicate. Samples of media were diluted (1:20 to 1:200) with buffer A and added to the wells in duplicate so that the resulting albumin concentrations fell within the standard curve. The plate was incubated for 1 hour at room temperature. The well was then washed as described above. Two hundred microliters of buffer A containing polyclonal anti-human-albumin antibody conjugated to horseradish peroxidase was added to each well and incubated for 1 hour, and the wells were washed again. Color development was started by adding 100 μL substrate solution, which was prepared by adding (at final concentration) 3,3′,5,5′-tetramethylbenzidine (0.2 mg/mL) and H_2O_2 (0.3 mg/mL) to 0.1 mol/L Na_2HPO_4 and citric acid buffer (pH 4.3). The reaction was stopped by adding 100 μL 1 mol/L H_2SO_4. The absorbance was determined at 450 nm using a Titertek Multiscan MCC/340 plate reader.

Electron Microscopy. Medium VLDL particles were isolated from d $<$ 1.006 g/mL fraction of the medium exposed to 1.8×10^7 hepatocytes for 24 hours in the presence of 1 mmol/L OA, by centrifuging 24 hours at 41,000 rpm in a Beckman SW41 rotor. The VLDL fractions obtained were treated for electron microscopy within 1 hour. The particles were allowed to adhere to hydrophilic carbon films and immersed in 2% potassium phosphotungstate (pH 7.4) as a negative stain. Electron micrographs were obtained in a Philips EM208 transmission electron microscope. Size distribution of VLDL particles was determined using Quantimet 520+ software (Leica, Cambridge, England).

Miscellaneous Methods. Protein was measured according to Lowry et al.,[33] using BSA as standard. Lactate dehydrogenase (LDH) activity in media and cells were determined as reported previously.[34] The leakage of LDH from cells is presented as LDH-leakage percent, which was calculated according to the formula: (LDH activity in medium/LDH activity in cells + medium) $\times$ 100%. The [^{3}H]leucine incorporation into the trichloroacetic acid–precipitable protein was investigated to determine the total protein synthesis and secretion. Hepatocytes were cultured for 5 hours with or without bile acids in the same medium as for TG synthesis measurement, and [^{3}H]leucine (5 μCi/mL per well) was added 1 hour before termination of the cell incubation.

Calculations and Statistical Analysis. Data were normalized to the amount of cellular protein. Statistical differences were assessed using paired Student's t test. Values of $P < .05$ were considered significant. Regression analysis was performed using a least-square method.

RESULTS

VLDL Secretion by Human Hepatocytes in Primary Culture. The VLDL (d $<$ 1.006 g/mL) fraction was isolated from media of 24-hour incubations of human hepatocytes and examined by negative stain electron microscopy (Fig. 1A). VLDL size distribution (Fig. 1B) shows that human hepatocytes in primary culture secrete nascent VLDL particles with an average size of about 40 nm, which falls into the size range (30 to 80 nm) of this lipoprotein fraction in human serum. Lipid composition of the nascent VLDL particles of two separate cell preparations is given in Table 2. The relative amounts of TG, CH, and PL on the particles are comparable to those reported for human serum VLDL, whereas the relative amount of CHE is markedly lower.

Effect of Bile Acids on VLDL Secretion and Cellular Lipid Content. The presence of physiological concentrations of taurocholic acid in incubation media of primary human hepatocytes caused a marked reduction of VLDL-associated [^{3}H]TG secretion after labeling of the cells with ^{3}H-glycerol. Results obtained from a representative experiment (donor c) are shown in Fig. 2. ^{3}H-TG accumulated linearly in medium for at least 3 hours when human hepatocytes were incubated with 1 mmol/L OA. Taurocholic acid dose-dependently suppressed [^{3}H]TG secretion by human hepatocytes during 3-hour incubations as compared with control.

The taurocholic acid–induced suppression of [^{3}H]TG secretion showed large variations in seven independent experiments in which human hepatocytes were incubated with 10 to 200 μmol/L of the bile acid, as summarized for incubations with 100 μmol/L taurocholic acid in Table 3. On average, [^{3}H]TG secretion at 3 hours was reduced by 1%, 35%, and 47% in the presence of 10, 100, and 200 μmol/L taurocholic acid, respectively (see legend, Table 3). Cellular bile acid content, as calculated from cell-associated [^{14}C]taurocholic acid after 1 to 3 hours of incubation with 10, 100, or 200 μmol/L taurocholic acid, varied between 0.74 and 19.94 nmol/mg cell protein among different hepatocyte isolations. Regression analyses were carried out to evaluate the relationship between cellular taurocholic acid content and the degree of inhibition of [^{3}H]TG secretion. Figure 3 shows the strong relationship between [^{3}H]TG secretion and cellular taurocholic acid content during 1 hour ($y = -3.649x + 104.12$, $r = .84$, $P < .01$) and 3 hours ($y = -3.379x + 102.60$, $r = .88$, $P < .01$) incubations. For the combined data, the equation obtained is $y = -3.452x + 103.18$, $r = .87$ ($P < .01$).

Incubation of human hepatocytes with taurocholic acid in the presence of [^{3}H]glycerol did not affect cellular [^{3}H]TG content or TG mass (Table 3). Likewise, the cellular concentration of CH was unaffected, but the CHE content was markedly decreased in the presence of the bile acid (Table 4). To investigate whether taurocholic acid suppresses TG secretion under OA-stimulated conditions only, human hepatocytes were incubated with 100 μmol/L taurocholic acid in medium with or without 1 mmol/L OA. Table 5 shows that OA increased [^{3}H]TG secretion eightfold as compared with that in fatty acid–free incubations, which is in agreement with data obtained with rat hepatocytes.[35] In fatty acid–free medium, taurocholic acid suppressed [^{3}H]TG secretion to a similar extent as in the presence of 1 mmol/L OA.

The effect of a limited number of other bile acid species on [^{3}H]TG secretion was investigated in two separate experiments, summarized in Fig. 4. Both

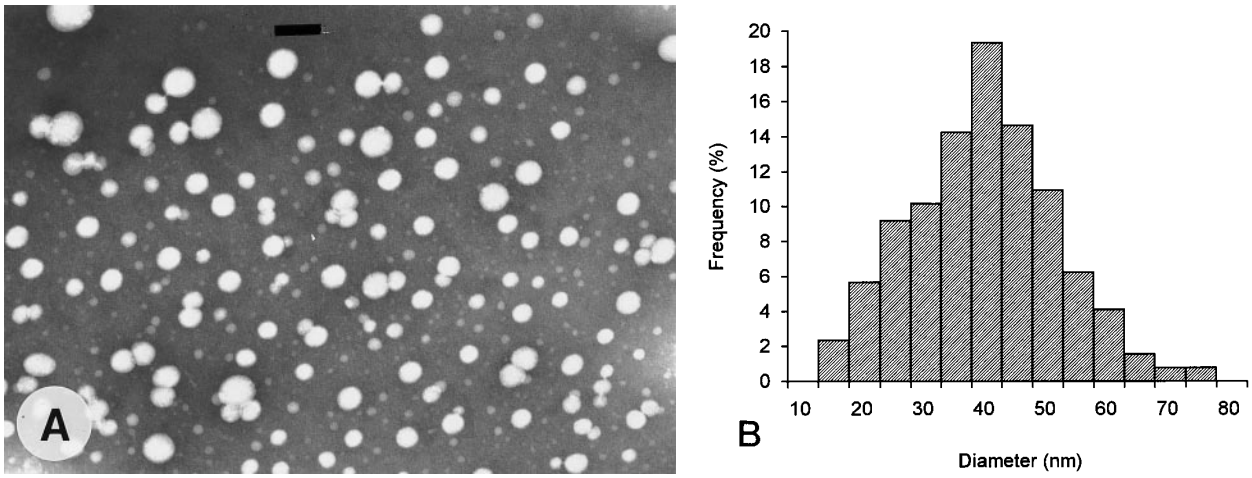

FIG. 1. Electron micrograph of VLDL particles (A) and their size distribution (B). VLDL particles were isolated from d < 1.006 fraction by ultracentrifugation from FCS hormone–free medium exposed to human hepatocytes (donor a and b) for 24 hours and examined by negative-staining electron microscopy. (Bar = 100 nm.) The size distribution of VLDL particles was obtained by evaluation of 500 particles.

taurochenodeoxycholic and tauroursodeoxycholic acids showed a tendency to reduce [^{3}H]TG secretion, whereas unconjugated ursodeoxycholic acid did not. Uptake and subsequent metabolism of the dihydroxy bile acids that may have occurred were not evaluated in these studies.

Effect of Taurocholic Acid on Apolipoprotein B Secretion and VLDL Lipid Composition. Cellular and medium apoB abundance was semiquantitatively determined by Western blotting. Taurocholic acid decreased medium apoB abundance without altering the amount of cellular apoB (109% ± 1.8% of control, mean ± SD of six independent experiments). In Fig. 5, the change in medium apoB abundance relative to control values is plotted against the change induced in [^{3}H]TG secretion for six individual experiments. A strong linear correlation exists between changes in apoB and [^{3}H]TG secretion ($P < .01$).

TABLE 2. Lipid Composition of Nascent VLDL Secreted by Human Hepatocytes

	TG	CH	CHE	PL
VLDL Source	nmol/mg cell protein/24 hours (%)			
Hepatocyte (a)	38.4 (63.6)	5.7 (9.4)	0.3 (0.5)	16.0 (26.5)
Hepatocyte (b)	22.2 (72.7)	2.6 (8.5)	0.3 (1.0)	5.4 (17.8)
Serum*	(62.0)	(11.2)	(10.3)	(16.5)

NOTE. Human hepatocytes were isolated from liver tissue of donors a and b described in Table 1. Cells were incubated with FCS-containing medium for 12 hours and then changed to FCS–hormone-free medium containing 1 mmol/L OA. VLDL was isolated from this FCS–hormone-free medium exposed to the cells for 24 hours. Media from 12 wells (1 mL/well) were pooled, and VLDL was isolated by ultracentrifugation for 24 hours at 41,000 rpm in a Beckman SW 41 rotor. VLDL-lipids were determined as described in Materials and Methods. Data represent means of two samples worked up in parallel. The numbers in parentheses indicate the percentage of total lipids.

* Calculated from published results.[60]

Anti-human-apoB antibody was used to precipitate apoB-containing lipoproteins present in medium after incubation with [^{3}H]OA for 3 hours in two separate experiments. The total amount of [^{3}H]OA labeled apoB-associated lipid in medium was suppressed by taurocholic acid to 62% of the control value in these experiments, mainly due to reduction of [^{3}H]TG secretion.

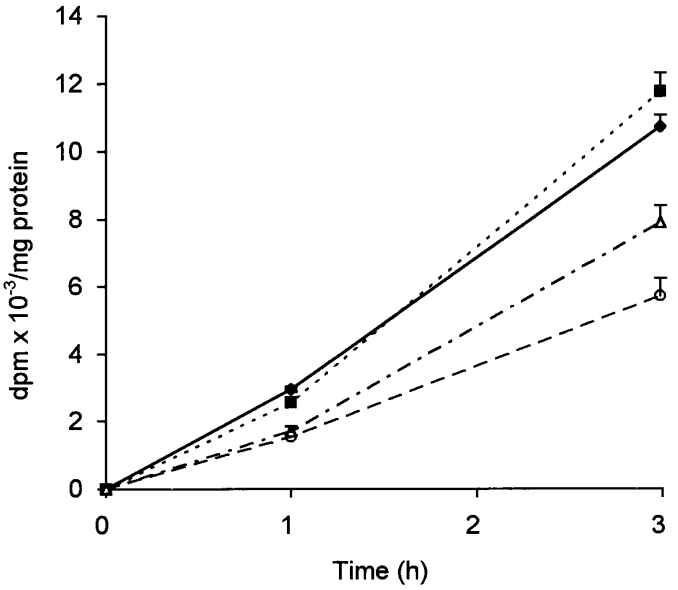

FIG. 2. Time course of the effects of taurocholic acid on [^{3}H]TG secretion. Human hepatocytes were incubated in Williams' E medium containing 1 mmol/L OA in the presence of 10 (■), 100 (△), 200 (○) μmol/L taurocholic acid or in its absence (——, control). [^{3}H]glycerol was added to the media (4.4 μCi/mL, 25 μmol/L at final concentration). At the times indicated, medium was harvested and centrifuged to remove suspended cells. Medium lipids were extracted and purified by TLC. [^{3}H]TG was determined by scintillation counting. Data represent the mean ± SD of triplicate incubation of hepatocytes isolated from the liver of donor c.

TABLE 3. Effects of Taurocholic Acid on Cellular and Medium TG Mass and [^{3}H]TG in Individual Experiments

	Cell TG Mass (nmol/mg protein)		Cell [^{3}H]TG (dpm $\times 10^{-5}$/mg protein)		Medium [^{3}H]TG (dpm $\times 10^{-3}$/mg protein)	
Donor	Control	Taurocholic Acid	Control	Taurocholic Acid	Control	Taurocholic Acid
c	133	129 (97)	4.66	4.61 (99)	10.7	7.9 (74)
d	224	210 (94)	7.08	6.65 (94)	24.9	19.1 (77)
e	128	125 (98)	5.38	4.65 (92)	18.5	5.9 (32)
f	122	115 (94)	5.51	5.23 (95)	42.9	44.6 (108)
g	164	166 (101)	4.49	3.98 (89)	7.2	2.7 (38)
h	111	102 (92)	5.05	5.83 (106)	6.1	3.8 (62)
i	112	113 (101)	5.50	5.38 (98)	33.7	20.5 (61)
Mean ± SD		97 ± 3		96 ± 5		65 ± 23*

NOTE. Human hepatocytes were incubated for 3 hours in the absence (control) or presence of 100 μmol/L taurocholic acid. [^{3}H]Glycerol (4.4 μCi/mL, 25 μmol/L at final concentration in medium) incorporated into cell and medium [^{3}H]TG was determined by scintillation counting. Data represent mean of triplicate incubations. The numbers in parentheses indicate the percent of control. Average values of medium [^{3}H]TG secretion for incubations with 10 μmol/L and 200 μmol/L taurocholic acid are 99% ± 9% (n = 7) and 53% ± 24% (n = 4, donors c, d, e, and g) ($P < .05$) of control, respectively.

* Significant difference compared with control ($P < .05$).

However, the relative composition of apoB-associated ^{3}H-labeled lipid was similar in the presence and absence of taurocholic acid (Table 6). Also in these experiments, taurocholic acid did not affect TG synthesis from the labeled precursor, as appears from the very similar values for cellular [^{3}H]TG content in both conditions.

Effect of Bile Acids on LDH Release and Protein Secretion. Table 7 shows that 3-hour incubations of human hepatocytes with taurocholic or taurochenodeoxycholic acids (10 to 200 μmol/L) did not result in increased release of LDH into the medium when compared with control incubations. The incorporation of [^{3}H]leucine into cellular protein and secretion of labeled protein by human hepatocytes were also similar across all groups. In addition, 100 μmol/L taurocholic acid had no adverse effects on albumin secretion. These results indicate that, under the conditions employed, bile acids did not exert distinct cytotoxic effects on human hepatocytes nor did they affect protein synthesis and secretion in an aspecific manner.

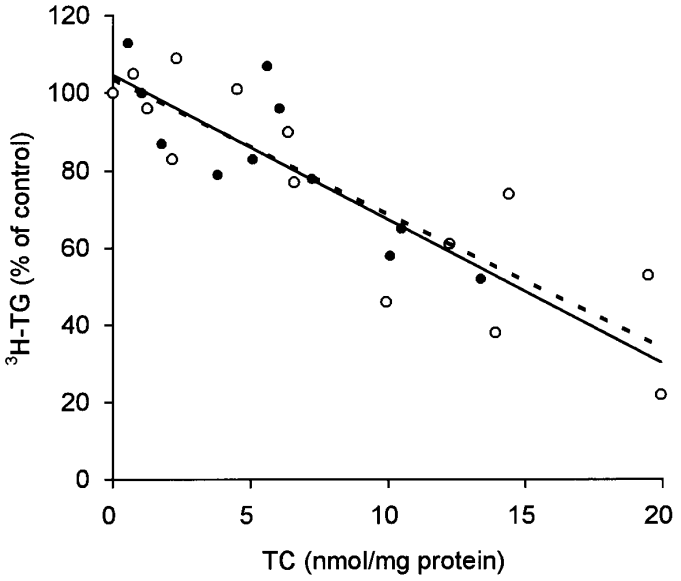

FIG. 3. Relationship between intracellular [^{3}H]taurocholic acid content and change in [^{3}H]TG secretion. Human hepatocytes were incubated as described in Fig. 2. Data shown are from five individual experiments (donors c through f and i). Each symbol represents mean of triplicate incubation per experiment. The regression lines are described by $y = -3.649x + 104.12$ and $y = -3.379x + 102.60$ for 1- (●) and 3-hour (○) incubations, respectively ($P < .01$).

DISCUSSION

Regulation of the secretion of TG-rich VLDL particles by the liver is crucial for the maintenance of whole-body energy balance in the fed and the fasted state. The current study was undertaken to further characterize the role of bile acids in this regulatory process. We have used human hepatocytes in primary culture for this purpose to circumvent potential drawbacks related to species and tumor specificity of the VLDL production process.[21,22] In a previous study, for instance, we observed marked quantitative and qualitative differences in the effects of eicosapentaenoic acid on glyc-

TABLE 4. Effects of Taurocholic Acid on Cellular CH and CHE Content

	CH (nmol/mg protein)	CHE (nmol/mg protein)
Control	17.9-31.8	5.4-8.1
Taurocholic acid	18.1-28.5 (93.5%)	3.0-3.1 (46.3%)

NOTE. Human hepatocytes were incubated for 3 hours in medium containing 1 mmol/L OA in the presence of 100 μmol/L taurocholic acid or in the absence of bile acid (control). Cells were harvested and cellular lipids were extracted. CH and CHE masses were determined as described in Materials and Methods section. Data are from two independent experiments (liver donors h and i), each performed in triplicate. The numbers in parentheses indicate the mean of the percentage of control.

TABLE 5. Effects of Taurocholic Acid (TC) on [^{3}H]TG Secretion by Human Hepatocytes in the Presence or Absence of OA

	0 mmol/L OA		1 mmol/L OA	
	(dpm/mg)	(% of control)	(dpm/mg)	(% of control)
Control	3,990 ± 942	100.0	33,655 ± 1,116	100.0
100 μmol/L TC	1,929 ± 599	48.1	20,447 ± 1,354	61.0

NOTE. Human hepatocytes were incubated for 3 hours in medium in the presence or absence of taurocholic acid and oleic acid. [^{3}H]Glycerol was added to the media (4.4 μCi/mL, 25 μmol/L final concentration) to quantify lipid secretion. Medium lipids were extracted and purified by TLC. [^{3}H]TG was determined by scintillation counting. Data represent the mean ± SD of triplicate incubation of the cells isolated from liver tissue of donor i.

erolipid metabolism and VLDL secretion between human hepatocytes and rat hepatocytes or HepG2 cells.[35] Cultured human hepatocytes produce nascent VLDL particles with a mean diameter of 40 nm, which is in the range of that of particles isolated from fasted human serum. The size of nascent human VLDL particles is also similar to that of VLDL produced by rat hepatocytes under similar culture conditions.[30] Furthermore, TG synthesis and VLDL production by human hepatocytes are stimulated by the presence of OA in the incubation medium to an extent similar as observed in rat hepatocytes.[35] Analysis of lipid composition showed a relatively low CHE content of nascent VLDL as compared with human serum VLDL (Table 2). This indicates that virtually all CHE present in serum VLDL must be acquired from other circulating lipoproteins, mainly from HDL, in exchange for VLDL-TG.[36]

We demonstrate a rapid, dose-dependent, parallel inhibition of VLDL-associated TG and apoB secretion by human hepatocytes in primary culture in the presence of physiological (i.e., portal) concentrations of bile acids.[37] Because VLDL particles contain a single apoB molecule,[3,4] this finding implies that bile acids inhibit hepatocytic lipid secretion by reducing the number of secreted particles rather than by altering their composition. This is further corroborated by the observation that incubation with taurocholic acid did not significantly alter the relative proportion of [^{3}H]OA derived TG, CH, CHE, and PL in the particles formed. The inhibitory effects of bile acids are not attributable to aspecific actions on apoB secretion, because the secretion of newly synthesized proteins and of albumin was not affected in these experiments. Our data clearly demonstrate that the intracellular concentration of bile acids is an important factor in determining the degree of inhibition. The different preparations of human cells

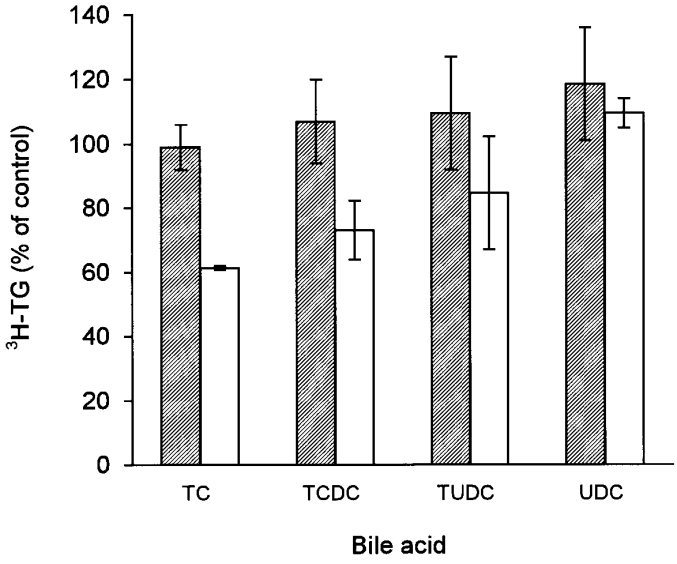

FIG. 4. The effects of individual bile acids on the incorporation of [^{3}H]glycerol into cellular (▨) and medium [^{3}H]TG (□). Human hepatocytes were incubated with taurocholic (TC), taurochenodeoxycholic (TCDC), tauroursodeoxycholic (TUDC), or ursodeoxycholic acids (UDC), all at a concentration of 100 μmol/L, for 3 hours. [^{3}H]-Glycerol (4.4 μCi/mL, 25 μmol/L at final concentration in medium) incorporated into cellular and medium [^{3}H]TG were determined by scintillation counting. Data are expressed as percentage of control incubations without bile acids added and represent mean of two independent experiments (donors h and i). The error bars indicate the range of these two experiments (triplicate measurements).

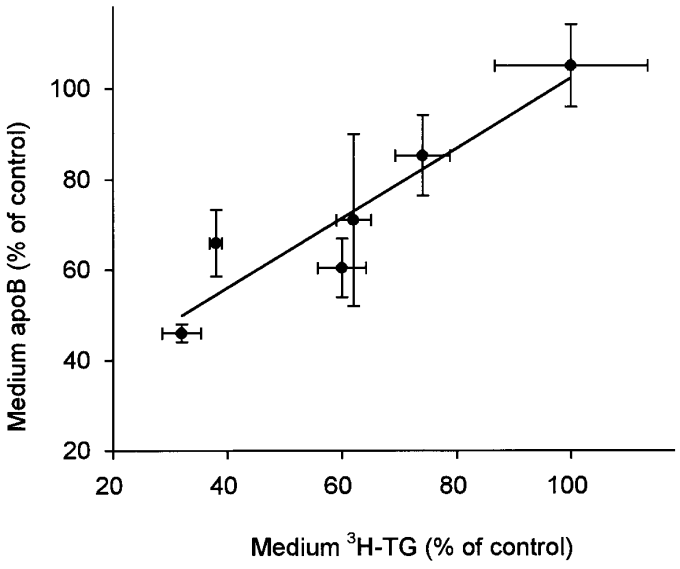

FIG. 5. Relationship between changes in apoB and [^{3}H]TG abundances in medium. Human hepatocytes were incubated for 3 hours as described in Fig. 2 in the absence (control) or presence of 100 μmol/L taurocholic acid. Medium apoB abundance was determined by immunoblot and semiquantified by densitometry. Medium [^{3}H]TG was determined by scintillation counting. Each symbol represents mean ± SD of triplicate incubation carried out in individual cell isolations from liver donors c, e through i.

TABLE 6. Effects of Taurocholic Acid on [^{3}H]OA Labeled VLDL and Hepatocyte Lipid Components

[^{3}H]Lipid dpm × 10^{-3}/mg protein (%)	VLDL		Hepatocyte	
	Control	Taurocholic Acid	Control	Taurocholic Acid
TG	10.5 ± 4.0 (79.7)	6.9 ± 1.9 (83.7)	800 ± 85 (85.5)	775 ± 182 (87.2)
CH	1.7 ± 0.5 (13.2)	0.8 ± 0.2 (10.3)	17 ± 7 (1.8)	10 ± 8 (1.2)
CHE	0.07 ± 0.00 (0.5)	0.08 ± 0.00 (1.0)	1.1 ± 0.9 (0.1)	1.0 ± 0.8 (0.1)
PL	0.86 ± 0.01 (6.5)	0.40 ± 0.02 (4.9)	118 ± 31 (12.6)	103 ± 39 (11.5)

NOTE. Human hepatocytes were incubated for 3 hours in medium containing 1 mmol/L OA with or without (control) addition of 100 μmol/L TC. [^{3}H]OA (10 mCi/mL medium) was added to monitor the composition of newly synthesized lipids in hepatocytes and in secreted VLDL particles. Cells were harvested and cellular lipids were extracted. Anti–human-apoB antiserum was added to fresh medium samples (1:200) and incubated overnight while shaking. Fifty milliliters 12% (wt/vol) protein A beads was added to each sample and incubated for another 2 hours. Protein A–antibody-apoB complexes were collected by centrifugation. After washing, lipids were extracted from the beads. Individual lipids were purified by TLC and determined by scintillation counting. Data represent mean ± SD of triplicate measurements in two independent experiments (donors h and i). The numbers in parentheses represent the percent of total ^{3}H-labeled lipids.

used showed a large variation in equilibrium taurocholic acid uptake values, with intracellular concentrations ranging from virtually zero to 20 nmol/mg protein. Recent studies by Sandker et al.[25] showed that taurocholic acid uptake in freshly isolated human hepatocytes as well as in basolateral plasma membrane vesicles prepared from human liver takes place by a sodium-dependent mechanism, i.e., analogous to the situation reported in rats,[38] and that the kinetic parameters (Km and V_{max}) calculated for individual human preparations show large variations. The sodium-dependent bile acid transporters of rat[39] and human[40] liver, ntcp and NTCP (Na–taurocholic acid cotransporting protein), respectively, have recently been cloned and functionally characterized. Ntcp messenger RNA levels rapidly decline during culture in rat hepatocytes; expression is reduced to virtually zero within 2 days.[41] Regulation of expression of the human system has to our knowledge not been studied so far. It is likely that the large variation in final taurocholic acid content between the cell preparations is due to differences in the expression of the sodium-dependent uptake system, but differences in efflux rates may also have contributed.[25]

Irrespective of the underlying mechanisms, these interindividual variations allowed us to demonstrate a clear inverse relationship between intracellular bile acid concentration, as determined from [^{14}C]taurocholic acid content of the cells and VLDL production by these human cells. Inhibition of VLDL-associated [^{3}H]TG synthesized from [^{3}H]glycerol and from [^{3}H]OA during these relatively short-term experiments occurred in the presence of unchanged intracellular content of TG mass and [^{3}H]TG, indicative for an unaltered TG synthesis under these conditions. TG mass secretion could not adequately be determined in our short-term experiments, because of low concentrations in the media. However, Del Pozo and Barth[19] were able to demonstrate a parallel decline in ^{3}H-labeled and mass TG secretion under the influence of taurocholic acid in freshly isolated rat hepatocytes. We have recently found the same in cultured rat hepatocytes incubated with taurocholic acid for 8 or 24 hours (Lin et al., Unpublished results, 1995). This indicates that the reduced secretion of radiolabeled VLDL-associated TG is due to "real inhibition" of VLDL production rather than to relative alterations in the pool size(s) of the labeled precursor ([^{3}H]glycerol), at least in rat cells. For human cells, this is further supported by the observation (Table 5) that taurocholic acid inhibited VLDL secretion to a similar extent in the presence and in the absence of OA in the incubation media. Our data can thus be interpreted to indicate that a decreased availability of TG does not directly underlie the observed inhibition of lipoprotein secretion. ApoB secretion, not determined in the previously mentioned report using rat hepatocytes,[19] was inhibited to the same extent as that of [^{3}H]TG in the presence of unaltered cellular apoB content. Whether bile acids affect the kinetics of intracellular apoB metabolism cannot be deduced from these experiments and awaits further studies.

TABLE 7. Lactate Dehydrogenase Release, Protein Synthesis, and Secretion by Human Hepatocytes

	LDH Leakage (%) (n = 3)*	^{3}H-Labeled Protein Synthesis (n = 5)†	^{3}H-Labeled Protein Secretion (n = 5)†	Albumin Secretion (n = 4)‡
		% of Control		
Control	6.6 ± 1.4			
10 μmol/L TC	6.6 ± 1.7	ND	ND	ND
100 μmol/L TC	7.3 ± 0.6	91.4 ± 3.3	103.3 ± 15.4	93.0 ± 4.9
200 μmol/L TC	6.2 ± 2.0	103.3 ± 2.4	99.2 ± 3.5	ND
100 μmol/L TCDC	6.5 ± 1.5	96.7 ± 5.8§	112.0 ± 4.3§	ND

NOTE. Human hepatocytes were incubated for 3 hours in hormone-free medium containing 1 mmol/L OA with or without bile acid. LDH was measured as described in Materials and Methods. LDH content in medium is expressed as a percentage of cellular LDH. ^{3}H-labeled protein synthesis and secretion were estimated by determining [^{3}H]leucine (5 mCi/mL) incorporation into trichloroacetic acid–precipitable cellular and medium proteins, respectively, which were quantitated by scintillation counting. The range of control values from different experiments were 18.6 to 158.5 and 5.0 to 51.6 dpm × 10^{-3}/mg cell protein for ^{3}H-labeled protein synthesis and secretion, respectively. Albumin secretion was determined by enzyme-linked immunosorbent assay (ELISA) with human albumin as standard. The control values ranged from 340 to 1190 ng/mg cell protein. Data are expressed as mean ± SD of numbers (n) of separate experiments, each experiment performed in triplicate. No statistically significant differences were observed between control and bile acid incubation groups.

* Donors c, e, and f.
† Donors c, d, e, g, and i.
‡ Donors e, f, g, and h.
§ Donors e, g, and i.

What are potential means by which intracellular bile acids modulate VLDL production? Recent studies by Björnsson et al.[42,43] have demonstrated that this process can be modulated by intracellular messengers Ca^{2+} and cyclic adenosine monophosphate (cAMP). It is well known that certain bile acids are able to affect cytosolic calcium concentrations in rat hepatocytes by mobilizing IP_3-sensitive Ca^{2+} stores or inducing influx of extracellular Ca^{2+}.[44,45] However, taurocholic acid, which in our hands strongly suppresses VLDL secretion, has been reported to have only small or no effects on intracellular Ca^{2+} in rat hepatocytes.[44,45] Tauroursodeoxycholic acid, however, only has a relatively small effect on VLDL production in human hepatocytes but has been reported to lead to a very strong increase in Ca^{2+} levels.[45] Another mode of regulation of rat cell VLDL metabolism acts through cAMP and cAMP-dependent protein kinases. It has been shown that a transient increase in cellular cAMP levels, for instance induced by glucagon, is sufficient to maintain a long-term inhibitory effect on the assembly and secretion of VLDL.[43] Yet, very recent studies by Bouscarel et al.[46,47] have shown that bile acids have no direct effect on cAMP production in isolated hamster hepatocytes[46] and in cells of nonhepatic origin,[47] and, in fact, inhibit hormone-induced cAMP production. Thus, it seems unlikely that modulation of VLDL production by bile acids is mediated by these second messenger systems.

It is also possible that bile acids interfere with VLDL production by influencing the availability of essential lipid constituents of the VLDL particles. Extensive studies by the group of Vance[48-51] have shown that *de novo* synthesis of phosphatidylcholine is required for VLDL formation and secretion by rat hepatocytes, possibly by providing this phospholipid to a specific (microsomal) pool destined for assembly into VLDL particles. In preliminary studies, we did not observe significant effects of taurocholic acid on PL synthesis from [^{14}C]-acetate and from [^{3}H]OA, which, obviously, does not exclude the possibility of an effect on the synthesis of a specific subpool of phosphatidylcholine. It may be, however, that taurocholic acid does not affect phosphatidylcholine availability by altering synthesis but by altering the intracellular destiny of newly synthesized molecules. As mentioned in the introductory section, bile acids strongly stimulate secretion of biliary PL, which almost exclusively consists of phosphatidylcholine, at the canalicular pole of the hepatocytes. Available evidence, reviewed by Verkade et al.,[1] indicates that quantitative regulation of this bile acid effect is exerted at the level of the bile canalicular membrane. Nevertheless, bile acids may also interact with lipids at the endoplasmic reticulum. Interactions of bile acids with endoplasmic reticulum have been described, in particular when the liver is exposed to high bile acid loads.[52,53] It may be that, at the endoplasmic reticulum, bile acids direct phosphatidylcholine from a "VLDL-destined pool" to a "bile-destined pool," but this remains momentarily speculative.

Hepatocytic CHE synthesis and CHE levels, under the control of acyl-coenzyme A:cholesterol acyltransferase, have been proposed to play a critical role in the regulation of apoB and VLDL secretion based on studies with HepG2 cells,[54,55] rabbit hepatocytes,[56] and perfused rat[57] and African Green monkey livers.[58] We found that all bile acids tested thus far in human cells cause a marked reduction of cellular CHE content (Table 4 for taurocholic acid); the mechanism responsible for this effect as well as its causal relationship to the suppression of VLDL secretion remains to be defined. The very low CHE content of nascent VLDL particles produced by these cells makes it, in our opinion, unlikely that packing of the VLDL core with CHE during lipoprotein formation at the endoplasmic reticulum represents a rate-limiting step in the production process. It may be, as hypothesized by Tanaka et al.,[56] that higher CHE formation at the rough endoplasmic reticulum, i.e., the site of both CHE and apoB synthesis, prevents apoB from entering a degradation pathway at an early stage of the assembly process, thereby permitting the formation of a "complete" particle at the endoplasmic reticulum and its eventual secretion.

It is evident that more studies are required to elucidate the mechanisms by which bile acids interfere with VLDL production by human liver cells. However, our results clearly demonstrate that inhibition of hepatocytic TG synthesis is not involved. Because the effects of bile acids on serum TG levels *in vivo* and on VLDL secretion *in vitro* are very pronounced, it appears that the enterohepatic circulation of bile acids may play a physiological role in the regulation of serum lipoprotein levels by inhibiting VLDL production. Because the exposure of liver cells to bile acids is largely determined by gallbladder contraction in response to a fat-containing meal, it seems reasonable to assume an active role of bile acids in the regulation of VLDL production in the postprandial phase, probably in a concerted action with hormones such as insulin and glucagon.[3] As recently reviewed by Havel,[59] it is now recognized that hepatogenous VLDL may contribute to postprandial hyperlipidemia. Whether this reflects impaired lipolysis of "endogenous" lipids due to the abundant presence of "exogenous" lipids during the postprandial phase or an actual increase in VLDL efflux under these conditions is not yet clear. From a viewpoint of metabolic efficiency, it would be beneficial to suppress VLDL secretion after a meal, a process in which bile acids may well play a role. The relevance of such a regulatory process is delineated by recent data indicating that postprandial TG levels are more closely related to risk for coronary heart disease than fasting levels,[59] and by the notion that defective enterohepatic circulation of bile acids due to intestinal malabsorption may underly familial hypertriglyceridemia.[16,17]

Acknowledgment: The authors thank Bert Blaauw and Dr. Marjan van Luyn (Laboratory for Cell Biology and Electron Microscopy, University of Groningen, Groningen, Germany) for their excellent technical assistance and advice.

REFERENCES

1. Verkade HJ, Vonk RJ, Kuipers F. New insights in the mechanism of bile acid-induced biliary lipid secretion. HEPATOLOGY 1995;21:1174-1189.
2. Oude Elferink RPJ, Ottenhof R, van Wijland M, Smit JJM, Schinkel AH, Groen AK. Regulation of biliary lipid secretion by mdr2 P-glycoprotein in the mouse. J Clin Invest 1995;95:31-38.
3. Gibbons GF. Assembly and secretion of hepatic very-low-density lipoprotein. Biochem J 1990;268:1-13.
4. Yao Z, McLeod RS. Synthesis and secretion of hepatic apolipoprotein B-containing lipoproteins. Biochim Biophys Acta 1994; 1212:152-166.
5. Smit MJ, Verkade HJ, Havinga R, Vonk RJ, Scherphof GL, In't Veld G, Kuipers F. Dietary fish oil potentiates bile acid-induced cholesterol secretion into bile in rats. J Lipid Res 1994;35:301-310.
6. Stone BG, Evans CD. Evidence for a common biliary and VLDL cholesterol precursor pool in rat liver. J Lipid Res 1992;33:1665-1675.
7. Marzolo MP, Amigo L, Nervi F. Hepatic production of very low density lipoprotein, catabolism of low density lipoprotein, biliary lipid secretion, and bile salt synthesis in rats fed a bean (Phaseolus vulgaris) diet. J Lipid Res 1993;34:807-814.
8. Grundy SM, Ahrens EH, Salen G. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. J Lab Clin Med 1971;78:94-121.
9. Beil U, Crouse JR, Einarsson K, Grundy SM. Effects of interruption of the enterohepatic circulation of bile acids on the transport of very low density lipoprotein triglycerides. Metabolism 1982; 31:438-444.
10. Angelin B, Einarsson K, Hellstrom K, Leijd B. Effects of cholestyramine and chenodeoxycholic acid on the metabolism of endogenous triglyceride in hyperlipoproteinemia. J Lipid Res 1978; 19:1017-1024.
11. Angelin B, Leijd B, Hulcrantz R, Einarsson K. Increased turnover of very low density lipoprotein triglyceride during treatment with cholestyramine in familial hypercholesterolaemia. J Intern Med 1990;227:201-206.
12. Akerlund JE, Björkhem I, Angelin B, Liljeqvist L, Einarsson K. Apparent selective bile acid malabsorption as a consequence of ileal exclusion: effects on bile acid, cholesterol, and lipoprotein metabolism. Gut 1994;35:1116-1120.
13. Angelin B, Leijd B. Effects of cholic acid on the metabolism of endogenous plasma triglyceride and on biliary lipid composition in hyperlipoproteinemia. J Lipid Res 1980;21:1-9.
14. Miller NE, Nestel PJ. Triglyceride-lowering effect of chenodeoxycholic acid in patients with endogenous hypertriglyceridemia. Lancet 1974;2:929-931.
15. Tint GS, Ginsberg H, Salen G, Lee NA, Shefer S. Chenodeoxycholic acid normalizes elevated lipoprotein secretion and catabolism in cerebrotendinous xanthomatosis. J Lipid Res 1989; 30:633-640.
16. Angelin B, Hershon KS, Brunzell JD. Bile acid metabolism in hereditary forms of hypertriglyceridemia: evidence for an increased synthesis rate in monogenic familial hypertriglyceridemia. Proc Natl Acad Sci U S A 1987;84:5434-5438.
17. Duane WC. Abnormal bile acid absorption in familial hypertriglyceridemia. J Lipid Res 1995;36:96-107.
18. Angelin B, Einarsson K. Bile acids and lipoprotein metabolism. Atheroscler Rev 1986;15:41-66.
19. Del Pozo R, Barth CA. Bile acids inhibit secretion of very low density lipoprotein by rat hepatocytes. Biol Chem Hoppe-Seyler 1987;368:887-893.
20. Marchegiano P, Carubbi F, Tiribelli C, Amarri S, Stebel M, Lunazzi GC, Levy D, et al. Transport of sulfobromophthalein and taurocholate in the HepG2 cell line in relation to the expression of membrane carrier proteins. Biochem Biophys Res Commun 1992;183:1203-1208.
21. Javitt NB. HepG2 cells as a resource for metabolic studies: lipoprotein, cholesterol, and bile acids. FASEB J 1990;4:161-168.
22. Dixon JL, Ginsberg HN. Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: information obtained from cultured liver cells. J Lipid Res 1993;34:167-179.
23. Moshage H, Yap SH. Primary cultures of human hepatocytes: an unique system for studies in toxicology, virology, parasitology and pathophysiology in man. J Hepatol 1992;15:404-413.
24. Kalayoglu M, Sollinger HW, Stratta RJ, D'Allessandro AM, Hoffman RM, Pirsch JD, Belzer FO. Extended preservation of the liver for clinical transplantation. Lancet 1988;1:617-619.
25. Sandker GW, Weert B, Olinga P, Wolters H, Slooff MJH, Meijer DKF, Groothuis GMM. Characterization of transport in isolated human hepatocytes: a study with the bile acid taurocholic acid, the uncharged ouabain and the organic cations vecuronium and rocuronium. Biochem Pharmacol 1994;47:2193-2200.
26. Princen HMG, Huijsmans CMG, Kuipers F, Vonk RJ, Kempen HJM. Ketoconazole blocks bile acid synthesis in hepatocyte monolayer cultures and in vivo in rat by inhibiting cholesterol 7α-hydroxylase. J Clin Invest 1986;78:1064-1071.
27. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Biophys 1959;37:911-917.
28. Gamble W, Vaughan M, Kruth HS, Avigan J. Procedure for determination of free and total cholesterol in micro- or nanogram amounts suitable for studies with cultured cells. J Lipid Res 1978;19:1068-1070.
29. Böttcher CFJ, van Gent CM, Pries C. A rapid and sensitive submicrophosphorous determination. Anal Chim Acta 1961;24:203-204.
30. Davis RA, Engelhorn SC, Pangburn SH, Weinstein DB, Steinberg D. Very low density lipoprotein synthesis and secretion by cultured rat hepatocytes. J Biol Chem 1979;254:2010-2016.
31. Rusiñol A, Verkade HJ, Vance JE. Assembly of rat hepatic very low density lipoprotein in the endoplasmic reticulum. J Biol Chem 1993;268:3555-3562.
32. Vance DE, Weinstein DB, Steinberg D. Isolation and analysis of lipoproteins secreted by rat liver hepatocytes. Biochim Biophys Acta 1984;792:39-47.
33. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with folin reagent. J Biol Chem 1951;193:265-275.
34. Smit MJ, Beekhuis H, Duursma AM, Bouma JMW, Gruber M. Catabolism of circulating enzymes: plasma clearance, endocytosis, and breakdown of lactate dehydrogenase. Clin Chem 1988;34:2475-2480.
35. Lin Y, Smit MJ, Havinga R, Verkade HJ, Vonk RJ, Kuipers F. Differential effects of eicosapentaenoic acid on glycerolipid and apolipoprotein B metabolism in primary human hepatocytes compared to HepG2 cells and primary rat hepatocytes. Biochim Biophys Acta 1995;1256:88-96.
36. Dullaart RPF, Groener JFM, Erkelens DW. Cholesterylester transfer between lipoproteins. Diab Nutr Metab 1991;4:329-343.
37. Angelin B, Björkhem I, Einarsson K, Ewerth S. Hepatic uptake of bile acids in man: fasting and postprandial concentrations of individual bile acids in portal venous and systemic blood serum. J Clin Invest 1982;70:724-731.
38. Zimmerli B, Valantinas J, Meier PJ. Multispecificity of Na^+-dependent taurocholate uptake in basolateral (sinusoidal) rat liver plasma membrane vesicles. J Pharmacol Exp Ther 1989;250:301-308.
39. Hagenbuch B, Stieger B, Foguet M, Lübbert H, Meier PJ. Functional expression cloning and characterization of the hepatocyte Na^+/bile acid cotransport system. Proc Natl Acad Sci U S A 1991;88:10629-10633.
40. Hagenbuch B, Meier PJ. Molecular cloning, chromosomal localization, and functional characterization of a human liver Na^+/bile acid cotransporter. J Clin Invest 1994;93:1326-1331.
41. Liang D, Hagenbuch B, Stieger B, Meier PJ. Parallel decrease of Na^+-taurocholate cotransport and its encoding mRNA in primary cultures of rat hepatocytes. HEPATOLOGY 1993;18:1162-1166.
42. Björnsson OG, Sparks JD, Sparks CE, Gibbons GF. Prostaglandins suppress VLDL secretion in primary rat hepatocyte cultures: relationships to hepatic calcium metabolism. J Lipid Res 1992;33:1017-1027.
43. Björnsson OG, Sparks JD, Sparks CE, Gibbons GF. Regulation

of VLDL secretion in primary culture of rat hepatocytes: involvement of cAMP and cAMP-dependent protein kinases. Eur J Clin Invest 1994;24:137-148.
44. Zimniak P, Little JM, Radominska A, Oelberg DG, Anwer MS, Lester R. Taurine-conjugated bile acids act as Ca^{++} ionophores. Biochemistry 1991;30:8598-8604.
45. Beuers U, Nathanson MH, Boyer JL. Effects of tauroursodeoxycholic acid on cytosolic Ca^{2+} signals in isolated rat hepatocytes. Gastroenterology 1993;104:604-612.
46. Bouscarel B, Gettys TW, Fromm H, Dubner H. Ursodeoxycholic acid inhibits glucagon-induced cAMP formation in hamster hepatocytes: a role for PKC. Am J Physiol 1995;268:G300-G310.
47. Bouscarel B, Ceryak S, Gettys TW, Fromm H, Noonan F. Alteration of cAMP-mediated hormonal responsiveness by bile acids in cells of nonhepatic origin. Am J Physiol 1995;268:G908-G916.
48. Yao Z, Vance DE. The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. J Biol Chem 1988;263:2998-3004.
49. Vance JE. The use of newly synthesized phospholipids for assembly into secreted hepatic lipoproteins. Biochim Biophys Acta 1989;1006:59-69.
50. Yao Z, Vance DE. Reduction in VLDL, but not HDL, in plasma of rats deficient in choline. Biochem Cell Biol 1990;68:552-558.
51. Verkade HJ, Fast DG, Rusiñol AE, Scraba DG, Vance DE. Impaired biosynthesis of phosphatidylcholine leads to formation of abnormal very low density lipoproteins with fewer particles in the Golgi. J Biol Chem 1993;268:24990-24996.
52. Lamri Y, Roda A, Dumont M, Feldmann G, Erlinger S. Immunoperoxidase localization of bile salts in liver cells: evidence for a role of the Golgi apparatus in bile salt transport. J Clin Invest 1988;82:1173-1182.
53. Crawford JM, Barnes S, Stearns RC, Hastings CL, Godleski JL. Ultrastructural localization of a fluorinated bile salt in hepatocytes. Lab Invest 1994;71:42-51.
54. Cianflone KM, Yasruel Z, Rodriguez MA, Vas D, Sniderman AD. Regulation of apo B secretion from HepG2 cells: evidence for a critical role for cholesterylester synthesis in the response to a fatty acid challenge. J Lipid Res 1990;31:2045-2055.
55. Cianflone KM, Dahan S, Monge JC, Sniderman AD. Pathogenesis of carbohydrate-induced hypertriacylglycerolemia using HepG2 cells as a model system. Arterioscler Thromb 1992; 12:271-277.
56. Tanaka M, Jingami H, Otani H, Cho M, Ueda Y, Arai H, Nagano Y, et al. Regulation of apolipoprotein B production and secretion in response to the change of intracellular cholesterylester contents in rabbit hepatocytes. J Biol Chem 1993;268:12713-12718.
57. Fungwe TV, Cagen LM, Wilcox HG, Heimberg M. Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. J Lipid Res 1992;33:179-191.
58. Carr TP, Hamilton RL, Rudel LL. ACAT inhibitors decrease secretion of cholesteryl esters and apolipoprotein B by perfused livers of African green monkeys. J Lipid Res 1995;36:25-36.
59. Havel RJ. Postprandial hyperlipidemia and remnant lipoproteins. Curr Opin Lipidol 1994;5:102-109.
60. Miller KW, Small DM. Surface-to-core and interparticle equilibrium distributions of triglyceride-rich lipoprotein lipids. J Biol Chem 1983;258:13772-13784.