Coordinate Regulation of Lipogenesis, the Assembly and Secretion of Apolipoprotein B-containing Lipoproteins by Sterol Response Element Binding Protein 1*

(Received for publication, February 24, 1997, and in revised form, May 27, 1997)

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Stable plasmid-driven expression of the liver-specific gene product cholesterol 7α-hydroxylation (7α-hydroxylase) was used to alter the cellular content of transcriptionally active sterol response element binding protein 1 (SREBP1). As a result of stable expression of 7α-hydroxylase, individual single cell clones expressed varying amounts of mature SREBP1 protein. These single cell clones provided an opportunity to identify SREBP1-regulated genes that may influence the assembly and secretion of apoB-containing lipoproteins. Our results show that in McArdle rat hepatoma cells, which normally do not express 7α-hydroxylase, plasmid-driven expression of 7α-hydroxylase results in the following: 1) a linear relationship between (i) the cellular content of mature SREBP1 and 7α-hydroxylase protein, (ii) the relative expression of 7α-hydroxylase mRNA and the mRNA's encoding the enzymes regulating fatty acid, i.e. acetyl-CoA carboxylase and sterol synthesis, i.e. HMG-CoA reductase, (iii) the relative expression of 7α-hydroxylase mRNA and microsomal triglyceride transfer protein mRNA, a gene product that is essential for the assembly and secretion of apoB-containing lipoproteins; 2) increased synthesis of all lipoprotein lipids (cholesterol, cholesterol esters, triglycerides, and phospholipids); and 3) increased secretion of apoB100 without any change in apoB mRNA. Cells expressing 7α-hydroxylase contained significantly less cholesterol (both free and esterified). The increased cellular content of mature SREBP1 and increased secretion of apoB100 were concomitantly reversed by 25-hydroxycholesterol, suggesting that the content of mature SREBP1, known to be decreased by 25-hydroxycholesterol, mediates the changes in the lipoprotein assembly and secretion pathway that are caused by 7α-hydroxylase. These data suggest that several steps in the assembly and secretion of apoB-containing lipoproteins by McArdle hepatoma cells may be coordinately linked through the cellular content of mature SREBP1.

* This work was supported by National Institutes of Health Grants HL52005 and HL51643. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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Apolipoprotein B100 (apoB) is an unusually large (>500 kDa) amphipathic protein responsible for the assembly and secretion of plasma lipoproteins by the liver and intestine (reviewed in Refs. 1–3). Its concentration in plasma, as a component of LDL, is a major determinant of susceptibility to the development of atherosclerotic cardiovascular disease (4, 5). Hepatic derived apoB100-containing lipoproteins are the precursors of plasma LDL (6). Hepatic assembly and secretion of apoB-containing lipoproteins require an orchestration of many seemingly independent processes as follows: 1) the production of component lipids (cholesterol, cholesterol esters, triglycerides, and phospholipids); 2) the synthesis of apoB, a uniquely large polypeptide containing multiple amphipathic structural domains that irreversibly associate with phospholipids (7); 3) translocation across the endoplasmic reticulum that requires an intraluminal protein complex consisting of MTP and PDI (8); and 4) the assembly of VLDL particle within the endoplasmic reticulum (1, 2, 9).

Previous studies showed a coordinate induction of the synthesis of all VLDL lipids and the assembly and secretion of apoB-containing lipoproteins in response to changes in metabolic state (e.g. during dietary carbohydrate overload (10) and fetal development (11)). In contrast, fasting caused a coordinate repression of all of these processes (12, 13). These findings led us to propose that in a coordinate manner, metabolic signals control multiple steps of the VLDL assembly/secretion pathway. Using a somatic cell genetic approach, we examined this possibility. Previous experiments showed that expression of the liver-specific gene 7α-hydroxylase (EC 1.14.13.17) in non-hepatic Chinese hamster ovary cells increased the transcription of the LDL receptor gene (14), a process now known to be regulated by the SREBP family of transcription factors (15, 16). Sterol-responsive transcriptional regulation is invoked by a proteolytic cleavage of the initial translational SREBP gene product that resides in the endoplasmic reticulum membrane as a trans-membrane loop (17, 18). When the sterol content of cells is insufficient to meet metabolic demands, proteolysis of the N-terminal domain releases the DNA binding domain of SREBP(s) (mature forms), allowing them to enter the nucleus and activate transcription of genes having cognitive promoter elements. Expression of 7α-hydroxylase in cells that do not normally express it increases the cellular content of mature SREBP1 (see “Results”). Moreover, since McArdle rat hepatoma cells express all of the genes required to assemble and secrete apoB-containing lipoproteins (19–21), but lack 7α-hydroxylase (see “Results”), by stably expressing 7α-hydroxylase via plasmid transfection in McArdle hepatoma cells, we were able to increase the cellular content of mature SREBP1 to different extents in single cell clones. These cells provide a low density lipoprotein; VLDL, very low density lipoprotein; BSA, bovine serum albumin.
unique opportunity to examine how SREBP1 may affect each of the steps of the VLDL assembly/secretion pathway. The results indicate that SREBP1 coordinately regulates many of the individual lipogenic and protein processing steps.

MATERIALS AND METHODS
All chemicals used for biochemical techniques were purchased from Sigma, VWR, Fisher, or Boehringer Mannheim. Cell culture medium was obtained from Life Technologies, Inc. and fetal bovine serum from Gemini. Restriction enzymes were purchased from New England Biolabs or Promega. The cDNA probes were obtained from the following: hamster MTP was a generous gift from Drs. Richard Gregg, John Wetterau, David Gordon, and their colleagues at Bristol-Myers Squibb (22); hamster HMG-CoA reductase was obtained from ATCC; rat 7α-hydroxylase was a generous gift from Dr. David Russell (23); and acetyl-CoA carboxylase was a generous gift from Dr. Tim Osborne (24). A mouse hybridoma cell line producing monoclonal antibody against human SREBP-1 (a generous gift from Dr. Tim Osborne) was propagated in mice, and the resulting IgG from the ascites fluid was obtained (25). A rabbit polyclonal antibody produced from a synthetic SREBP2 peptide was a generous gift from Dr. Michael Briggam.

Cell Culture and Transfection—McArdle RH-7777 hepatoma cells, a gift from Dr. Tom Innerarity, were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 25 mm glucose, 15% heat-inactivated fetal bovine serum, and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml; and fungizone, 50 μg/ml). The cells were grown at 37 °C in a humidified atmosphere of air with 5% CO2. A plasmid was prepared from pcDNA3 (Invitrogen) in which the coding region of rat 7α-hydroxylase was placed in the EcoRI-Digested linker region. This plasmid contains a cytomegalovirus promoter and encodes neomycin resistance. Stable expression of the plasmid in McArdle RH-7777 cells was achieved by calcium phosphate precipitation and selection for neomycin resistance (G418, 400 μg/ml). Single cell clones of neomycin-resistant cells were obtained.

Northern Blot Analysis—Poly(A) RNA was isolated from cells using a modification of the guanidinium isothiocyanate method, as described (27). Two to 5 μg of the resulting mRNA was separated by 0.8% agarose gel electrophoresis, transferred to a nylon membrane, and probed with nick-translated 32P-DNA probes prepared from gel-purified inserts.

SREBP Analysis of Nuclei and Membrane Fractions—Cells were harvested on ice in cold phosphate-buffered saline containing a protease inhibitor mixture (1 mM phenylmethylsulfonyl fluoride, 10 μM aprotinin, and 50 μg/ml leupeptin) using a rubber policeman. Nuclei and membrane fractions were obtained using the method described by Wang et al. (17). Cells were centrifuged at 1000 rpm for 10 min, and the pellets were resuspended in 10 volumes of cell homogenization buffer (20 mM HEPES-KOH at pH 7.6, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol, 1 mM EDTA, and proteinase inhibitors). The cells were disrupted by passage through a 22-gauge needle (15 times) and then centrifuged at 1000 rpm for 10 min. The crude nuclear pellet was extracted with an equal volume of nuclear extraction buffer (20 mM HEPES-KOH at pH 7.6, 25% glycerol, 0.5 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, and proteasome inhibitors) and centrifuged at 12,000 rpm for 30 min. The supernatant was used as extract for immunoblotting analysis. The micromolar membrane fraction was obtained by further centrifugation of the supernatant obtained from the nuclear pellet by ultracentrifugation at 45,000 rpm for 2 h using a TLA45 rotor (Beckman).

Western Blot Analysis—Western blotting was performed as described (28). Following SDS-PAGE (1–15% gradient), the gels were electroblotted onto nitrocellulose membranes. The nonspecific binding sites of the membranes were blocked using 10% defatted dried milk, followed by addition of the appropriate primary antibody. The relative amount of primary antibody bound to the proteins on the nitrocellulose membrane was detected with the species-specific horseradish peroxidase-conjugated IgG. Blots were developed using the enhanced chemiluminescence detection kit (Amersham Corp.).

RESULTS
Expression of 7α-Hydroxylase in McArdle Cells—McArdle cells were transfected with a plasmid conferring neomycin resistance and expression of rat 7α-hydroxylase. Following selection for G418 resistance, single cell clones were grown. One clone (SLW-1 cells) was used to examine the expression of 7α-hydroxylase mRNA (A) and protein (B) using Northern and Western blot analyses, respectively. The migration of molecular mass markers corresponding to BSA and ovalbumin are indicated in B.

Analysis of Lipid Biosynthesis—Cells were incubated in serum-containing medium with [1-14C]acetate (5 μCi, specific activity 47 mCi/mmol) for 2 h, extracted with chloroform/methanol (2:1, v/v), separated on silica gel TLC plates, and the radioactivity in phospholipids, free cholesterol, triglycerides, and cholesterol esters was quantitated (29). The cellular content of free and esterified cholesterol was determined in cells cultured to ~85% confluency by gas liquid chromatography, as described (14).

Statistics Analysis—Results are given as means ± S.D. Linear regression analysis was determined by Sigma Plot computer program, and significance of correlation constants was determined by Student’s t test. Values of p < 0.05 were considered to be significant. The number of data points obtained from individual single cell clones used for linear correlations differed from experiment to experiment due to loss of a particular single cell clone. All data points were used and none were selectively deleted.

Expression of 7α-Hydroxylase mRNA or Protein (A and B) in McArdle cells. McArdle (McA) cells were transfected with a plasmid conferring neomycin resistance and expression of rat 7α-hydroxylase. Following antibiotic selection, single cell clones were picked and grown. One single cell clone of McArdle cells stably expressing 7α-hydroxylase mRNA or protein (A and B) was used for detailed metabolic studies, whereas single cell clones were used to establish metabolic relationships (see below). Single cell clones of transfected cells expressed 7α-hydroxylase mRNA and protein of the size expected from the plasmid used (Fig. 1, A and B). While wild-type McArdle rat hepatoma cells express all of the gene products required for VLDL assembly and secretion (19–21), they show no detectable expression of 7α-hydroxylase mRNA or protein (Fig. 1, A and B).

Cellular Content of Mature SREBP1 Varies Linearly with 7α-Hydroxylase Protein—Single cell clones of McArdle cells transfected with pcDNA-7α were subjected to Western blot analysis using repetitive immunodetection with antibodies to SREBP1, 7α-hydroxylase, and albumin. Since the amount of albumin in either the cell or medium was unchanged (see below), albumin served as an internal standard for recovery and blotting. The cellular content of mature SREBP1 was increased, whereas the content of the precursor form was decreased in SLW-1 cells stably expressing 7α-hydroxylase (Fig. 2A). Incubating SLW-1 cells and wild-type McArdle cells with 25-hydroxycholesterol, an established inhibitor of SREBP1 proteolytic processing (17), decreased the content of mature...
SREBP1 by about 90%, whereas the precursor form was slightly increased (Fig. 2B). Moreover, analysis of all of the single cell clones showed that the cellular content of mature SREBP1 varied as a linear function of 7α-hydroxylase protein (Fig. 2C). Additional studies show that the cellular content of SREBP2 was not significantly affected by the expression of 7α-hydroxylase (data not shown).

**Secretion of ApoB100 Varies as a Linear Function of 7α-Hydroxylase: Increased Secretion Is Caused by a Post-transcriptional Mechanism**—The secretion of apoB100, apoB48, and albumin by McArdle and SLW-1 cells was compared by Western blotting of culture medium (Fig. 3A). The blots were scanned by densitometry, and the amount of apoB100 secreted by SLW-1 cells was increased by 2-fold, compared with wild-type McArdle cells (p < 0.05). There were no significant differences in the secretion of either apoB48 or albumin by the two different cell types. Since the expression of apoB mRNA was unaffected (Fig. 3B), the increased secretion of apoB100 was the result of a post-transcriptional event. Analysis of the single cell clones showed that the secretion of apoB100 varied as a linear function of 7α-hydroxylase protein (Fig. 3C).

**Expression of MTP and 7α-Hydroxylase mRNA Varies as a Linear Function**—The content of MTP mRNA was significantly increased in SLW-1 cells expressing 7α-hydroxylase as compared with wild-type McArdle cells. Using three individual plates of cells from each group, there was a 2.2-fold increase in the content of MTP mRNA relative to β-actin in SLW-1 cells compared with wild-type McArdle cells (Fig. 4A, p < 0.05). Moreover, the relative abundance of MTP mRNA varied in proportion to the relative abundance of 7α-hydroxylase mRNA (Fig. 4B). These data indicate that expression of 7α-hydroxylase in McArdle cells induces the expression of MTP mRNA.

**Expression of 7α-Hydroxylase in McArdle Cells Cooperatively Induces the Synthesis and Secretion of All VLDL Lipids and Decreases Cellular Cholesterol Content**—Compared with wild-type cells, in SLW-1 cells the synthesis of all VLDL lipids (cholesterol, cholesterol esters, triglycerides, and phospholipids) was significantly increased as determined by the incorporation of [14C]acetate (Fig. 4A). Since the cellular content of mature SREBP is sensitive to the sterol content of cells (15–18), we determined the cellular content of free and esterified cholesterol in McArdle and SLW-1 cells (Fig. 4B). In SLW-1 cells, expressing 7α-hydroxylase, the cellular content of both free and esterified cholesterol was 80 and 50% of the levels in McArdle cells (Fig. 4B). This experiment was repeated two times and similar results were obtained.

**Expression of HMG-CoA Reductase and Acetyl-CoA Carboxylase Varies as a Linear Function of 7α-Hydroxylase mRNA**—Recent reports indicate that several genes, whose products control both sterol and fatty acid synthesis, are targets for SREBP-induced transcription (15, 24, 30–33). The expression of the mRNA’s encoding HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, and acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid synthesis, was increased in SLW-1 cells compared with wild-type McArdle cells (Fig. 6A). In contrast, the relative abundance of SREBP1 mRNA was similar in both cell types. Moreover, the expression of HMG-CoA reductase (Fig. 6B) and acetyl-CoA carboxylase mRNA (Fig. 6C) varied as a linear relationship with the expression of 7α-hydroxylase mRNA. These data suggest that the expression of genes responsible for the biosynthesis of cholesterol and the fatty acid components in VLDL is coordinately influenced by the expression of 7α-hydroxylase in McArdle cells.
Expression of 7α-Hydroxylation in McArdle Cells Blocks Oleic Acid-stimulated ApoB Secretion—Oleic acid stimulates the synthesis of glycerolipids and increases the secretion of apoB100 when added to the culture medium of McArdle hepatoma cells (2). To examine if 7α-hydroxylation-mediated induction of glycerolipid synthesis (Fig. 5) "saturates" the lipid requirement for maximal secretion of apoB100, we examined the effect of oleic acid on the cellular content and secretion of apoB100 in wild-type and SLW-1 cells (Fig. 7). In McArdle and SLW-1 cells, oleic acid increased the cellular content of apoB100 by 2.5-fold (p < 0.01) and 1.5-fold (p < 0.05), respectively (Fig. 7A). Adding oleic acid to the serum-free BSA-containing medium increased the secretion of apoB100 by McArdle cells (Fig. 7B). The oleic acid stimulation of apoB100 secretion by McArdle cells was specific since it did not significantly affect the secretion of apoB48 or total protein (data not shown). These data confirm those of others showing that in McArdle hepatoma cells, oleic acid stimulates apoB100 secretion but not apoB48 secretion (34). Moreover, although oleic acid increased the secretion of apoB100 by wild-type McArdle cells, it had no effect on the secretion of apoB100 by wild-type McArdle cells, it had no effect on the secretion of apoB100 in wild-type SLW-1 cells expressing 7α-hydroxylation (Fig. 7B). Since oleic acid did significantly increase the cellular content of apoB100 in SLW-1 cells, albeit less than the increase observed in wild-type McArdle cells (Fig. 7A), the unaltered apoB100 secretion in SLW-1 cells cannot be due to impaired apoB100 synthesis. It appears that SLW-1 cells respond similarly to oleic acid as do primary cultured hepatocytes: increased content of cellular apoB but no change in secretion (35, 36).

Increased Secretion of ApoB100 in SLW-1 Cells Is Blocked by 25-Hydroxycholesterol—To examine the possibility that 7α-hydroxylation increases apoB100 secretion through a SREBP1-mediated process, McArdle and SLW-1 cells were cultured with and without 25-hydroxycholesterol (2 μg/ml) plus cholesterol (10 μg/ml) for 24 h. The proteolytic processing of transmembrane SREBP precursors to the mature transcriptionally active forms is inhibited by 25-hydroxycholesterol (17). Similar to its ability to decrease the cellular content of mature SREBP1 in SLW-1 cells (Fig. 2B), 25-hydroxycholesterol also decreased the secretion of apoB100 to levels that were similar to those of wild-type McArdle cells (Fig. 8). In the absence of 25-hydroxycholesterol, SLW-1 cells secreted 3.1-fold more apoB100 (p < 0.05) than McArdle cells. In the presence of 25-hydroxycholesterol, both cell types secreted similar amounts of apoB100. Although 25-hydroxycholesterol did not significantly affect the secretion of apoB100 in wild-type McArdle cells, it decreased the secretion of apoB100 in SLW-1 cells by 71% (p < 0.02). The secretion of apoB48 and albumin by both groups of cells was similar and unaffected by 25-hydroxycholesterol. This experiment was repeated twice and similar results were obtained. The combined data suggest that the augmented secretion of apoB100 displayed by McArdle cells expressing 7α-hydroxylation can be reversed to levels detected in wild-type McArdle cells by 25-hydroxycholesterol. The additional finding that 25-hydroxy-
cholesterol also restores the level of mature SREBP1 in SLW-1 cells to those of McArdle cells suggests that SREBP1 mediates the augmented secretion of apoB100 caused by expression of 7α-hydroxylase.

**DISCUSSION**

SREBP was first identified as the sterol-responsive transcription factor that mediates the regulation of human LDL receptor gene (15). Its rat homologue ADD1 was identified as a transcription factor involved in adipocyte differentiation (37), suggesting that it may play a diverse role in regulating the expression of genes involved in the biosynthesis and metabolism of several classes of lipids. Subsequent studies show that many of the genes encoding isoprenoid/cholesterol biosynthetic enzymes are SREBP-responsive: HMG-CoA synthase (17), HMG-CoA reductase (31), isopentenylfarnesyl diphosphate synthase (30), and squalene synthase (32). More recently, it has been reported that the genes for fatty acid synthase (33) and acetyl-CoA carboxylase (24) are both SREBP targets. The data derived from our studies now extend the metabolic regulatory importance of SREBP(s) to having a significant influence on controlling several steps of the VLDL assembly/secretion pathway.

Through the expression of 7α-hydroxylase in McArdle hepatoma cells, we were able to obtain single cell clones having a fairly large variation in the cellular content of mature SREBP1 that varied as a linear function of 7α-hydroxylase protein content (Fig. 2C). It is interesting to note that in vivo the cellular content of mature SREBP2 is increased in the livers of hamsters treated with bile acid sequestrants, which induce the expression of 7α-hydroxylase (38). The mechanism through which 7α-hydroxylase expression increases the cellular content of mature SREBP1 (this study) or SREBP2 in hamsters is not firmly established but may involve altering the signaling of SREBP cleavage by regulatory sterols (39).

The following data and interpretations support the conclusion that SREBP1 coordinately regulates the assembly and secretion of apoB-containing lipoproteins. Since 7α-hydroxylase expression via plasmid transfection is the manipulation we employed to obtain SLW-1 cells, changes in lipoprotein assembly and secretion are the resulting consequence of this liver-

**Fig. 4.** Expression of MTP mRNA correlated with 7α-hydroxylase mRNA. A, McArdle (McA) and SLW-1 cells were cultured in DMEM to 85% confluency. Poly(A)+ RNA was isolated and subjected to Northern blot analysis. Membranes were probed with 32P-nick-translated cDNAs encoding MTP and β-actin. B, poly(A)+ RNA was isolated from eight single cell clones obtained after the transfection of wild-type McArdle cells with the plasmid conferring neomycin resistance and expression of rat 7α-hydroxylase. Northern blots were probed with 32P-nick-translated cDNAs encoding MTP and 7α-hydroxylase. The Northern blot was quantitated for the amount of MTP mRNA relative to 7α-hydroxylase mRNA by PhosphorImaging. This relationship analyzed by linear least squares was calculated to be significant (p < 0.01).

**Fig. 5.** Lipid biosynthesis from [14C]Acetate and cellular cholesterol content. A, McArdle (McA) and SLW-1 cells were cultured to 85% confluency and incubated with [14C]acetate for 2 h. The lipids were extracted from the cells and separated on TLC plates. Free cholesterol, cholesterol ester (CE), triglyceride (TG), and phospholipids (PL) were scraped off and assayed for 14C radioactivity using a Beckman scintillation counter. Each value represents the mean ± S.D. of triplicate plates of cells. For each individual lipid class, SLW-1 cells showed greater levels of biosynthesis than McArdle cells (p < 0.01). B, cells cultured as described in A were harvested and extracted with chloroform/methanol, and the amount of free and esterified cholesterol was quantitated by gas/liquid chromatography. Each value represents the mean ± S.D. of triplicate plates of cells. SLW-1 cells contained significantly less free and esterified cholesterol (p < 0.01).
specific gene (23). These consequences include linear relationships between 7α-hydroxylase mRNA and the expression of mRNAs encoding the enzymes regulating fatty acid and sterol synthesis (acetyl-CoA carboxylase and HMG-CoA reductase) and MTP. As a result of the increased expression of these mRNAs and perhaps others (not studied), SLW-1 cells display increased synthesis of all lipid classes (cholesterol, cholesteryl esters, triglycerides, and phospholipids). These changes in lipid biosynthesis and MTP expression may be responsible for the increased secretion of apoB100. Moreover, as shown by the direct linear relationship between the cellular content of 7α-hydroxylase and mature SREBP1 (Fig. 3C), it is likely that the changes in the expression of genes regulating sterol and fatty acid biosynthesis and the assembly of apoB-containing lipoproteins (i.e. MTP) caused by 7α-hydroxylase expression are mediated through the variation of the cellular content of mature SREBP1. The additional findings that 1) expression of 7α-hydroxylase (SLW-1 cells) decreased the cellular content of both free and esterified cholesterol (Fig. 5B), and 2) 25-hydroxycholesterol concomitantly decreases the cellular content of mature SREBP1 in SLW-1 cells and decreases the secretion of apoB100 to the same levels exhibited by wild-type McArdle cells provide compelling evidence that the cellular content of mature SREBP1 is responsible for these changes. Based on this rationale, we propose that SREBP1 is a common regulatory mediator responsible for coordinate regulation of the lipoprotein assembly and secretion pathway in McArdle hepatoma cells. It is important to point out that in other cell types (e.g. liver in vivo (38)), SREBP2 may mediate changes in lipoprotein assembly and secretion. Recent studies in our lab show that in a differentiated hepatoma cell line found to express liver-specific genes not expressed by McArdle cells, SREBP2 induces the expression of the MTP gene. Thus, depending upon the cell type and physiologic conditions, SREBP1 or SREBP2 may act to mediate changes in lipoprotein assembly and secretion. This coordinate regulation provides a means for the efficient utilization of both lipids and apoB100 for transport from the liver to peripheral tissues.

It is now established that under most conditions, hepatic secretion of apoB is regulated post-transcriptionally by a process that determines the portion of de novo synthesized apoB that either enters the lipoprotein assembly/secretion pathway or is degraded in the endoplasmic reticulum (1, 2, 9). Studies using cultured hepatocytes and perfused livers of rats showed that the translocation of apoB across the endoplasmic reticulum is inefficient (40). One pool consisting of incompletely translocated apoB appeared to be diverted into the degradation pathway, whereas the pool of apoB that is completely translocated into the lumen can ultimately be secreted as a lipoprotein particle (40). Additional studies using human hepatoma HepG2 cells (41) and McArdle rat hepatoma cells (42) showed that the availability of glycerolipids (triglycerides and phospholipids) affects the efficiency of apoB translocation across the endoplasmic reticulum. It appears that when the availability of lipid is insufficient, translocation of de novo synthesized apoB is disrupted causing it to be degraded. The majority of the degradation of translocation-arrested apoB occurs co-translationally in HepG2 cells (43). While the availability of lipid is required for efficient translocation and utilization of apoB (9), the presence

2 J. K. Bonnardel and R. A. Davis, unpublished observations.
changed to serum-free medium for 24 h. Oleate (1 mM) conjugated with apoB100 secretion (

There was no difference in the amount of [35S]Met-labeled trichloroacetic acid-precipitated and quantitated by liquid scintillation using a PhosphorImager. The total amount of radiolabeled protein was the mean ± S.D. *Significant difference between McA cells incubated with and without oleic acid (p < 0.01). **Significant difference between SLW-1 cells incubated with and without oleic acid (p < 0.001). *Significant difference between SLW-1 cells incubated with and without oleic acid (p < 0.01).

and activity of MTP is essential. In Chinese hamster ovary cells, which do not normally express MTP, essentially all of the apoB synthesized from a stably expressed plasmid is translocation arrested and rapidly degraded by a ALLN-inhibitable process (26, 28). In addition, blocking the functional activity of MTP also blocks the secretion of apoB by hepatoma cells, which do express MTP (44). Our results showing that the increased secretion of apoB100 correlated with MTP and mature SREBP1, but was not associated with any detectable change in apoB mRNA (Fig. 3B), suggest that a post-transcriptional mechanism is responsible. The additional finding that oleic acid increases the secretion of apoB100 by wild-type McArdle hepatoma cells, but has no effect in McArdle cells expressing 7α-hydroxylase (Fig. 7), supports the proposal that SREBP1 induction of genes regulating lipogenesis saturates the requirement for lipids in the lipoprotein assembly pathway of SLW-1 cells. Our findings that in SLW-1 cells oleic acid increased the cellular content of apoB100, but not its secretion, suggest that the intracellular degradation was blocked. The inability of oleic acid to increase the secretion of apoB100 in SLW-1 cells suggests that one or more of the processes required to assemble and secrete apoB100 lipoproteins other than lipid availability is limiting. Based on these data it is tempting to speculate that in McArdle cells, when the availability of lipids is in excess of what is required for lipoprotein assembly, MTP becomes rate-limiting for apoB100 translocation and the subsequent assembly and secretion of apoB100-containing lipoproteins.

Our results may explain why oleic acid stimulation of lipogenesis is not associated with increased apoB secretion in primary rat hepatocytes (35, 36), whereas oleic acid increases the secretion of apoB by hepatoma cells (HepG2 (45–47) and McArdle (34, 48, 49). In hepatoma cells, glycerolipid biosynthesis may be limiting for apoB secretion, whereas in primary hepatocytes other processes may be limiting.

There are other examples in which limitations in the availability of lipids in addition to triglycerides restrict the ability of the liver cell to assemble and secrete apoB-containing lipoproteins. Decreased production of cholesterol and cholesterol esters caused by drugs that competitively inhibit HMG-CoA reductase and/or ACAT also decrease the assembly and secretion of apoB-containing lipoproteins in some (50, 51) but not all (52) studies. Decreased production of phosphatidylcholine (via choline deficiency (53) and altered head-group precursors (54)) also blocks the secretion of apoB-containing lipoproteins. It seems reasonable to propose that a deficiency in the availability of one or more of the lipid components required for the intracellular processing of apoB and assembly of the lipoprotein particle can impair the overall pathway. Optimal utilization of lipids and apoB for lipoprotein assembly requires that each essential protein and lipid component be in sufficient supply relative to the others to assemble the lipoprotein particle.

Coordinate changes of the individual processes required for VLDL assembly and secretion are invoked in response to physiologic state (reviewed in Ref. 55). For example, carbohydrate “overload” leads to a coordinate increase in the synthesis of all lipoprotein lipids and an amplification of all of the processes required to increase the secretion of apoB-containing lipoproteins (10). Conversely, fasting leads to the opposite coordinated responses: decreased apoB translocation, increased apoB degradation, decreased lipid biosynthesis, and decreased lipoprotein secretion (12, 13). Fetal nutritional development also shows a coordinated change in apoB processing, lipogenesis, and lipoprotein secretion (11). These findings have led us to propose that one or more metabolic signals is responsible for the coordinate regulation of lipid biosynthesis and the intracellular processing of apoB.

Our present data show that expression of 7α-hydroxylase in McArdle cells alters the cellular content of mature SREBP1 in a manner that coordinately changes the availability of lipids and the processes required to efficiently assemble apoB-containing lipoproteins. We also observed that in McArdle cells expressing 7α-hydroxylase, 25-hydroxycholesterol decreases both the cellular content of mature SREBP1 and the secretion of apoB100. Our findings may explain the well-established observation that in patients treated with bile acid sequestrants, known to increase the expression of 7α-hydroxylase, there is a concomitant increase in the secretion of VLDL triacylglycerides (56, 57). Furthermore, in some patients, there appears to be a direct correlation between the rate of bile acid synthesis, a parameter linked to 7α-hydroxylase activity, and VLDL-triaclyceride secretion (56, 57). The combined data suggest that changes in cholesterol metabolism may alter VLDL assembly and secretion by 1) affecting the amount of cholesterol and cholesterol esters that are available for VLDL assembly and 2) by altering the cellular content of mature SREBP. It
is through the second parameter (cellular content of mature SREBP) that coordinate response of the gene products controlling the availability of glycerol lipids (i.e. triglycerides and phospholipids) and MTP is linked to the first parameter (cholesterol metabolism). Optimal production of VLDL in mammals may require coordinate induction of all of the gene products necessary for the synthesis of individual lipids and for their assembly with apoB (e.g. MTP). This may be comparable to other species (e.g. birds) in which VLDL secretion by the liver is optimized by a coordinate regulation of gene expression by estrogen (58–61). However, it is important to emphasize that mammalian VLDL production may not be optimal under all conditions. Variation in physiologic conditions may determine which of the many processes are rate-limiting for VLDL assembly and/or secretion.

Acknowledgments—We gratefully acknowledge the following people who provided reagents for these studies: Drs. Timothy Osborne (antibody to SREBP1 and cDNA for acetyl-CoA carboxylase), Mike Briggs (antibody to SREBP2), Mike Davis, Paul Buzeo, and their colleagues at Bristol-Myers Squibb for the MTP cDNA. We thank Drs. Huda Shubeita and John Trawick for help in preparing the plasmid used for newly constructed expression plasmid), Aldons Lusis (antibody to SREBP2), David Russell (original cDNA for rat 7 alpha-hydroxy-}

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