methods

Huh-7 or HepG2 cells: which is the better model for studying human apolipoprotein-B100 assembly and secretion?[S]

Steven J. R. Meex,*,† Ursula Andreo,* Janet D. Sparks,§ and Edward A. Fisher[1],*

Departments of Medicine (Leon H. Charney Division of Cardiology) and Cell Biology and the Marc and Ruti Bell Vascular Biology and Disease Program,* New York University School of Medicine, New York, NY 10016; Department of General Internal Medicine and the Cardiovascular Research Institute,† Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands; and Department of Pathology and Laboratory Medicine,§ University of Rochester School of Medicine, Rochester, NY 14642

Abstract Apolipoprotein-B100 (apoB100) is the essential protein for the assembly and secretion of very low density lipoproteins (VLDL) from liver. The hepatoma HepG2 cell line has been the cell line of choice for the study of synthesis and secretion of human apoB-100. Despite the general use of HepG2 cells to study apoB100 metabolism, they secrete relatively dense, lipid-poor particles compared with VLDL secreted in vivo. Recently, Huh-7 cells were adopted as an alternative model to HepG2 cells, with the implicit assumption that Huh-7 cells were superior in some respects of lipoprotein metabolism, including VLDL secretion. In this study we addressed the hypothesis that the spectrum of apoB100 lipoprotein particles secreted by Huh-7 cells more closely resembles the native state in human liver. We find that Huh-7 cells resemble HepG2 cells in the effects of exogenous lipids, microsomal triglyceride transfer protein (MTP)-inhibition, and proteasome inhibitors of apoB100 secretion, recovery, and degradation. In contrast to HepG2 cells, however, MEK-ERK inhibition does not correct the defect in VLDL secretion. Huh-7 cells do not appear to offer any advantages over HepG2 cells as a general model of human apoB100-lipoprotein metabolism.—Meex, S. J. R., U. Andreo, J. D. Sparks, and E. A. Fisher. **Huh-7 or HepG2 cells: which is the better model for studying human apolipoprotein-B100 assembly and secretion?** ***J. Lipid Res.*** **2011.** 52: **152–158.**

Supplementary key words very low density lipoproteins • Huh-7 cells • HepG2 cells

Apolipoprotein-B100 (apoB100) is the essential protein for the assembly and secretion of very low density lipoproteins (VLDL) from liver. From a clinical perspective, plasma apoB100 levels and the apoB100/apoA1 ratio are superior to any other lipoprotein-related indices used to estimate risk of acute myocardial infarction (1). This illustrates the need for a deep understanding of the cellular mechanisms that regulate apoB100 production and secretion. Although rat hepatoma McA-RH7777 cells secrete much of their apoB100 as buoyant VLDL, it would be desirable to have a similar cell line of human origin. Many studies of human apoB100 metabolism have used the hepatoma HepG2 cell line. Despite their general use, however, HepG2 cells secrete relatively dense, lipid-poor apoB100-containing particles, unlike the buoyant VLDL particles secreted in vivo by mammalian liver. An alternative human cell model with a more native level of VLDL secretion would strongly benefit the lipoprotein field and might advance novel insights into apoB100 metabolism.

Recently, Huh-7 cells were proposed as a superior human hepatic cell model for the study of apoB100 metabolism and VLDL secretion [see references 2, 3)] and are becoming more widely used for these purposes [see references (4, 5)]. To our knowledge, however, there have been no published studies of the basic characteristics of Huh-7 cells with regard to apoB100 and VLDL metabolism. In the present report, we have filled this gap in knowledge and also have compared the results to those with HepG2 cells. Based on the available evidence, Huh-7 cells resemble HepG2 cells in many respects, with neither cell

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Abbreviations: apoB100, apolipoprotein-B100; MEK-ERK, mitogen-activated protein kinase kinase-extracellular signal regulated kinase; MTP, microsomal triglyceride transfer protein; OA, oleic acid.

[1]To whom correspondence should be addressed.
email: edward.fisher@nyumc.org

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of two figures.

line having obvious superiority as the model of normal human liver apoB100 and VLDL metabolism.

MATERIALS AND METHODS

Reagents

Dimethyl sulfoxide (DMSO) and the proteasomal inhibitor MG132 (Z-Leu-Leu-Leu-al) were obtained from Sigma (St. Louis, MO). Complete Protease Inhibitor Cocktail Tablets were obtained from Roche (catalog no. 1836153; Indianapolis, IN). Protein A Sepharose was obtained from GE Healthcare (Uppsala, Sweden). The [^{35}S]methionine/cysteine protein labeling mixture was obtained from Perkin-Elmer Life Sciences (Waltham, MA). Goat anti-human apoB100 polyclonal antibody was from Calbiochem (catalog no. 178467). Goat anti-human albumin antibody was obtained from Midland Bioproducts Corp. (catalog no. 71907). The microsomal triglyceride transfer protein (MTP) inhibitor was provided by BristolMyers-Squibb (designated compound no. 9 in the study by Wetterau et al.) (6). The mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK-ERK) inhibitor PD98059 was purchased from Calbiochem (San Diego, CA).

Cell culture

HepG2 cells were obtained from American Type Culture Collection (Manassas, VA). Huh-7 cells were a kind gift from Dr. Z. Yao (University of Ottawa, ON, Canada). HepG2 and Huh-7 cells were maintained in Dulbecco's modification of Eagle's medium (DMEM; Cellgro, Manassas, VA) containing 1% L-glutamine, 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin in 5% Co_2 at 37°C. The medium was changed every 3 days.

Density gradient separation of apoB100-containing lipoproteins

HepG2 and Huh-7 cells were grown on 100-mm tissue culture dishes and preincubated for 1 h in low-serum medium (1% fetal bovine serum, 1% L-glutamine). Cells were labeled with 150–200 μCi of sulfur-35 protein labeling mixture/ml of medium for 3 h. To promote lipid loading of apoB100-containing lipoproteins, cells were incubated with oleic acid (OA) complexed to BSA (0.6 mM OA; OA/BSA molar ratio 5:1) or incubated with BSA as a control during the metabolic labeling period. To examine the effect of MEK-ERK inhibition on VLDL assembly, cells were preincubated overnight with 5 μM PD98059 inhibitor in DMSO. The same concentration of PD98059 was present in the medium during the 3-h labeling period. Equal volumes from each dish of conditioned medium were harvested, and 0.5 ml of human plasma (from outdated plasma obtained from the Tisch Hospital Blood Bank) was added as a carrier. A total of 4 ml of the sample was then adjusted to a density (d) of 1.2 g/l with KBr and loaded onto the bottom of a Beckman model SW41 centrifuge tube. The sample was overlaid with 2.5 ml KBr at d = 1.065, 2.5 ml of KBr at d = 1.02, and 2.5 ml of KBr at d = 1.006. All solutions contained 2 mM EDTA. After ultracentrifugation (20 h, 15°C, 173,000 *g*), lipoproteins were collected from top to bottom in 11 fractions. ^{35}S-apoB100 in each fraction was immunoprecipitated, as described below.

Pulse-chase experiments

Pulse-chase studies of HepG2 and Huh-7 cells were performed to investigate the effects of various metabolic perturbations on the synthesis and degradation of apoB100. The time required to reach maximal incorporation of radioactive isotope in apoB100 protein varies from 15 min in rat hepatoma McA-RH7777cells to 20 min in primary rat hepatocytes and 30 min in primary mouse hepatocytes (7). In order to accurately determine the peak incorporation of radioactive isotope in apoB100 in HepG2 and Huh-7 cell lines, we varied our initial chase point from 5 to 30 min, with sampling at 5-min intervals (supplemental Fig. SI). The peak amount of radiolabeled apoB100 in both types of cells was recovered at 10–15 min. Accordingly, we chose 13 min as our standard initial chase point for all pulse-chase experiments with HepG2 and Huh-7 cells.

For subsequent experiments in this study, cells were preincubated for 1 h (37°C, 5% Co_2) in low-serum DMEM (1% fetal bovine serum, 1% L-glutamine), washed twice with ice-cold PBS, and then labeled for 15 min with methionine/cysteine-free DMEM (1% fetal bovine serum, 1% L-glutamine) supplemented with ~300 μCi of ^{35}S protein labeling mixture/ml of medium at 37°C, under 5% Co_2. After the labeling period, the medium was removed, and cells were washed twice with ice-cold PBS. Cells were subsequently incubated with chase medium (Met/Cys-free DMEM, 1% fetal bovine serum, 1% L-glutamine) supplemented with an excess amount of unlabeled methionine (1.5 mg/ml) and cysteine (0.5 mg/ml). The durations of the chase periods are shown in the appropriate figure legends. When the OA stimulation of lipid synthesis and lipoprotein lipid loading were assessed, 0.6 mM OA complexed to BSA (molar ratio, 5:1) was provided throughout the course of the experiment. In some experiments, 25 μM MG132 (Sigma) and 10 nM of an MTP inhibitor (provided by Bristol-Myers-Squibb; designated compound no. 9 in the study by Wetterau et al. (6)) were present throughout the course of the experiment as indicated in Results and shown in appropriate figures.

Immunoprecipitation and quantification of labeled apoB100

At the end of the chase period, medium samples were collected, supplemented with fresh PMSF (1 mM), and centrifuged at 10,000 rpm for 5 min in a table-top centrifuge to remove debris. Cells were washed twice with ice-cold PBS and lysed in cell lysis buffer (10 mM PBS, pH 7.4, 125 mM NaCl, 36 mM lithium dodecyl sulfate, 24 mM deoxycholate, and 1% Triton X-100) freshly supplemented with protease inhibitor cocktail (commercially available from Roche) and 1 mM PMSF. Lysed cells, still in their original 6-well plates, were gently shaken on ice for 30 min, then pulled 7–10 times through a 25-G needle, transferred to an Eppendorf tube, and centrifuged at 10,000 rpm for 5 min in a table-top centrifuge. To immunoprecipitate ^{35}S-apoB100, cell lysate or conditioned medium was mixed with NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris [pH7.4], 1% Triton X-100, and 0.1% SDS), 5 μl of anti-apoB serum, and protein A Sepharose. A 5× NET buffer was mixed with cell lysate or conditioned medium to reach a final concentration of 1× NET buffer in the immunoprecipitation mixture.

The mixture was incubated overnight with shaking at 4°C. The next morning, the beads were washed three times with NET buffer, and proteins were released with sample buffer (0.125M Tris HCl, pH 6.8, 4%SDS, 6 M urea, 1 mM EDTA, 10 mM DTT, 25 mM β-mercaptoethanol) by heating the samples to 95°C for 5 min. Quantification of labeled apoB100 was performed by SDS-PAGE, fluorography, and densitometry. Total protein synthesis was measured by determination of trichloroacetic acid precipitable radioactivity in aliquots of cell lysates and conditioned medium. Quantitative results are displayed as means ± SEM. For comparisons, two-tailed Student's *t*-tests were used.

Relative secretion of ApoB100 mass by HepG2 and Huh-7 cells

ELISA assays were performed to measure the mass amounts of apoB100 secreted by HepG2 and Huh-7 cells. Cells were plated in

15-cm dishes. At the time of the experiment (80% confluency), cells were washed twice with PBS and incubated for 3 h in 15 ml of DMEM and 1% fetal bovine serum. At the end of the experiment, apoB100 content in the conditioned medium was determined using an ELISA kit from ALerCHECK, Inc. (Portland, ME). The content of apoB100 was normalized to the protein content in the cell lysate determined by Lowry assay.

RESULTS

ApoB100 secreted from HepG2 and Huh-7 is predominantly in the LDL range

We first investigated the hypothesis that the buoyant density of apoB100-containing lipoprotein particles secreted by Huh-7 cells more closely resembles that of VLDL particles secreted by human liver in vivo, in contrast to the LDL-like particles secreted by HepG2 cells. For this purpose, HepG2 and Huh-7 cells were isotopically labeled with [^{35}S]methionine/cysteine for 3 h in the presence or absence of OA, complexed to BSA. Equal volumes of conditioned medium were then subjected to density gradient ultracentrifugation, and apoB100 was recovered from each fraction **(Fig. 1)**. Under basal conditions, HepG2 cells secreted 70% of their apoB100 as lipoproteins with density of ⩽1.06 g/ml (LDL-sized), compared with 95% secreted by Huh-7 cells. The amount of apoB100 secreted as VLDL-sized particles (⩽1.006 g/ml) was insignificant (<5%) in both HepG2 and Huh-7 cells under basal conditions. Upon lipid loading with OA (3 h, 0.6 mM), both HepG2 and Huh-7 cells increased their apoB100 secretion by more than 100%. ApoB100 in the two lightest fractions (VLDL and intermediate density lipoprotein [IDL]-sized particles) increased from 4% to 27% upon lipid loading in HepG2 cells but only from 0% to 9% in Huh-7 cells. Thus, lipid loading induced a greater density shift of secreted particles in HepG2 cells than in Huh-7.

Despite the generally darker apoB100 bands in the density fractions from the conditioned medium of HepG2 cells, when normalized to cell protein, Huh-7 cells actually secrete more apoB100 mass (0.59 ± 0.11 ng/μg cell protein/h) than HepG2 cells (0.27 ± 0.03 ng/μg cell protein/h). Note that the band intensities reflect the content of radiolabeled apoB100 in equal volumes of medium and are not corrected for differences in cell protein or number, as the mass data are.

An additional comparison between the two cell types was the density distribution in conditioned medium samples of apoE, which can associate with lipoproteins of all densities. The apoE secretion patterns were identical in HepG2 and Huh-7, with the highest apoE levels in the dense fractions (**Fig. 2**).

Proteasomal degradation of apoB100 in HepG2 and Huh-7 cells

Secretion of apoB100 is regulated primarily at the level of degradation (8, 9). In HepG2 cells the ubiquitin proteasome pathway has been firmly established in the degradation of apoB100 (9). Under conditions of relative lipid insufficiency the "nascent" apoB100 molecule is cotranslationally ubiquitinylated and targeted to the proteasome for degradation (10). In contrast, administration of an inhibitor of the proteasomal degradation pathway increases apoB100 recovery from many transformed cells, whereas exogenous supply of OA strongly stimulates apoB100 secretion (as reviewed in reference 8). We wished to directly compare the effects of OA and a proteasomal inhibitor on apoB100 secretion and degradation in HepG2 and Huh-7 cells.

The pulse-chase experiments depicted in **Fig. 3** show that under conditions of relative lipid insufficiency, most apoB100 (65%–85%) is degraded in both HepG2 and Huh-7 cells (Fig. 3, lanes 1 and 2; compare the amount of apoB100 in the cell at 13 min chase with the amount of apoB100 in cell plus medium at 180 min chase). Secretion efficiency, i.e., the percentage of apoB100 that is secreted after 3 h of chase compared with the peak amount of apoB100 recovered from the cell lysate, is only about 10% in both HepG2 and Huh-7 cells under standard culture conditions. (Fig. 3, lanes 1 and 2; compare the amount of apoB100 at 13 min of chase with the amount of apoB100 in the medium at 180 min of chase).

The degree of proteasomal degradation is strongly regulated by the availability of lipid-ligands for apoB100 (11). Consistent with previous reports (12), we found that OA stimulated the apparent net synthesis (∼30%, $P = 0.1$) and secretion (∼400%, $P < 0.05$) of apoB100 and rescued twice as much apoB100 from degradation ($P < 0.05$) (Fig. 3 left panel; compare lanes 3 and 4 to lanes 1 and 2). A similar effect was seen in Huh-7 cells (Fig. 3, right panel; compare lanes 3 and 4 to lanes 1and 2). The proteasome inhibitor MG132 increased apparent net apoB100 synthesis 2–3-fold ($P < 0.05$), as well as the recovery from media and cell lysates in HepG2 ($P < 0.05$) and Huh-7 cells ($P = 0.16$), consistent with the presence of proteasomal degradation in both cell lines (Fig. 3; compare lanes 9–12 to lanes 1–4). For the increases in apparent net synthesis, these data are consistent with decreased cotranslational proteasomal degradation (reviewed in reference 9).

Another way to promote apoB100 degradation, independent of the level of lipid synthesis, is to prevent the transfer of lipid-ligands to the nascent apoB100 polypeptide by pharmacological inhibition of MTP (13–15) Accordingly, using both HepG2 and Huh-7 cells, we tested the effects of a specific MTP inhibitor on apoB100 secretion and intracellular apoB100 degradation and the extent by which apoB100 degradation could be prevented when cells were cotreated with an inhibitor of the proteasome. To this end, we first determined which concentration of MTP inhibitor efficiently abolished apoB100 secretion in HepG2 and Huh-7 cells. In both cell types, 10 nM Bristol-Meyers Squibb compound no. 9 completely prevented secretion of apoB100 in the medium after 3 h of chase (supplemental Fig. II).

As expected, inhibition of MTP tended to decrease apparent net apoB100 synthesis (∼65%, $P < 0.05$ in HepG2; ∼30% in Huh-7 [$P = 0.06$]), most likely through increased proteasomal cotranslational degradation (10), virtually abolished apoB100 secretion, and increased apoB100

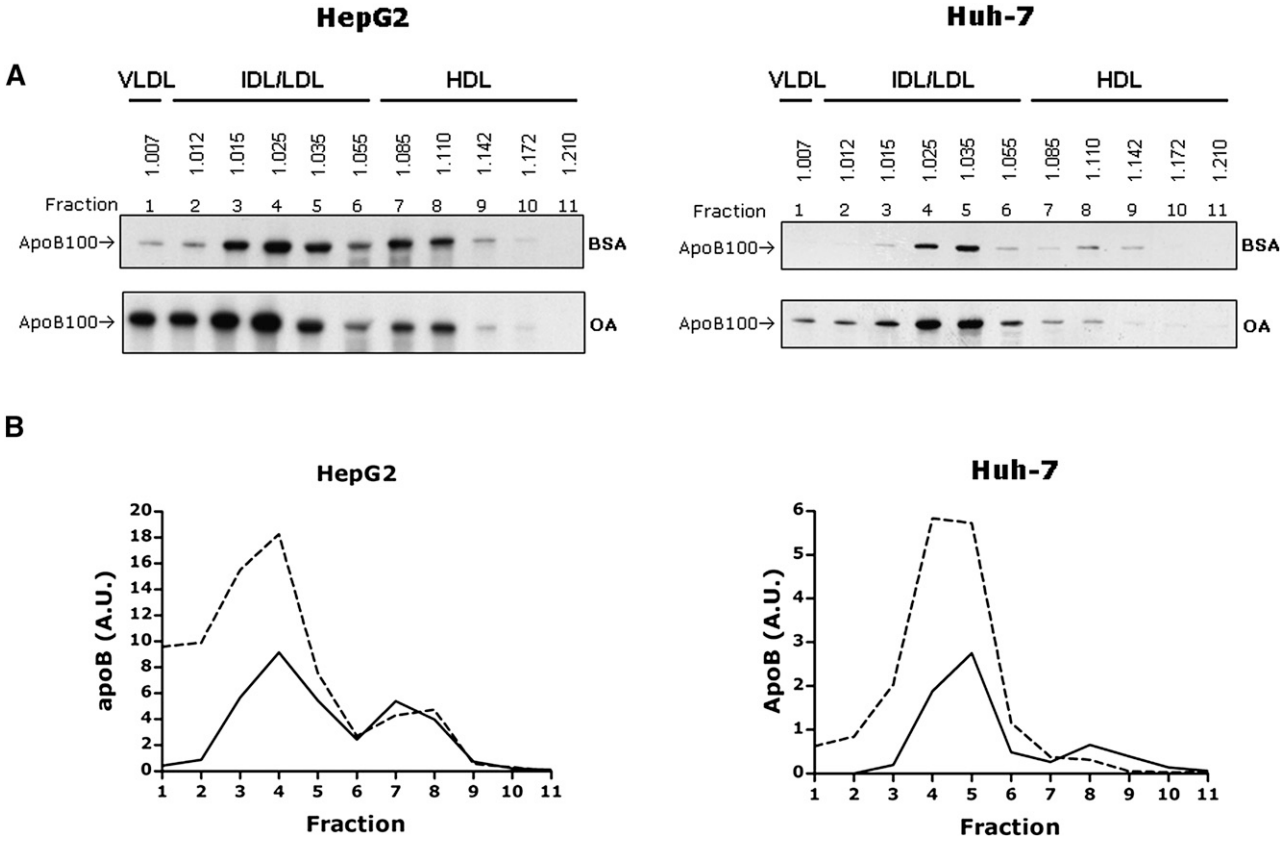


Fig. 1. Density distribution of apoB100 secreted by HepG2 cells and Huh-7 cells. HepG2 cells and Huh-7 cells were metabolically labeled for 3 h with [^{35}S]methionine/cysteine in the presence of BSA (solid line) or BSA-OA (dotted line). Conditioned medium samples were subjected to density gradient centrifugation. A: ApoB100 was immunoprecipitated from each fraction, separated by SDS-PAGE, and detected by fluorography. B: Densitometric quantification and graphic representation of apoB100 in each fraction (means ± SEM). Labels at the top indicate the fraction number, the corresponding measured density of each fraction (g/ml), and the expected distributions of the indicated lipoproteins.

intracellular degradation (up to 90%–95% in HepG2 and ~75% in Huh-7 cells), both in the presence and absence of OA (Fig. 3, lanes 5–8). Simultaneous administration of proteasomal inhibitor MG132 strongly increased the apparent net synthesis of apoB100 (4–7-fold in HepG2, and 3–4-fold in Huh-7, $P < 0.05$) and doubled the amount of apoB100 that could be recovered from media and cells after 3 h of chase ($P < 0.05$) (Fig. 3, lanes 12–16), again

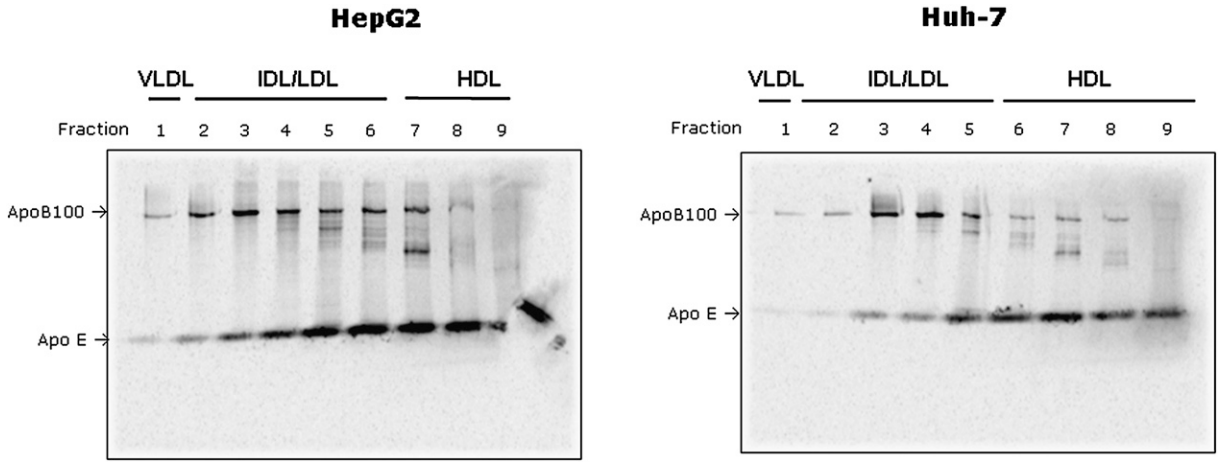
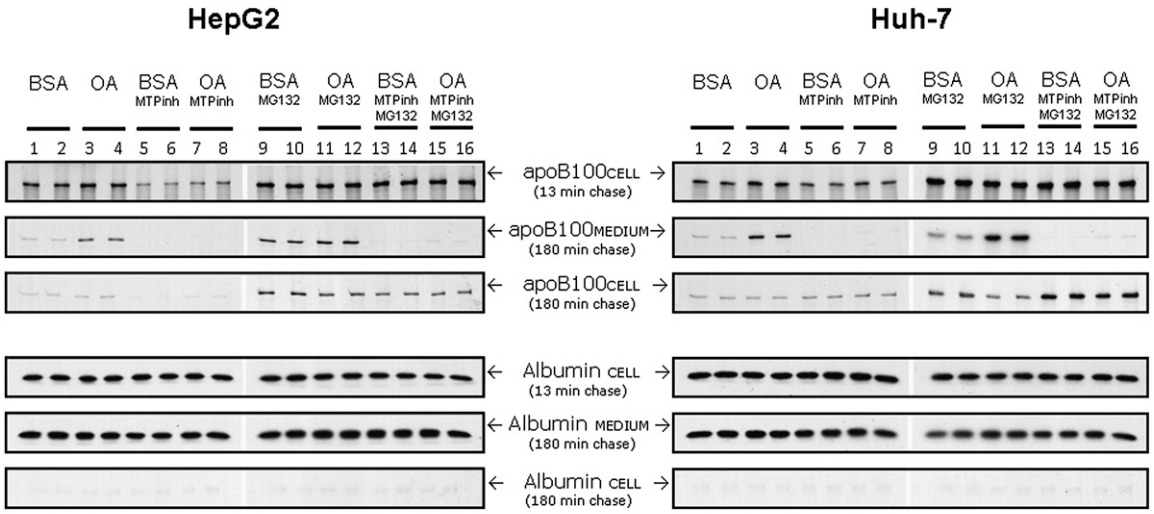


Fig. 2. Density distribution of apoE secreted by Huh-7 cells and HepG2 cells. HepG2 cells and Huh-7 cells were metabolically labeled for 3 h with [^{35}S]methionine/cysteine. Conditioned medium samples were subjected to density gradient centrifugation. The centrifuged fractions were directly separated by SDS-PAGE. ApoB100 and apoE bands are indicated on the resulting fluorograms. Labels at the top indicate the fraction number.



HepG2	Secretion efficiency (% ± SD)	Recovery (% ± SD)
BSA	7±1	16±2
OA	30±1	37±1
BSA + MTPinh.	0±0	5±1
OA + MTPinh.	0±0	11±2
BSA + MG132	28±1	58±2
OA + MG132	46±3	69±3
BSA + MTPinh. + MG132	0±0	17±1
OA + MTPinh. + MG132	1±0	19±2

Huh-7	Secretion efficiency (% ± SD)	Recovery (% ± SD)
BSA	15±2	35±4
OA	63±7	74±8
BSA + MTPinh.	0±0	26±0
OA + MTPinh.	0±0	26±4
BSA + MG132	19±1	43±4
OA + MG132	71±4	85±7
BSA + MTPinh. + MG132	0±0	53±4
OA + MTPinh. + MG132	1±0	62±9

Fig. 3. Effects of proteasome and MTP inhibition on the secretion and recovery of apoB100 in HepG2- and Huh-7-cells. Cells were preincubated for 60 min and then pulse labeled with [^{35}S]methionine/cysteine for 15 min, followed by a 3-h chase. At the beginning and end of the chase, cells and conditioned media samples were subjected to anti-apoB immunoprecipitation and SDS-PAGE and detected by fluorography. The indicated compounds were present throughout the course of the experiment. All bands were densitometrically quantified to calculate apoB100 secretion efficiency (means ± SD) and apoB100 recovery (means ± SD). Samples in lanes 1–8 and lanes 9–16 were run on separate gels for practical reasons, but all samples derive from the same experiment, and can be directly compared. "Secretion efficiency" is calculated as the percentage of apoB100 secreted in the medium after 3 h of chase compared with the peak amount of apoB100 in the cell lysate, i.e., at 13 min of chase. "Recovery" is defined as the percentage of apoB100 at the end of the chase in medium and cell lysate combined, relative to the peak amount of apoB100 in the cell lysate, i.e., at 13 min of chase. MTP inhibitor, 10nM.

confirming the participation of the proteasome in the degradation of apoB100, both co- and posttranslationally (10,16).

To ensure that the observed effects of OA, proteasomal inhibition, or MTP inhibition were specific, we also immunoprecipitated albumin as a control secretory protein from the same samples. None of the above interventions affected secretion, degradation, or recovery of albumin from HepG2 or Huh-7 cells (Fig. 3).

MEK-ERK inhibition corrects the defect in VLDL secretion in HepG2- but not in Huh-7-cells

Hyperactivity of the MEK-ERK signaling pathway was previously identified as a contributing factor to defective VLDL secretion in HepG2 cells (17). We investigated whether this effect of MEK-ERK inhibition is restricted to HepG2 or is applicable to Huh-7 cells as well. As shown in **Fig. 4**, PD98059 treatment induced a pronounced increase in VLDL secretion in HepG2 cells . Under identical experimental conditions, this shift was not observed in Huh-7 cells.

DISCUSSION

It is known that the standard cell model for the study of human apoB100-lipoprotein metabolism, HepG2, secretes predominately LDL and higher density apoB100-containing particles, unlike normal human liver, which secretes apoB100 associated mainly with VLDL particles. In this study, we addressed the hypothesis that the density of apoB100 lipoprotein particles secreted by Huh-7 cells more closely resembles that of VLDL. The study was motivated by the increasing use of this cell line as an alternative model to HepG2 cells (2–5, 18), with the implicit assumption that Huh-7 are more native in their characteristics.

Unlike most secretory proteins, apoB100 levels are regulated primarily by degradation (8).The characteristics of apoB100 degradation have been comprehensively studied in HepG2 cells. Previous studies (15, 19) and our present data show that HepG2 cells strongly depend on exogenous fatty acids to maintain lipid synthesis and availability for lipoprotein assembly/secretion. Under relative lipid insufficiency, the majority of newly synthesized apoB100 is

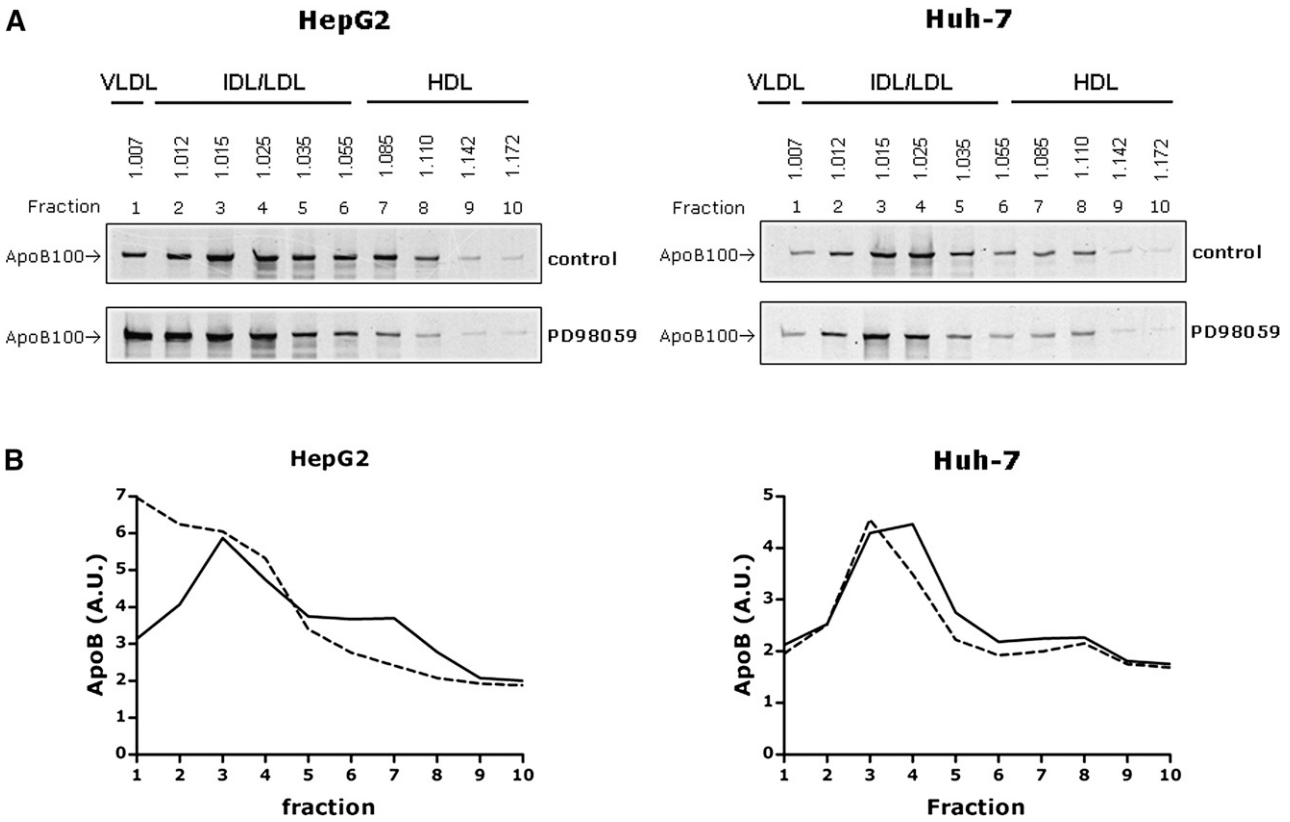


Fig. 4. Effect of MEK-ERK inhibition on the density profile of secreted apoB100 containing lipoproteins. HepG2 cells and Huh-7 cells were pretreated with 5 μmol/l PD98059 and metabolically labeled to steady-state with [^{35}S]methionine/cysteine in the presence of PD98059 dissolved in DMSO or control (DMSO). Conditioned medium samples were subjected to density gradient centrifugation. A: ApoB100 was immunoprecipitated from each fraction, separated by SDS-PAGE, and detected by fluorography. B: Densitometric quantification and graphic representation of apoB100 in each fraction (means ± SEM). Labels at the top indicate the fraction number, the corresponding measured density of each fraction (g/ml), and the expected distributions of the indicated lipoproteins. PD98059, dotted line; DMSO control, solid line.

co- and posttranslationally targeted for ubiquitinylation and degradation by the proteasome (10). OA rescues part of the newly synthesized apoB100 from proteasomal degradation and allows it to be secreted. A major shortcoming of HepG2 cells as a model of human apoB100 metabolism is their limited ability to fully lipidate apoB100 and secrete VLDL-sized particles. Consistent with this is our finding that using density gradient ultracentrifugation, in the absence of exogenous lipids, most of the secreted apoB100 had the density of LDL, and only ~1% of secreted apoB100 was fully lipidated to mature VLDL. Lipid loading increased the apoB100 in the VLDL fraction to ~13%. A similar increase from 1% to 13% was observed in the IDL-sized fraction.

Huh-7 cells also secreted almost all apoB100 (~80%) as LDL density particles under basal conditions. OA, again, strongly promoted apoB100 secretion, but unlike in HepG2 cells, it did not induce a significant density shift of secreted particles in Huh-7 cells: only ~3%–4% of the apoB100 was fully lipidated and secreted as mature VLDL and another 5% as IDL. Hence, the efficiency of lipidation of apoB100 is lower in Huh-7 cells than in HepG2 cells

We initially thought that Huh-7 cells secreted less apoB100 than HepG2 cells did. This was based on Fig. 2, which shows the recoveries of radiolabeled apoB100 from equal volumes of conditioned medium samples taken from cultures of both cell types. As shown, there were darker gel bands of apoB100 in the HepG2 samples. In contrast, the mass data (normalized to cell protein) showed less apoB100 secretion from HepG2 cells. Based on TCA precipitable radioactivity, HepG2 protein synthesis was lower than that in Huh-7 cells, consistent with apoB100 mass data. Typically, however, fewer cells were found in Huh-7 culture wells, so that by taking equal volumes of conditioned media samples for analysis, the true relationship between the two cell types in the production of radiolabeled apoB100 was obscured; although HepG2 cells have a lower production of apoB100 on a per cell basis, the greater number of HepG2 cells resulted in the secretion of a relatively larger amount of radiolabeled apoB100 per culture well.

Lipid insufficiency or the prevention of transfer of "lipid-cargo" to the nascent apoB100 molecule in all hepatic cells studied to date (20) causes degradation of the majority of apoB100, which could be partially reversed by cotreatment with an inhibitor of the proteasome. The ubiquitin-proteasome pathway was previously identified as a dominant cellular degradation process for apoB100 during and after its translation (10, 12, 13, 16, 21). These observations were confirmed in the present study for HepG2 cells and further extended to Huh-7 cells. In particular, there

were apparent increases in apoB100 synthesis when MG132 was present during the pulse-labeling period, which we have previously shown to be from reduced cotranslational degradation (10, 13), as well as an overall increase in apoB100 at the end of the chase periods.

Recently, hyperactive MEK-ERK signaling was found to contribute to defective VLDL secretion in HepG2 cells. Consistent with that original report (17), we show that pharmaceutical inhibition of MEK shifts the distribution of secreted apoB-lipoproteins to lower-density particles, with the most pronounced effect on VLDL. No effect was seen on the lipoprotein secretion pattern in HUH-7 cells, suggesting a distinct fundamental difference in signaling pathways relevant to VLDL formation or secretion between the two cell lines.

In summary, we find that Huh-7 cells do not appear to offer any advantages over HepG2 cells as a general model of human apoB100-lipoprotein metabolism. In fact, VLDL secretion was, in general, better supported in HepG2 cells.

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