Phosphatidylcholine biosynthesis is required for secretion of truncated apolipoprotein Bs from McArdle RH7777 cells only when a neutral lipid core is formed

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Abstract Decreased phosphatidylcholine biosynthesis inhibits the secretion of very low density lipoproteins from hepatocytes (Yao, Z. and D. E. Vance. 1988. *J. Biol. Chem.* **263:** 2998–3004). We have now investigated whether or not phosphatidylcholine biosynthesis is required for secretion of carboxyl-truncated apoBs 15, 18, 23, 28, and 37 from transfected McArdle RH7777 cells. Transfected cells were choline-deprived for 1 day and then choline-supplemented or maintained choline-deficient. Pulse-chase experiments showed that in the presence of 100 μM choline, the secretion of apoBs 28 and 37 was increased by up to 50 and 45%, respectively, whereas the secretion of apoBs 15, 18, and 23 was not affected by the addition of choline. Immunoblot analyses also showed that choline in the medium increased the secretion of apoBs 28 and 37 by 30–50% whereas the secretion of apoBs 15, 18, and 23 was unaffected over 24 h. As only carboxyl-terminal truncations with a size greater than 23% of apoB-100 are able to assemble a neutral lipid core (McLeod, R. S., Y. Zhao, S. L. Selby, J. Westerlund, and Z. Yao. 1994. *J. Biol. Chem.* **269:** 2852–2862), we conclude that only apoB-truncations that assemble a neutral lipid core require phosphatidylcholine synthesis. Other experiments established that the requirement of phosphatidylcholine for apoB secretion is related to the presence of neutral lipid associated with a truncation, rather than the length of apoB.—**Vermeulen, P. S., S. Lingrell, Z. Yao, and D. E. Vance.** Phosphatidylcholine biosynthesis is required for secretion of truncated apolipoprotein Bs from McArdle RH7777 cells only when a neutral lipid core is formed. *J. Lipid Res.* 1997. **38:** 447–458.

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Atherosclerosis is closely correlated with high levels of low density lipoprotein in the blood stream. The plasma concentration of this lipoprotein is determined by its rate of removal via receptor-mediated endocytosis and by the rate of secretion of its precursor, very low density lipoprotein (VLDL). In mammalian liver, the assembly of VLDL particles requires the synthesis of lipids and apolipoproteins (1–5). Indispensable for the formation of VLDL and other triacylglycerol (TG)-rich lipoproteins are apolipoprotein Bs (apoBs). These large proteins have numerous amphipathic domains and provide a framework for assembly of TG-rich lipoproteins. Although it has been shown that apoB-mRNA levels can regulate apoB secretion from McArdle cells (6), under most conditions studied the steady state levels of apoB are not altered (7). It, therefore, is generally believed that the secretion of apoB, unlike most other secretory proteins, is regulated at a post-transcriptional level (1, 3, 8). ApoB is synthesized in excess of the amount secreted and is either secreted or degraded depending on the lipid availability (9–11). Regulation of VLDL secretion might occur at translocation of apoB across the endoplasmic reticulum (ER), assembly of the lipid core and/or intracellular transport from the ER to the plasma membrane.

Phopholipids play important roles in the above-described processes. It has previously been reported that decreased PC biosynthesis specifically inhibits the secretion of VLDL, but not high density lipoprotein (HDL), from cultured rat hepatocytes (12, 13). How-

Abbreviations: apoB(s), apolipoprotein(s) B; CD, choline-deficient; CS, choline-supplemented; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; EST, (2S-3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester; HDL, high density lipoprotein; MTP, microsomal triacylglycerol transport protein; PC, phosphatidylcholine; RIPA, radio immunoprecipitation assay; TG(s), triacylglycerol(s); VLDL, very low density lipoprotein.

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ever, these studies did not show at what stage impaired PC biosynthesis interfered with the process of assembly and secretion of VLDL. In vitro translation–translocation studies with carboxyl-truncated forms of apoB with lengths of 15, 29, and 48% of the full-length apoB-100 (designated as apoB-15, B-29, and B-48, respectively) showed no defect in the ability of the three apoproteins to translocate into the lumen of liver microsomes from rats that were fed a choline-deficient (CD) diet (A. E. Rusiñol, P. S. Vermeulen and D. E. Vance, unpublished results). Moreover, the numbers of nascent apoB-containing particles were almost equal in the luminal fractions of ER of liver from choline-supplemented (CS) and CD rats (14). However, the number of apoB-containing lipoprotein particles in a liver Golgi fraction from CD rats was significantly lower than that in rats fed a CS diet. This result implies that impaired PC synthesis leads to enhanced degradation of apoB in a post-ER compartment. As the lipid composition of the lipoprotein might be a determining factor in proteolytic degradation (15), we investigated the role of PC synthesis in lipid core formation in McArdle cell lines that secrete carboxyl-terminal truncations of human apoB-100 that differ in size and ability to assemble a stable lipid core. McArdle cells secreting apoB-15, -18, or -23 were selected for their presumed inability to assemble a neutral lipid core, whereas cells secreting apoB-28 and -37 were chosen for their ability to form a stable lipid core (16). The results show that only apoBs that assemble a lipid core require PC biosynthesis to achieve a rate of secretion equal to that in CS cells.

EXPERIMENTAL PROCEDURES

Materials

McArdle RH7777 cells were stably transfected as previously described (17). Anti-mouse and -rabbit IgG-horseradish peroxidase, ECL™ Western blot detection reagents, L-[4,5-^{3}H]leucine (2.26 TBq/mmol), Aqueous Counting Scintillant and Amplify™ were from Amersham Canada, Oakville, ON. Sheep anti-human apoB was obtained from Boehringer Mannheim, Germany. Culture medium, horse serum, fetal bovine serum, penicillin–streptomycin, and G418 sulfate were from GIBCO BRL, Life Technologies Inc., Grand Island, NY. 1-Bromodecane was purchased from Aldrich Chemicals Incorporated, Milwaukee, WI. The 1D1 monoclonal antibody against human apoB was a generous gift from Drs. R. W. Milne and Y. L. Marcel. Rabbit antiserum against rat apoA-I was prepared and characterized in our laboratory as previously described (18). All other reagents were from Sigma Chemical Co., St. Louis, MO.

Growth of hepatoma cells

Transfected McArdle RH7777 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum, 10% (v/v) heat-inactivated horse serum, penicillin–streptomycin (40 units/ml each) and 0.02% (w/v) G418. Cells were maintained in 75-cm^2 vented flasks (Corning) at 37°C in humidified air (89–91% of saturation) containing 5% CO_2. Cells were subcultured at a 1:5 ratio after having reached a confluency of approximately 80%.

Induction of choline deficiency in McArdle RH7777 cells

Transfected hepatoma cells were plated in 3 ml of the above described culture medium at 1.0–1.5 × 10^5 cells per 60-mm dish (Falcon 3002, Becton Dickinson, Lincoln Park, NJ) and grown under the conditions described above. After 4 days, 40–50% confluent cells were washed with 2 ml of DMEM that lacked choline and incubated for 20 or 24 h in the same medium containing 1% (w/v) bovine serum albumin, 0.75 mM oleic acid, and 1 μM dexamethasone. Oleic acid and dexamethasone were added to boost the secretion of VLDL. Cells reached a CD state as judged by a 50% decrease in cellular PC content.

Immunoblot analyses

Choline deficient cells were washed with 2 ml DMEM that lacked choline, then incubated with 1.3 ml DMEM containing 1 μM dexamethasone in the presence or absence of 100 μM choline. Media were harvested at the times indicated, layered on a 90 μl 1:1 (v/v) mixture of 1-bromodecane and 1-bromododecane and centrifuged for 2 min at 10,000 *g* to remove cellular debris. Aliquots of the media were diluted 1:1 (v/v) with sample buffer [6 M urea, 100 mM dithiothretiol, 2% (v/v) β-mercaptoethanol, 10% (v/v) glycerol, 2% (w/v) SDS, 0.002% (w/v) bromophenol blue, and 6.25 mM Tris-HCl, pH 6.8] and heated at 80°C for 15 min. Aliquots of medium, corrected for amounts of cellular protein, were loaded onto a 3–15% polyacrylamide gradient gel that contained 0.1% (w/v) SDS. After electrophoresis, proteins were transferred (6–7 h at 600 mA) to Immobilon™ polyvinylidene difluoride membranes (Millipore, Bedford, MA) using 20% (v/v) methanol, 2.5% (v/v) 2-propanol, 192 mM glycine, and 25 mM Tris as a transfer medium and blocked for 2 h with blocking buffer [0.8 M NaCl, 20 mM $CaCl_2$, 10% (w/v) skimmed milk powder, 0.1% (v/v) Tween-20, and 0.5 M Tris-HCl, pH 8.8]. To detect truncated apoBs, membranes were incubated overnight at 4°C with the 1D1 antibody in blocking buffer. After three washes for 10 min with the same buffer, membranes were incubated for 3 h at room temperature with anti-mouse IgG-horseradish peroxidase,

diluted 1:10,000 in blocking buffer. Membranes were washed three times with blocking buffer and twice with Tris-buffered saline (0.5 M NaCl, 20 mM Tris-HCl, pH 7.5). Finally, membranes were incubated with ECL reagents and immediately exposed for 1 to 10 s to Kodak XAR-5 X-ray film.

Immunoblot analysis of secreted endogenous apoA-I

To determine whether the effect of choline supplementation on the secretion of truncated apoBs was specific, we used endogenous apoA-I levels in the medium as a marker of protein secretion. To monitor apoA-I levels, membranes described in the preceding section were equilibrated in Tris-buffered saline and incubated for 3 h with rabbit antiserum against apoA-I. After 3 washes for 10 min each with the same buffer, membranes were incubated for 3 h at room temperature with a 1:10,000 dilution of anti-rabbit IgG-horseradish peroxidase in blocking buffer. Membranes were further treated as described in the above section except the exposure time was 5 min.

Pulse-chase analysis

CD cells were washed with 2 ml DMEM that lacked choline and leucine, and preincubated for 45 min with 2 ml of the same medium that contained 1 μM dexamethasone. Medium was removed and cells were labeled for 2 h with 250 μCi [^{3}H]leucine per dish supplied in 2 ml of the latter medium that lacked or contained 100 μM choline. Radioactive medium was removed, cells were washed with 2 ml of DMEM either containing or lacking 100 μM choline, and subsequently incubated with 1.3 ml DMEM that contained 50 mM leucine, 1 μM dexamethasone and, where indicated, 100 μM choline. At the times indicated, cells and media were harvested.

Immunoprecipitation and quantitation

Cells were washed with 1 ml of ice-cold phosphate-buffered saline (135 mM NaCl, 2.7 mM KCl, 11.5 mM Na_2HPO_4–KH_2PO_4, pH 7.2) and scraped into RIPA buffer (radio immunoprecipitation assay buffer) [1% (v/v) Triton X-100, 1% (w/v) deoxycholic acid, 0.15 M NaCl, and 50 mM Tris-HCl, pH 8.0] that contained 1% (w/v) SDS. Afterwards, 2 ml of RIPA buffer was added and samples were heated at 90°C for 30 min. Five hundred μl of this solution was diluted 1:1 with RIPA buffer containing 2 mM dithiothreitol and incubated overnight with 20 μl of sheep anti-human apo-B serum. The samples were mixed for 3 h with 50 ml of a 1:7 (w/v) protein A-Sepharose CL-4B slurry before centrifugation. The pellet was washed 5 times with RIPA buffer that contained 1 mM dithiothreitol and 0.1% SDS. ApoB was eluted from the protein A-Sepharose by heating the pellet in 200 μl of denaturing buffer (2% (w/v) SDS, 10% glycerol, 8 M urea, 5% (v/v) β-mercaptoethanol, 10 mM Tris-glycine, pH 8.3) and incubated for 20 min at 90°C. After centrifugation, supernatants with equal amounts of protein were loaded on a 3–15% polyacrylamide gradient gel that contained 0.1 (w/v) % SDS. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250, destained, soaked in 10% (per volume) glycerol for 45 min, soaked in Amplify™ for 30 min, dried under vacuum, and exposed to X-ray film for 2 or 3 days. The identified apoB bands were excised, gel slices were digested with 23% perchloric acid/20% hydrogen peroxide for 3 h at 70°C, and radioactivity was determined by liquid scintillation spectrometry.

Media were layered on a 90-μl 1-bromodecane and 1-bromododecane (1:1 per volume) mixture and centrifuged for 2 min at 10,000 *g* to remove cellular debris. Eight hundred μl aliquots of medium were mixed with 20 μl sheep anti-human apoB and 80 μl of a 10 × concentrated RIPA buffer containing 1% (w/v) SDS and 10 mM dithiothreitol. After incubation overnight, samples were further identically treated as described above in this section.

Trichloroacetic acid precipitation and quantification of total radiolabeled protein

To determine the flow of all secreted labeled proteins during pulse-chase studies, parallel cultures of cells were treated as described in the pulse-chase section, except that 25 μCi instead of 250 μCi of [^{3}H]leucine was used for labeling. At indicated times, media were taken and cells were washed with phosphate-buffered saline. The cells were then scraped into 2 ml of distilled water and sonicated. Five hundred μl of either the sonicated cell suspension or medium was mixed with 300 μl of 10% (w/v) bovine serum albumin and diluted 1:1 (v/v) with 20% (w/v) ice-cold trichloroacetic acid. After centrifugation, the pellets were washed 4 times with 5% (w/v) ice-cold trichloroacetic acid and resuspended in 0.8 ml of 0.1 M NaOH and 0.2 ml of 0.5 M HCl-0.12 M Tris. Radioactivity was determined by liquid scintillation spectrometry.

Density fractionation studies

Pulse-chase experiments were performed in the presence or absence of choline exactly as described above. After 90 min of chase, CD and CS media were each taken from 4 dishes and centrifuged to remove cellular debris. Subsequently, 0.5-ml portions of fresh rat plasma were added to 3.0-ml fractions that were taken from the combined media of three matching dishes. Samples were adjusted to a final density of 1.21 g/ml with solid KBr. Subsequently, samples were centrifuged at 4°C for 24 h in a SW 60 Ti-rotor at 485,000 *g*. After centrifugation, the upper 40% and the lower 60% of the gradient were dialyzed overnight at 4°C against 0.15

M NaCl and 0.1 mM EDTA. Subsequently, 500-μl aliquots were taken from upper and lower fractions and mixed with 20 μl sheep anti-human apoB and 50 μl of a 10 × concentrated RIPA buffer containing 1% (w/v) SDS and 10 mM dithiothreitol. After incubation overnight, samples were treated as described above in the pulse-chase analysis section.

Other methods

Protein was determined by the bicinchoninic acid method (Pierce, Rockford, IN), using bovine serum albumin as a standard. Lipids were extracted from cells by the method of Sundler, Åkesson, and Nilsson (19), and phospholipids were subsequently separated by thin-layer chromatography on silica gel G60 plates with chloroform–methanol–acetic acid–formic acid–water 70:30:12:4:1 (v/v) as solvent. Phospholipid concentrations were determined by phosphorous analysis according to Ames (20) except for the ashing procedure that was replaced by acid hydrolysis.

RESULTS

Establishment of choline deficiency in rat hepatoma cells

In primary rat hepatocytes, a CD state can be achieved by omitting choline from the diet of young rats for 3 days and subsequent isolation of the cells (12). This protocol could not be adapted to our cultured hepatoma cells for three reasons. First, unlike hepatocytes in vivo, cultured cells in serum-free CD medium are not exposed to circulatory choline. Second, cultured hepatoma cells divide more rapidly than hepatocytes in vivo. Third, unlike rat hepatocytes, McArdle RH7777 cells lack a phosphatidylethanolamine methylation pathway to synthesize PC (21). For these reasons a CD state is reached more rapidly in cultured hepatoma cells than in primary hepatocytes. Because long-term choline deprivation might induce cellular death (22), it was important not to deprive cultured cells of choline longer than necessary. We initially cultured the cells in CD and serum-free medium for 36 h. Although the concentration of cellular PC was decreased by 55%, there was no significant accumulation of TGs within the cells. This was unexpected because a marked cellular accumulation of TGs is commonly used as a marker for choline deficiency in rat hepatocytes (12). We considered that the supply of fatty acids might limit the synthesis of TG and included 1% (w/v) bovine serum albumin and 0.75 mM oleic acid in our CD medium. Under these conditions,

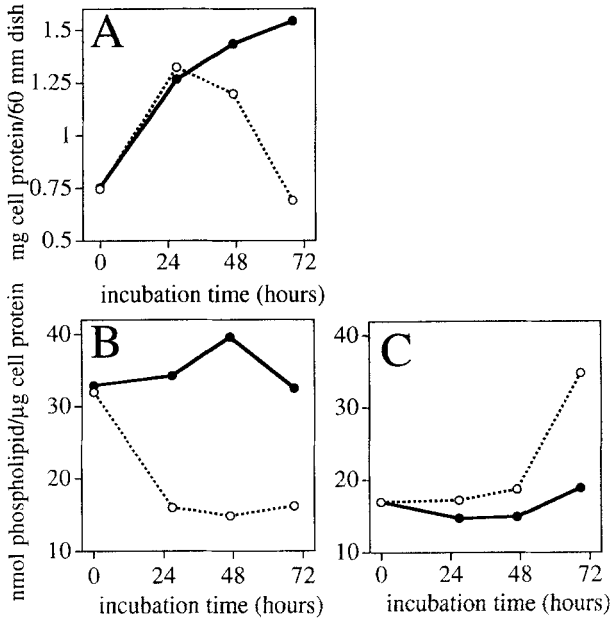

Fig. 1. The effect of choline deficiency on cell growth (A), phosphatidylcholine (B), and phosphatidylethanolamine (C) levels. McArdle RH7777 cells, stably transfected with apoB-15 cDNA were initially grown to ~50% confluency. Cells were cultured in DMEM that contained 0.75 mM oleic acid, 1% (w/v) bovine serum albumin, and 1 μM dexamethasone that lacked (○) or contained (●) 100 μM choline. At times indicated, cellular protein and phospholipid levels were measured. Growth curves were also determined for McArdle cells that were transfected with cDNAs for apoB-18, -23, and -28. All cell lines studied gave essentially the same growth curves and phospholipid profiles.

we found a 2- to 3-fold accumulation of TGs in the CD cells.

To determine a suitable length of deprivation necessary to achieve a CD state, we measured the cellular PC levels of cells, grown in the absence or presence of 100 μM choline, as a function of time. After 1 day of choline deprivation, the cellular PC levels were decreased by 52% in CD compared to CS cells (**Fig. 1B**). Prolonged choline deprivation did not decrease the cellular PC levels further. Cellular phosphatidylethanolamine levels were not decreased in CD compared to CS cells over the first 2 days (Fig. 1C), indicating that in the absence of choline PC synthesis was specifically inhibited. We also measured the amount of cellular protein in CD and CS cells as a function of time. As the absence of choline became unfavorable for cellular growth after 2 days (Fig. 1A), we decided to deprive our cells of choline for only 24 h prior to pulse chase experiments or 20 h prior to longer time studies (immunoblot analyses).

Impaired PC biosynthesis decreases the secretion of C-terminally truncated apoBs that form a lipid core

After induction of choline deficiency for 24 h, cells were depleted of leucine for 45 min, in the absence of

choline. Subsequently, cells were pulse-labeled with [^{3}H]leucine for 2 h in the absence or presence of 100 μM choline. The radioisotope was chased with 20 mM leucine, in the absence or presence of 100 μM choline. Cells and media were harvested at various times; truncated apoBs were immunoprecipitated, separated on SDS gels, and visualized by fluorography. The flow of labeled apoB-15, -18, -23, -28, and -37 from cells to medium is shown in **Fig. 2**. There was no significant effect of choline on the secretion of apoB-15, -18, and -23, whereas the secretion of apoB-28 and -37 was stimulated by the presence of choline in the medium (Fig. 2A). Quantitation of the bands revealed that choline in the medium stimulated apoB-28 secretion by 54% after 2 h of chase, whereas the maximum effect on apoB-37 secretion was 46%, reached after 1 h of chase.

Although choline stimulated the secretion of apoB-28 and -37 to a similar degree, different effects on the intracellular levels of both core-forming truncations were observed. For apoB-37, the intracellular apoB levels did not significantly differ in CD compared to CS cells (Fig. 2A). In CD cells, the fraction of apoB that cannot be secreted is quantitatively degraded. In contrast, the intracellular levels of apoB-28 inversely correlated with the amounts secreted by CD and CS cells (Fig. 2A).

The total amount of labeled apoB recovered from the five cell lines is shown in Fig. 2B. The loss of radioactivity after time 0 reflects apoB degradation. Thus, it is apparent in all cell lines except those that secrete apoB-18, that only a small percentage of apoB is degraded. Why so much of apoB-18 (>60%) is degraded is not known. Enhanced degradation of apoB occurs in CD compared to CS cell lines that secrete apoB-28 or apoB-37. At 6 h the total amount of labeled apoB recovered was similar ± choline supplementation. This appears to occur because at 6 h the total amount of apoB has started to exceed the maximal binding capacity of the antiserum added. Thus, in the CS secreting cells, there is less radioactivity immunoprecipitated due to competition for the antibody by unlabeled apoB that has been secreted in the later time periods. This explanation was supported by studies with a cell line that secreted abundant amounts of apoB-37. A CD effect was evident during 2 h, but not 4 h, of chase when 20 μl of antiserum was incubated with 500 μl of medium. When the experiment was repeated with 250 μl of medium, the CD effect was still apparent at 4 h in the chase period.

In addition to immunoprecipitation and quantitation of labeled apoproteins, we also measured the secretion of total labeled proteins after trichloroacetic acid precipitation. The presence of choline had no effect on the secretion of total labeled proteins from cells secreting apoB-28 (**Fig. 3A**), whereas the secretion of apoB-28 was specifically stimulated when choline was present (Fig. 3B). Similarly, the secretion of total labeled proteins was unaffected by the presence of choline in the other cell lines investigated (data not shown).

Different effects of choline on the secretion of core-forming and non-core-forming truncations are not due to clonal differences

The cell lines differed in efficiency of label incorporation, rates of basal degradation (i.e., degradation of apoB in the presence of choline) and efficiency of secretion (Fig. 2). The following experiments suggest that none of these differences was a determining factor in the differential effects of choline on secretion of truncated apoBs.

To determine whether choline supplementation affected the rate of [^{3}H]leucine incorporation into apoB in our experiments, we compared the rate of apoB synthesis in cell lines that secreted truncated, core-forming particles with cell lines that secreted apoB truncations without a core. The incorporation of [^{3}H]leucine into apoBs was similar in control and CD cells for all cell lines studied (Fig. 2A). This result is consistent with the report that the number of apoB-containing lipoproteins is similar in the ER of rats that were fed a CD or CS diet (14).

To exclude the possibility that the rate of apoB synthesis might have been responsible for our results, we compared the secretory characteristics of a high apoB-18 expressor (4830 dpm per h per μg cell protein) with the secretory characteristics of two cell lines that incorporated [^{3}H]leucine into apoB-18 at a much lower rate (402 and 503 dpm per h per μg cell protein). For the high expressor, 5.2 and 6.1% of the total label incorporated into apoB-18 was secreted after 1 h from CD and CS cells, respectively. These numbers were 6.3 compared to 6.4 and 2.19 compared to 2.00 in CD and CS cells, respectively, for the cell lines that expressed apoB-18 at a slower rate. As the level of basal degradation only marginally differed for the various cell lines (varying from 47.4 to 57.8%), we conclude that the rate of apoB synthesis was not a determining factor in the requirement for choline.

Possibly, the reason why secretion of apoB-28 and -37 was decreased by choline deficiency was that these cell lines were more efficient in the secretion of truncated apoBs than lines that secreted the shorter truncations. Thus, we compared secretion from a cell line that efficiently secreted apoB-28 (**Fig. 4A,** 66.2% of total label in apoB-28 after 4 h of chase), with that of a cell line that secreted apoB-28 less efficiently (Fig. 4B, 12.7% of total label in apoB-28 after 4 h of chase). The low secretor (Fig. 4B) had an apoB-28 secretory profile ± choline similar to that of the apoB-28 high secretor ± cho-

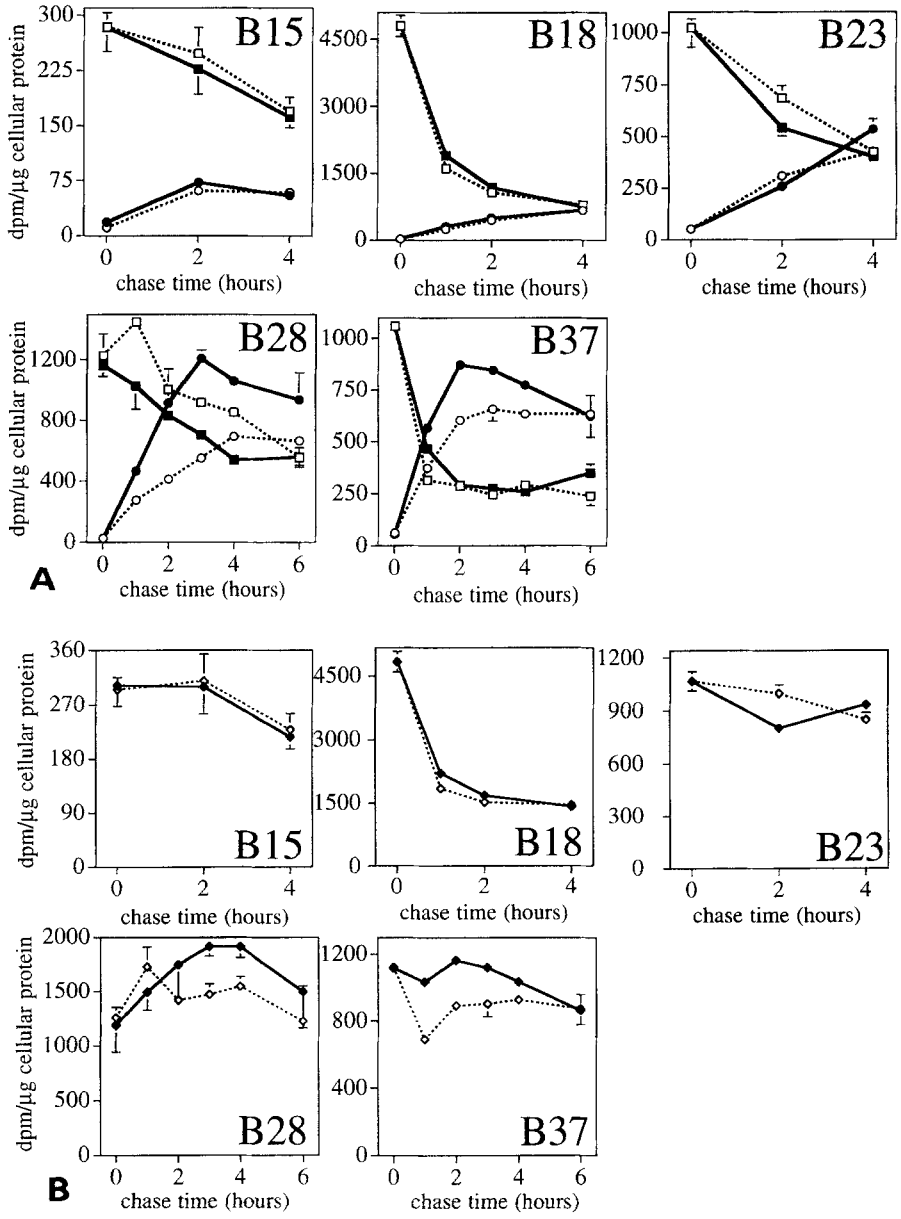

Fig. 2. Secretory kinetics of apoB-15, B-18, B-23, B-28, and B-37. A: McArdle cells were labeled with [^{3}H]leucine, in the presence (closed symbols) or absence (open symbols) of 100 μM choline. At the indicated times, cells (squares) and media (circles) were harvested, apoBs were immunoprecipitated, separated by gel electrophoresis, and monitored by fluorography. Gel bands that contained labeled apoB were excised and digested and radioactivity was determined by liquid scintillation counting. For every cell line, the analyses were done in duplicate and the experiment was repeated at least 3 times with similar results. Data shown are the means of the individual measurements of one typical experiment. The apparent diminishing effect of choline on apoB secretion after a 3-h chase is explained by dilution of labeled apoB in the medium with non-radioactive apoB. When the amount of total apoB exceeds the maximal binding capacity of the anti-serum, a smaller fraction of the labeled apoB in the medium will be bound to the anti-serum. The amount of labeled apoB immunoprecipitated will be diluted by the increased amount of apoB secreted from the CS cells. B: The results in panel A for cells and medium were summed and are plotted for each cell line to display the total amount of apoB recovered at each time point. Open and closed symbols represent the results in the absence and presence of choline, respectively.

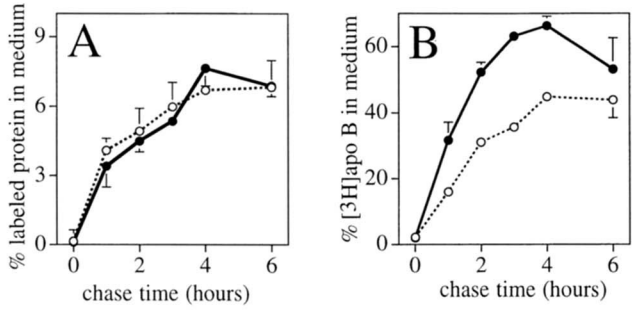

Fig. 3. Secretion of total labeled proteins (A) and apoB-28 (B) from a stably transfected McArdle cell line. Pulse-chase experiments were performed in the presence (●) or absence (○) of 100 μM choline as briefly described in the legend to Fig. 2. Total secretion of proteins was assessed by trichloroacetic acid-precipitation of proteins from cells and media. Secretion is expressed as the amount of labeled protein in medium as a percentage of total amount of labeled protein recovered from cells and media. The measurements were performed in duplicate and the experiment was repeated with similar results. Data shown are the means of the individual measurements of one typical experiment.

line (Fig. 4A). Thus, differences in secretory efficiency did not determine the effectiveness of the choline deficiency.

The extent of intracellular basal degradation (the degradation of apoB truncations that occurs in CS hepatocytes) was not the reason for the CD effect as cell lines secreting non-core-forming truncations strongly differed in the extent of basal degradation. Although basal degradation was high for all B-18 secretors (47.4 to 57.8% after 1 h of chase), basal degradation in B-23- and B-15-expressing cells was much lower (16.4% to virtually none after 2 h of chase, respectively). The addition of choline to CD cells did not affect the secretion of truncated apoBs in any of these cell lines. We conclude that basal degradation is not a determining factor in the differential CD effect on core- versus non-core-forming apoB truncations. As apoB-37 secreting cells degrade apoB-37 extensively, whereas intracellular degradation of apoB-28 in the apoB-28 high secretor was virtually absent, we further deduce that the choline effect is not determined by differences in basal intracellular degradation.

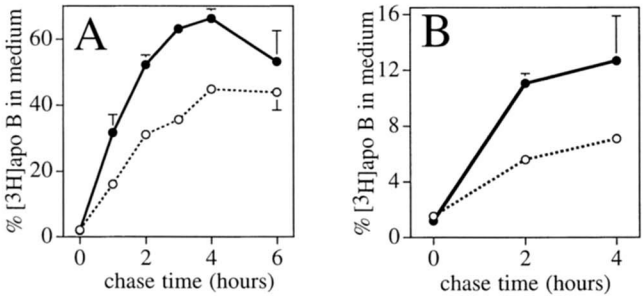

Fig. 4. Secretion of apoB-28 from cell lines that secrete truncated apoB with high (A) and low (B) secretory efficiency. A pulse-chase experiment was performed in the presence (●) or absence (○) of 100 μM choline as briefly described in the legend to Fig. 2. The two cell lines differed in the rate of label incorporation (745 and 212 dpm per mg cell protein per h, for the high and low secretor, respectively). The measurements were performed in duplicate and the experiment was repeated with similar results. Data shown are means of the individual measurements of one typical experiment.

Effect of impaired PC biosynthesis on continuous secretion of carboxyl-terminally truncated apoBs

Transfected cells were deprived of choline for 20 h. Cells were then incubated for up to 24 h in the absence or presence of 100 μM choline. The amounts of truncated apoBs in the cells and secreted were determined by immunoblot analysis. The secretion of non-core-forming truncations, B-15, B-18, and B-23, was not affected by the addition of choline to the medium (**Fig. 5**). On the other hand, the secretion of core-forming apoB truncations, B-28 and B-37 (Fig. 5), was markedly increased by the presence of choline in the medium. Density integration of scans of Western blots showed that the secretion of apoB-28 and B-37 was, on average, increased by 49 and 37%, respectively, when choline was present in the medium.

The intracellular levels of apoB-28 and B-37 remained relatively constant over a long period of time

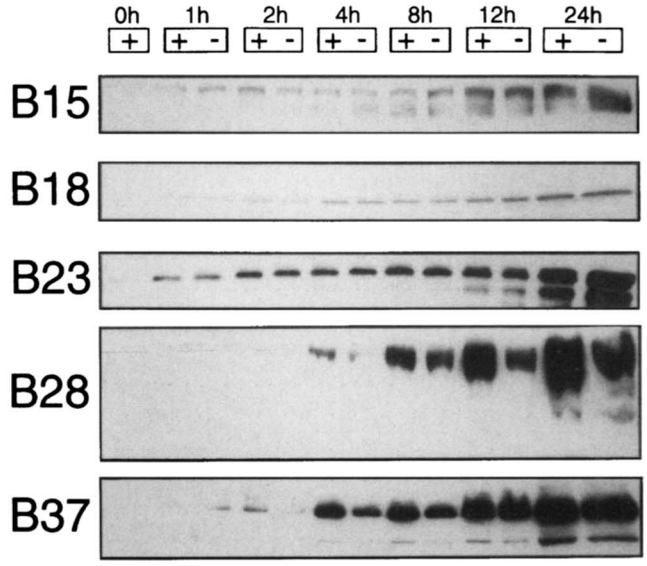

Fig. 5. Immunoblot analysis of secreted apoB-15, B-18, B-23, B-28, and B-37. CD and CS McArdle cells were incubated in medium that lacked (−) or contained (+) 100 μM choline. At the indicated times, media were harvested and aliquots were subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes and apoBs were analyzed by immunoblot analysis. The 1D1 mouse monoclonal antibody was used as primary antibody. Immunoblot analysis was repeated at least twice for all cell lines indicated, with essentially the same results.

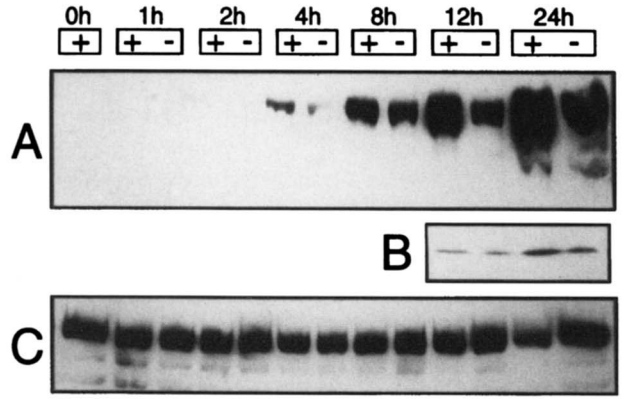

Fig. 6. Immunoblot analysis of apoB-28 from cells (C) and medium (A); comparison with the secretion of apoA-I (B). The experiment was performed basically as described in the legend to Fig. 3. At the indicated times, media and cells were harvested in sample buffer and diluted 1:1 (v/v) with water. The proteins were separated by SDS-polyacrylamide gel electrophoresis. For immunoblot analysis of apoA-I, rabbit anti-rat apoA-I antiserum was used as primary antibody. Fig. 6A is the same as the B-28 experiment presented in Fig. 5.

(**Fig. 6C** and **Fig. 7C,** 1–8 h) after choline supplementation. However, choline supplementation for 24 h did cause a decrease in intracellular apoB-28. We also examined the level of apoA-I in the medium of apoB-28- and B-37-secreting cells by immonoblot analysis. After 12 and 24 h, the secretion of apoA-I was not significantly affected by the presence of choline in the medium (Figs. 6B and 7B), indicating that the effect of choline is specific for apoB. Taken together, our results from pulse-chase and immunoblot analysis indicate that only apolipoproteins that assemble a neutral lipid core require PC synthesis for normal secretion from McArdle cells.

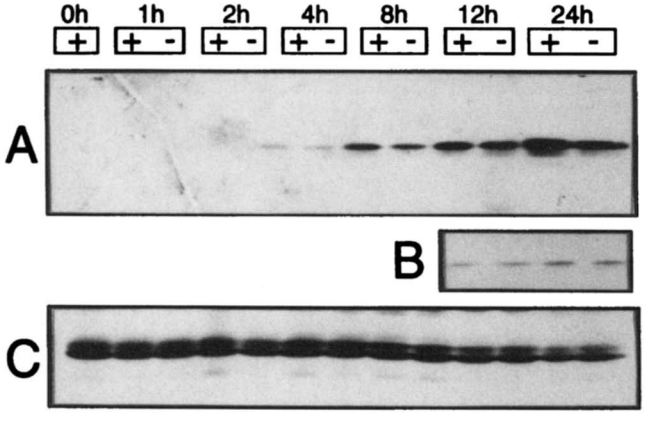

Fig. 7. Immunoblot analysis of apoB-37 from cells (C) and medium (A); comparison with the secretion of apoA-I (B). The experiment is essentially the same as described in Fig. 6 for apoB-28-secreting cells. In panel C both bands are thought to be apoB-37 but the reason for the difference in electrophoretic mobility is not presently understood.

Other evidence for the choline effect being specific for core-forming apoBs comes from the secretion of endogenously synthesized apoB-48 and -100 from cells secreting a non-core-forming truncation. When we overexposed the apoB-23 autoradiograms we found that the secretion of endogenous apoB-48 and -100 was inhibited by 37 and 52%, respectively, as determined by densitometric scanning. However, the secretion of apoB-23 was not affected. Hence, the CD effect depends on features of individual apoB-containing lipoproteins and not on the nature of the cell secreting them.

Impaired PC biosynthesis and the density distributions of secreted apoB-28 and -37

To find a relationship between length, buoyancy, and/or lipid binding ability of apoB truncations, we fractionated media from cell lines secreting apoB-28 or -37 on the basis of density. ApoB-28 from the medium of CD cells was equally present in two forms, one of which floated at $d < 1.21$ g/ml and one that did not (**Fig. 8**). In contrast, apoB-37 secreted from CD cells appeared to be present only in the low density fraction (Fig. 8). When cells were supplied with choline, the level of apoB-28 in the fraction with density < 1.21 g/ml increased (Fig. 8), whereas the amount of apoB-28 in the fraction with a density > 1.21 g/ml was not affected by the presence of choline (Fig. 8). Supplementation of choline only increased the levels of apoB-37 in the fraction at the top of the gradient (Fig. 8). As buoyancy of a lipoprotein particle is related to the amount of lipid assembled with apoB (16), we conclude that the CD effect on apoB secretion is related to the amount of lipid bound to the apoprotein, rather than to the length of the truncation. Moreover, the lack of

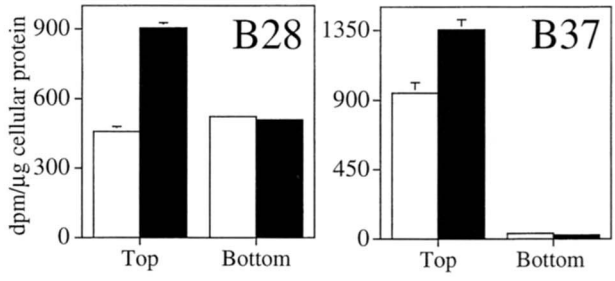

Fig. 8. The effect of choline supplementation on the density distribution of secreted apoB-28 and B-37. Pulse-chase experiments were performed, basically as described in the legend to Fig. 2. After 90 min of chase, media from CD (open bars) and CS (filled bars) cells were harvested and carrier lipoproteins and solid KBr were added before samples were subjected to density fractionation by ultracentrifugation. After centrifugation for 24 h at 485,000 *g*, the upper 40% (referred to as the top fraction) and lower 60% (referred to as the bottom fraction) were collected separately. Aliquots were subjected to immunoblotting and the amount of apoB-28 was quantitated by densitometric scanning of the autoradioagram. Values are means ± standard error of at least three separate experiments.

an effect of choline on the apoB fraction that bound relatively low amounts of lipid underscores the result that PC synthesis is only required for secretion when a neutral lipid core is formed.

DISCUSSION

Association with lipid components, the requirement of the microsomal TG transfer protein (MTP) for VLDL secretion (2, 23, 24), and the existence of default pathways for degradation of incorrectly assembled apoB, distinguish the secretion of this unusually large amphipathic protein from other secretory proteins. In the present study we investigated the importance of PC biosynthesis for the secretion of carboxyl-truncated apoBs from stably transfected McArdle RH7777 cells. The truncated forms differed in length and ability to assemble a neutral lipid core. Our approach was to inhibit selectively PC synthesis in transfected cell lines by depriving them of choline for 1 day. Cells were then supplemented with choline or maintained CD. The results from both pulse-chase experiments and immunoblot analyses indicate that the secretion of apoB is dependent on PC synthesis only when an apoB truncation is longer than 23% of the N-terminus of full-length apoB-100. Somewhere between apoB-23 and apoB-28 there appears to be a transition where the requirement for PC becomes manifest. The experiment with apoB-28 (Fig. 8) showed that the presence of neutral lipid associated with a truncated apoB, rather than the length of apoB, determined the requirement for PC.

Is PC biosynthesis required for translocation of apoB or core lipid assembly?

We have recently shown that translocation of truncated apoB-15, -29, and -48 into the lumen of hepatic microsomes from CD rats during in vitro translation, in the presence of lipid precursors, is not impaired (A. E. Rusinol, P. S. Vermeulen, and D. E. Vance, unpublished results). Therefore, the length of apoB does not appear to limit the translocation of apoB in microsomes from CD rat livers. Moreover, the number of particles recovered in the lumen of microsomes from CD rat livers was the same as from CS rat livers, but fewer particles are recovered from the Golgi of CD livers (14). However, the particles from both the Golgi and ER from CD livers were shown to be deficient in PC (15). Thus, VLDL particles are formed in normal amounts in CD hepatic cells despite impaired PC biosynthesis; translocation is not impaired. However, phospholipids may modulate apoB translocation, as enrichment of microsomal membranes with phosphatidylmonomethylethanolamine inhibits the translocation of apoB-100 and B-48 (18) as well as shorter apoB variants (B-15, B-18, B-23, and B-28) (11).

Our studies have shown that apoB-28 is secreted in two forms: a highly lipidated form (buoyant at a density of 1.21 g/ml) and an under-lipidated form (not buoyant at the same density). Enhanced PC biosynthesis stimulated the secretion of the buoyant, but not the non-buoyant, form. One possibility is that the non-buoyant, luminal form of apoB-28 is a precursor of the buoyant form of apoB-28 as has been suggested to occur for conversion of the apoB-48-HDL particle to the apoB-48-VLDL particle (25, 26). PC biosynthesis might be required for assembly of the lipidated form but not the high density form. However, a precursor–product relationship between the two apoB-28 forms, or between the two luminal forms of apoB-48, has not been established.

What is the relationship between apoB secretion and intracellular degradation? A proposed role for choline deficiency-related intracellular degradation

At least two types of intracellular degradation of apoB have been observed. Under normal conditions excess apoB that is not assembled with lipids into a lipoprotein particle is degraded. In HepG2 cells (9, 10) and McArdle cells (11) translocation of apoB, and not the ER-localized degradation, regulates the amount of apoB secreted. Translation-coupled degradation in these cells might play a role in clearing unused apoB molecules from the secretory pathway. ApoB molecules that have failed to traverse correctly the ER membrane, or that are produced in excess, would be targets for proteolysis. When hepatocytes are deprived of choline, an additional level of apoB degradation occurs. Possibly a quality control protease in a post-ER compartment degrades apoB-containing lipoprotein particles that are defective so that secretion of apoB is inhibited during choline deficiency (14, 15).

We observed that under CD conditions the secretion of apoB-28 and -37 was decreased by approximately 50% compared to that in CS cells (Fig. 2). However, choline deficiency had different effects on the intracellular levels of the truncated apoB forms. The labeling of apoB-37 was practically equal in CD and CS cells (Fig. 2), indicating that apoB-37 that was not secreted was rapidly degraded (Fig. 2B). ApoB-28 accumulated intracellularly followed by more rapid degradation in CD cells (Fig. 2B). Proteolysis of apoB in abnormal particles might prevent apoB accumulation which might block the normal transit of apoB through its secretory pathway.

In cultured rat hepatocytes, in contrast to HepG2 cells, the major part of apoB degradation has been re-

ported to occur in a post-ER compartment (possibly Golgi) via the action of a cysteine protease (27). This conclusion was drawn from experiments with a calpain protease inhibitor (2S-3S)-*trans*-epoxysuccinyl-L-leucyl-amido-3-methylbutane ethyl ester (EST) in cultured hepatocytes and isolated hepatic Golgi fractions. Our preliminary observation that EST suppressed enhanced degradation of apoB-37 in CD McArdle cells (P. S. Vermeulen and D. E. Vance, unpublished results) is an indi cation that choline deficiency-related degradation also involves a cysteine protease. However, at this juncture, the subcellular localization of the choline deficiency-related degradation in McArdle cells has not been established. In rat hepatocytes, the CD-related degradation of apoB is in a post-ER compartment (14, 15).

How do our choline deficiency results compare with the effects of oleate on the secretion of apoB truncations from McArdle cells?

In primary rat hepatocytes, oleate does not significantly stimulate the secretion of apoB whereas the amount of TG secreted is substantially increased (28). However, oleate supplementation of McArdle cells stimulates the secretion of apoBs with a minimum length of 23% of apoB-100 (29). We found a stimulatory effect of choline on secretion of apoBs at a length of 28% and higher of apoB-100. Oleate is known to stimulate PC synthesis through the activation of CTP:phosphocholine cytidylyltransferase (30, 31). If oleate stimulation of PC biosynthesis were of primary importance, the stimulatory effect of choline should have occurred with the same length of apoB truncation as with oleate supplementation (i.e., apoB-23). Thus, PC availability would not seem to be predominantly responsible for the stimulatory effect of oleate on apoB secretion. The stimulatory effect of oleate on apoB secretion may be more related to the stimulation of TG synthesis (32). Supply of TG is recognized as an important component in regulating apoB secretion, as Triacsin D, an inhibitor of TG synthesis, can fully counteract the oleate-stimulated apoB secretion in HepG2 cells (33). Possibly, the stimulation of TG and PC synthesis by oleate might alter apoB conformation and, thereby, promote the assembly of TG into the nascent particle (34–36). Taken together, the data suggest that the stimulatory effect of oleate on apoB secretion may be related to the synthesis and availability of both TG and PC.

One reason why the oleate and choline stimulatory effects became apparent at different apoB sizes is that the amounts of PC and TG that are required are different. As PC forms a monolayer around the hydrophobic core, the PC requirement increases as a function of the surface area. TGs, however, are present in the lipoprotein interior. Therefore, the TG requirement increases with apoB size as a function of volume of the lipoprotein particle. Furthermore, differences in availability, rates of synthesis and delivery of TG and PC might play a role.

Relationship among MTP, PC, and VLDL assembly

The inability of non-hepatic cells to secrete truncated apoBs such as apoB-28 and longer is ascribed to these cells lacking MTP (23, 24, 37–39). There is striking similarity among the lengths of apoB where the smallest detectable buoyant particle is formed (16), where oleate (29) and choline stimulatory effects become apparent, where n–3 fatty acids limit the secretion of apoB (40), and where non-hepatic cells become unable to secrete apoB truncations (37). MTP is reported to be involved in the co-translational addition of lipid to apoB (2, 24). COS cells secrete apoB-17 without expression of MTP but require MTP for the secretion of apoB-41 (23). Moreover, co-expression of apoB and MTP in COS cells demonstrated that MTP is only required for the secretion of core-forming species (equal to or longer than apoB-29) (39). As the effect of choline deficiency on apoB secretion is dependent on formation of a neutral lipid core, the process in lipoprotein assembly that involves MTP might be the same process that is impaired as a result of choline deficiency. An important function of MTP might be to transfer PC from the luminal ER membrane to the surface of the particle as it matures with a lipid core.

Conclusion

Our results show that apoB secretion is dependent on PC synthesis only when a neutral core is formed. If PC were required in the translocation of apoB into the lumen of the ER, sufficient PC appears to be available even though the ER membranes are deficient in PC in CD cells (15). PC biosynthesis might also be required to expand the lipoprotein surface to accommodate the TG in the core. In CD hepatocytes, this process would result in the appearance of particles that are deficient in PC in the lumen of the ER (15). A quality control protease has been postulated to degrade the apoB associated with the PC-deficient particles (15), hence, fewer buoyant particles are secreted when PC biosynthesis is limiting. The identification and purification of this putative quality control protease will be the next important step in understanding why lipoprotein particles are not secreted normally from CD cells.

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REFERENCES

1. Davis, R. A., and J. E. Vance. 1996. Structure, assembly and secretion of lipoproteins. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. E. Vance, editors. Elsevier, Amsterdam. 473–493.
2. Gordon, D. A., J. R. Wetterau, and R. E. Gregg. 1995. Microsomal triglyceride transfer protein: a protein complex required for the assembly of lipoprotein particles. *Trends Cell Biol.* **5:** 317–321.
3. Innerarity, T. L., J. Borén, S. Yamananaka, and S-O. Olofsson. 1996. Biosynthesis of apolipoprotein B-48-containing lipoproteins. Regulation by novel post-transcriptional mechanisms. *J. Biol. Chem.* **271:** 2353–2356.
4. Sparks, J. D., and C. E. Sparks. 1994. Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochim. Biophys. Acta.* **1215:** 9–32.
5. Yao, Z., and R. S. McLeod. 1994. Synthesis and secretion of hepatic apolipoprotein B-containing lipoproteins. *Biochim. Biophys. Acta.* **1212:** 152–166.
6. Selby, S. L., and Z. Yao. 1995. Level of apolipoprotein B mRNA has an important effect on the synthesis and secretion of apolipoprotein B-containing lipoproteins. Studies on transfected hepatoma cell lines expressing recombinant human apolipoprotein B. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1900–1910.
7. Pullinger, C. R., J. D. North, B-B. Teng, V. A. Rifici, A. E. Ronhild de Brito, and J. Scott. 1989. The apolipoprotein B gene is constitutively expressed in HepG2 cells: regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J. Lipid Res.* **30:** 1065–1077.
8. Dashti, N., D. L. Williams, and P. Alaupovic. 1989. Effects of oleae and insulin on the production rates and cellular mRNA concentrations of apolipoproteins in HepG2 cells. *J. Lipid Res.* **30:** 1365–1372.
9. Bonnardel, J. A., and R. A. Davis. 1995. In HepG2 cells, translocation, not degradation, determines the fate of the de novo synthesized apolipoprotein B. *J. Biol. Chem.* **270:** 28892–28896.
10. Sakata, N., X. Wu, J. L. Dixon, and H. N. Ginsberg. 1993. Proteolysis and lipid-facilitated translocation are distinct but competitive processes that regulate secretion of apolipoprotein B in Hep G2 cells. *J. Biol. Chem.* **268:** 22967–22970.
11. Rusiñol, A. E., and J. E. Vance. 1995. Inhibition of secretion of truncated apolipoproteins B by monomethylethanolamine is independent of the length of the apolipoprotein. *J. Biol. Chem.* **270:** 13318–13325.
12. Yao, Z., and D. E. Vance. 1988. The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J. Biol. Chem.* **263:** 2998–3004.
13. Yao, Z., and D. E. Vance. 1989. Head group specificity in the requirement of phosphatidylcholine biosynthesis for very low density lipoprotein secretion from cultured hepatocytes. *J. Biol. Chem.* **264:** 11373–11380.
14. Verkade, H. J., D. G. Fast, A. E. Rusiñol, D. G. Scraba, and D. E. Vance. 1993. Impaired biosynthesis of phosphatidylcholine causes a decrease in the number of very low density lipoprotein particles in the Golgi but not in the endoplasmic reticulum of rat liver. *J. Biol. Chem.* **268:** 24990–24996.
15. Fast, D. G., and D. E. Vance. 1995. Nascent VLDL phospholipid composition is altered when phosphatidylcholine biosynthesis is inhibited: evidence for a novel mechanism that regulates VLDL secretion. *Biochim. Biophys. Acta.* **1258:** 159–168.
16. McLeod, R. S., Y. Zhao, S. L. Selby, J. Westerlund, and Z. Yao. 1994. Carboxyl-terminal truncation impairs lipid recruitment by apolipoprotein B-100 but does not affect secretion of the truncated apolipoprotein B-containing lipoproteins. *J. Biol. Chem.* **269:** 2852–2862.
17. Yao, Z., B. D. Blackhart, F. L. MacRae, S. M. Taylor, S. G. Young, and B. J. McCarthy. 1991. Expression of carboxyl-terminally truncated forms of human apolipoprotein B in rat hepatoma cells. Evidence that the length of apolipoprotein B has a major effect on the buoyant density of the secreted lipoproteins. *J. Biol. Chem.* **266:** 3300–3308.
18. Rusiñol, A. E., E. Y. W. Chan, and J. E. Vance. 1993. Movement of apolipoprotein B into the lumen of microsomes from hepatocytes is disrupted in membranes enriched in phosphatidylmonomethylethanolamine. *J. Biol. Chem.* **268:** 25168–25175.
19. Sundler, R., B. Åkesson, and A. Nilsson. 1974. Effect of different fatty acids on glycerolipid synthesis in isolated hepatocytes. *J. Biol. Chem.* **249:** 5102–5107.
20. Ames, B. N. 1966. Assay of inorganic phosphate, total phosphate and phosphatases. *Methods Enzymol.* **8:** 115–118.
21. Cui, Z., M. Houweling, and D. E. Vance. 1994. Suppression of rat hepatoma cell growth by expression of phosphatidylethanolamine N-methyltransferase-2. *J. Biol. Chem.* **269:** 24531–24533.
22. Ghoshal, A. K., and E. Farber. 1993. Choline deficiency, lipotrope deficiency and the development of liver disease including liver cancer: a new perspective. *Lab. Invest.* **68:** 255–260.
23. Leiper, J. M., J. D. Bayliss, R. J. Pease, D. J. Brett, J. Scott, and C. C. Shoulders. 1994. Microsomal triglyceride transfer protein, the abetalipoproteinemia gene product, mediates the secretion of apolipoprotein B-containing lipoproteins from heterologous cells. *J. Biol. Chem.* **269:** 21951–21954.
24. Gordon, D. A., H. Jamil, B. D. Shan, D. Mullaney, Z. Yao, R. E. Gregg, and J. R. Wetterau. 1994. Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability. *Proc. Natl. Acad. Sci. USA.* **91:** 7628–7632.
25. Borén, J., S. Rustaeus, and S-O. Olofsson. 1994. Studies on the assembly of apolipoprotein B-100- and B-48-containing very low density lipoproteins in McARH7777 cells. *J. Biol. Chem.* **269:** 25879–25888.
26. Rustaeus, S., K. Lindberg, J. Borén, and S-O. Olofsson. 1995. Brefeldin A reversibly inhibits the assembly of apoB-containing lipoproteins in McA-RH7777 cells. *J. Biol. Chem.* **270:** 28879–28886.
27. Wang, C-N., T. C. Hobman, and D. N. Brindley. 1995. Degradation of apolipoprotein B in cultured rat hepatocytes occurs in a post-endoplasmic reticulum compartment. *J. Biol. Chem.* **270:** 24924–24931.
28. Davis, R. A., and J. R. Boogaerts. 1982. Intrahepatic assem-

bly of very low density lipoproteins. Effects of fatty acids on triacylglycerol and apolipoprotein synthesis. *J. Biol. Chem.* **257:** 10908–10913.
29. White, A. L., D. L. Graham, J. LeGros, R. J. Pease, and J. Scott. 1992. Oleate-mediated stimulation of apolipoprotein B secretion from rat hepatoma cells. A function of the ability of apolipoprotein B to direct lipoprotein assembly and escape pre-secretory degradation. *J. Biol. Chem.* **267:** 15657–15664.
30. Cornell, R., and D. E. Vance. 1987. Translocation of CTP: phosphocholine cytidylyltransferase from cytosol to membranes in HeLa cells: stimulation by fatty acids, fatty alcohol, mono- and diacylglycerol. *Biochim. Biophys. Acta.* **919:** 26–36.
31. Weinhold, P. A., L. Charles, M. E. Rounsifer, and D. A. Feldman. 1991. Control of phosphatidylcholine synthesis in HepG2 cells. Effect of fatty acids on the activity and immunoreactive content of choline phosphate cytidylyltransferase. *J. Biol. Chem.* **266:** 6093–6100.
32. Gibbons, G. F., and F. J. Burnham. 1991. Effect of nutritional state on the utilization of fatty acids for hepatic triacylglycerol synthesis and secretion as very-low-density lipoprotein. *Biochem. J.* **275:** 87–92.
33. Wu, X., N. Sakata, J. L. Dixon, and H. N. Ginsberg. 1994. Exogenous VLDL stimulates apolipoprotein B secretion from HepG2 cells by both pre- and post-translational mechanisms. *J. Lipid Res.* **35:** 1200–1210.
34. Furukawa, S., and T. Hirano. 1993. Rapid stimulation of apolipoprotein B secretion by oleate is not associated with cholesteryl ester biosynthesis in HepG2 cells. *Biochim. Biophys. Acta.* **1170:** 32–37.
35. Borén, J., M. Wettesten, S. Rustaeus, M. Anderson, and S-O. Olofsson. 1993. The assembly and secretion of apoB-100-containing lipoproteins. *Biochem. Soc. Trans.* **21:** 487–493.
36. Dixon, J. L., S. Furukawa, and H. N. Ginsberg. 1991. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from HepG2 cells by inhibiting early intracellular degradation of apolipoprotein B. *J. Biol. Chem.* **266:** 5080–5086.
37. Thrift, R. N., J. Drisko, S. Dueland, J. D. Trawick, and R. A. Davis. 1992. Translocation of apolipoprotein B across the endoplasmatic reticulum is blocked in a non-hepatic cell line. *Proc. Natl. Acad. Sci. USA.* **89:** 9161–9165.
38. Du, E. Z., J. Kurth, S-L. Wang, P. Humiston, and R. A. Davis. 1994. Proteolysis-coupled secretion of the N terminus of apolipoprotein B. Characterization of a transient, translocation arrested intermediate. *J. Biol. Chem.* **269:** 24169–24176.
39. Wang, S., R. S. McLeod, D. A. Gordon, and Z. Yao. 1996. The microsomal triglyceride transfer protein facilitates assembly and secretion of apolipoprotein B-containing COS-7 cells. *J. Biol. Chem.* **271:** 14124–14133.
40. Wang, H., Z. Yao, and E. A. Fisher. 1994. The effects of n–3 fatty acids on the secretion of carboxyl-terminally truncated forms of human apoprotein B. *J. Biol. Chem.* **269:** 18514–18520.