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Fatty acids alter time dependent loss of apolipoprotein E expression by primary cultures of rat hepatocytes

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Although the phenomenon of intracellular apolipoprotein E (apoE) degradation has been reported in other cell types, the fate of newly synthesized apoE in the liver is not well understood. In the present study, we examined the expression (the balance of synthesis, secretion, and degradation) of apoE in primary cultures of rat hepatocytes and compared it with albumin, a typical secretory protein. Synthesis and secretion of [^{35}S]apoE was diminished in primary hepatocytes cultured for more than 2 days, in agreement with an observed decrease in apoE mRNA. Cells cultured for 1 day and labeled for up to 4 hours secreted total protein, apoE, and albumin, linearly. The apparent rates of synthesis for apoE and albumin were similar (1,158 vs. 1,334 dpm/mg/min) but rates of their secretion differed significantly (225 vs. 1,159 dpm/mg/min). Pulse-chase experiments indicated that cell-associated [^{35}S]albumin was secreted without degradation, whereas significant quantities of newly synthesized apoE were degraded. The overall synthesis and secretion of total proteins, including secretion of apoE, was enhanced by oleic acid (1 mmol/L). However, this effect may not be limited to oleic acid because other fatty acids showed a similar effect on apoE mRNA abundance. In control cells, apoE was found to associate with high density lipoproteins predominantly, although the fraction associated with very low density lipoprotein was increased in hepatocytes incubated with oleic acid. Overall, the findings from this study suggest that the level of apoE expression by primary hepatocytes is dependent on the age of the culture. The study also indicates that the phenomenon of apoE degradation occurs in primary hepatocytes. (J. Nutr. Biochem. 10:306–313, 1999)

Keywords: oleic acid; synthesis and secretion; protein degradation

Introduction

Apolipoprotein E (apoE), a component of circulating cholesterol-rich lipoproteins,[1] functions as a recognition ligand for the removal of cholesterol from the circulation.[2–4] It is synthesized mainly in the liver, although nonhepatic tissues also express apoE.[4,5] Diet has been shown to have a major influence on plasma concentrations,[6–13] but other factors that regulate overall expression of apoE (i.e., synthesis, secretion, and degradation) are still under active investigation. The regulation of apoE expression may occur transcriptionally by cholesterol[11,14] and posttranscriptionally at the levels of translation[15] and sorting.[16] Stimulation of apoE secretion by high density lipoprotein (HDL) in macrophages and by low density lipoprotein (LDL) in HepG2 cells at a posttranscriptional locus has been reported.[16,17] However, only cholesterol supplementation or the availability of sterol-rich lipoproteins (LDL and HDL) has been shown to affect apoE expression in the liver.[17–23]

Intracellular degradation is one form of regulation of protein expression. At least in some cells, the association of apoE with lipoproteins may influence the extent to which apoE is degraded intra- or extracellularly.[17] Intracellular degradation of newly synthesized apoE in HepG2 cells occurs in a post-Golgi compartment;[24] recent studies from our laboratory demonstrate that intracellular degradation of apoE in macrophages occurs in the lysosomes.[25]

To determine if apoE is similarly degraded prior to or after secretion by the liver we examined the expression of

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apoE by primary rat hepatocytes and compared it with albumin, a typical secretory protein. Our findings suggest that apoE and albumin are synthesized by primary hepatocytes at similar rates, but differ in the rates of secretion due to a greater level of degradation of newly synthesized apoE. In addition, the inclusion of fatty acids in the incubation medium led to increased recovery of apoE. A preliminary report on this work has appeared.[26]

Materials and methods

Materials

Collagenase was obtained from Worthington Biochemical Corporation (Freehold, NJ USA). Bovine serum albumin (BSA, fatty acid free), insulin, and antibiotics were obtained from Sigma Chemical Co. (St. Louis, MO USA). Collagen (rat-tail, Type I) was obtained from Collaborative Research (Bedford, MA USA). William's E, Dulbecco's minimum essential medium (DMEM), and fetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY USA). ApoE and β-actin cDNA probes were obtained as previously described.[16] [^{35}S]methionine and [^{32}P]dCTP were obtained from NEN (Boston, MA USA). All other chemicals were reagent grade and were obtained from Sigma.

Hepatocyte culture

In this study, male Sprague-Dawley rats, (300–350 g) were used in a protocol for which we received prior institutional approval and which followed the *Guide for the Care and Use of Laboratory Animals*[27]. The animals had free access to a nonpurified Purina rodent diet, #5001 (TMI Nutrition, St. Louis, MO USA). Livers were surgically isolated and the hepatocytes were obtained as previously described.[28,29] Briefly, hepatocyte suspensions were prepared using collagenase and were separated from other cell types by four cycles of low speed centrifugation ($50 \times g$ for 3 minutes), followed by resuspension in the plating medium. The plating medium consisted of an arginine-free, L-glutamine containing DMEM supplemented with 0.64 mmol/L L-ornithine, 38 mmol sodium bicarbonate, 10 mmol HEPES, 10 mmol/L dextrose, and 20% (v/v) FBS. Cell viability was assessed by trypan blue exclusion and was consistently in excess of 95%. Cells were resuspended at a density of 4.0×10^6 cells/well and seeded on 60-mm dishes (3 mL/dish) precoated with rat-tail collagen (0.25–3.5 mg). After 4 hours, the plating medium and nonadherent cells were aspirated and replaced with William's E medium (3 mL/plate). In some experiments the feeding medium was further supplemented with BSA or oleic acid or other fatty acids (complexed to BSA, 1:2), giving a final concentration of 1 mmol/L. For cultures that proceeded beyond 24 hours following seeding, medium was aspirated and replaced daily.

Measurement of apoE mRNA content

Total hepatocyte RNA was extracted by the method of Chomczinski and Sacchi—described previously.[15] The ratio of absorbency (OD_{260}:OD_{280}) averaged 1.8 or greater. Total RNA (1–2 μg) was fractionated according to size by formaldehyde-containing agarose (1.2%) gel electrophoresis,[12] and the separated RNA was transferred to nitrocellulose membranes (Schleicher and Schuell Inc., Keene, NH USA) overnight. For the detection of apoE mRNA, a nearly full-length apoE probe was used.[15] The β-actin probe was a DNA fragment of the chicken β-actin gene.[30] DNA probes were labeled with ^{32}P by nick translation to a specific activity of more than 10^8 dpm/ng DNA (1.67 Mbq/ng DNA), using [^{32}P]dCTP (s.a. >3,000 Ci/mmol/L). The membranes were autoradiographed using XAR-5 film (Eastman Kodak Co., Rochester, NY USA). Autoradiograms were evaluated by densitometry.

Pulse-chase studies

Initially, cells were cultured for up to 5 days and three to five plates were labeled daily with [^{35}S]methionine for 3 hours to determine the best culture conditions suited for studies involving the synthesis and secretion of apoE. Rates of specific protein synthesis and secretion were determined by continuous labeling (30–240 minutes) of hepatocytes with 50 μCi/mL of [^{35}S]-methionine. Incorporation of the label into immunoprecipitable rat apoE and albumin or trichloroacetic acid (10%) precipitable total proteins was determined in both cells and medium. Apparent rates of synthesis were determined from the slope of the earliest linear portion of the curve of label incorporated into cellular proteins.[14] Apparent secretion rates were determined similarly, based on the slope of the line showing accumulation of [^{35}S]-labeled protein in the medium. Previous work showed that, after an initial lag period of 30 minutes, accumulation of proteins in the medium was linear for several hours.[15]

For pulse-chase experiments, hepatocytes briefly incubated (10 minutes) in methionine-free DMEM were pulsed for 60 to 120 minutes with [^{35}S]methionine (50 μCi/mL). Cells then were washed twice and chased for periods of up to 3 hours. At the end of each chase period, cell-associated and medium [^{35}S] apoE and albumin were quantitatively immunoprecipitated. The sum of cell-associated and medium proteins of interest at each time point represented total recovery and was expressed as a percentage of the cell-associated amount at the initiation of the chase period (0 time chase). Differences between total recovery at given times and that present in cells at zero time was considered to be degradation.

The immunoprecipitates were washed as previously described[15] and then dissolved in 50 μL of Laemmli gel loading buffer, heated, and resolved by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (10% acrylamide, 0.1% SDS).[31] ApoE and albumin bands were identified by Coomassie blue staining using molecular weight markers, purified albumin, and very low density lipoprotein (VLDL)-associated apolipoproteins as standards. The relevant bands were cut out and solubilized overnight in 0.4 mL of 30% hydrogen peroxide at 60°C, and radioactivity was determined by scintillation counting. For flourography, gels were stained and de-stained in 10% acetic acid and treated with 1 M of sodium salicylate for 30 minutes. Dried gels were exposed to X-ray film at −70°C. The extent of secretion and the half-life ($T_{1/2}$) of [^{35}S]-apoE and albumin were determined based on the differences between cell-associated levels at the initiation of chase (zero time) and subsequent recoveries within the cell or in the medium.

Total labeled proteins were determined by a modification of trichloroacetic acid precipitation.[15] Five microliters of cell lysate or 25 μL of medium was applied onto Whatman #5 filter paper discs. Samples were air dried for 1 hour and soaked in 10% trichloroacetic acid overnight. Samples were then washed twice in 10% trichloroacetic acid overnight, twice in 100% ethanol for 30 minutes each, and once in 100% anhydrous ether for 10 minutes. The filter papers were air-dried for 1 hour. Scintillation cocktail (10 mL) was added to each sample and counted. A modified Lowery (Bio-Rad) was used to determine total cell protein.[32]

Agarose column chromatography

The distribution of newly secreted apoE among lipoproteins in the hepatocyte-conditioned media was examined by agarose (A-5M) column chromatography.[15,33] Hepatocyte-conditioned media from cells incubated in the presence or absence of oleic acid were concentrated five-fold by centrifugation. The column was stan-

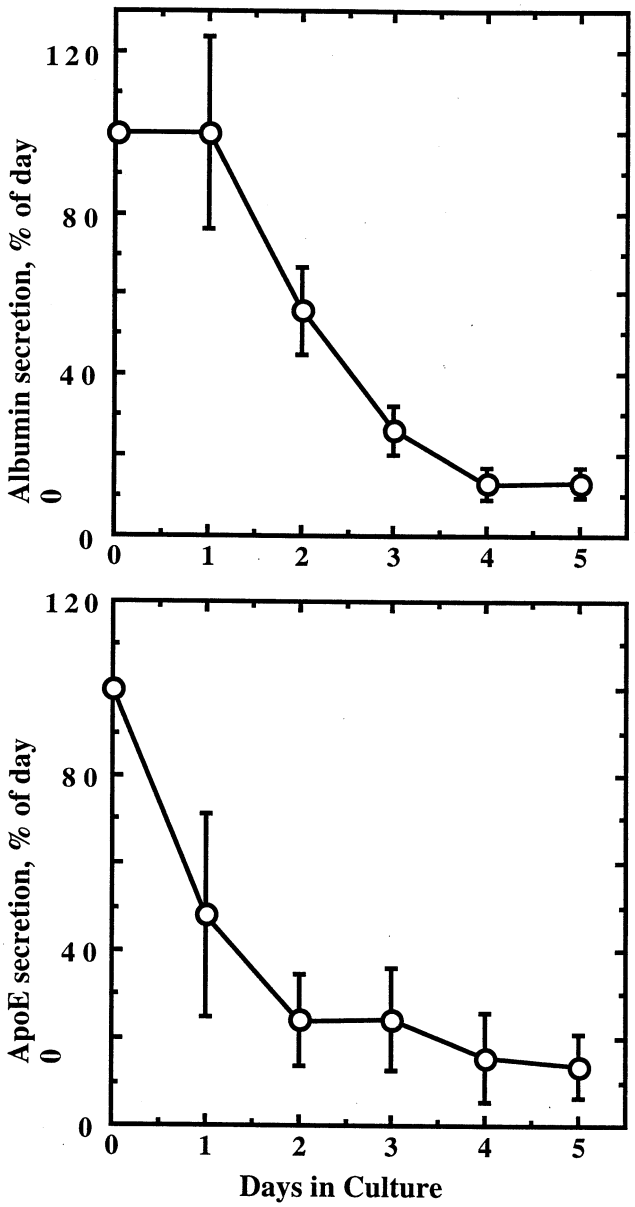

Figure 1 Apparent rates of albumin and apolipoprotein E (apoE) secretion by rat hepatocytes as a function of days in culture. Hepatocytes were seeded for 4 hours in medium containing 20% fetal bovine serum (FBS). At 4 hours the medium was replaced with control medium (William's E with no FBS or oleic acid) on a daily basis, as described in Materials and methods. Each day a set of cells was labeled with [^{35}S]methionine (1.85 Bq/L) in met-free Dulbecco's modified Eagle's medium for 3 hours. Secretion of newly synthesized albumin and apoE during the 3-hour period was determined by quantitative immunoprecipitation. The data are expressed as percent of day 0 secretion. Each point represents an average ± SEM of a minimum of three plates in each of two experiments.

dardized with rat plasma lipoproteins ($d < 1.006–1.121$) and albumin. Each column fraction was assayed for total protein and aliquots were subjected to quantitative immunoprecipitation of apoE and albumin. HDL containing fractions were identified by the presence of immunoprecipitable apolipoprotein A-I (apoA-I).

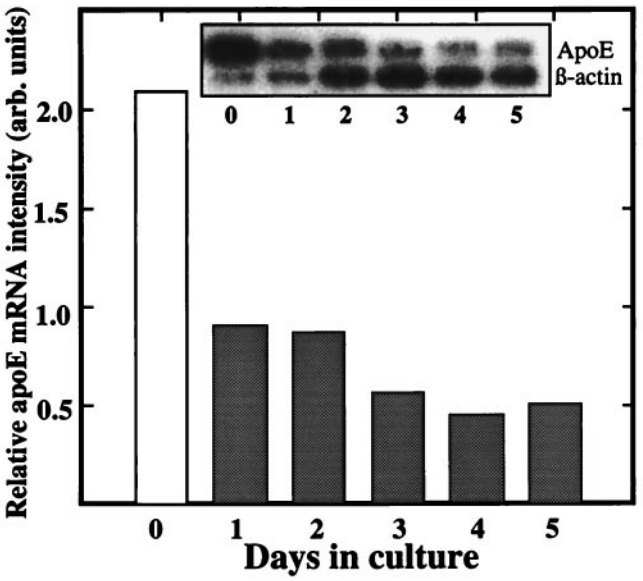

Figure 2 Rat hepatocyte apolipoprotein E (apoE) mRNA abundance as a function of days in culture. Cells were cultured as described in *Figure 1*. Each day a set of cells was washed with cold phosphate buffered saline, total RNA was extracted, and apoE mRNA levels were determined as described in Materials and methods. At each time point apoE mRNA (as determined by densitometry) is expressed relative to day 0. The insert is a representative autoradiogram of apoE and β-actin mRNA blots at indicated age of culture. Based on ethidium bromide staining of ribosomal RNA, RNA loading was equal in all lanes. ApoE mRNA levels were not expressed relative to β-actin mRNA, because β-actin mRNA levels increased with time.

Results

ApoE expression by primary hepatocytes as a function of time

First, we examined the secretion of apoE and albumin as a function of period of culture. Cells were cultured for up to 5 days and each day separate dishes were labeled with [^{35}S]methionine for 3 hours. Apparent rates of secretion were determined by quantitative immunoprecipitation of [^{35}S]-apoE and [^{35}S]albumin from the medium. The results are shown in *Figure 1*. Secretion of albumin was constant during the first 24 hours, followed by a gradual reduction of 50% per day, up to day 4 (*Figure 1A*). Overall, rate of albumin secretion decreased by 85% between days 1 and 4. By contrast, a 50% decrease in apoE secretion was observed in 24-hour-old hepatocytes relative to day 0 (*Figure 1B*). A more gradual decrease followed, and by day 2 rates of apoE secretion stabilized at approximately 20 to 25% of the secretory rate observed on day 0.

To examine the declining expression of apoE more closely, mRNA levels of apoE were examined on each day of culture. Total hepatocyte RNA was extracted from identically-treated cells 0 to 5 days in culture and the accumulation of apoE mRNA by Northern blot analysis was examined. ApoE mRNA level decreased by 50% by day 1 (*Figure 2*) and remained relatively stable thereafter (approximately 40% of day 0). The rapid and profound decline in apoE mRNA level corresponded well with the observed decreases in apoE secretion (*Figure 1B*). β-Actin mRNA

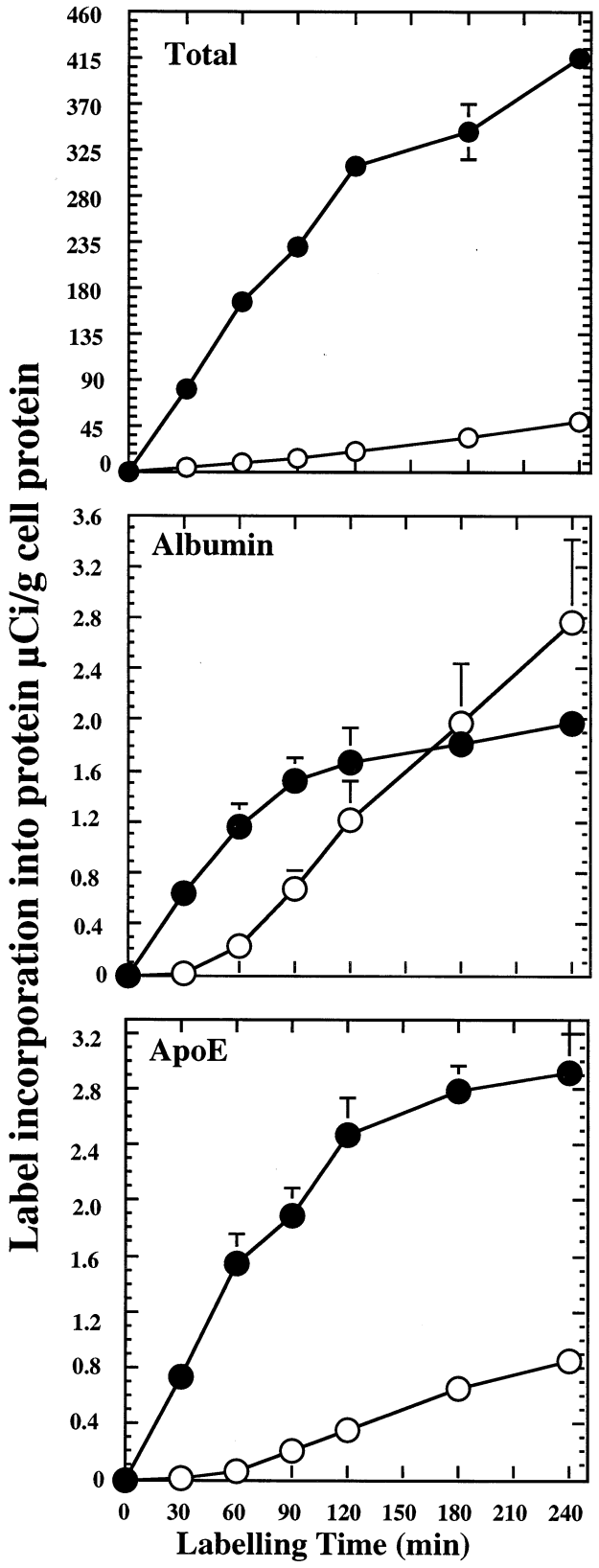

Figure 3 Synthesis and secretion of cellular and medium [^{35}S]-total proteins, albumin, and apolipoprotein E (apoE). One-day-old hepatocytes were labeled continuously for periods up to 4 hours. At indicated time points, label incorporated into cellular (synthesis, closed circles) and medium (secretion, open circles) albumin and apoE was determined by quantitative immunoprecipitation, and total proteins were determined by tricholoroacetic acid precipitation as described in Materials and methods. Apparent rates of synthesis and secretion were determined from the linear portion of the curves. The data represent the average ± SEM (may be smaller than symbol) of three separate experiments.

levels continued to increase during the period of culture, suggesting that the cells continue to undergo de-differentiation as they age, which is a characteristic of primary cultures.[34,35] The remainder of these studies utilized 1-day-old (20–24 hours) cultured hepatocytes.

Kinetics of synthesis and secretion of apoE and albumin

To determine the relative rates of synthesis and secretion of apoE and albumin, continuous labeling studies were carried out in 1-day-old hepatocytes. These experiments allowed for a direct comparison of the synthetic and secretory rates of each protein examined. Cells were continuously labeled with [^{35}S]methionine (1.85 Bq/L) for periods of 15 to 240 minutes. The results, summarized in *Figure 3*, indicated that synthesis of total proteins was linear during the initial 3 hours, whereas secretion remained linear during the 4-hour experimental period. Secretion of apoE and albumin also remained linear for at least 4 hours. Apparent rates of synthesis and secretion for apoE and albumin were obtained by extrapolating the linear portions of the respective curves. As expected for a secretory protein, the rates of synthesis and secretion of albumin were similar (1,334 and 1,158 dpm/mg cell protein per minute, respectively). In contrast, the rate of apoE synthesis (1,316 dpm/mg cell protein per minute) far exceeded its apparent rate of secretion (225 dpm/mg cell protein per minute). Cell-associated apoE and albumin reached a steady state plateau at 2 to 3 hours, the result of the complete labeling of the cellular pool of these proteins.

Turnover of apoE

To further investigate the disparity between the rates of apoE synthesis and secretion, turnover of apoE was examined in 1-day-old cells by a pulse-chase experimental design. To label the intracellular pool of apoE and albumin, cells were pre-labeled with [^{35}S]methionine for 120 minutes. The fate of labeled proteins in these cells was followed during chase periods of up to 120 minutes. The fate of labeled proteins in these cells was followed during chase periods of up to 120 minutes. Results from these experiments are summarized in *Figure 4*. Loss of cell-associated albumin was accompanied by the appearance of a corresponding amount of albumin in the medium. Recovery was complete and therefore reflected a precursor-product relationship expected of a secretory protein. Because label re-utilization may be significant with this major product of hepatocyte protein synthesis, the $T_{1/2}$ of albumin (26 minutes) was estimated from the initial 30-minute chase period only, and was within the expected value.[35] $T_{1/2}$ of apoE was approximately 60 minutes but, in contrast to albumin, the loss of cell-associated apoE was not accompanied by an

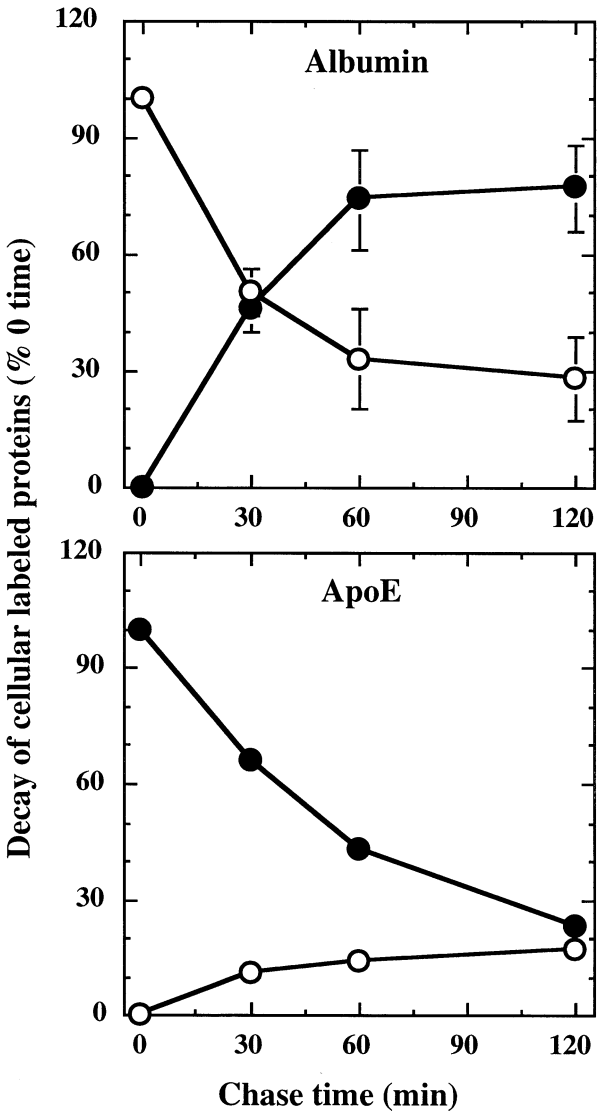

Figure 4 Turnover of [^{35}S]-apolipoprotein E (apoE) and albumin in primary hepatocyte culture. One-day-old hepatocytes were labeled for 120 minutes in met-free Dulbecco's modified Eagle's medium containing 1.85 Bq/L [^{35}S]methionine. Following the pulse period, hepatocytes were chased for the indicated periods of time. At each time point of the chase, both medium (open circles) and cell-associated (closed circles) apoE and albumin were quantitatively immunoprecipitated and quantified as described in Materials and methods. Each time point represents a mean ± SEM (may be smaller than symbol) of a minimum of three plates in each of two experiments.

equivalent recovery of apoE in the medium. In fact, by 120 minutes of chase, nearly 60% of apoE was unaccounted for and apparently degraded. These results were confirmed in shorter pulse-chase experiments using a 60-minute pulse and chase period.

Cellular re-uptake and degradation in the medium of secreted apoE

To examine apoE degradation more closely, we determined the extent of apoE re-uptake or extracellular degradation. The cells were labeled for 2 hours with [^{35}S]methionine (50 μCi/mL), followed by a 2-hour incubation in label-free media. This hepatocyte-conditioned medium containing newly secreted [^{35}S]proteins then was incubated in the absence or presence of hepatocytes for 60 minutes. The results showed cellular uptake of apoE to be approximately 7%, whereas degradation in the medium was 20%, which suggests that a higher proportion of apoE is degraded extracellularly. However, the hourly rate of degradation in the medium and re-uptake by the cells accounted for approximately 75% of the total apoE loss observed in pulse-chase studies (see *Figure 4*). The remainder (approximately 25%) was likely due to intracellular degradation.

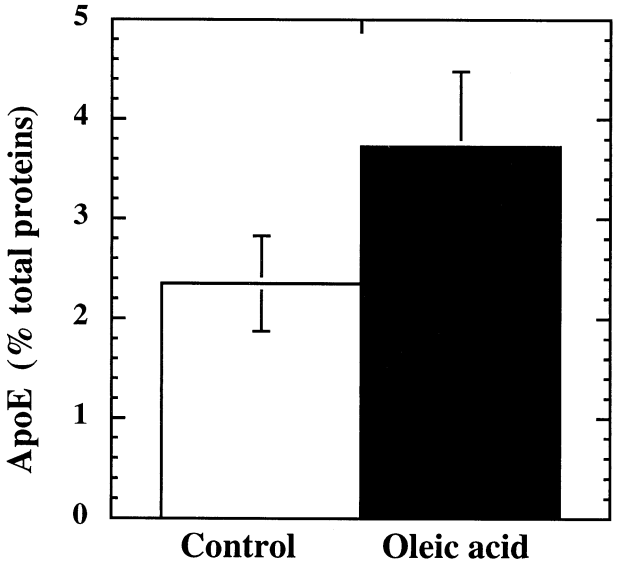

Figure 5 The effect of oleic acid on secretion of [^{35}S]-apolipoprotein E (apoE) by primary hepatocytes. Hepatocytes were cultured overnight in the presence or absence of oleic acid. Plates of cells were labeled with [^{35}S]methionine (1.85 Bq/L) for 3 hours. Label incorporation into media total proteins, apoE, and albumin was determined as described in Materials and methods. Secretion of apoE is expressed as a percentage of total secretory proteins.

Secretion and distribution of apoE in the medium: Effect of fatty acid

The secretion and distribution of apoE was examined in medium from normal cells and from cells treated with fatty acid to determine the nature of secreted apoE and whether lipogenesis had any effect on this process. Hepatocytes were incubated overnight in the presence or absence of oleic acid. The cells then were continuously labeled for up to 240 minutes and rates of synthesis and secretion of albumin and apoE were determined. In the presence of oleic acid, the apparent rates of synthesis for total proteins, apoE, and albumin were 1.6×10^5, 2,337, and 2,453 dpm/mg protein per minute, respectively, which is an overall 50% increase in protein synthesis.

To determine if the observed increase in protein synthesis also is reflected in apoE secretion, separate experiments were carried out. The results are summarized in *Figure 5*. Net secretion of apoE was determined following incubations with and without oleic acid over a 24-hour period.

When secreted apoE was expressed as a fraction of total secreted proteins, more apoE was secreted in the presence of oleic acid than in its absence. This observation was further investigated in the presence of other fatty acids to determine if the 50% decline in apoE mRNA (*Figure 2*) after 1 day of culture is influenced by fatty acids. As shown in *Figure 6*, incubation of hepatocytes with various fatty acids (1 mmol/L of butyric, lauric, palmitic, stearic, oleic, elaidic, and linoleic acids) improved the ability of these cells to synthesize and secrete apoE at levels significantly higher than controls. Although the stability of albumin secretion also was maintained, the effect of these fatty acids did not influence its synthesis and secretion to the same extent as apoE. These results also suggest that the influence of fatty acids on the synthesis and secretion of apoE is the result of increased levels of apoE mRNA, as shown in *Figure 7*. The increase in the abundance of apoE mRNA ranged from 11% greater than controls with palmitic acid to 33% greater than controls with oleic acid.

We also examined the nature of secreted apoE by 1-day-old hepatocytes incubated in the presence or absence of oleic acid. Cells were continuously labeled for 4 hours and the medium was pooled and concentrated five-fold. The concentrated medium was fractionated by agarose column chromatography, followed by SDS-PAGE. The results are summarized in *Figure 8*. In the absence of oleic acid, newly secreted apoE eluted in fractions that coincided with HDL particles (measured by the presence of apoA-I). We observed a single apoE peak eluting in the area similar to the first HDL peak. In the presence of oleic acid, a second and larger apoE peak was found, which was consistent with the increased secretion of apoE described previously (see *Figure 5*). In addition, medium from oleic acid treated cells contained measurable amounts of apoE associated with VLDL-sized particles.

Discussion

The knowledge of regulation of apoE expression is essential for understanding the degree and significance to which nutrients, hormones, and other substances may regulate production and circulating levels of this protein. The intracellular concentration of apoE is affected by at least three separate events: synthesis, secretion, and degradation. How these events are regulated in the hepatocyte is not well understood. To gain an understanding of how apoE is expressed by the liver and compare our observations with a typical secretory protein, such as albumin, we measured the rates of synthesis, secretion, and degradation of these proteins by cultured rat hepatocytes.

Initially, we determined the rates of secretion of apoE by hepatocytes cultured under basal conditions over a 5-day period and observed that the extent to which apoE was secreted significantly diminished with the age of the culture. On the other hand, secretion of albumin remained constant during the first 48 hours of culture, followed by a decline similar to apoE. Rates of apoE secretion correlated well with apoE mRNA levels. Because the mRNA for β-actin actually increased with age of the culture under the same conditions, we concluded that de-differentiation of hepatocytes occurred. This observation is contrary to Kim et al.[36] who found that apoE mRNA was stable in a cultured hepatocyte system void of hormones, serum, fat, or carbohydrate, and with amino acids as the only source of energy. Our observations, however, are similar to the reported time-dependent decline in apoE mRNA in cultured aortic smooth muscle cells[37] and consistent with the decrease in tissue-specific expression in cultured primary hepatocytes.[34,35,38–40] An important observation from this study is that the level of expression of apoE in primary hepatocytes is dependent on the age of the culture. This is important because cultures less than 1-day-old may lack cell surface receptors necessary for the metabolism of lipoproteins.

The secretory pattern and intracellular decay of apoE and albumin were monitored by continuous labeling and pulse-chase studies. Using an antibody that recognized intact albumin, we observed no degradation of albumin, either intracellularly or in the medium. In contrast to albumin, degradation of newly synthesized apoE occurred both intra- and extracellularly. Intracellular degradation of apoE prior to secretion is a phenomenon reported previously in primary macrophages[16] and in HepG2[17] cells in a post-Golgi compartment.[24] Deng et al.[25] have demonstrated that in primary macrophages this post-Golgi compartment is the lysosome. In the current study, the loss of immunoprecipitable apoE in pulse chase experiments is due mostly to extracellular degradation. Intracellular degradation prior to secretion accounts for approximately 25% whereas degradation secondary to re-uptake accounts for approximately 7%. Al-

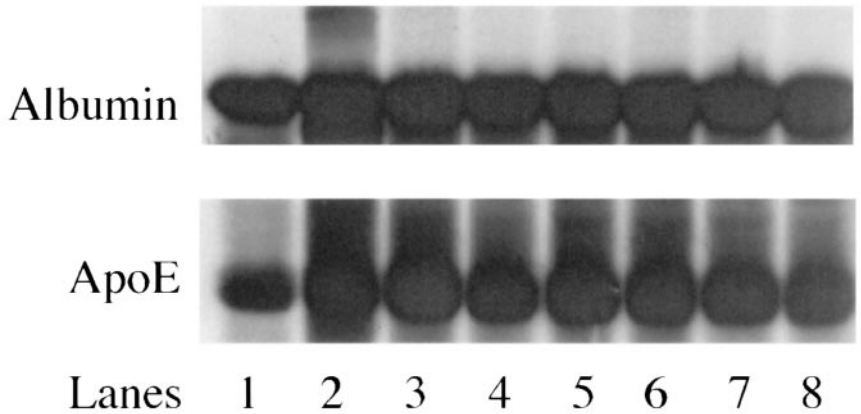

Figure 6 The effect of fatty acids on secretion of [^{35}S]-apolipoprotein E (apoE) and albumin by primary hepatocytes. Hepatocytes were cultured overnight in the presence or absence of indicated fatty acids. Lanes represent *(1)* control (no fatty acid) and *(2)* butyric, *(3)* lauric, *(4)* palmitic, *(5)* stearic, *(6)* oleic, *(7)* elaidic, and *(8)* linoleic acids. Plates of cells were labeled with [^{35}S]methionine (1.85 Bq/L) for 3 hours. Label incorporation into media apoE and albumin were determined as described in Materials and methods. Secretion of apoE and albumin is expressed as shown on the representative autoradiogram.

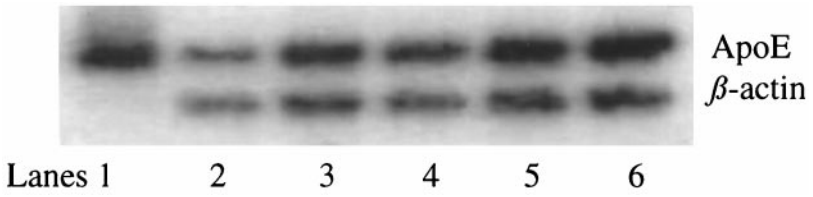

Figure 7 Rat hepatocyte apolipoprotein E (apoE) mRNA abundance as a function of fatty acids. Cells were cultured as described for *Figure 1*. Twenty-four hours after incubation with the selected fatty acids total RNA was extracted and apoE mRNA levels were determined as described in Materials and methods. A representative autoradiogram of apoE and Beta-actin mRNA blots is shown. Lanes represent *(1)* rat liver, *(2)* control (no fatty acid), *(3)* butyric, *(4)* lauric, *(5)* stearic, and *(6)* oleic acids. RNA loading was equal in all lanes (5 μg), confirmed by ethidium bromide staining of ribosomal RNA.

though the extent of intracellular degradation of apoE in hepatocytes appears to represent a far smaller portion of the newly synthesized apoE than was observed in primary macrophages,[16] it still represents a substantial amount.

The current study shows that in hepatocytes newly synthesized apoE is degraded intra- and extracellularly. In contrast, essentially complete secretion of newly synthesized albumin is observed. Preincubation of hepatocytes with oleic acid increased total protein synthesis, which led to increased apoE secretion and its detection in VLDL and HDL. It is not clear when apoE and these lipoproteins become associated. However, our finding is in agreement with that of Reardon et al.,[41] who reported a similar association in McA RH7777 cells transfected with human apoE. In the current study the overall increases in total protein synthesis in the presence of oleic acid may be, in part, the result of a decreased rate of cell de-differentiation and/or the availability of an extra energy source for the cells.

We find that 1-day-old hepatocytes provide an appropriate model for studies of regulation of apolipoprotein expression; longer culture periods result in a substantial transcriptional down-regulation of apoE expression. In addition, fatty acids may improve apoE expression by increasing its mRNA levels. It is generally known that incubation of liver cells with fatty acids increases triglyceride synthesis with concurrent stimulation of VLDL output. The stimulatory effects of VLDL on apoE mRNA has been demonstrated previously by Feng and coworkers.[42] In that study, mouse peritoneal macrophages were incubated with similar concentrations of apoE-poor VLDL or apoE-rich VLDL for 24 hours. The apoE mRNA content of the cells was elevated in both groups although the effect of apoE-poor VLDL was more pronounced in stimulating apoE mRNA. We speculate that the incorporation of newly synthesized apoE into VLDL is facilitated when fatty acids are present, which could account for some of the rescue of apoE. In addition, it is plausible that by stimulating lipoprotein production fatty acids also may promote the recycling of apoE.

Overall, this study demonstrates that degradation of apoE previously reported in hepatoma cell lines and macrophages

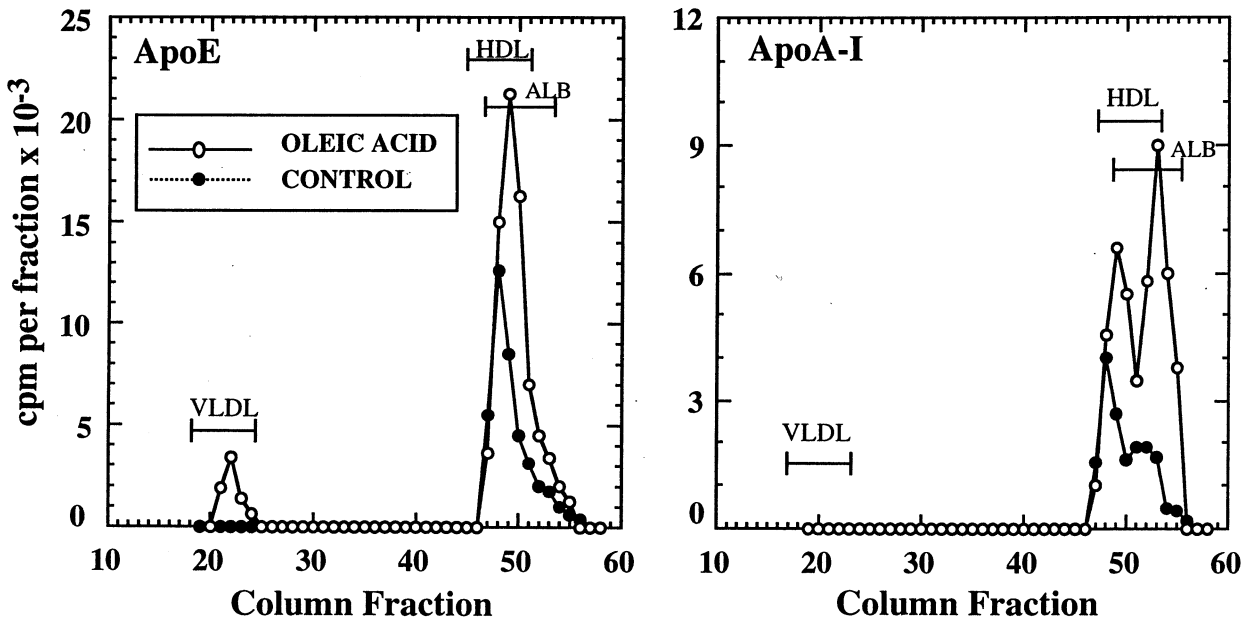

Figure 8 Distribution of secreted [^{35}S]-apolipoprotein E (apoE) and albumin (ALB) in hepatocyte-conditioned medium. One-day-old hepatocytes incubated in the presence (open circle) or absence of oleic acid (closed circle) were labeled continuously for 4 hours and the media were collected. The distribution of apoE and albumin was examined by agarose column chromatography, followed by immunoprecipitation of column fractions. The agarose column was initially standardized with rat plasma lipoproteins and albumin to identify all peaks associated with lipoproteins [very low density lipoproteins (VLDL), and high density lipoproteins (HDL)]. [^{35}S]-apolipoprotein A-I (apoA-I) was measured as a marker for HDL-containing fractions.

is also present in the primary hepatocyte. In light of our observations of an "across-the-board" stimulation of protein synthesis by hepatocytes cultured in the presence of fatty acid, the increased apparent rate of apoE secretion under this condition is most likely due to increased rates of secretion and not to diminished degradation.

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