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Enhanced Secretion of ApoB by Transfected HepG2 Cells Overexpressing Fibrinogen[1]

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HepG2 cells stably transfected with cDNA-encoding single fibrinogen chains overexpress fibrinogen and have increased (4-fold) secretion of apolipoprotein B. Overexpression of fibrinogen does not affect the secretion of three representative acute-phase proteins but causes a small increase in albumin secretion. Enhanced apolipoprotein B secretion is due to less intracellular degradation and not to increased expression. The increased secretion of apolipoprotein B is independent of the acute-phase response, since stimulation of fibrinogen gene expression by interleukin 6 did not affect secretion. HepG2 cells overexpressing fibrinogen chains had increased 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels, enhanced cholesterol production but normal levels of triglyceride and phospholipid synthesis and of sterol response binding proteins. These results, that associate overexpression of fibrinogen with enhance apolipoprotein B secretion, may be significant since epidemiological studies indicate that elevated levels of fibrinogen and lipids are independent risk factors in coronary artery disease.

Key Words: **fibrinogen; apoB; cholesterol; secretion; proteolysis.**

Fibrinogen is an acute-phase plasma glycoprotein with diverse physiological functions, although its primary role is in the final stages of blood coagulation when it forms a fibrin clot and promotes platelet aggregation. Fibrinogen also, directly and indirectly, participates in a number of other biological processes such as wound healing, development of blood vessels and also serves as a component of the extracellular matrix. This topic has been reviewed (1, 2). Apolipoprotein B (apoB) is a large (540 kDa) protein that is the principal structural protein in human LDL and VLDL particles. ApoB, an amphipathic protein, assembles lipids in the liver and intestine and the resulting lipoprotein particles are secreted (3, 4). A number of epidemiological studies have shown that elevated plasma levels of fibrinogen and LDL-cholesterol are associated with an increased risk for coronary artery disease (5–7). However, the reasons for the correlation of elevated plasma fibrinogen levels and vascular disease are not known. Recent studies with transgenic mice indicate that an elevated level of plasma fibrinogen, by itself, may not induce coronary artery disease (8).

Fibrinogen (340-kDa), a dimer composed of 2 pairs of non-identical polypeptide chains, Aα, Bβ and γ, is mainly expressed in hepatocytes and reaches wide-ranging values, with a mean average of about 2.5 mg/ml in the plasma of normal healthy adults. Fibrinogen is part of a subset of hepatic proteins whose expression and secretion may be increased, 2- to 10-fold, in response to tissue injury or inflammation (9). Thus the expression of fibrinogen is regulated at both the basal or constitutive level and as a member of the family of acute-phase proteins. In the acute phase response, expression of fibrinogen, together with that of C-reactive protein, α_1-antitrypsin, α_1-antichymotrypsin, α_2-macroglobulin, haptoglobulin, α_1-acid glycoprotein and the third-factor of complement, is elevated while those of albumin and transferrin may remain unaffected or decrease (10). Classified as a class II reactant, fibrinogen gene expression during the acute phase response is mainly mediated by interleukin 6 (IL-6) and by glucocorticoids (11–13). IL-6 acts on the Jak-STAT signaling pathway to phosphorylate, and activate, STAT-3, a transcription factor, that binds to a consensus sequence in the promoter region of all three fibrinogen genes (14, 15). In contrast to the other coagulation proteins, fibrinogen is expressed at relatively high levels under normal basal conditions and there may be different mechanisms for regulating the consti-

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ApoB, apolipoprotein B; LDL, low density lipoprotein; VLDL, very low density lipoprotein; IL-6, interleukin 6; SREBP, sterol responsive element binding protein; ER, endoplasmic reticulum; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin; DTT, dithiothreitol.

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tutive expression of the three fibrinogen genes. For example, HepG2 cells, a human hepatocellular carcinoma cell line, has surplus intracellular Aα and γ and smaller amounts of Bβ chains. The different amounts of intracellular fibrinogen chains could be due to a combination of different rates of expression and intracellular degradation (16–19). In HepG2 cells, overexpression of any one fibrinogen gene, elicited by transfection, leads to the concurrent up-regulation of the other 2 genes, suggesting coordinate gene expression (20, 21). However, the molecular basis of this coordinated expression is not understood. Characterization of the promoter regions of the three fibrinogen has not shown striking homology, suggesting that the genes may be independently expressed. The three fibrinogen genes however have many cis-acting elements in common (14, 22–27). In addition to these identified regulatory elements, there may be a number of other, yet unidentified, factors that are involved in regulating transcription of the three genes.

Epidemiological evidences support a direct correlation between certain polymorphisms in the 5′ flanking region of the Bβ gene and elevated circulating fibrinogen levels (28–30). A transactivation protein complex that associates with the Bβ gene promoter containing the $^{-455}$G/A polymorphism and affects transcription of the Bβ gene has been described (31) and $^{-455}$G/A and $^{-854}$G/A polymorphisms show increased basal rates of expression of the Bβ fibrinogen chain (32). These relatively common Bβ chain polymorphisms are associated with increased levels of plasma fibrinogen (33).

Regulation of apoB secretion is mostly a post-transcriptional event brought about by proteolytic degradation of unassembled apolipoprotein. The translocation of apoB and the availability of lipid substrates are two important factors in regulation of apoB secretion. Both translocation and lipid addition require the presence of microsomal triglyceride transfer protein (MTP) in the ER and increased supplies of lipid can inhibit the degradation of translocation-arrested apoB (34–37). In absence of either sufficient lipid (38, 39) or MTP (34–36), apoB translocation and lipoprotein assembly are blocked and the unassembled apoB is rapidly degraded by a ubiquitin-dependent proteasome pathway (40–42). Thus, synthesis of lipid is one of the important regulatory steps in apoB secretion. VLDL is rich in neutral lipids, mainly triacylglycerol, cholesterol and cholesterol esters. Hepatic cholesterol level is controlled through feedback mechanisms that endogenously regulate 2 enzymes, HMG-CoA synthase and HMG-CoA reductase, in the mevalonate pathway and by regulating the expression of LDL receptors which are involved in the uptake of exogenous cholesterol (43). Enzymes and receptors involved in synthesis and uptake of cholesterol and fatty acid are regulated by the sterol responsive element binding protein (SREBP) family of transcription factors which emanate from an ER membrane protein following sterol-regulated proteolysis. This topic has been reviewed (44).

In this study we show that transfection of HepG2 cells with cDNA that causes overexpression of fibrinogen is accompanied by increased apoB secretion. Enhanced apoB secretion is due to inhibition of apoB degradation, which may be promoted by increased cholesterol synthesis. This finding may be significant given the epidemiological data, which indicate that elevated plasma levels of fibrinogen and cholesterol are independent risk factors in coronary artery disease.

MATERIALS AND METHODS

Antibodies. A rabbit polyclonal antibody that recognizes the 3 component chains of fibrinogen was purchased from Dako Corp (Carpenteria, CA). Also purchased from Dako Corp. were rabbit polyclonal antibodies to human albumin, α_1-antichymotrypsin and α_2-macroglobulin. Polyclonal rabbit antibody to human apoB was obtained from Calbiochem (La Jolla, CA) and a goat antiserum to α_1-antitrypsin was from ICN (Costa Mesa, CA). A rabbit polyclonal antibody to the precursor (p125) and mature forms (p68) of SREBP-1 and a goat polyclonal IgG to SREBP-2 were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The rabbit anti-angiotensinogen serum (45) was a generous gift from Drs. X. Jeunemaitre and J. Celerier.

Transfection and selection of stable cell lines. HepG2 cells were transfected by the calcium precipitation method with an expression vector (pRSVNeo) which contains full-length Aα, Bβ or γ cDNA and a geneticin resistant gene. The control cells were transfected with the same expression vector, lacking cDNA. The vectors and procedures have been previously described (20, 21). Stable cell lines were selected with 0.6 mg/ml geneticin. The amount of fibrinogen produced by the selected cell lines was measured by metabolically labeling the cells with L-[^{35}S]methionine (NEN, Boston, MA) for 2 h and determining the amount of radioactive fibrinogen secreted into the medium. The cells transfected with fibrinogen cDNA are termed Aα-HepG2, Bβ-HepG2 and γ-HepG2 cells and the control cells Neo-HepG2.

Metabolic labeling and isolation of fibrinogen, apoB, and other secreted proteins. Aα-HepG2, Bβ-HepG2, γ-HepG2 and Neo-HepG2 cells were incubated for 2 h at 37°C in methionine-free minimal essential medium containing approximately 500 μCi/ml of L-[^{35}S]methionine. The incubation media was collected and the radioactivity in total trichloroacetic acid insoluble proteins and in specific secreted proteins was determined. Secreted proteins were isolated, as previously described from the incubation media by immunoprecipitation (17, 18), separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), detected by autoradiography and the relative amounts measured by densitometry.

In some cases the HepG2 cells were pulse-labeled with L-[^{35}S]methionine for 15 min as described above and then "chase" incubated by first washing with phosphate-buffered saline and then incubating for 2 h at 37°C with 20 mM L-methionine in minimal essential medium. Secreted and retained apoB were isolated separately from the medium and from the cell lysate. When determining apoB synthesis, the cells were pulse-labeled for 15 min with L-[^{35}S]methionine and intracellular apoB was isolated from the cell lysate. For a time course of apoB secretion, HepG2 cells were pulse-labeled for 15 min with [^{35}S]methionine and then chase incubated for periods up to 2 h. At the end of each chase period the incubation medium was collected and replaced with fresh medium. ApoB was isolated by immunoprecipitation from the collected medium.

Treatment with interleukin-6. Bβ-HepG2, and Neo-HepG2 cells were preincubated for 16 h with, or without, 10 ng/ml IL-6 purchased

from Gibco Life Technologies (Gaithersburg, MD). The cells were then incubated for 2 h with L-[^{35}S]methionine, as described above, again without or with 10 ng/ml IL-6.

Northern blot analysis. Total RNA was isolated from Neo-HepG2 and Bβ-HepG2 cells and 10 μg RNA was separated by electrophoresis on 0.8% agarose gels. The RNA was transferred to nylon membrane and hybridized with the appropriate ^{32}P-labeled cDNA. Human apoB and HMG-CoA reductase cDNA probes were obtained from the American Type Culture collection (Rockville, MD) and β-actin cDNA was purchased from Clontech (Palo Alto, CA).

Western blot of SREBP. Total proteins from Neo- and Bβ-HepG2 cells, separated by SDS–PAGE, were electroblotted onto nitrocellulose membranes. The nonspecific binding sites of the membranes were blocked using 7% BSA, followed by addition of the polyclonal rabbit antibodies to SREBP-1or SREBP-2. The amount of primary antibody bound to the proteins was detected using an Immun-star chemiluminescence kit.

Determination of lipid biosynthesis. Neo- and Bβ-HepG2 cells were incubated in serum-containing medium with 5 μCi/ml [^{3}H]acetate for 2 h. Lipids were extracted from the cells with chloroform/methanol (2:1 v/v), and separated on silica gel thin layer chromatography (TLC) plates. The lipids were identified with iodine vapor and the spots on the TLC plates were cut and radioactivity determined.

RESULTS

Increased Secretion of Fibrinogen and ApoB, but Not of Acute-Phase Proteins, by HepG2 Cells Transfected with Fibrinogen cDNA

Control (Neo-HepG2) cells and those transfected with a single fibrinogen chain cDNA (Aα-HepG2, Bβ-HepG2 and γ-HepG2) were metabolically labeled for 2 h with L-[^{35}S]methionine and the amount of radioactive fibrinogen and apoB secreted into the incubation media was measured. As previously reported, HepG2 cells transfected with any one of the fibrinogen chain cDNAs resulted in an increased synthesis and secretion of fibrinogen and Bβ-HepG2 cells secreted more fibrinogen than Aα-HepG2 and γ-HepG2 cells (20, 21) (see Fig. 1, lanes 1 and 2). All of the transfected cells that overexpressed fibrinogen secreted more apoB than the control Neo-HepG2 cells (Fig. 1, lanes 3 and 4).

Since Bβ-HepG2 cells were more effective than Aα-HepG2 and γ-HepG2 cells in overexpressing fibrinogen, subsequent experiments were only performed with Bβ-HepG2 cells. The amount of 3 representative acute-phase proteins (α_1-antitrypsin, α_1-antichymotrypsin, and α_2-macroglobulin) secreted into the medium was measured. Also determined was the secretion of angiotensinogen, which differs from the other acute-phase protein in that it is a class I reactant protein and its expression is affected by NFκB (46). Compared to the control Neo-HepG2 cells, there was no difference in the amounts of α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin secreted by Bβ-HepG2 cells but there was a small, 1.3-fold, increase in the amount of albumin secreted (Fig. 2). There was no difference in the amount of angiotensinogen secreted by Neo- and Bβ-HepG2 cells (data not shown).

To exclude the possibility that the correlation between enhanced fibrinogen expression and increased apoB secretion is due to an artifact of transfection, or was due to selection of a special cell line, we measured 11 different clones which were transfected with Bβ cDNA, each of which over-expressed fibrinogen. All 11 of the clonal cell lines that over-expressed fibrinogen exhibited enhanced secretion of apoB, when compared to 3 control clones and to the pool of HepG2 cells transfected with the empty vector. Some Hep-G2 cells transfected with Bβ cDNA did not over-express fibrinogen and these cells did not have enhanced secretion of apoB. Thus the correlation of enhanced fibrinogen expression and increased apoB secretion is specific and reproducible in 11 different clones. These data demonstrate that increased fibrinogen levels in HepG2 cells, elicited by over-expression of a single fibrinogen chain, is associated with enhanced apoB secretion.

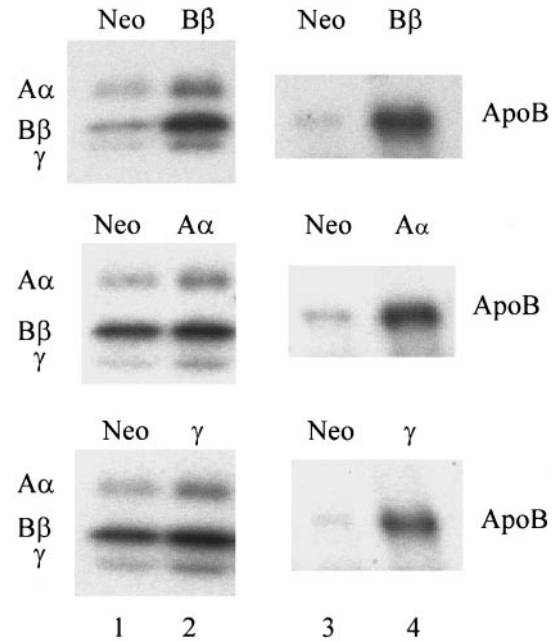

FIG. 1. Increased apoB secretion accompanies overexpression of fibrinogen. Neo-HepG2, Aα-HepG2, Bβ-HepG2, and γ-HepG2 cells were metabolically labeled with L-[^{35}S]methionine for 2 h and secreted radioactive fibrinogen and apoB were isolated from the incubation medium by immunoprecipitation and reduced SDS–PAGE. Radioactivity was detected by autoradiography and the areas containing the fibrinogen chains and apoB are shown. The top panels compare Neo-HepG2 and Bβ-HepG2 cells; the middle panels compare Neo- and Aα-HepG2 cells, and the bottom panels compare Neo- and γ-HepG2 cells. Lanes 1 and 2 show the component chains of secreted fibrinogen and lanes 3 and 4 show secreted apoB. Lanes 1 and 3 are from Neo-HepG2 cells and lanes 2 and 4 are from HepG2 cells overexpressing fibrinogen.

Enhanced Synthesis of Fibrinogen, Elicited by Interleukin-6, Does Not Affect apoB Secretion

Fibrinogen is an acute-phase protein and the synthesis of its 3 component chains is induced by IL-6. To determine if stimulation of fibrinogen synthesis, as

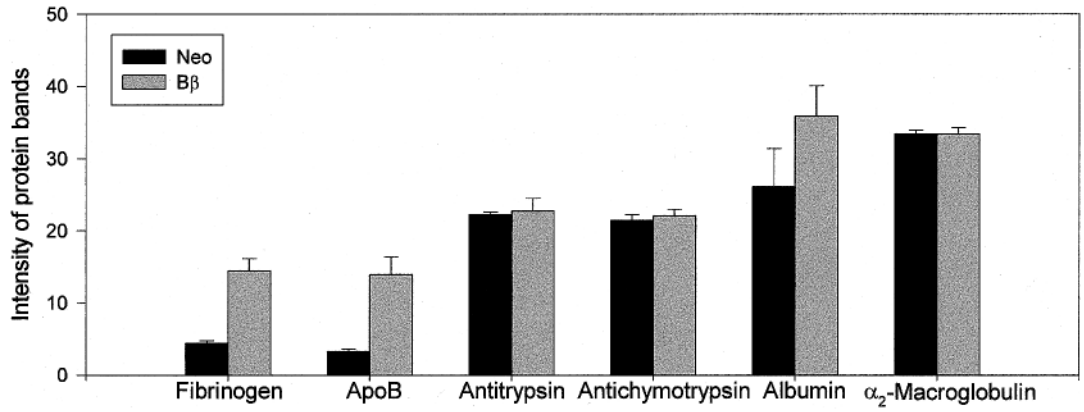

FIG. 2. Secretion of fibrinogen, apoB, albumin, and three acute-phase proteins. Neo-HepG2 and Bβ-HepG2 cells were metabolically labeled as described in the legend to Fig. 1. Secreted proteins were isolated by immunoprecipitation and SDS–PAGE, detected by autoradiography, and quantified by densitometry. Values represent the means ± SD of protein intensity from 3 different cell clones in each group of cells.

part of the acute-phase response, also increases apoB secretion, both Neo-HepG2 and Bβ-HepG2 cells were treated with IL-6 and the amount of secreted fibrinogen and apoB determined.

Treatment of both Neo-HepG2 and Bβ-HepG2 cells with IL-6 resulted in increased secretion of fibrinogen. IL-6 however had no effect on the secretion of apoB either by Neo-HepG2 or by Bβ-HepG2 cells (Fig. 3).

Overexpression of Fibrinogen Leads to Increased Secretion, Not Expression, of ApoB

Expression of apoB by Neo-HepG2 and Bβ-HepG2 cells was measured by determining mRNA levels by Northern blots and determining initial protein synthesis, utilizing a 15-min pulse-incubation with L-[^{35}S]methionine. Northern blot analysis of apoB mRNA indicated that there was no difference in the mRNA levels of Neo-HepG2 and Bβ-HepG2 cells. Figure 4 shows an autoradiogram of the Northern blots for apoB and actin. Measurements of the densities of the apoB bands, normalized for equal amounts of actin, showed near equal levels of apoB mRNA in Neo-HepG2 and Bβ-HepG2 cells.

ApoB is an unusually large protein and after 15 min of pulse-incubation with L-[^{35}S]methionine there is a mixture of complete and incomplete chains detected intracellularly. Because of this, isolated radioactive apoB migrates as a streak on SDS–PAGE. There was little or no difference, in the amounts of apoB synthesized by Neo-HepG2 and Bβ-HepG2 cells (Fig. 5A, lanes 1 and 2). Because of streaking it is difficult to quantitate the amounts by densitometry, but measurements of the amount of radioactive protein isolated by immunoprecipitation with antibody to apoB confirmed that there was no difference.

Although both Neo-and Bβ-HepG2 cells synthesize equal amounts of apoB after 15 min, there was a large difference in the amount of apoB secreted after a 2-h chase incubation. The Bβ-HepG2 cells secreted 3-times more apoB than the Neo-HepG2 cells (Fig. 5A, lanes 3 and 4). Quantitation of the amount of radioactive apoB

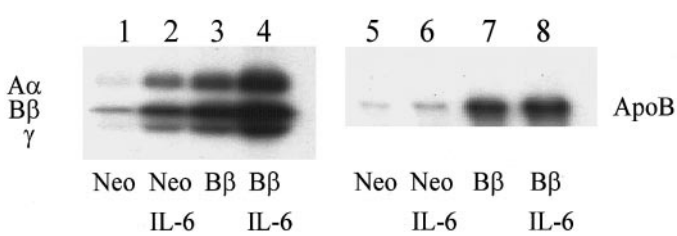

FIG. 3. IL-6 increases fibrinogen secretion but does not affect apoB. Neo-HepG2 and Bβ-HepG2 cells were preincubated with or without 10 ng/ml of IL-6 for 16 h prior to a 2-h incubation with L-[^{35}S]methionine also in the presence or absence of IL-6. Secreted apoB and fibrinogen were determined as in Fig. 1. Autoradiograms are shown. Lanes 1–4 contain fibrinogen and lanes 5–8 apoB. Lanes 1, 2, 5, and 6 are from Neo-HepG2 and lanes 3, 4, 7,and 8 are from Bβ-HepG2 cells. IL-6 treated cells are in lanes 2, 4, 6, and 8.

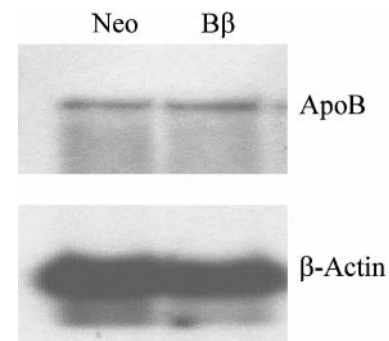

FIG. 4. Northern blot analysis of apoB. Total RNA of Neo- and Bβ-HepG2 cells was analyzed for apoB mRNA by Northern blot. As a control the amount of actin mRNA was also determined. The upper autoradiogram shows the amount of apoB and the lower panel the amount of actin.

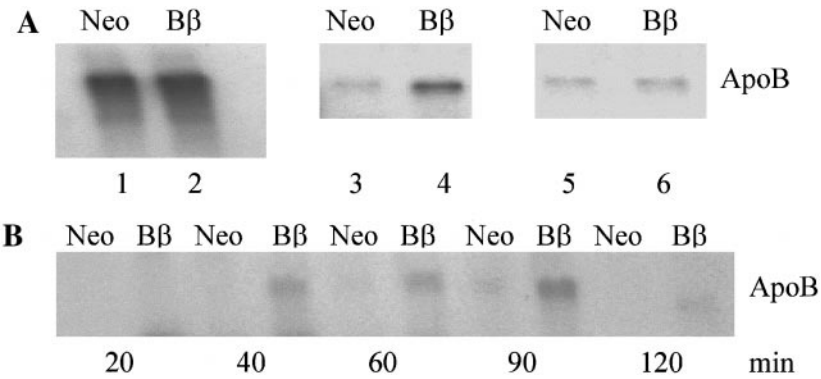

FIG. 5. Overexpression of fibrinogen affects the secretion but not the initial synthesis of apoB. In A, Neo- and Bβ-HepG2 cells were pulse-labeled for 15 min with L-[^{35}S]methionine and chase incubated for 2 h. The amounts of radioactive intracellular apoB at 15 min and after 2 h chase are shown in lanes 1 and 2 and lanes 5 and 6, respectively. ApoB secreted into the medium after 2-h chase is shown in lanes 3 and 4. Lanes 1, 3, and 5 are from Neo-HepG2 and lanes 2, 4, and 6 from Bβ-HepG2 cells. In B, Neo- and Bβ-HepG2 cells were pulse-chase incubated as described above. At the indicated chase times, the medium was collected and replaced with fresh medium.

synthesized at the end of the 15 min pulse period and that recovered intracellularly and secreted at the end of the 2-h chase period, showed that, as has been noted by others (47), the large majority of nascent apoB is degraded intracellularly and not secreted. In Neo-Hep G2 cells 86% of apoB was proteolytically degraded compared to 78% in Bβ-Hep G2 cells. Because normally a large percent of apoB is not secreted, the relatively small percent inhibition of degradation that occurs in Bβ Hep G2 cells is reflected as a large percent increase in the amount of apoB secreted.

A time course of secretion, in which the incubation media were collected and replaced with fresh media at set intervals, demonstrated that there was about a 40 min intracellular transit time for apoB, with maximum secretion occurring between 60 and 90 min. After 2 h of chase, secretion of pulse-labeled apoB had nearly ceased (Fig. 5B). The time course of secretion of Bβ-HepG2 and Neo-HepG2 cells were similar, except that the Bβ-HepG2 cells secreted more apoB at all times.

Degradation of apoB occurs through the ubiquitin-proteasome pathway (40–42). To determine if direct inhibition of degradation with a specific inhibitor of the proteasome system affects apoB secretion in both Bβ-HepG2 and Neo-HepG2 cells; the cells were treated with MG132 for 4 h and apoB secretion was determined. Both Bβ- and Neo-HepG2 cells, treated with MG132, secreted more apoB than untreated cells indicating that inhibition of degradation, by itself, is sufficient to allow undegraded apoB to be secreted (Fig. 6).

mRNA Levels of HMG-CoA Reductase in Neo- and Bβ-HepG2 Cells

Regulation of apoB secretion is influenced by the availability of lipids destined to be secreted as components of LDL and VLDL particles. HMG-CoA synthase and HMG-CoA reductase are 2 key enzymes that are sensitive to feedback mechanisms regulating cholesterol synthesis via the mevalonate pathway (43). We chose to determine the mRNA levels of HMG-CoA reductase in Neo- and Bβ-HepG2 cells as an index of cholesterol synthesis. Northern blot analysis showed that Bβ-HepG2 cells had 3 times more HMG-CoA reductase mRNA than Neo-HepG2 cells (Fig. 7).

Overexpression of Fibrinogen Bβ Chain Increases the Synthesis of Cholesterol Lipid but Does Not Affect Sterol Response Element Binding Protein Levels

Since the cellular levels of mature SREBP-1 and SREBP-2 influence the expression of the mRNA encoding HMG-CoA reductase (43), we measured SREBP-1 and SREBP-2 by Western immunoblotting. Protein fractions from Neo- and Bβ-HepG2 cells were analyzed. There was no difference in the amounts of the 125-kDa precursor or of the 68 kDa matures SREBP-1 present in Bβ-HepG2 and Neo HepG2 cells. An antibody to SREBP-2, with epitope mapping at the amino terminus of SREBP-2, also did not show any difference in the amounts of SREBP-2 (data not shown).

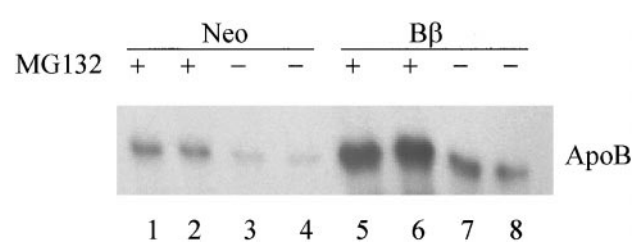

FIG. 6. MG132 enhances apoB secretion. Neo- and Bβ-HepG2 cells were pretreated with or without MG132 for 2 h prior to a 2-h incubation with L-[^{35}S]methionine, also in the presence or absence of MG132. Lanes 1, 2, 5, and 6 (+) contained MG132 and lanes 3, 4, 7, and 8 (−) did not. Lanes 1, 2, 3, and 4 are from Neo-HepG2 and lanes 5, 6, 7, and 8 are from Bβ-HepG2 cells.

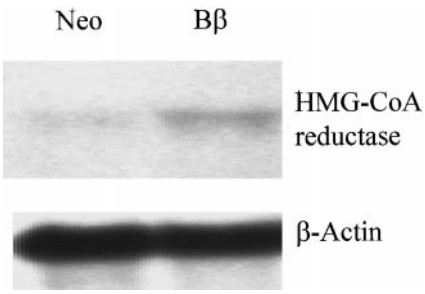

FIG. 7. Expression of HMG-CoA reductase. Total RNA was analyzed for mRNA levels of HMG-CoA reductase and actin by Northern blot analysis. ^{32}P-labeled probes for HMG-CoA reductase and actin were used. Autoradiograms are shown. The upper panel shows HMG-CoA reductase and the lower panel shows actin.

To determine lipid synthesis, Bβ-HepG2 and Neo-HepG2 cells were incubated with [^{3}H]acetate for 2 h and radioactivity in various lipid fractions was determined (Fig. 8). Compared with Neo-HepG2 cells, Bβ-HepG2 cells synthesized 3 times more cholesterol and 1.6 fold more cholesterol esters. However, there were no significant difference in the synthesis of triglycerides and phospholipids.

DISCUSSION

Elevated blood levels of fibrinogen are correlated with several risk factors for coronary artery disease, including LDL cholesterol (5–7, 48, 49). ApoB is the principal structural protein in LDL and its secretion is regulated by intracellular proteolysis, which depends on the availability of lipids destined to be secreted. Proteolysis of nascent apoB and unassembled fibrinogen chains (19) is mediated by the cytosolic ubiquitin-proteasome system (41, 42). Hepatic cholesterol synthesis is balanced by the supply of external cholesterol, obtained through the LDL receptor, and by compensatory synthesis, through the mevalonate pathway. The expression of both LDL receptor and of at least 2 key regulatory enzymes in the mevalonate pathway, HMG-CoA reductase and HMG-CoA synthase, are in turn regulated by a family of transcription factors, SREBPs, that emanate from an ER membrane protein by sterol-regulated proteolysis (44). Our studies show that increased fibrinogen production, in HepG2 cells, elicited by transfection of fibrinogen cDNA, but not by the acute-phase response, increases cholesterol lipid synthesis and apoB secretion. Although the SREBPs are essential transcription factors necessary for activation of HMG CoA reductase expression, there was no detectable increase in the amount of these proteins present in Bβ-HepG2 cells, suggesting that co-regulatory factors may be primarily affected.

The role of hepatic free and esterified cholesterol in influencing degradation, assembly and secretion of apoB-containing lipoproteins, is controversial. ApoB secretion is affected both *in vitro* (50–53) and *in vivo* (54–56) by the rate of cholesterol synthesis and esterification. However, several studies with HepG2 cells argue against the regulation of apoB secretion by the availability of free cholesterol and/or cholesterol esters (57–59). A recent study has shown that proteasome degradation of translocation-arrested apoB can be regulated by a sterol-sensitive polyubiquitin conjugation step (37). Our studies are complementary, showing that Bβ-HepG2 cells have increased cholesterol synthesis and that degradation of apoB, via the ubiquitin-proteasome pathway, is inhibited.

The association of increased fibrinogen expression and enhanced apoB secretion is not due to the selection, by chance, of a cell line that over secretes apoB, since 11 different clones, all of which over-expressed fibrinogen, had enhanced apoB secretion. Also, cell lines that were derived from cells transfected with the same expression vector containing Bβ cDNA, but did not over-express fibrinogen, did not have enhanced apoB secretion. Further evidence against the notion that the increased apoB secretion is a transfection artifact is that others have transfected HepG2 cells with an expression vector containing a cDNA different to fibrinogen, a minigene for apolipoprotein(a), and those stably transfected HepG2 cells did not exhibit increased secretion of apoB (60).

Fibrinogen expression is regulated at different two levels. At the level of constitutive or basal expression and during the acute-phase response, principally induced by IL-6 and glucocorticoids, when there is a coordinate enhanced expression of the 3 genes. Increased fibrinogen production, caused by IL-6, did not affect apoB secretion clearly showing that increased fibrinogen expression, as part of the acute phase re-

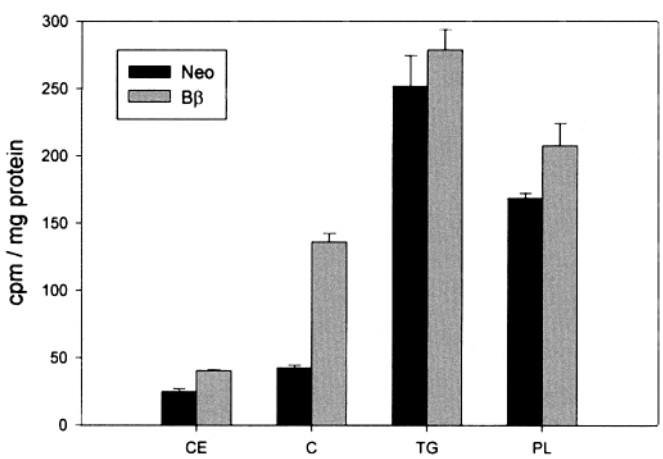

FIG. 8. Lipid biosynthesis. Neo- and Bβ-HepG2 cells were incubated with [^{3}H]acetate (5 μCi/ml) for 2 h. Radiolabeled lipids were extracted with chloroform/methanol, and cholesterol ester (CE), cholesterol (C), triglyceride (TG), and phospholipids (PL) were separated by TLC plates and quantitated by determining radioactivity. Values represent the means ± SD of triplicate plates of cells.

sponse, does not cause enhanced apoB secretion. On the other hand, transfection with Bβ cDNA did not affect other acute-phase proteins, as determined by measuring secretion of α1-antichymotrypsin, α1-antitrypsin and α2-macroglobulin. Earlier studies also showed that over-expression of fibrinogen did not increase the amounts of C-reactive protein secreted (20, 21). Thus, under these experimental conditions, apoB secretion is enhanced primarily due to increased expression of basal levels of fibrinogen brought about by transfection of individual fibrinogen cDNAs. Increased basal expression of fibrinogen, reflected in elevated plasma fibrinogen levels, has been documented in some genetic variants of fibrinogen which have polymorphisms in the promoter region of the Bβ gene (30, 32, 61). Thus, although basal over-expression of fibrinogen, caused by transfection, is not a physiological event this procedure may expose underlying mechanisms that are normally in play, perhaps at a lower level, in genetic variations that affects basal expression of fibrinogen.

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