# Effects of overexpression of the amino-terminal fragment of apolipoprotein B on apolipoprotein B and lipoprotein production

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**Abstract** **In vitro studies have shown that the binding site for microsomal triglyceride transfer protein (MTP) is within the first 17% of apoB (apoB-17). Expression of apoB-48 in McArdle cells decreases endogenous lipoprotein production; however, overexpression of human apoB in transgenic mice does not decrease endogenous mouse apoB expression. To assess this inconsistency, adenoviruses expressing human apoB-17 (AdB17) or apoB-17-β (which contains apoB-17 plus a small lipid-binding β-sheet region of apoB, AdB-17β) were produced. Hepatoma cells were infected with AdB17 or AdB17-β with AdLacZ, an adenovirus expressing β-galactosidase, as a control. Overexpression of apoB-17 and apoB-17-β in hepatoma cells to levels 2- to 3-fold greater than that of endogenous apoB did not alter endogenous apoB production. This was also true in the presence of oleic acid and *N*-acetyl-leucyl-leucyl-norleucinal. High levels of apoB-17 or β-galactosidase expression reduced apoB-100 production; however, control protein production was also reduced. To assess the effects of apoB-17 expression in vivo, mice of three different strains were injected with AdB17. Two days after injection, plasma apoB-17 was approximately 24 times the amount of endogenous apoB in the C57BL/6 mice, 2 times the apoB-100 in human apoB transgenic mice, and 4 times the apoB-48 in apoE knockout mice. Overexpression of apoB-17 did not decrease apoB-100 or apoB-48 concentrations in mouse plasma as assessed by Western blot analysis. These results demonstrate that although the apoB-17 binds to MTP in vitro, it does not alter endogenous apoB expression in mice or in hepatoma cells.**—Li, Z., Y. Kako, L. Pang, M. W. Freeman, J. M. Glick, X. Wang, and I. J. Goldberg. **Effects of overexpression of the amino-terminal fragment of apolipoprotein B on apolipoprotein B and lipoprotein production.** ***J. Lipid Res.*** **2000.** 41: **1912–1920.**

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Apolipoprotein B (apoB) is the major protein component of the two known atherogenic lipoproteins, low density lipoproteins (LDL) and remnants of triglyceride-rich lipoproteins. Because of the importance of apoB in lipid metabolism and in the pathology of atherosclerosis, apoB production has been intensively studied. Two different sized isoforms of the apoB polypeptide are found in animal plasma. One is apoB-100, with a full-length apoB protein of 4,536 amino acids; the other is apoB-48, with 48% of the molecular weight of apoB-100 (1). In humans, apoB-100 is primarily expressed in the liver, and is secreted into the plasma. It is the major protein in very low density lipoproteins and LDL. ApoB-48 is secreted by human and mouse intestines in large triglyceride-rich particles called chylomicrons. Mouse liver, however, secretes both apoB-100 and apoB-48 (2).

Although produced from different tissues and on lipoproteins with different sizes, the synthesis of both apoB-48 and apoB-100 is mainly regulated at the postranslational level (3). After apoB translation, nascent apoB is translocated into the endoplasmic reticulum (ER) lumen. It is widely, although not universally, believed that apoB translocation transiently halts because of pause transfer sequences (4). Complete apoB translocation requires correct folding of its N-terminal region and an association with lipid. Microsomal triglyceride transfer protein (MTP), a 97-kDa

Abbreviations: AdB17, adenovirus expressing apoB-17; AdB17-β, adenovirus expressing apoB-17-β; AdLacZ, adenovirus expressing β-galactosidase; ALLN, *N*-acetyl-leucyl-leucyl-norleucinal; apoB, apolipoprotein B; apoB-17, N-terminal 17% of apoB; apoB-17-β, apoB-17 fused with β sheet of apoB; apoE, apolipoprotein E; CHO cell, Chinese hamster ovary cell; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; ER, endoplasmic reticulum; FBS, fetal bovine serum; FPLC, fast protein liquid chromatography; LDL, low density lipoproteins; LpL, lipoprotein lipase; MEM, minimum essential medium; MOI, multiplicity of infection; MTP, microsomal triglyceride transfer protein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; VLDL, very low density lipoproteins.

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subunit of a heterodimer with protein-disulfide isomerase, is needed to add this lipid to newly synthesized apoB. A second lipid addition is required to produce fully mature lipoproteins. A deficiency of either MTP or available lipid leads to an increase in apoB degradation (5–7).

The amino-terminal 17% of apoB (apoB-17) is crucial for apoB translocation, assembly, and secretion. In vitro data generated by Hussain, Bakillah, and Jamil (8) indicated that binding of apoB to MTP decreases as the length of apoB increases. They also showed that amino acids 430–570, which fall within apoB-17, contain the MTP-binding region (9). Overexpression of apoB or the MTP-binding portion of apoB contained within apoB-17 should eventually produce a situation in which MTP is rate limiting for lipoprotein production in cells or animals. This may have been the case when apoB-48 was expressed in rat hepatoma cells by transfection (10), where it was found that the endogenous apoB-100 production was decreased by 50%. However, when Nicodeme et al. (11) stably transfected McArdle cells with constructs expressing various length of apoB, only apoB fragments larger than B-53 were inhibited by a low dose of an MTP inhibitor. They suggested that MTP interacts with a region of apoB between apoB-48 and B-53. Other data suggest that intracellular MTP is not rate limiting for apoB production. Transgenic mice overexpressing human apoB did not decrease their secretion of endogenous mouse apoB (12). These apparently disparate results could be due to the different systems used. In addition, the in vivo experiments may not have been under limiting conditions, that is, these investigations were performed in mice that consumed a diet with an excess of fat and cholesterol, as well as cholic acid. Cholic acid, by itself, decreases apoB-100 production (12) and might produce conditions in which neither MTP nor the movement through the translocation channel is limiting.

To assess the effects of apoB-17 overexpression, recombinant adenoviruses were produced that expressed apoB-17 or apoB-17 fused to a lipid-binding region from amino acids 1333 to 1569 (B-29 to -34). Despite the marked overexpression of the MTP-binding portion of apoB, no effect on apoB-100 or apoB-48 expression was found in cells or mice. Therefore, although MTP deficiency will decrease apoB production, under normal conditions even marked overexpression of MTP-binding regions of apoB will not inhibit apoB-100 production.

## MATERIALS AND METHODS

### Cells

HepG2 cells were grown in minimum essential medium (MEM); containing 10% fetal bovine serum (FBS). 293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, and McArdle-RH7777 cells were grown in DMEM containing 10% FBS and 10% horse serum. All cells were cultured at 37°C with 5% $CO_2$.

### Mice

C57BL/6J and apoE knockout mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Human apoB transgenic mice were a gift from S. Young (Gladstone Institute of Cardiovascular Disease, San Francisco, CA). All mice were housed in a conventional facility with a 12-h light/dark cycle and temperature of 25°C. They were given free access to water and chow diet (PMI Nutrition International, St. Louis, MO). All mice were 10-week-old males and were fasted for at least 5 h before blood was drawn by retroorbital phlebotomy. Blood samples were collected into tubes containing 1 mM ethylenediaminetetraacetic acid (EDTA) and plasma samples were separated by centrifugation.

### Production of AdB17 and AdB17-β

To generate the adenovirus expressing apoB-17, the cDNA encoding this fragment was cloned into the vector pACE, which contains sequence homologous to the adenoviral genome. Plasmid DNA was cotransfected with adenoviral DNA in 293 cells to generate replication-defective adenovirus expressing apoB-17 by homologous recombination. Plaques were picked, expanded, and screened by polymerase chain reaction (PCR) and Western blotting for production of apoB-17. The virus was further subjected to two rounds of plaque purification and rechecked for transgene expression prior to large-scale production (13). To generate the adenovirus expressing apoB-17-β, a segment of apoB cDNA encoding the β-sheet region was amplified by PCR with primers 5′-cagccctccaattcaagtctgatcgattccat and 5′-ggcaaagt tcttatacttcccattgtcgaccta. This PCR product was digested with *Xho*I and blunted with T4 DNA polymerase followed by *Sal*I digestion. The construct containing apoB-17 (13) was digested with *Cla*I and filled in and then digested with *Sal*I. These two pieces of DNA were ligated with T4 DNA ligase. This new construct (pACEB17-β) encoding apoB-17-β was sequenced at the junction between apoB-17 and β sheet to confirm that it was in frame. Adenoviruses carrying the sequence encoding apoB-17 with β sheet (AdB17-β) were constructed in a two-cosmid vector system developed at the Nessel Gene Therapy Center (Massachusetts General Hospital, Boston, MA). ApoB sequences were excised from pACEB17-β using *Age*I and *Sal*I, after blunt end filling of the *Age*I site. This fragment was ligated into a *Hin*dIII (blunt end filled) and *Sal*I-digested AdV vector plasmid (pLEP4) containing the left end of the adenoviral genome. This plasmid was directly ligated to a cosmid containing the right end of the adenoviral genome, pREP7, that contained an E3 gene deletion. The ligation mixture was packaged using bacteriophage lambda cos sites engineered into the vectors and then cloned in bacteria. The resultant cosmid DNA was used to transfect 293 cells in order to generate recombinant adenoviruses. The recombinant adenoviruses were expanded in 293 cells, purified by cesium chloride centrifugation, and stored in phosphate-buffered saline (PBS) with 10% glycerol at −80°C (14).

### Infection of cells with adenovirus

Cells were cultured in six-well plates until a confluent monolayer was formed. After removing the medium, 0.1 ml containing differing amounts of viral particles was added to each well. After a 1-h incubation, 2 ml of conditioned medium with 2% FBS was overlaid. The cells were then grown for 24 h before experiments were performed.

### Radioactive labeling and use of oleate and ALLN

After a 24-h incubation, the cells were washed with PBS. Leucine-free medium containing [$^3$H]leucine and 2% bovine serum albumin was added to each well and newly synthesized apoB protein was assessed, usually after a 2-h incubation. For oleate treatment, 0.4 mM oleate was added in the labeling medium. In some experiments, the proteolysis inhibitor *N*-acetyl-leucyl-leucyl-norleucinal (ALLN, 2 μg/ml) was used in the labeling medium.

### Cell lysis and immunoprecipitation

In all experiments, media were collected in a similar manner. Cells were washed twice with PBS and harvested in lysis buffer [62.5 mM sucrose, 0.5% sodium deoxycholate, 0.5% Triton X-100, leupeptin (50 μg/ml), pepstatin A (50 μg/ml), phenylmethylsulfonyl fluoride (PMSF, 150 μg/ml), 5 mM EDTA, 50 mM Tris-HCl (pH 7.4), and 150 mM NaCl].

Immunoprecipitation of apoB in cell lysates and media was performed as follows: Samples (0.5 ml) were mixed with 2× NET buffer to make final 1× NET [150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.4), 0.5% Triton X-100, and 0.1% sodium dodecyl sulfate (SDS)]. Protease inhibitors were added to the medium samples, with final concentrations of 1 mM benzamidine, 0.86 mM PMSF, and aprotinin (100 kallikrein-inactivation units/ml) in 10 mM *N*-2-hydroxylethylpiperazine-*N*′-2-ethanesulfonic acid, pH 8.0. Two microliters of anti-human apoB antiserum purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN) was added to each sample and the mixture was incubated on a shaker for 10 h at 4°C. Protein A-Sepharose CL-4B (Amersham Pharmacia Biotech, Piscataway, NJ) was added to the mixture and the incubation was continued for an additional 3 h. The beads were washed three times with 1× NET. ApoB was separated from the protein A pellet with sample buffer [0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, and 10% mercaptoethanol] by boiling for 2 min. In some experiments using McArdle cells, parallel experiments were performed with the antiserum produced by Sparks et al. (15).

### SDS-PAGE and Western blot analysis

An aliquot of each sample from immunoprecipitation or mouse plasma was separated by 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). For the labeled samples, the gel was treated with autofluor (National Diagnostics, Atlanta, GA) and, after drying, was exposed to X-Omat AR film (Eastman Kodak, Rochester, NY) at −80°C. For those samples without radioactive labeling, Western blot analysis was performed with sheep anti-human apoB (Boehringer Mannheim Biochemicals). The second antibody was rabbit anti-sheep IgG. Western blotting, membrane treatment, and second antibody detection were performed according to the procedure provided with the ECL system manufactured by Amersham Pharmacia Biotech.

### Injection of adenovirus into mice

Mice were warmed by a heating lamp before injection. Each mouse was injected with $1 \times 10^{11}$ particles of adenovirus in 0.1 ml of PBS via the tail vein. Two to 3 days later, 200 μl of blood was collected by retroorbital bleeding and kept on ice with 1 mM benzamidine, 0.5 mM PMSF, aprotinin (100 kallikrein-inactivatin units/ml), and 5 mM EDTA. The inhibitors were required to prevent the degradation of plasma apoB. The plasma samples, in Eppendorf tubes, were separated from blood by spinning at 7,000 rpm for 3 min at 4°C.

### Lipid and lipoprotein analysis

To examine the lipid profile of mice injected with AdB17, plasma was analyzed by fast protein liquid chromatography (FPLC) as described in another publication from this laboratory (16). The triglycerides and cholesterol of each lipoprotein species were analyzed by standard methods, using commercially available kits.

### Analysis of plasma apoB and its fragments

The endogenous apoB production was analyzed by immunoprecipitation followed by Western blot analysis. The plasma was separated from 200 μl of blood by centrifugation at 10,000 rpm for 5 min at 4°C. Then, 0.5 μl of plasma from each mouse was analyzed by SDS-PAGE and Western analysis was performed.

### Analysis of postheparin lipoprotein lipase activity

Postheparin plasma was obtained from fasting mice to measure lipoprotein lipase (LpL) activity. The animals were injected with 10 units of heparin (Elkins-Sinn, Cherry Hill, NJ) intravenously via the tail vein. Blood was obtained by retroorbital phlebotomy 5 min later and plasma was frozen at −70°C within 30 min. Postheparin lipase activities were measured using the glycerol-based emulsion described by Nilsson-Ehle and Schotz (17) with heat-inactivated human serum as a source of apoC-II. LpL was assessed with a chicken antiserum that inhibits mouse LpL, leaving the residual hepatic lipase (18). Plasmas from control and AdB17-injected mice were alternated and assayed at the same time to minimize intra- and interassay variability.

## RESULTS

### Expression of adenovirus apoB-17 in HepG2 cells and effects of oleate and ALLN on apoB-17 and production

ApoB-17-expressing viruses were used to infect HepG2 cells. The standard infection protocol involved using about $10^7$ virus particles for each 35-mm well, which is a multiplicity of infection (MOI) of approximately 10. As shown in **Fig. 1A**, apoB-100 migrating at approximately 550 kDa was found by Western blot analysis from cell lysates of all cells. Lanes 1 and 2 in Fig. 1A show lysates from uninfected cells and cells infected with adenovirus expressing β-galactosidase under the control of the cytomegalovirus promoter (AdLacZ). Infection with AdB17 and AdB17-β led to the expected molecular mass bands of approximately 93 and 120 kDa (lanes 3 and 4, respectively,

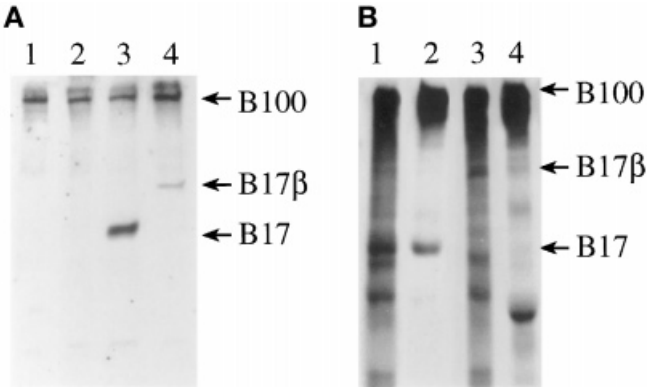


**Fig. 1.** Expression of apoB-17 and apoB-17-β by adenovirus-infected HepG2 cells. (A) Cell lysates were obtained 24 h after infected with or without adenovirus and Western blots were performed with anti-human apoB antiserum. Lane 1 is the control with no infection of adenovirus, whereas lane 2, 3, and 4 were infected with AdLacZ, AdB17, and AdB17-β, respectively. (B) After 24 h of infection, medium was changed to [$^3$H]leucine-containing labeling medium. After a 2-h incubation at 37°C, media were collected, and cells were lysed. Both cell lysates and media were immunoprecipitated with anti-human apoB antiserum, and the precipitated proteins were analyzed by SDS-PAGE. Lanes 1 and 2 show cell lysates and media from cells infected with AdB17 whereas lanes 3 and 4 show lysates and media from AdB17-β infection, respectively.

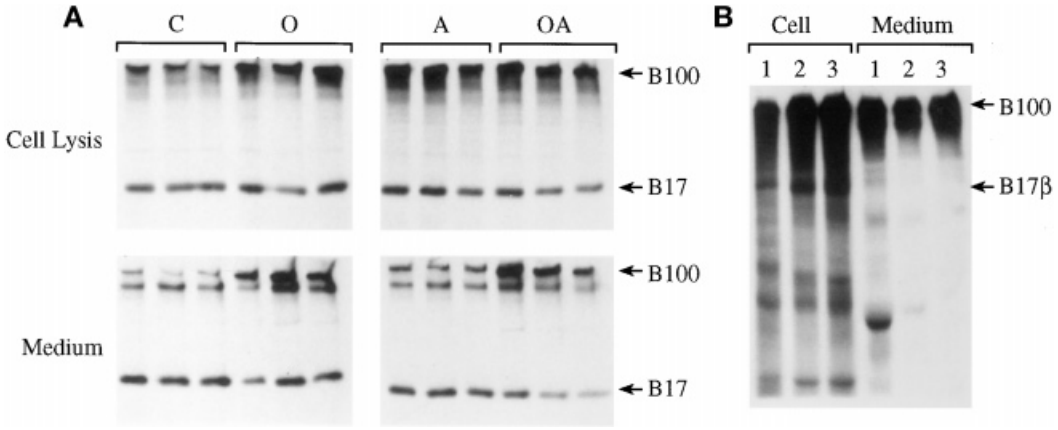


**Fig. 2.** ApoB-17 and apoB-17-β expression in HepG2 cells treated with oleate or ALLN. After 24 h of infection, cells were switched to [$^3$H]leucine-containing labeling medium with oleate, ALLN, or both. After an additional 2 h of incubation at 37°C, media were collected and cells were lysed. Both cell lysates and media were immunoprecipitated with anti-human apoB antiserum and the precipitated proteins were analyzed by SDS-PAGE. (A) Cells were infected with AdB17. Cell lysates are shown in the top panel and media are in the bottom panel. C, Control without treatment; O, oleate-treated cells; A, ALLN-treated cells; OA, cells treated with both oleic acid and ALLN. (B) Cells were infected with AdB17-β and treated as described above. The left panel presents cell lysates whereas the right panel presents the medium. Lane 1, proteins from control cells; lane 2, cells treated with oleate; lane 3, cells treated with both oleate and ALLN.

in Fig. 1A) when cell lysates of infected HepG2 cells were probed with anti-apoB antiserum.

The media from control and infected cells were used for immunoprecipitation and the immunoprecipitates were analyzed by SDS-PAGE and Western blotting. In addition, to increase detection of apoB, cells were grown in [$^3$H]leucine and the precipitated proteins were analyzed. Both methods gave the same results. As shown in Fig. 1B, AdB17 led to the detection of protein secreted into the medium (lanes 1 and 2). However, apoB-17-β was not secreted into the medium despite its presence in the cells (Fig. 1B, lanes 3 and 4). Thus, apoB-17 but not apoB-17-β was secreted from cells after adenoviral infection.

Inclusion of oleate in the medium or inhibition of proteolysis by ALLN has been shown to increase secretion of full-length apoB by liver cells (19). In contrast, studies by Thrift et al. (20) in Chinese hamster ovary cells (CHO cells) showed that apoB-15 production by these cells was not affected by either of these interventions. For this reason, we tested whether the production of apoB-17 in HepG2 cell was altered by oleate or ALLN. Although the variability in the infection rate led to some differences in apoB-17 expression between cells, as in CHO cells, when cells were labeled and immunoreactive apoB was assessed, apoB-17 production did not appear to be increased by either of these interventions (**Fig. 2A**). In contrast, apoB-100 production was dramatically increased with either oleate or ALLN or both treatments. Neither oleic acid nor ALLN allowed secretion of apoB-17-β from the cells (Fig. 2B).

### Effects of apoB-17 expression on apoB-100 production by HepG2 cells

We hypothesized that apoB-17, which contains the MTP-binding region of apoB, would inhibit apoB-100 production. As shown in **Fig. 3A**, cells infected with the lowest dose of control virus ($3 \times 10^7$ particles/35-mm well; MOI of approximately 30) showed no change in the amount of newly synthesized cellular apoB-100; compare untreated cells (Fig. 3A, lanes 1 and 2) and AdLacZ-treated cells (Fig. 3A, lanes 3 and 4). At higher doses of control virus ($\sim 3 \times 10^8$ and $3 \times 10^9$ particles per 35-mm well; Fig. 3A, lanes 5 and 6, and 7 and 8) an approximately 110-kDa band was seen that might represent a cross-reacting viral protein. At the highest dose of virus apoB-100 production decreased (see Fig. 3A, lanes 7 and 8). As shown in Fig. 3B, albumin production also decreased at this dose. Lanes 9–14 in Fig. 3B show cells infected with increasing amounts of AdB17. The marked increase in apoB-17 in the cells can readily be seen. As was found for the control virus, at the highest doses both apoB-100 and albumin production was decreased.

Media from the infected cells were analyzed for apoB-100 by Western blot to assess total production (Fig. 3C). There was no decrease in apoB-100 in the cells or medium on examining HepG2 cells infected with a standard dose of apoB-17 virus. We found a decrease in apoB-100 after adenovirus infection only when we used $3 \times 10^9$ particles per well of a six-well plate (MOI of 3,000). In this situation, however, cells that were infected with the same amount of control virus also showed decreased apoB-100 production. Furthermore, the production of albumin, a protein produced by HepG2 without interaction with MTP, was dramatically decreased in cells infected with either AdB17 or control virus (Fig. 3B).

### Effect of viral infection on apoB production by McArdle cells

Because other studies assessing apoB-100 production by transfected hepatoma cells were performed in McArdle cells (10, 11), similar viral infection studies were also done in this cell line. Identical results were obtained by infection of McArdle cells; only the higher doses of virus de-

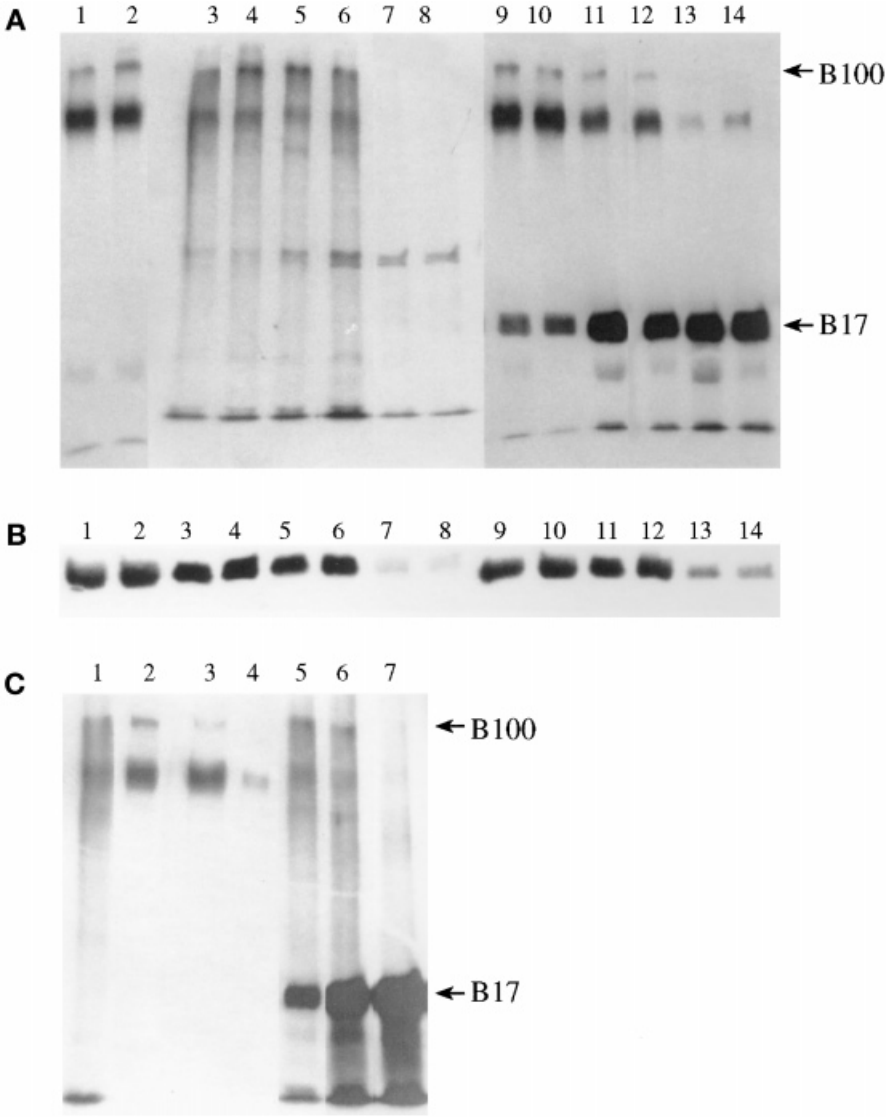


**Fig. 3.** Dosage effect of AdB17 on endogenous apoB production in HepG2 cells. After 24 h of infection, medium was changed to MEM containing [$^3$H]leucine. After 2 h of incubation at 37°C, media were collected and cell lysates were prepared. Cell lysates and media were immunoprecipitated with anti-human apoB antiserum. Lysates were also immunoprecipitated with anti-human albumin antiserum. The precipitated proteins were separated by SDS-PAGE. (A) ApoB precipitated from cell lysates of uninfected cells (lanes 1 and 2); increasing doses of AdLacZ (particles per well): $10^7$ (lanes 3 and 4), $10^8$ (lanes 5 and 6), and $10^9$ (lanes 7 and 8); and increasing doses of AdB17 (particles per well): $10^7$ (lanes 9 and 10), $10^8$ (lanes 11 and 12), and $10^9$ (lane 13 and 14). (B) Human albumin precipitated from cell lysates of uninfected cells (lanes 1 and 2); increasing doses of AdLacZ (particles per well): $10^7$ (lanes 3 and 4), $10^8$ (lanes 5 and 6), and $10^9$ (lanes 7 and 8); and increasing doses of AdB17 (particles per well): $10^7$ (lanes 9 and 10), $10^8$ (lanes 11 and 12), and $10^9$ (lanes 13 and 14). (C) ApoB precipitated from medium without infection (lane 1); increasing doses of AdLacZ (particles per well): $10^7$ (lane 2), $10^8$ (lane 3), and $10^9$ (lane 4); and increasing doses of AdB17 (particles per well): $10^7$ (lane 5), $10^8$ (lane 6), and $10^9$ (lane 7).

creased endogenous apoB-100 production (**Fig. 4**). We conclude that adenovirus expression of apoB-17 does not alter apoB-100 production by liver cells unless the level of expression is so great that it leads to a generalized decrease in protein synthesis.

### Expression of apoB-17 in apoE knockout mice

ApoB-17 expression in vivo and its association with circulating lipoproteins was next assessed. Several apoE knockout mice were injected with apoB-17 virus and apoB-17 expression was confirmed (see below). Plasma was taken from mice 3 days after injection and used for gel-filtration chromatography. As shown in **Fig. 5**, the majority of cholesterol and apoB-48 coeluted from the column. An intense apoB-17 band was found eluting in later fractions containing high density lipoprotein and proteins not associated with lipids (fractions 37 to 42). Thus, most apoB-17 was not associated with apoB-48-containing lipoproteins and

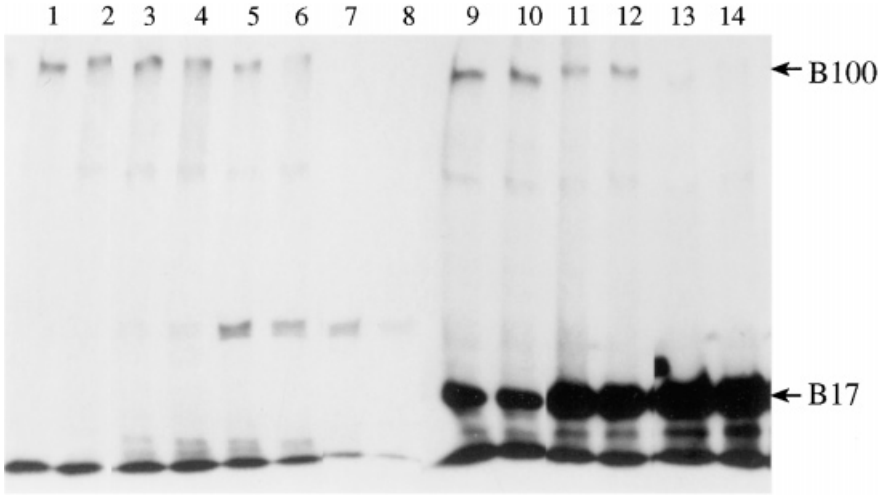


**Fig. 4.** Dosage effect of AdB17 on endogenous apoB production in McArdle cell. After 24 h of infection, medium was changed to medium containing [$^3$H]leucine. After 2 h of incubation at 37°C, media were collected and cells were lysed. Cell lysates were immunoprecipitated with anti-human apoB antiserum, and the precipitated proteins were analyzed by SDS-PAGE. ApoB precipitated from lysates of uninfected cells was loaded in lane 1 and 2. ApoB precipitated from lysates of cells infected by AdLacZ with increasing dosage was loaded in lanes 3 and 4 ($10^7$ particles/well), lanes 5 and 6 ($10^8$ particles/well), and lanes 7 and 8 ($10^9$ particles/well). Precipitates from cells infected with AdB17 were loaded in lanes 9 and 10 ($10^7$ particles/well), lanes 11 and 12 ($10^8$ particles/well), and in lane 13 and 14 ($10^9$ particles/well).

was, therefore, not associated with the majority of lipid-carrying particles in the plasma.

### In vivo effects of adenoviruses on apoB-100 production

To assess whether apoB-17 production in vivo alters lipoprotein and apoB-100 secretion from liver, wild-type, human apoB transgenic, and apoE knockout mice were injected with $10^{11}$ particles of apoB-17 virus. As shown on the Western blots in **Fig. 6**, the intensity of plasma apoB-17 in the apoB-17-expressing mice markedly exceeded that of either apoB-100 or apoB-48 in all infected mice. In the wild-type mice, this band was 24-fold more intense than apoB-48 and apoB-100 (Fig. 6A, lanes 3 and 4), in the human apoB-100 transgenic mice the apoB-17 band was 2- to 7-fold more intense than either the apoB-100 or apoB-48 bands (Fig. 6B), and in the apoE knockout mice apoB-17 was 3- to 5-fold more intense than the apoB-48 band (Fig. 6C). Despite this marked overproduction of apoB-17, no detectable decrease in the plasma apoB-48 or apoB-100 bands was found in any of the three strains of mice (compare lanes 1 and 2 with lanes 3 and 4). Moreover, as shown in **Table 1**, despite the intensive expression of apoB-17 in these three different strains of mouse, the lipid profile was similar between mice expressing apoB-17 and mice expressing control virus.

### Effects of apoB-17 on postheparin plasma lipases

Previous work from this laboratory showed that LpL would associate with apoB-17. For this reason, we assessed postheparin LpL activity in control and AdB17-infected mice. LpL was specifically assessed by difference, using a polyclonal antibody that inhibits mouse LpL. Four control and three apoB-17-expressing mouse postheparin plasma samples were assayed and no differences were found in either LpL or hepatic lipase (data not shown). Therefore, consistent with the lack of changes in plasma lipids, postheparin LpL was unaffected by marked overexpression of apoB-17.

## DISCUSSION

Adenoviruses were used to markedly overexpress apoB-17 in two different lines of hepatoma cells, and in wild-type, apoE knockout, and human apoB-expressing transgenic mice. Despite evidence that MTP binds to this region of apoB and previous studies showing that fragments of apoB decrease apoB-100 production in McArdle cells, we were unable to show a reduction in apoB-100 production with apoB-17 overexpression. Similarly, apoB-17 with a lipid-binding β sheet did not alter apoB-100 production by the cells. ApoB-100 production was decreased when high levels of virus were used to infect the cells, but these effects were nonspecific; control virus led to similar reductions in albumin secretion. Therefore, apoB-17 expression decreases apoB-100 production only if the virus leads to a general impairment in cellular protein production.

Results obtained by co-immunoprecipitation suggested that MTP and apoB physically interact with each other during apoB synthesis, although this interaction is transient (21). In a previous study using McArdle cells, Hussain et al. (10) showed that overexpression of apoB-48 decreases synthesis of endogenous apoB-100. They hypothesized that the apoB-48 competed at the translation-translocation step of apoB-100 synthesis by targeting to specific channels. However, they did not study whether this competi-

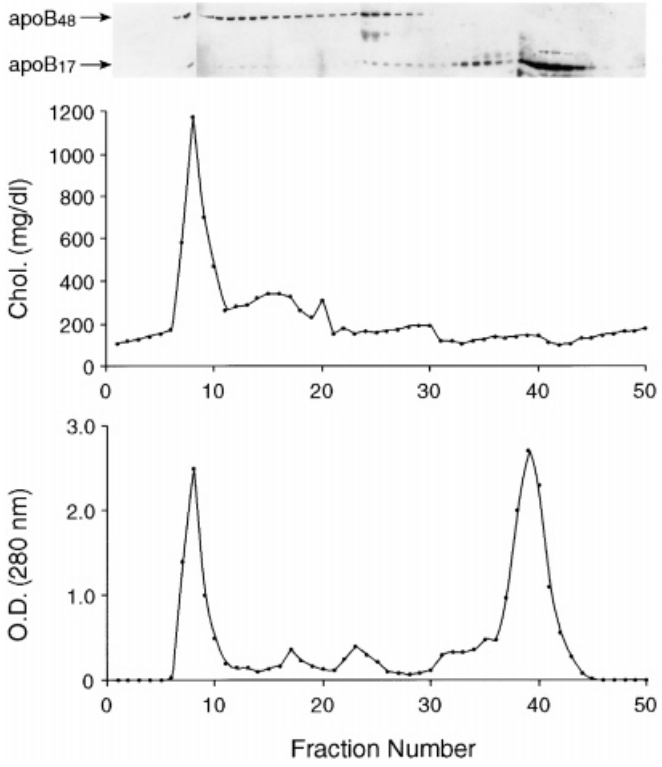


**Fig. 5.** FPLC analysis of the plasma of mice injected with AdB17. ApoE knockout mice were injected with $10^{11}$ particles of AdB17, and plasma was obtained on day 3. Two hundred microliters of the plasma was used for gel-filtration chromatography as described in Materials and Methods. Fifty fractions were collected and were analyzed by Western blot with anti-human apoB antiserum (top panel). Cholesterol and protein concentrations of each fraction were analyzed and the results are shown in the middle and bottom panels, respectively.

TABLE 1. Lipids in mice injected with AdLacZ or AdB17

| | TG | | TC | |
|---|---|---|---|---|
| | AdLacZ | AdB17 | AdLacZ | AdB17 |
| | *mg/dl* | | *mg/dl* | |
| C57/pre | 105 ± 10 | 103 ± 9 | 88 ± 10 | 83 ± 12 |
| C57/post | 96 ± 21 | 62 ± 8 | 73 ± 25 | 70 ± 9 |
| hApoB/pre | 175 ± 17 | 154 ± 49 | 162 ± 38 | 122 ± 19 |
| hApoB/post | 151 ± 29 | 141 ± 26 | 134 ± 24 | 115 ± 21 |
| ApoE(KO)/pre | 114 ± 44 | 84 ± 24 | 448 ± 25 | 380 ± 77 |
| ApoE(KO)/post | 117 ± 59 | 91 ± 19 | 505 ± 104 | 483 ± 106 |

Data were obtained from mice before injection (pre) and 3 days after injection (post) with either AdLacZ or AdB17 virus ($10^{11}$ particles per mouse). C57, C57BL/6J mice; hApoB, human apoB transgenic mice; ApoE(KO), ApoE knockout mice; TG, triglyceride; TC, total cholesterol. Values are reported as means ± SD (five mice in each group).

tion involves the interaction of MTP with apoB. Although they subsequently showed that the interaction region between MTP and apoB is located within the amino-terminal 17% of apoB (9), other laboratories reported that regions that are downstream of apoB-17 also interact with MTP (11, 22). Our results demonstrate that apoB-17 does not compete with apoB for the actions of MTP in lipoprotein assembly. It should be noted that our results were obtained not only from cell experiments but also from in vivo experiments, whereas all the experiments mentioned above were performed either in vitro or ex vivo. Thus, under physiological conditions (in vivo), apoB-17 might not interact with MTP in a manner that blocks MTP actions on endogenous apoB.

In some situations apoB production is modulated by the amount of cellular lipid. ApoB degradation in HepG2 cells occurs in two steps: step 1 is when apoB is partially translocated, and step 2 is when apoB is in the ER lumen (23). Oleate increases translocation of nascent apoB into the ER lumen of HepG2 cells, blocks the first step of degradation, and markedly increases cellular apoB secretion (24–26). In other cells the effects of oleate are less impressive (15), perhaps because these cells contain more endogenous lipid. For this reason, we performed studies of apoB-17 overexpression in two different cell types, as well as with and without addition of oleic acid and ALLN. Even under conditions of greater lipid flux, when MTP might be more rate limiting, apoB-17 did not alter apoB-100 production. To determine whether apoB-17 might affect this process, ALLN, which blocks proteosomal degradation of apoB (20), was used to be better able to assess post-ER degradative processes. However, apoB-17 expression

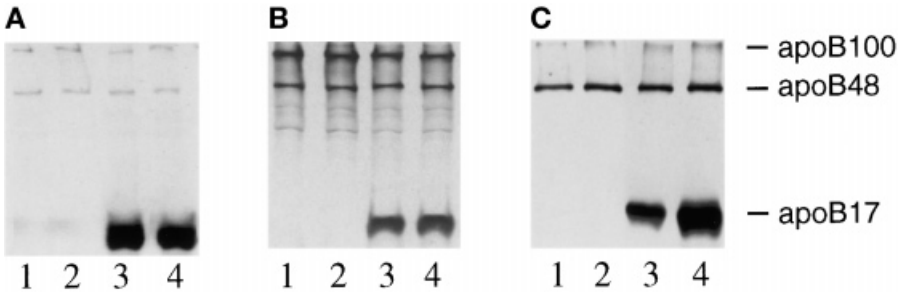


**Fig. 6.** Effect of overexpression of apoB-17 in mice. AdLacZ or AdB17 ($10^{11}$ particles) was injected into mice of three different strains: C57BL/6J mice (A), human apoB transgenic mice (B), and apoE knockout mice (C). Plasma samples were obtained 3 days after injection and analyzed by Western blot with anti-human apoB antiserum. Plasma from control mice injected with AdLacZ was loaded in lanes 1 and 2, whereas plasma from AdB17-injected mice was loaded in lanes 3 and 4 (0.5 μl of plasma per mouse per lane).

did not alter apoB-100 in ALLN-treated cells, consistent with a lack of effect on MTP.

Although AdB17 led to secretion of apoB-17, apoB-17-β produced intracellular but not secreted protein. This was true even in the presence of oleic acid and ALLN, which increase apoB-100 and prevent its intracellular degradation. Why was apoB-17-β not secreted from the cells when apoB-48 and other apoB-17 lipid-binding constructs are? Liang et al. (27) compared secretion of two apoB-16 fragments in tandem with or without the same β-sheet region. They found that secretion of the fragment containing a β sheet was much less than that of the tandem apoB-16. In contrast to our findings, however, oleic acid and ALLN did increase apoB-16 apoB-16-β-sheet secretion. Thus, the size of the peptide as well as the presence of a lipid-binding region appear to regulate the secretion of the amino-terminal region of apoB. Because this was not the focus of our experiments, further analysis of the relationship between apoB-17 and its secretion was not done. We could conclude, however, that apoB-17-β does not affect apoB-100 production in hepatoma cells.

Using several lines of genetically altered mice we were able to assess plasma apoB-100 in mice with apoB-17 expression. Human apoB transgenic mice are animals in which the excess liver apoB production might have made MTP more limiting. ApoE knockout mice contain predominantly apoB-48 lipoproteins in their circulation and allowed us to assess the effects of apoB-17 on these lipoproteins. We also studied wild-type mice fed chow diets, when LDL receptors would not be downregulated by dietary fat or hyperlipidemia. Under none of these situations did apoB-17 alter plasma apoB-100 or apoB-48 levels.

Another potential effect of apoB-17 on lipoprotein metabolism that we examined was on LpL. We had previously demonstrated that apoB-17 associates with LpL on ligand blots (28, 29), and, moreover, that addition of apoB-17 to purified LpL decreased LpL binding to cultured endothelial cells. Thus it was of interest to determine whether the expression of high levels of apoB-17 in vivo might alter the location or activity of LpL. However, neither pre- nor postheparin LpL activities were altered in the mice expressing apoB-17. This finding was consistent with one aspect of our prior in vitro studies, namely that the addition of apoB-17 to cultured endothelial cells containing associated LpL did not lead to release of bound LpL.

Overall, our results demonstrating a lack of effect of apoB-17 on endogenous apoB are consistent with several observations in the literature. Expression of fragments of apoB by Liang et al. (27) led to no change in apoB-100 production by cultured HepG2 cells. Similarly, in vivo studies also suggest that expression of apoB or apoB fragments does not alter production of endogenous apoB-100 or apoB-48 (30). Expression of human apoB (12, 31) or truncated fragments of apoB (32) did not affect endogenous mouse apoB production. There are several types of data on cells and animals suggesting that MTP is rate limiting for production of lipoproteins. Using a photo-affinity inhibitor in rat hepatoma cells, Jamil et al. (7) indicated that the MTP-mediated lipid transfer step was the rate-limiting step in the production of apoB-containing lipoproteins. By using an MTP inhibitor, Wetterau et al. (33) showed that apoB-containing lipoproteins within the plasma of experimental animals were decreased proportionally with drug-induced MTP inhibition. This study implied that MTP was not in great excess within cells and animals. However, this study did not measure MTP activity directly. Thus, the dose-response relationship between changes in lipoprotein secretion and MTP activity cannot be understood from these experiments. Other data, however, do not support this hypothesis. Complete knockout of the MTP gene in mice led to an embryonic lethality, while the heterozygous knockout mice had a 28%, not 50%, reduction in plasma apoB levels (34). A study by Leung et al. (35) with transgenic and knockout mice concluded that the MTP-to-apoB ratio is not critical for apoB production. In other words, decreased apoB-containing lipoprotein secretion is not proportional to the decrease in amount of MTP. Abetalipoproteinemia is a human disease associated with low plasma levels of apoB-containing lipoproteins. These patients cannot secrete apoB either in the liver or intestine and have a defect in absorption of fat-soluble vitamins. Abetalipoproteinemia is caused by a mutation in the gene encoding the 97-kDa catalytic subunit of MTP (36). Heterozygous carriers of this mutation, however, have normal plasma lipoproteins (37). Taken together, the data suggest that MTP is limiting only under certain conditions. This occurs when more than 50% of the MTP is inhibited; even dramatic overproduction of apoB or its MTP-binding fragments does not appear to make the MTP-mediated step in lipoprotein assembly limiting.

In summary, under a variety of conditions we were unable to affect the production of apoB by marked overexpression of apoB-17, a fragment of apoB that contains an MTP-binding region. Parallel experiments using adenovirus to express this fragment of apoB in vivo also did not alter apoB-100 or apoB-48 levels in the mice. The suggestion that MTP interaction with apoB is limiting provides a rationale for gene therapy approaches to limit lipoprotein production by the liver. Neither our studies nor those produced by the laboratories of Young and Rubin (12, 31) lead to this conclusion. Thus, the kinetics or biological significance of apoB-MTP interaction may be complicated and, hence, blocking this interaction might not lead to the same effects as directly inhibiting MTP (33). Although reductions of MTP appear to be a target for therapies aimed at reducing plasma levels of apoB-containing lipoproteins, MTP interaction with apoB-17 is not a competitive inhibitor of MTP.

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