Adenovirus-Mediated Expression of Caveolin-1 in Mouse Liver Increases Plasma High-Density Lipoprotein Levels†

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ABSTRACT: Caveolae are 50−100 nm plasma membrane invaginations, which function in cell signaling and transcytosis, as well as in regulating cellular cholesterol homeostasis. These subcompartments of the plasma membrane are characterized by the presence of caveolin proteins. Recent studies have indicated that caveolae may be involved in the regulation of cellular cholesterol efflux to HDL, as well as selective uptake mediated by SR-BI. In the present study, we have determined the effect of caveolin-1 overexpression in mouse liver on plasma lipoprotein metabolism. We evaluated this effect using an adenovirus-mediated gene delivery system. C57BL/6J mice were injected with adenoviruses encoding either caveolin-1 (Adcav-1) or green fluorescent protein (AdGFP) together with a transactivator adenovirus (AdtTA). We found that, after adenovirus injection, caveolin-1 was overexpressed in hepatocytes. Moreover, the recombinant protein was localized to the plasma membrane. We also found that caveolin-1 overexpression induced a marked change in the lipoprotein profile of injected animals. In caveolin-1 overexpressing animals, plasma HDL−cholesterol levels were found to be ∼2-fold elevated, as compared with control animals. To determine the effect of caveolin-1 on SR-BI-mediated selective uptake, we infected murine hepatocytes in culture with an adenoviral vector carrying the caveolin-1 cDNA or GFP as a control protein. We show that, in primary cultures of hepatocytes, caveolin-1 inhibits DiI−HDL uptake mediated by SR-BI. This result would mechanistically explain the increased plasma HDL−cholesterol levels we observed in caveolin-1 adenovirus-injected animals. In addition, caveolin-1 expression increased the secretion of apolipoprotein A-I in cultured hepatocytes and increased apolipoprotein A-I plasma levels in mice. Our study therefore demonstrates an important role for caveolin-1 in regulating HDL metabolism.

Caveolae are 50−100 nm plasma membrane invaginations that participate in cell signaling (*1*) and transcytosis (*2*, *3*), as well as in regulating cellular cholesterol homeostasis (*4*). These subcompartments of the plasma membrane are characterized by the presence of caveolin proteins (*5*, *6*). Several isoforms of caveolin have been identified (*7*−*11*). Caveolins are expressed principally in terminally differentiated cell types such as fibroblasts, adipocytes, smooth and striated muscle cells, and endothelial cells (*12*).

In nonmuscle cells, caveolin-1 is the main component of caveolae. Caveolin-1 drives caveolae formation through oligomerization (with itself and with caveolin-2) and by interacting with cholesterol (*13*−*15*). The formation of this functional assembly unit may facilitate targeting of numerous constituents to caveolae, including proteins involved in signal transduction or cholesterol homeostasis (reviewed in refs *16*−*18*).

Caveolae have a very specific lipid composition; they are highly enriched in cholesterol and sphingomyelin. This has led to the hypothesis that caveolae may play a role in the regulation of cellular cholesterol homeostasis. The observation that caveolin-1 binds cholesterol specifically (*19*, *20*) suggests that caveolin-1 may play a direct role in this process. Recently, Smart et al. demonstrated that caveolin-1, coupled with other chaperone proteins, facilitates transport of cholesterol from the ER to the plasma membrane (*21*). Studies from Fielding et al. (*22*) have indicated that free cholesterol (FC) selectively transferred from LDL to cells appears first in clathrin-coated pits and is eventually transferred to caveolae via the trans-Golgi network. Furthermore, it has been demonstrated that plasma membrane caveolae can mediate cellular cholesterol efflux to plasma (*23*). Additionally, caveolin-1 expression is under the positive control of cellular cholesterol levels (*24*).

The importance of HDL[1] in cholesterol elimination has been suggested by several epidemiological studies that show an inverse correlation between the development of coronary artery disease and HDL−cholesterol levels (*25*−*29*). This has led to the concept of reverse cholesterol transport, a process by which HDL removes excess peripheral cholesterol and transfers it to the liver for degradation and removal from

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the body (*30*). HDL particles are believed to function as the primary acceptors of cellular cholesterol. Following cellular cholesterol efflux to HDL, cholesterol is esterified and transferred to apolipoprotein B-containing lipoproteins via the protein cholesterol ester transfer protein (*30*). Alternatively, cholesterol ester from HDL (HDL-CE) may be transferred to hepatocytes via the scavenger receptor class B type I (SR-BI), which mediates selective uptake of CE without HDL−particle uptake (*31*). This pathway may be of special importance in the case of mouse plasma, which does not display cholesterol ester transfer activity and, therefore, contains CE-enriched HDL particles (*31*). The crucial role of liver SR-BI has been demonstrated by several studies using transgene expression or adenovirus-mediated gene delivery in mouse models (*32*−*41*). In these studies, it was shown that hepatic expression levels of SR-BI are inversely correlated with plasma HDL−cholesterol (HDL-C) levels. Consequently, SR-BI mediates HDL-CE uptake and appears to allow recycling of HDL for efficient reverse cholesterol transport. This hypothesis is supported by the observation that liver expression levels of SR-BI are negatively correlated with atherosclerosis in mouse models (*32*, *37*, *39*).

Caveolin-1 is expressed in the liver, including the sinusoidal plasma membrane of hepatocytes (*42*, *43*), where it may play a role in potocytosis of small molecules such as folate (*44*) or retinol (*45*). Interestingly, SR-BI is associated with caveolae (*46*). Selective HDL-CE uptake mediated by SR-BI was recently shown to be mediated through caveolae in THP-1 macrophages (*47*). This group also showed that caveolin-1 expression might alter selective HDL-CE uptake in macrophages (*48*). However, these effects appear to be dependent on the cell type used (*49*). In steroidogenic tissues, the relationship between caveolin-1- and SR-BI-mediated selective HDL-CE uptake is unclear (*50*). In rat ovarian granulosa cells, upon luteinization, SR-BI does not colocalize with caveolae, and increases in SR-BI expression and selective CE uptake are associated with a decline in caveolin-1 expression (*51*). However, in similar studies with mouse ovarian granulosa cells, luteinization was associated with a marked increase in SR-BI expression but only a modest increase in SR-BI-mediated HDL-CE selective uptake. However, caveolin-1 expression in mouse granulosa cells was not affected by luteinization (*52*).

Here, we have employed an adenovirus-mediated gene transfer approach to examine the effects of hepatic overexpression of caveolin-1 on lipoprotein metabolism. We show that hepatic overexpression of caveolin-1 leads to increased plasma HDL-C levels. In addition, we find that caveolin-1 inhibits selective HDL-CE uptake mediated by SR-BI in cultured hepatocytes.

EXPERIMENTAL PROCEDURES

Materials. Antibodies and their sources were as follows: anti-caveolin-1 IgG (mAb 2297; gift of Dr. Roberto Campos-Gonzalez, BD Transduction Laboratories) (*9*); anti-caveolin-2 IgG (mAb 65, gift of Dr. Roberto Campos-Gonzalez, BD Transduction Laboratories) (*53*); rabbit polyclonal anti-GFP and anti-caveolin-1 IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); rabbit anti-mouse apolipoprotein A-I, E, B48, and B100, mouse anti-human apolipoprotein A-I (Biodesign International, Saco, ME). The cDNA for caveolin-1 was as we described previously (*54*, *55*). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was obtained from Molecular Probes (Eugene, OR). All other reagents were of analytical grade.

Adenoviral Vectors. Caveolin-1, green fluorescent protein (GFP), and transactivator recombinant adenoviruses were generated by our group, as previously described (*56*). The C-terminally Myc-tagged canine caveolin-1 cDNA or GFP cDNA was placed under the control of a CMV immediate early (IE) minimal promoter preceded by a heptamer of tetO sequences. Protein expression in mouse livers and primary cultures was then induced by co-infection of the caveolin-1 (or GFP) adenovirus with a second adenovirus containing a constitutive CMV-driven transactivator (AdtTA).

Animals and Adenovirus Injections. C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were maintained on a 12 h light/ 12 h dark schedule on a normal chow diet (LabDiet, Richmond, IN) or an atherogenic diet composed of 15% fat, 1.25% cholesterol, and 0.5% cholate (LabDiet, Richmond, IN), as indicated. All mice used in this study were 7-week-old females. Mice were injected with 0.1 mL of 0.9% sterile saline solution containing 5×10^{10} PFU (10^{11} particles) of caveolin-1 (or GFP) adenovirus and 5×10^{10} PFU (10^{11} particles) of transactivator adenovirus into the tail vein of C57BL/6J female mice. Experiments were carried out 72 h postinfection. Mice were fasted 5−7 h prior to plasma and liver analysis. Mice were anesthetized by intraperitoneal injection of 136 mg of pentobarbital (Abbott Laboratories, North Chicago, IL) per gram mouse weight before liver and blood collection.

Protein Expression in Mouse Liver. Seventy-two hours postinfection, mouse livers were collected and frozen in liquid N_2 with or without prior fixation in 4% paraformaldehyde. For Western blot analysis, 100 mg of each tissue sample (unfixed) was solubilized using a tissue homogenizer in 1 mL of lysis buffer [20 mM Tris (pH 8), 150 mM NaCl, 1% Triton X-100, 60 mM *n*-octyl glucoside, complete miniprotease inhibitor cocktail tablets]. Samples were then spun for 10 min at 14000*g* at 4 °C, and the supernatants were collected. The protein concentration was measured using the BCA protein assay (Bio-Rad Laboratories, Hercules, CA), with bovine serum albumin as the protein standard. Equal amounts of protein for each liver sample were loaded and run on 12% SDS−polyacrylamide gels. After transfer to nitrocellulose, caveolin-1 and GFP expression levels were examined using specific antibodies.

Analysis of Mouse Liver Sections by Immunofluorescence Microscopy. Frozen sections (1 μm thickness) of liver were prepared from adenovirus-infected animals and placed on polylysine-coated slides. For immunofluorescence analysis, tissue sections were pretreated with PBS containing 10% horse serum. The primary antibody (rabbit polyclonal anti-Cav-1 IgG or anti-GFP IgG) was incubated for 1 h with the sections in the presence of PBS containing 10% horse serum. After three washes with PBS, tissue sections were incubated

[1] Abbreviations: Adcav-1, adenoviruses encoding caveolin-1; AdGFP, adenoviruses encoding green fluorescent protein; AdtTA, adenoviruses encoding a constitutive CMV-driven transactivator; apoA-I, apolipoprotein A-I; CE, cholesteryl ester; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; HDL, high-density lipoproteins; SR-BI, scavenger receptor class B type I.

for 1 h with secondary antibody in PBS [Rhodamine red-X-labeled goat F(ab′)$_2$ anti-rabbit IgG (Jackson Immunoresearch laboratory)]. After being rinsed three times with PBS, slides were mounted with Slow-Fade antifade reagent (Molecular Probes). Sections were observed using an Olympus IX70 microscope equipped with a Photometrics CCD camera.

Plasma Lipoprotein Analysis. Blood samples were collected into EDTA(K_3) vacutainer tubes following a laparotomy and subsequent clipping of the descending aorta. Plasma (200 μL) was isolated and loaded onto two Superose 6 columns (analytical grade, Amersham Pharmacia Biotech) connected in series to achieve a total bed volume of approximately 50 mL and void volume of 15 mL. Plasma was passed over the columns at a flow rate of 0.5 mL/min, and 0.3 mL fractions were collected. Total cholesterol content of each fraction was determined and plotted against elution volume (Sigma total cholesterol kit).

Discontinuous Gradient Density Ultracentrifugation and Sample Analysis. Plasma samples were subjected to discontinuous gradient density ultracentrifugation, as described (*57*). After ultracentrifugation, fractions (1 mL) were collected from the top to the bottom, yielding a total of 11 fractions. The densities of the fractions were determined, and samples of these fractions (dialyzed against PBS) were resolved by SDS−PAGE. After transfer to nitrocellulose, the distribution of apoB100/B48, apoA-I, and apoE was examined using rabbit polyclonal antibodies (Biodesign International, Saco, ME).

DiI−HDL Uptake by Murine Hepatocytes. For DiI uptake studies, DiI-labeled HDL particles were obtained from Intracel Corp. (Rockville, MD). Primary cultures of murine hepatocytes were prepared from C57BL/6J female mice as described by Neufeld (*58*). Isolated cells were seeded in six-well plates in RPMI 1640 containing 10% FBS, 10 μg/mL insulin, and 0.1 μM dexamethasone. The next day, hepatocytes were infected with different combinations of adenovirus (Adcav-1-AdtTA or AdGFP-AdtTA, MOI of 10 PFU/cell for each virus). DiI−HDL uptake experiments were performed as described by Acton and Rigotti (*59*). Twenty-four hours postinfection, cells were washed with media alone and incubated with DiI−HDL (20 μg/mL) for 5 h at 37 °C. Cells were subsequently washed with PBS, and DiI incorporation was determined after solubilization of the cells with DMSO. Fluorescence of the different extracts was then quantified using a spectrofluorometer. In parallel wells, protein content was determined by solubilizing the cells in 0.5 N NaOH.

Effect of Caveolin-1 on Apolipoprotein Secretion. Primary cultures of murine hepatocytes were seeded in six-well plates and grown in RPMI 1640 containing 10% FBS, 10 μg/mL insulin, and 0.1 μM dexamethasone. After 2 days, cells were infected with adenovirus (Adcav-1-AdtTA or AdGFP-AdtTA, MOI of 10 PFU/cell for each virus). The next day, the medium was replaced with RPMI 1640 containing 0.2% BSA. After 24 h, the medium was recovered and concentrated using Centriprep concentrators (Amicon, Beverly, MA). Equivalent volumes of media were analyzed by SDS−PAGE and immunoblotting. Levels of mouse apoA-I and apoE were detected using rabbit polyclonal antibodies (Biodesign International, Saco, ME).

HDL Turnover in Mouse Plasma. Three days postinfection with the different adenovirus combination, HDL was injected into the tail vein of mice. Since iodination of apoA-I has been shown to alter plasma clearance of HDL (*60*), unlabeled human HDL (d = 1.063−1.210 g/mL) particles were used in this experiment. Blood samples were subsequently collected by retro-orbital bleeding at different time. Samples were analyzed by SDS−PAGE followed by transfer to nitrocellulose. The amount of human apoA-I remaining in mouse plasma was determined by Western blot analysis using a specific mouse mAb against human apoA-I (Biodesign International, Saco, ME) that does not cross-react with mouse apoA-I.

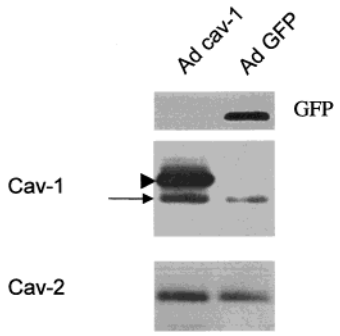

FIGURE 1: Recombinant expression of caveolin-1 in mouse liver. Seven-week-old C57BL/6J mice were injected with 0.1 mL of a 0.9% NaCl solution containing adenovirus (5×10^{11} Adcav-1 or AdGFP, with 5×10^{11} AdtTA). Three days postinfection, livers were collected and quickly frozen in liquid N_2. After extraction of the proteins (see Experimental Procedures), 20 μg of protein from each extract (Adcav-1- and AdGFP-infected animals) was analyzed by 12% SDS−PAGE. The expression of caveolin-1, caveolin-2, and GFP was detected after transfer to nitrocellulose using monospecific antibody probes. Recombinant Myc-tagged caveolin-1 (see arrowhead); endogenous caveolin-1 (see arrow). Note that recombinant expression of caveolin-1 stabilizes and increases the expression of endogenous caveolin-1 by ~2-fold.

RESULTS

Caveolin-1 Expression in Adenovirus-Infected Animals. C57BL/6J mice were infected by tail vein injection with two different combinations of adenoviruses (10^{11} particles for each virus): Adcav-1 plus AdtTA or AdGFP plus AdtTA (as a control). Caveolin-1 expression was monitored as a function of time following injection by Western blot analysis. We observed that expression was maximal after 3 days (not shown). Therefore, subsequent experiments were performed at 3 days postinjection. Typical expression levels are shown in Figure 1. Note that endogenous caveolin-1 is expressed at much lower levels than the recombinant form (an ~4−5-fold elevation). In all cases, hepatic caveolin-1 expression was determined by Western blotting, and only animals in which recombinant caveolin-1 expression was significantly higher than the endogenous form were included in the following experiments.

Caveolin-1 Localization in Murine Hepatocytes in Vivo. Expression of caveolin-1 in liver was also examined by immunofluorescence microscopy. Figure 2A,B summarizes the results obtained with anti-Cav-1 IgG and shows that caveolin-1 was overexpressed (Figure 2A), as compared with control AdGFP-infected livers (Figure 2B). Moreover, caveolin-1 was localized to the plasma membrane, including the basolateral (sinusoidal) membrane (Figure 2A). This observation is in contrast with that of AdGFP-infected livers, which present a diffuse expression pattern for GFP expression (Figure 2C).

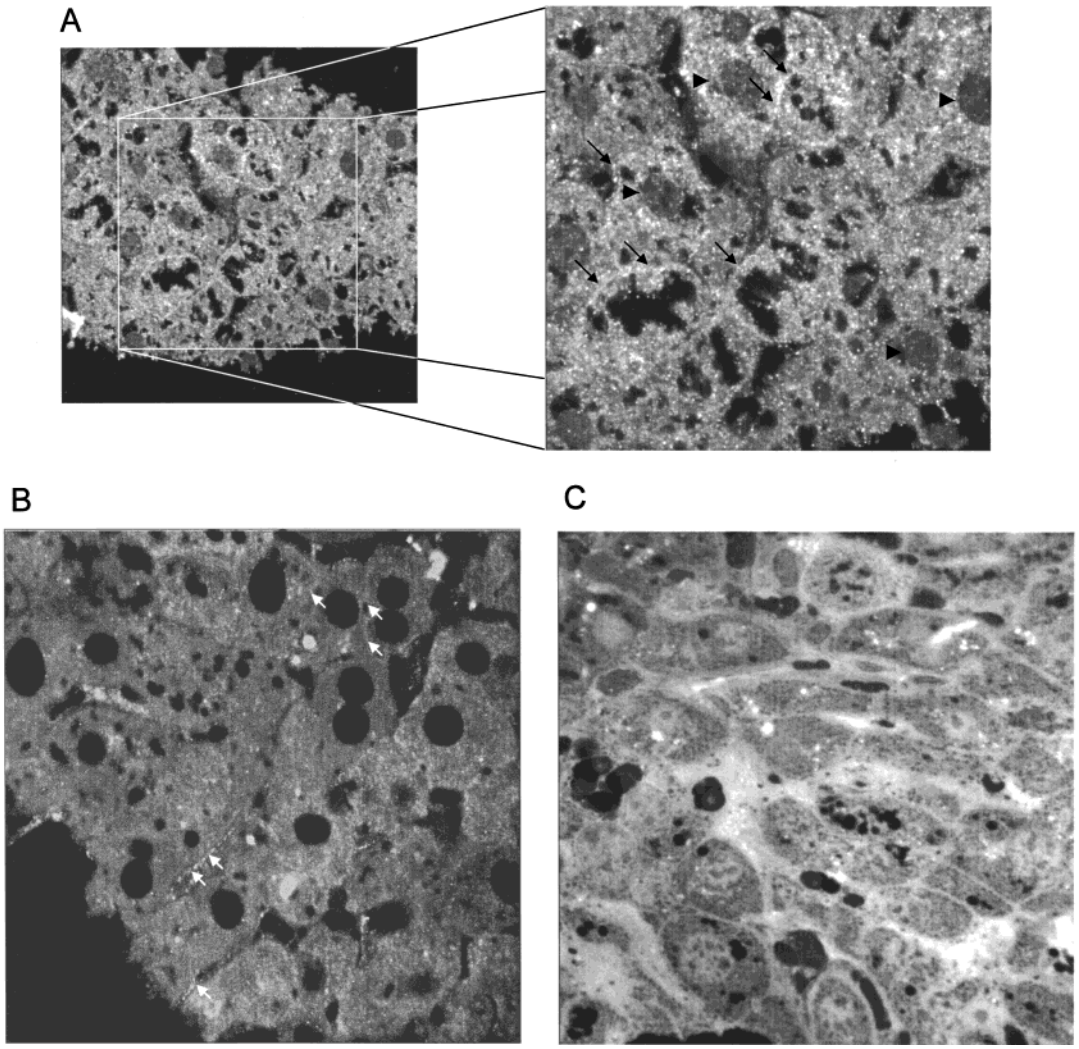

FIGURE 2: Immunofluorescent localization of recombinant caveolin-1 in mouse liver. Seven-week-old C57BL/6J mice were injected with 0.1 mL of a 0.9% NaCl solution containing adenovirus (5×10^{11} Adcav-1 or AdGFP, with 5×10^{11} AdtTA). Three days postinfection, the livers were collected, diced into small sections, and fixed in PBS/4% formaldehyde. After fixation, samples were quickly frozen in liquid N_2. Ultrathin frozen sections (1 μm thickness) were then prepared using an ultramicrotome. Caveolin-1 expression was detected using a rabbit polyclonal antibody, followed by a secondary antibody [Rhodamine red-X-labeled goat $F(ab')_2$ anti-rabbit IgG]. Sections were visualized using an Olympus IX70 microscope equipped with a Photometrics CCD camera. Representative sections of Adcav-1 (panel A, Cav-1 staining, the right-hand panel represents a 1.5× zoom of the boxed section in the left-hand panel) and AdGFP (panel B, Cav-1 staining, see arrows; panel C, GFP autofluorescence) infected livers are presented. Note that in panel A (Adcav-1 infected livers) arrowheads indicate the position of the nucleus, while arrows show the basolateral plasma membrane localization of caveolin-1.

Lipoprotein Profiles of Mice Recombinantly Expressing Caveolin-1 in the Liver. The localization of caveolin-1 and caveolae at the basolateral membrane of hepatocytes suggests a possible role for caveolin-1 in lipoprotein metabolism. Therefore, we examined the lipoprotein profiles of mice injected with adenovirus (Figure 3A). When compared to the control animals (AdGFP), note that animals overexpressing caveolin-1 in the liver show an ~2-fold increase in plasma HDL−cholesterol levels.

In addition, C57BL/6J female mice were also fed a high fat/high cholesterol diet for 4 weeks and then injected with adenovirus. Three days after injection, plasma was collected, pooled, and analyzed by gel filtration (Figure 3B). Note that, in Adcav-1-infected animals, HDL-C levels nearly double and VLDL levels are greatly reduced, as compared with AdGFP-infected control animals.

Apolipoprotein Composition of Fractions Obtained after Ultracentrifugation of Mouse Plasma. To determine the influence of caveolin-1 on apolipoprotein levels and distribution among lipoproteins, plasma from adenovirus-injected animals was subjected to discontinuous gradient density ultracentrifugation. Fractions were analyzed by 4−15% gradient polyacrylamide SDS−PAGE and transfer to nitrocellulose. Apoliprotein distribution was determined using anti-apoA-I, -apoE, and -apoB100/48 polyclonal antibodies. Figure 4 clearly demonstrates the effect of caveolin-1 liver overexpression on apolipoprotein B100/48. In Adcav-1 injected animals, we observed a reduction in the amount of apoB-containing lipoproteins in the low-density fraction range. In the case of apoA-I, we also observed an increase in mice injected with Adcav-1, as compared to AdGFP-injected animals.

Caveolin-1 Inhibits DiI Uptake in Cultured Murine Hepatocytes. Caveolin-1 has been implicated in SR-BI-mediated selective cholesterol ester uptake from HDL. To determine the role of caveolin-1 in this process, primary cultures of murine hepatocytes were obtained and infected with adenovirus. One day postinfection, cells were incubated with HDL

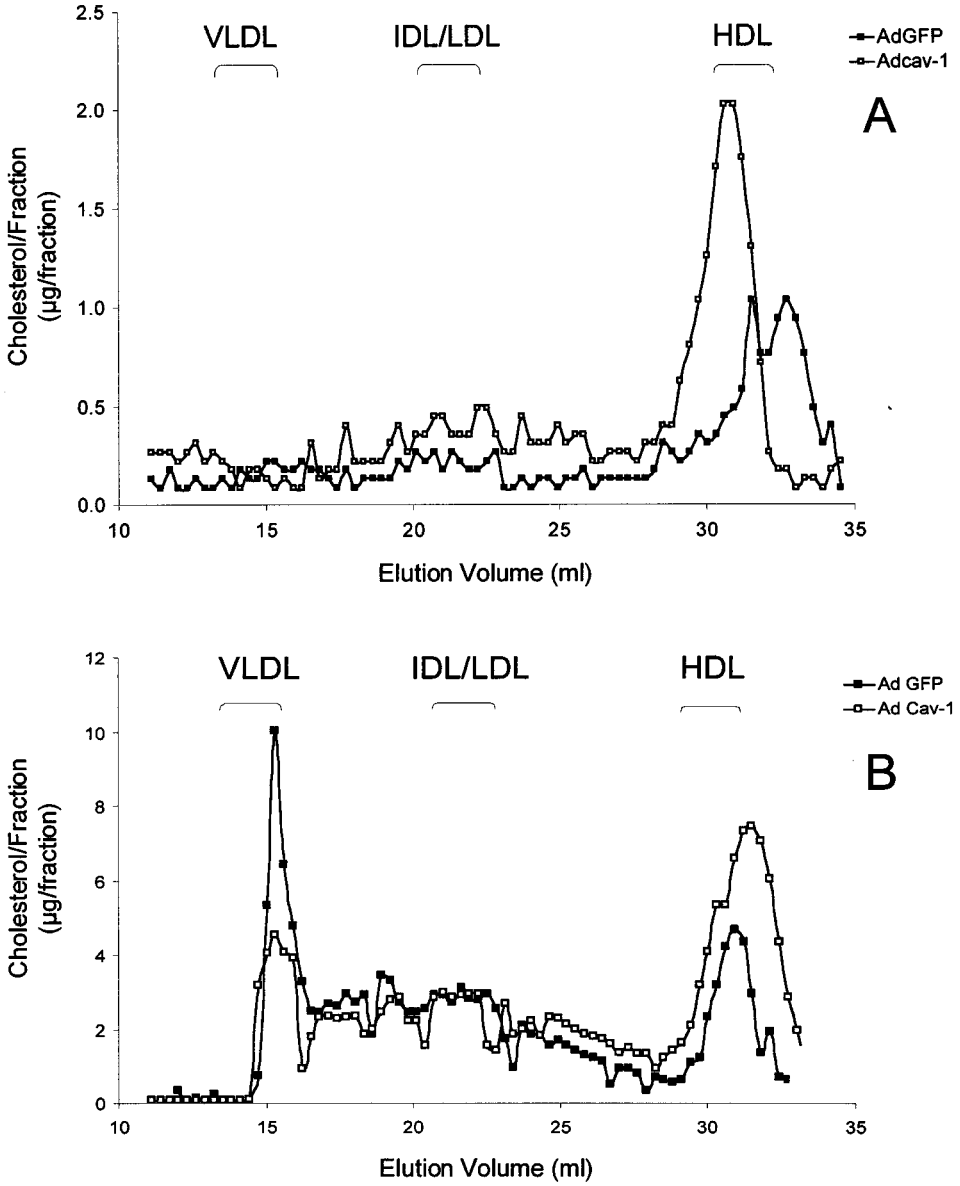

FIGURE 3: Effect of caveolin-1 expression on the lipoprotein profile of mouse plasma, as assayed by gel filtration chromatography. Seven-week-old C57BL/6J mice were injected with 0.1 mL of a 0.9% NaCl solution containing adenovirus (5×10^{11} Adcav-1 or AdGFP, with 5×10^{11} AdtTA). Plasma samples isolated from two mice from each group (Adcav-1- or AdGFP-infected animals) were pooled and loaded (200 μL) atop two Superose 6 columns (analytical grade, Amersham Pharmacia Biotech) connected in series with a total bed volume of approximately 50 mL and a void volume of 15 mL. Fractions collected were analyzed for their cholesterol content. The profiles presented were obtained after feeding the animals with normal chow diet (panel A) or a high fat/high cholesterol diet for 4 weeks (panel B). We have found that mice injected with AdtTA alone (adenovirus carrying the cDNA for the transactivator, present for both Adcav-1 and AdGFP injection) presented a profile similar to those of AdGFP-injected mice (data not shown). This result suggests that hepatic overexpression of caveolin-1 is responsible for the change in lipoprotein profile.

(using DiI as a fluorescent probe) for 5 h. Figure 5 shows that, in cells infected with Adcav-1 adenovirus, DiI uptake is significantly reduced, as compared to AdGFP-infected cells. It is important to note that caveolin-1 overexpression did not affect SR-BI expression levels (not shown).

Apolipoprotein A-I Secretion Is Increased in Cultured Murine Hepatocytes after Caveolin-1 Overexpression. To determine if the observed increase in plasma HDL-C levels in Adcav-1 injected mice could be due to a change in apoA-I secretion, primary cultures of hepatocytes were obtained from C57BL/6J mice. One day postinfection with adenovirus (Adcav-1-AdtTA or AdGFP-AdtTA), cells were incubated with RPMI containing 0.2% BSA. After 24 h, the medium was analyzed for apolipoprotein content, and the results are shown in Figure 6. Whereas apoE protein levels remained unchanged when caveolin-1 was overexpressed, apoA-I protein levels doubled as compared to the GFP controls. These results indicate that increases in plasma HDL-C levels in Adcav-1-injected animals may, at least in part, be due to increases in apoA-I secretion from hepatocytes.

Effect of Caveolin-1 on the in Vivo Turnover of HDL. Human HDL particles were injected 3 days post-adenovirus injection. Plasma samples were collected and analyzed for apoA-I content by Western blot analysis. For this purpose, we used a specific mouse mAb directed against human apoA-I that does not cross-react with mouse apoA-I.

Results presented in Figure 7 show a marked decrease in the rate of plasma clearance for apoA-I in Adcav-1-injected

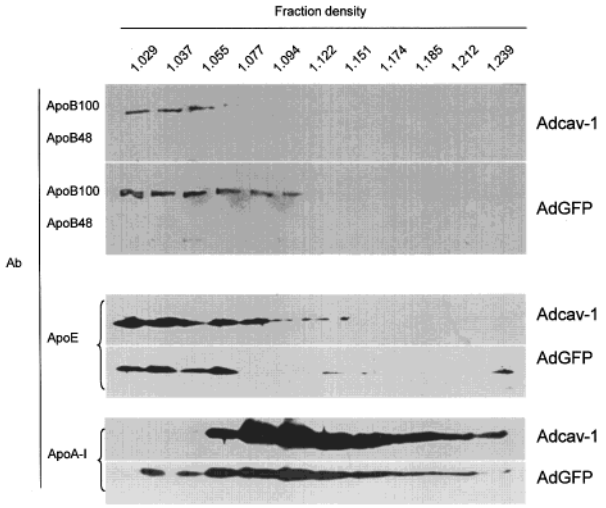

FIGURE 4: Effect of caveolin-1 expression on the lipoprotein profile of mouse plasma as assayed by ultracentrifugation. Seven-week-old C57BL/6J mice were injected with 0.1 mL of a 0.9% NaCl solution containing adenovirus (5×10^{11} Adcav-1 or AdGFP, with 5×10^{11} AdtTA). Three days postinfection, blood samples were collected into EDTA-containing tubes. Isolated plasma (500 μL per analysis, pooled plasma collected from two mice) was subjected to discontinuous gradient density ultracentrifugation, as previously described (*57*). After ultracentrifugation, fractions (1 mL) were collected from the top to the bottom of the gradient, yielding a total of 11 fractions. An aliquot of each fraction was then analyzed by SDS−PAGE (with a 4−15% polyacrylamide gradient). The presence of apoA-I, apoE, apoB100, and apoB48 was examined after transfer to nitrocellulose, using rabbit polyclonal rabbit antibodies specific for mouse apolipoproteins. Plasma samples from both Adcav-1- and AdGFP-infected mice were analyzed. The density of the fractions ranged from 1.029 to 1.24 g/mL and is indicated above each fraction. These results were obtained with mice fed a high fat/high cholesterol diet (4 weeks).

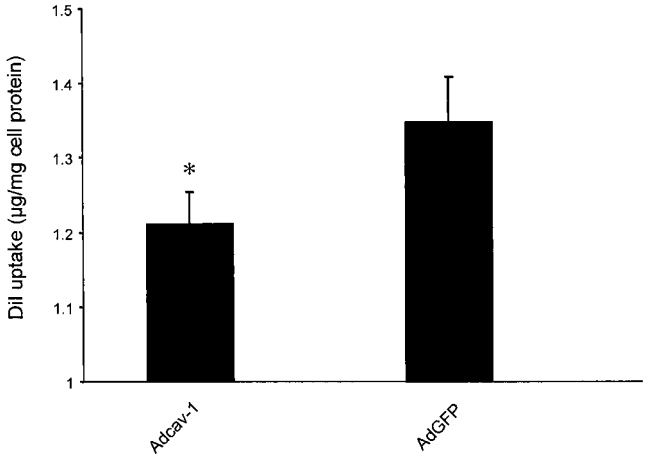

FIGURE 5: Caveolin-1 expression in murine hepatocytes inhibits DiI uptake from DiI-labeled HDL. Primary cultures of murine hepatocytes were obtained from seven-week-old C57BL/6J mice. They were grown in six-well plates. After 1 day of culture in RPMI 1640 media containing 10% FBS, dexamethazone, and insulin, cells were infected with Adcav-1-AdtTA or AdGFP-AdtTA (10 PFU/cell). Twenty-four hours postinfection, the cells were washed with PBS and incubated with DiI-labeled HDL for 5 h at 37 °C. Cells were subsequently washed with PBS, and DiI incorporation was determined after solubilization of the cells with DMSO. Fluorescence of the different extracts was then determined. In parallel wells, protein content was determined by solubilizing the cells in 0.5 N NaOH. An asterisk (*) indicates a significant difference, as compared with control cells ($P < 0.05$).

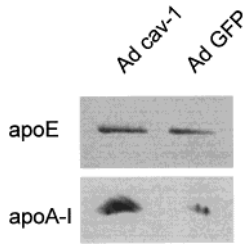

FIGURE 6: Effect of caveolin-1 on apolipoprotein secretion in primary cultures of murine hepatocytes. Primary cultures of murine hepatocytes were seeded in six-well plates and grown in RPMI 1640 containing 10% FBS, 10 μg/mL insulin, and 0.1 μM dexamethasone. After 2 days, cells were infected with adenovirus (Adcav-1-AdtTA or AdGFP-AdtTA, 10 PFU/cell for each virus). The next day, the medium was changed to RPMI 1640 containing 0.2% BSA. After 24 h, the medium was recovered and concentrated. Equivalent amounts of media were analyzed by SDS−PAGE and transferred to nitrocellulose. Mouse apoA-I and apoE were detected using rabbit polyclonal antibodies.

animals, as compared to the control AdGFP-injected animals. This decrease may, at least in part, be responsible for the increased plasma HDL-C in AdCav-1-injected animals, as compared to control animals.

DISCUSSION

Endogenous and Recombinant Expression of Caveolin-1 in Murine Hepatocytes. The endogenous expression of caveolin-1 in parenchymal liver cells has been demonstrated by others (*42*, *43*, *45*, *61*, *62*) and in the present study. Consistent with a role in uptake or secretion of lipoproteins in the space of Disse, caveolin-1 is expressed on the hepatocyte basolateral plasma membrane.

Our current study demonstrates that when caveolin-1 is recombinantly overexpressed using an adenovirus-mediated gene delivery system, caveolin-1 is notably localized to the hepatocyte basolateral membrane, in agreement with the findings of Calvo et al. (*63*). In addition, Cavo and Enrich have recently isolated and characterized a caveolae-enriched subcellular fraction from the sinusoidal membrane of hepatocytes (*43*). These authors have shown that this hepatocellular caveolar fraction contains specific known caveolar components (e.g., SR-BI, G_αs, MEK/ERK), as predicted.

Role of Caveolin-1 in the Hepatocyte. The functional role of caveolin-1 in hepatocytes is not yet well understood. It has been proposed that hepatocyte caveolae may be involved in the uptake of retinoic acid (*45*), a lipid that is usually associated with lipoproteins when transported in the plasma.

Caveolae may also play important roles in the uptake of proteins and possibly modified lipoproteins, such as oxidized LDL. Studies from Matveev et al. (*48*) have shown that, in THP-1 cells, caveolin-1 expression is upregulated during monocyte−macrophage differentiation. Moreover, these authors have also shown that upregulation of caveolin-1 expression is associated with an enhancement of selective HDL-CE uptake. However, studies with caveolin-1 transfected mouse macrophages have shown reduced HDL-CE uptake, as compared with control cells (*49*). Previous studies with steroidogenic cells have given contradictory results (*51*, *52*). The reasons for these discrepancies remain unknown but may be associated with the specific properties of THP-1 cells. However, in all cases, SR-BI appears to be present in a buoyant low-density fraction of the cell plasma membrane that is associated with caveolin-1 (*50*).

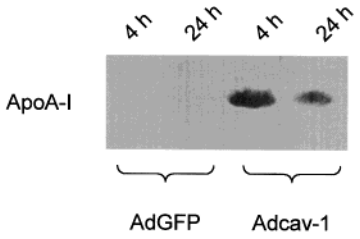

FIGURE 7: Effect of caveolin-1 on apoA-I turnover in vivo. Human HDL particles were injected into mice 3 days post-adenovirus injection. Plasma samples were collected and analyzed for apoA-I content by Western blot analysis. This blot shows the amount of apoA-I remaining 4 and 24 h post-HDL injection. Detection was performed by using a specific mAb directed against human apoA-I that does not cross-react with mouse apoA-I.

Here, we show that caveolin-1 overexpression in mouse liver is associated with increased plasma HDL−cholesterol levels. To determine the effect of caveolin-1 on SR-BI-mediated selective uptake, we infected murine hepatocytes in culture with an adenoviral vector carrying the caveolin-1 cDNA or GFP as a control protein. We show that, in cultured hepatocytes, caveolin-1 inhibits DiI−HDL uptake mediated by SR-BI (Figure 5). This result would mechanistically explain the increased HDL−cholesterol levels we observed in caveolin-1 adenovirus-injected animals. Impaired selective uptake of cholesteryl esters would result in an increase in HDL size as well as increases in plasma HDL-C levels. This result is observed in the case of hepatic overexpression of caveolin-1 in animals fed a chow diet. However, we did not observe any change in HDL size in the case of animals fed a high cholesterol diet. One possible explanation for this difference may be that caveolin-1-induced secretion of apoA-I can be affected by the cholesterol content of the diet. In that case, an increase in the dietary cholesterol content may induce a further increase in hepatic apoA-I secretion in caveolin-1 overexpressing animals, as compared to animals overexpressing caveolin-1 on a chow diet. Alternatively, caveolin-1 may be regulated at the posttranslational levels by cellular cholesterol levels. In either case, the resulting enhanced apoA-I secretion would be accompanied by an increase in the number of apoA-I-containing particles that could compensate for the increased HDL−cholesterol ester content and therefore prevents an increase in HDL size. This interpretation is also consistent with the lipoprotein profile shown in Figure 4; note that Adcav-1 induces an increase in plasma apoA-I without changing the lipoprotein density. We also observed that HDL size is the same for Adcav-1-infected animals on both chow and high cholesterol diets, possibly because the maximum size has been reached. In contrast, as expected, a cholesterol-rich diet increases HDL size in the control AdGFP animals.

Caveolin-1, Caveolae, and HDL Metabolism. Our current studies may imply that caveolin-1 regulates the activity of SR-BI, as SR-BI is the main protein responsible for HDL-CE selective uptake. Selective HDL-CE uptake occurs especially in cells requiring or excreting high levels of cholesterol (adrenal, ovaries, testis, liver). Since caveolin-1 expression is positively regulated by free cholesterol levels, it follows that high levels of caveolin-1 may indicate an excess of cellular cholesterol. Therefore, it would be important for cells to prevent any further accumulation of cholesterol.

SR-BI has been shown to colocalize and coimmunoprecipitate with caveolin-1 (*46*, *64*), and caveolin-1 is known to inhibit the activity of several proteins involved in various signaling cascades. One possibility is that the interaction of SR-BI with caveolin-1 inhibits SR-BI-mediated selective HDL-CE uptake, until cellular cholesterol decreases to acceptable levels. Interestingly, caveolin-1 expression is usually relatively low in tissues with high SR-BI activity (i.e., high selective HDL-CE uptake), such as liver. Caveolin-1 overexpression may block downstream effectors of SR-BI, for example, by decreasing the internalization of cholesteryl esters already associated with the plasma membrane.

Recent studies by Arakawa et al. (*65*) have suggested a role for caveolin-1 in cholesterol enrichment of HDL. These authors have shown that inhibition of caveolin-1 expression was associated with a reduced HDL−cholesterol enrichment when apoA-I was incubated with THP-1 macrophages. In this study, we show that hepatic caveolin-1 overexpression leads to increased secretion of hepatic apoA-I and a decreased rate of plasma HDL clearance. Caveolin-1 may enhance this process by modifying apoA-I lipidation and, more specifically, cholesterol association, which may allow its enhanced secretion. This hypothesis is further supported by the observation that apoA-I has a high affinity for cholesterol-enriched plasma membrane domains, like caveolae (*66*).

Functional Significance. Increased HDL−cholesterol levels are usually associated with a reduced risk of coronary artery disease. However, recent studies have indicated that, in some cases, high levels of HDL-C can lead to increased susceptibility to atherosclerosis (*67*). These cases are usually associated with a defect in reverse cholesterol transport.

Reverse cholesterol transport allows the elimination of excess peripheral cholesterol and its transport by HDL to the liver for elimination and degradation. Defects in HDL transport have been associated with atherosclerosis (*67*). For example, lack of hepatic SR-BI expression was associated with increased atherosclerosis in LDL-R-deficient mice despite a slight increase in HDL-C levels (*37*). However, LDL-C levels were also increased in that case. Other data have indicated that CETP genetic defects in humans are associated with increased risk of atherosclerosis despite increased HDL-C levels (*68*). Conversely, very efficient clearance of cholesterol can be obtained by overexpressing SR-BI. Experiments in transgenic mice overexpressing this receptor have shown that these mice not only present reduced risk of developing atherosclerosis but also reduced HDL-C. Therefore, increasing HDL-C by increasing hepatic expression of caveolin-1 may not lead to an athero-protective lipoprotein profile but could rather induce atherosclerosis in these animals. On the other hand, an increase in apoA-I secretion may be protective against atherosclerosis. One can also suggest that defective regulation of caveolin-1 expression in the liver could lead to abnormal lipoprotein profiles. Further studies will be required to directly test this hypothesis in genetically modified mice that lack caveolin-1 gene expression (Cav-$1^{-/-}$).

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