The severe depletion of cholesteryl ester (CE) in adrenergicortical cells of apoA-I−/− mice suggests that apolipoprotein (apo) A-I plays an important role in the high density lipoprotein (HDL) CE selective uptake process mediated by scavenger receptor BI (SR-BI) in vivo. A recent study showed that apoA-I−/− HDL binds to SR-BI with the same affinity as apoA-I+/+ HDL, but apoA-I−/− HDL has a decreased V_{max} for CE transfer from the HDL particle to adrenal cells. The present study was designed to determine the basis for the reduced selective uptake of CE from apoA-I−/− HDL. Variations in apoA-I−/− HDL particle diameter, free cholesterol or phospholipid content, or the apoE or apoA-II content of apoA-I−/− HDL had little effect on HDL CE selective uptake into Y1-BS1 adrenal cells. Lecithin cholesterol acyltransferase treatment alone or addition of apoA-I to apoA-I−/− HDL alone also had little effect. However, addition of apoA-I to apoA-I−/− HDL in the presence of lecithin cholesterol acyltransferase reorganized the large heterogeneous apoA-I+/− HDL to a more discrete particle with enhanced CE selective uptake activity. These results show a unique role for apoA-I in HDL CE selective uptake that is distinct from its role as a ligand for HDL binding to SR-BI. These data suggest that the conformation of apoA-I at the HDL surface is important for the efficient transfer of CE to the cell.

It is well established that the risk of developing coronary heart disease is inversely proportional to plasma HDL cholesterol levels (1). Decreased levels of apoA-I, the major protein of HDL, are also associated with an increased risk for coronary heart disease (2). The most widely accepted model explaining the anti-atherogenic properties of apoA-I is reverse cholesterol transport (3). In this process, poorly lipidated apoA-I first removes excess free cholesterol (FC) from peripheral cells through a mechanism dependent on ABCA1 (4). The apoA-I then acts as a co-factor for LCAT, which transforms the FC to cholesteryl ester (CE). This event initiates the conversion of the poorly lipidated apoA-I to a spherical HDL particle. After remodeling by plasma enzymes including cholesteryl ester transfer protein, hepatic lipase, and phospholipid transfer protein, the HDL finally delivers its CE either to the liver, where it can be excreted or repackaged into new lipoproteins, or to ovaries, testes, and adrenal glands, where it can be used in the production of steroid hormones.

ApoA-I has a number of important roles in HDL metabolism including activation of LCAT, determination of plasma HDL cholesterol levels, and interaction with the ABCA1 transporter and the HDL receptor, SR-BI. Mice deficient in apoA-I have a 70% reduction in total plasma cholesterol and HDL cholesterol (5–7), a 75% reduction in LCAT activity (8), and a severe depletion of cholesteryl ester stores in steroidogenic tissues (9). In these animals, the cortical cells of the adrenal gland, the luteal and interstitial cells of the ovary, and the Leydig cells of the testis all display diminished CE content, indicating that apoA-I is important for the SR-BI-mediated HDL CE selective uptake process (10, 11). This deficiency appears to be directly attributable to the absence of apoA-I because apoA-I deficient mice have a similar reduction in HDL cholesterol but do not show reduced CE reserves in their adrenals, ovaries, and testes (9).

In a recent study we addressed the role of apoA-I in HDL CE selective uptake by analyzing the structural, chemical, and functional properties of apoA-I−/− and apoA-I+/− HDL. Compared with the apoA-I+/− HDL, apoA-I−/− particles were larger, more heterogeneous in size, and enriched in apoA-II, apoCs, apoE, FC, and CE (12). Compared with apoA-I+/− HDL, CE selective uptake from apoA-I−/− HDL was significantly reduced into Y1-BS1 adrenal cells and F5UAH hepatoma cells, which naturally express SR-BI, and into ldla[SR-BI] cells, a Chinese hamster ovary cell line expressing SR-BI from a transfected cDNA. In Y1-BS1 and ldla[SR-BI] cells, the reduction in HDL CE selective uptake was attributed to a reduced V_{max} for CE transfer to the cell. Interestingly, in both cell types, apoA-I−/− HDL showed a lower K_{D} for HDL cell association, indicating that the absence of apoA-I did not reduce the affinity of HDL for SR-BI. These findings illustrate that HDL properties necessary for HDL binding to SR-BI are distinct from those properties necessary for the transfer of HDL CE to the cell membrane. Additionally, the V_{max} for endocytic uptake and degradation of HDL did not differ between apoA-I+/− and apoA-I−/− HDL.
apoA-I−/− HDL in either cell type. Thus, the absence of apoA-I on HDL particles selectively affected the SR-BI-mediated HDL CE selective uptake pathway.

In the current report we have explored the basis for the reduced selective uptake of CE from apoA-I−/− HDL. Variation in HDL particle size, cholesterol to phospholipid ratios, and apolipoprotein compositions had little effect on HDL CE selective uptake into Y1-BS1 adrenal cells. Addition of apoA-I to apoA-I−/− HDL also had little effect. However, addition of apoA-I to apoA-I−/− HDL in the presence of LCAT reorganized HDL structure and produced an HDL particle with increased CE selective uptake activity. These data suggest that the formation of apoA-I at the HDL surface is important for the efficient transfer of CE to the cell.

EXPERIMENTAL PROCEDURES

Materials—The following reagents used for culturing Y1-BS1 cells were purchased from the listed vendors: poly-n-lysine (Becton Dickinson), heat-denatured fetal bovine serum (Atlanta Biologicals), 100× penicillin/streptomycin/glutamine (Invitrogen), six-well plates (Costar), Cortrosyn (Organon), Ham’s F-10 medium, and heat-denatured horse serum (Sigma). Sodium [3H]cholesterol iodide and [3H]cholesterol ester were acquired from PerkinElmer Life Sciences and Amersham Bio- sciences, respectively.

Animals—apoA-I−/− C57BL/6J-Apoa1m1ts6 (apoA-I−/− C57BL/6J mice, and apoA-I−/− and apoA-I+/− mice on an 8:1 FVB/N: C57BL6 background, were obtained and maintained on a 12-h light/ 12-h dark cycle with standard rodent chow and water ad libitum (12). Housing and experimental procedures were approved by the State University of New York at Stony Brook Committee on Laboratory Animal Resources and the Scripps Research Institute Institutional Animal Care and Use Committee.

Isolation and Analysis of Wild Type and ApoA-I-deficient Lipoproteins—After an overnight fast, mice were anesthetized and exsanguinated by heart puncture, and blood cells were removed by centrifugation. After an overnight fast, mice were anesthetized and exsanguinated by heart puncture, and blood cells were removed by centrifugation. Plasma was adjusted to 0.05% NaN3, and 35.3–43.5 and 37–40 min for apoA-I+/− HDL and from 33–33.3, 33.3–35.3, and 35.3–40 min for apoA-I−/− HDL. Each fraction (10 μg of total cholesterol) was analyzed by a non-denaturing 4–25% polyacrylamide gradient gel as described above. The HDL apolipoproteins were then visualized using a 0.1% Coomassie Brilliant Blue R-250. After concentrating the HDL fractions using a Centricon 50 (Millipore), HDL apolipoproteins were radiolabeled using [125I]DLT as described above. Using 12% SDS-PAGE, the apolipoprotein complement of the [3H]COE-apoA-I−/− HDL was analyzed by separating an equal number of [3H] counts from each fraction. Following fixation of the gel with 40% methanol, 10% acetic acid, the [125I]DLT-labeled apolipoproteins were visualized by PhosphorImager analysis (Amersham Biosciences).

Treatment of ApoA-I+/− and ApoA-I−/− HDL—with d > 1.21 g/ml Plasma—Plasma was isolated from FVB/N mice as described above and subjected to ultracentrifugation (39,000 rpm, 24, 15°C) at d = 1.21 g/ml in a SW41 Ti rotor. The d > 1.21 g/ml fraction was washed by ultracentrifugation (39,000 rpm, 24, 15°C) at d = 1.21 g/ml and dialyzed against PBS-E supplemented with 0.02% NaN3.

[3H]COE apoA-I+/− and apoA-I−/− HDL (1.5 mg of protein) were incubated at 37°C for 16 h in 5.5 ml of PBS-E containing 50% by volume d > 1.21 g/ml plasma or d > 1.21 g/ml plasma plus 0.3 mg/ml lipid-free apoA-I, respectively. For controls, HDL (1.5 mg of protein) was incubated under similar conditions in PBS-E alone. The density of the sample after incubation was adjusted to 1.21 g/ml with KBr and subjected to ultracentrifugation (39,000 rpm, 19, 15°C) in a SW41 Ti rotor. The HDL was dialyzed against PBS-E, and apolipoproteins were radiola- beled using [125I]DLT as described above.

LCAT Treatment of [3H]COE-labeled ApoA-I+/− and ApoA-I−/− HDL—Recombinant human LCAT was purified as previously described and stored at −70°C in 50 μM imidazole, 10% glycerol (18). LCAT in the presence of fatty acid-free bovine serum albumin (BSA; Intergen) was concentrated at 4°C using an Ultrafree-15 centrifugal filter device with a 50,000 M cutoff (Millipore). The final concentrations of the LCAT and the BSA were 1.1–1.4 × 1010 units/nmol of CE formed/h of enzyme)/ml and 60 mg/ml, respectively. [3H]COE apoA-I+/− HDL (1.5 mg of protein) was incubated at 37°C for 24 h at 2.3 ml of reaction buffer (150 mM NaCl, 10 mM potassium phosphate, 1 mM EDTA, 0.1% poly-D-lysine). After 48 h, medium was replaced with 6 mg/ml BSA. The sample was adjusted to 1.21 g/ml with KBr, and HDL was isolated by ultracentrifugation (39,000 rpm, 19, 15°C) in a SW60 Ti rotor. Following dialysis against PBS-E, HDL were then radiolabeled using [125I]DLT as described above.

Using particles that were only [3H]COE-radiolabeled, the total cholesterol concentrations of the HDL samples were determined using the Cholesterol CII enzymatic assay (Wako). An equal amount of each sample was analyzed by 12% SDS-polyacrylamide electrophoresis (5 μg of total cholesterol) and non-denaturing 4–25% polyacrylamide gradient gels (5 and 10 μg of total cholesterol) as described above. Gels were stained with 0.1% Coomassie Brilliant Blue R-250 or evaluated by Western blot analysis as described above. Similar conditions were used to stain [3H]COE apoA-I+/− and apoA-I−/− HDL (1.2 mg of protein) were incubated under the same conditions in reaction buffer supplemented with 0.02% NaN3.

Size Fractionation of ApoA-I+/− and ApoA-I−/− HDL—Lipoproteins (1.02–1.21 g/ml radiolabeled with [3H]COE were separated using a Nephros column run at a flow rate of 0.4 ml/min. Following the initial separation, each sample was collected from 39,35.6, 35.3–37, and 37–40 min for apoA-I+/− HDL and from 33–33.3, 33.3–35.3, and 35.3–40 min for apoA-I−/− HDL. Each fraction (10 μg of total cholesterol) was analyzed by a non-denaturing 4–25% polyacrylamide gradient gel as described above. The HDL apolipoproteins were then visualized using a 0.1% Coomassie Brilliant Blue R-250. After concentrating the HDL fractions using a Centricon 50 (Millipore), HDL apolipoproteins were radiolabeled using [125I]DLT as described above. Using 12% SDS-PAGE, the apolipoprotein complement of the [3H]COE-[125I]DLT HDL was analyzed by separating an equal number of [3H] counts from each fraction. Following fixation of the gel with 40% methanol, 10% acetic acid, the [125I]DLT-labeled apolipoproteins were visualized by PhosphorImager analysis (Amersham Biosciences).

Isolation of Lipid-free Mouse ApoA-I—Lipid-free mouse apoA-I was isolated with a modification of a previously published protocol (16).  

HDL (1.063–1.21 g/ml) were isolated from apoA-I−/− FVB/N mouse plasma using sequential density ultracentrifugation. Following dialysis against PBS-E, HDL was incubated in 3 g guanidine HCl for 2 h at 37°C, dialyzed against PBS-E, and subjected to ultracentrifugation (39,000 rpm, 19 h, 15°C) at d = 1.21 g/ml in a SW60 Ti rotor. HDL remnants (d < 1.21 g/ml) were separated from the lipid-free apoA-I (d > 1.21) using a tube slicer. ApoA-I was dialyzed against PBS-E and stored under argon at 4°C. Because of the tendency of the protein to form multimers at concentrations above 1 mg/ml, the apoA-I was not concentrated after the final dialysis step (17).
with Ham's F-10 complete medium plus 100 ng/ml Cortrosyn, a 1-35ACTH synthetic analogue. All studies were conducted following a 24-h exposure to ACTH.

**Determination of Cell Association, Degradation, and Selective Uptake of HDL-CE—**After being seeded and treated as listed above, Y1-BS1 cells were washed and the medium replaced with serum-free Ham's F-10 medium. [3H]COE-[125I]DLT apoA-I/- or apoA-I/- HDL was added to the final concentration specified in the figure legends. Following a 4-h incubation at 37 °C, the cells were washed three times with phosphate-buffered saline plus 0.1% BSA, pH 7.4; one time with phosphate-buffered saline, pH 7.4; lysed with 1.25 ml of 0.1 M NaOH; and passed five times through a 23-gauge needle. The lysate was then processed to determine trichloroacetic acid-soluble and -insoluble 125I radioactivity and organic solvent-extractable 3H radioactivity as described (14, 19). Trichloroacetic acid-insoluble 125I radioactivity represents cell-associated HDL apolipoprotein that is the sum of cell surface-bound apolipoprotein and endocytosed apolipoprotein that is not yet degraded. Trichloroacetic acid-soluble 125I radioactivity represents endocytosed and degraded apolipoprotein that is trapped in lysosomes as a result of the dilactitol tyramine label (14, 20). The sum of the 125I-degraded and 125I-cell-associated undegraded apolipoprotein expressed as CE equivalents was subtracted from the CE measured as extractable 3H radioactivity to give the selective uptake of HDL-CE (14, 19). Values for these parameters are expressed as nanograms of HDL-CE/mg of cell protein.

**RESULTS**

**Role of Apolipoprotein Content and Particle Size in Reduced CE Selective Uptake Activity of apoA-I/- HDL—**Previous studies with reconstituted or modified HDL suggest that specific apolipoproteins, particularly apoA-II and apoE, may alter the efficiency of SR-BI-mediated HDL CE selective uptake. However, there is no clear consensus in the literature as to whether these proteins have inhibitory or stimulatory effects (21–25). To compare apoA-I/- HDL with different apoA-II and apoE contents, HDL were isolated from mice on FVB/N or C57BL/6 genetic backgrounds. Previous studies showed that apoA-I/- HDL from C57BL/6 mice are enriched in apoE (5, 7), and we noted that HDL from FVB/N mice are enriched in apoA-II. The SDS-PAGE analysis in Fig. 1A shows the relative enrichment of FVB/N apoA-I/- HDL in apoA-II in comparison to the enrichment of C57BL/6 apoA-I/- HDL in apoE. Each of these HDLs, along with the respective apoA-I/- HDLs were labeled with [3H]COE and [125I]DLT and tested in a standard selective uptake assay using ACTH-treated Y1-BS1 adrenocortical cells in which HDL CE selective uptake is primarily the result of SR-BI (26). Comparing particles isolated from mice of the same genetic strain, ~2-fold more selective CE uptake was observed from apoA-I/- than from apoA-I/- HDL (Fig. 1D). Additionally, HDL CE selective uptake was similar when comparing apoA-I/- HDL of both strains. This result indicates that apoA-I/- HDL, regardless of its apoA-II or apoE content, is less efficient than wild type HDL at selectively transferring its CE to the Y1-BS1 cells. Interestingly, the Y1-BS1 cells displayed similar HDL-CETL CE cell association and degradation of the different particles with the exception of the C57BL/6 apoA-I/- HDL (Fig. 1, B and C). The 2-fold increase in these parameters for the C57BL/6 apoA-I/- HDL is likely a result of this apoE-rich HDL being bound and internalized by proteoglycans or members of the LDL receptor family (27). In contrast, differences in the apoA-II and apoE content of the apoA-I/- HDL did not alter the ability of the Y1-BS1 cells to internalize CE via SR-BI-dependent selective uptake.

Several studies have shown that HDL particle size affects the ability of cells to selectively internalize HDL-CETL (28–30). To test whether the larger size and heterogeneity of the apoA-I/- HDL (5, 12) may explain its reduced selective uptake activity, [3H]COE-labeled apoA-I/- and apoA-I/- HDL were separated into three size fractions by gel exclusion chromatography (Fig. 2A). Analysis by nondenaturing gradient gel electrophoresis indicated that the HDL was separated into fractions with different mean particle diameters (Fig. 2B). Following radiolabeling of the HDL with [125I]DLT, the apolipoprotein complement of each fraction was determined by SDS-PAGE (Fig. 2C). As observed previously (12), apoA-II was enriched on smaller particles and apoE on larger particles of apoA-I/- HDL.

The functional properties of the size-fractionated HDL were
tested on ACTH-treated Y1-BS1 cells. Although the two types of unfractionated particles were bound and degraded to similar extents (Fig. 3, A and B, T columns), more selective CE uptake was seen with apoA-I<sup>+</sup> compared with the apoA-I<sup>−</sup> total HDL fraction (Fig. 3C, T columns). In contrast, cell association and degradation of the fractionated particles increased in proportion to their diameter for both apoA-I<sup>+</sup> and apoA-I<sup>−</sup> HDL (Fig. 3, A and B). These differences among the size-fractionated particles likely reflect the larger, apoE-rich HDL interacting with LDL receptor family members or proteoglycans. In contrast, little difference was seen for HDL CE selective uptake among the size-fractionated particles for either apoA-I<sup>+</sup> or apoA-I<sup>−</sup> HDL (Fig. 3C). Additionally, more selective CE uptake was seen with apoA-I<sup>+</sup> than apoA-I<sup>−</sup> HDL when particles of similar diameter were compared. For instance, the Y1-BS1 cells selectively internalized more HDL CE from apoA-I<sup>+</sup> fraction 2 than from the similar sized apoA-I<sup>+</sup> fraction 3. Similar results were obtained with size-fractionated particles from two independent HDL preparations. Several conclusions can be drawn. First, size subpopulations within the apoA-I<sup>+</sup> and apoA-I<sup>−</sup> HDL do not differentially transfer CE to the Y1-BS1 cells by selective uptake. Second, in agreement with the experiments comparing FVB/N and C57BL/6 apoA-I<sup>+</sup> HDL (Fig. 1), selective CE uptake from apoA-I<sup>+</sup> HDL is not significantly affected by the addition of apoA-II and apoE content. Third, the larger size and heterogeneity of apoA-I<sup>−</sup> HDL are not responsible for the diminished SR-BI-mediated selective CE uptake.

**Role of Free Cholesterol Content in Reduced CE Selective Uptake Activity of apoA-I<sup>−</sup> HDL.**—In a previous analysis, we noted that apoA-I<sup>−</sup> HDL has a significantly higher FC content than apoA-I<sup>+</sup> HDL, a factor that may reduce the fluidity of the PL monolayer and hinder SR-BI-mediated transfer of CE from the HDL core (12). To test the importance of the HDL FC content for CE selective uptake, <sup>3</sup>HCE apoA-I<sup>−</sup> HDL was incubated with apoA-I<sup>+</sup> or apoA-I<sup>−</sup> HDL that had been radiolabeled with <sup>3</sup>HCE and <sup>125</sup>I-DLT. The amounts of cell association (A), degradation (B), and selective uptake (C) of HDL-CE were determined as described under “Experimental Procedures.” Each column represents the mean of three samples (± S.E.). Note that the scale of the y axis for panels A and B is different from that in panel C. Similar results were seen using a separately isolated and radiolabeled batch of particles.
associated with the apoA-I−/− particles (data not shown). After radio-labeling with [3H]COE-[125I]DLT, the HDL were incubated with ACTH-treated Y1-BS1 cells. The selective CE uptake from the particles exposed to the d > 1.21 g/ml plasma was significantly increased compared with their respective mock-treated controls (Table I). This result suggested that the high FC content of the apoA-I−/− HDL may impede selective transfer of CE to Y1-BS1 cells. However, the functional properties of the apoA-I−/− HDL may also have been altered by the acquisition of apoA-I or modification by enzymes other than LCAT in the d > 1.21 g/ml plasma.

Role of ApoA-I and LCAT in Reduced CE Selective Uptake Activity of ApoA-I−/− HDL—To test more directly the impact of the FC content of apoA-I−/− HDL on selective CE uptake, apoA-I−/− particles were incubated with purified recombinant human LCAT. Regardless of the absence or presence of lipid-free mouse apoA-I, LCAT treatment significantly decreased the FC content of the apoA-I−/− HDL resulting in FC/protein and FC/PL ratios that were lower than those of apoA-I+/+ HDL (Table II). In addition, the size and the apolipoprotein composition of the apoA-I−/− particles were modified by exposure to LCAT. SDS-PAGE showed that apoA-I−/− HDL incubated with LCAT and apoA-I acquired apoA-I that was stable to re-isolation of the particles (Fig. 4A, lane 4). NDGGE of apoA-I−/− HDL that had been incubated with LCAT alone showed a minor reduction in particle mobility with no change in heterogeneity of the particles (Fig. 4B, lane 3). Western blot analysis showed that LCAT treatment released most of the apoE from apoA-I−/− HDL (Fig. 4B, lane 3). Incubation of apoA-I−/− HDL with LCAT plus apoA-I generated a smaller, more distinct particle population, the diameter of which was only slightly larger than that of apoA-I+/+ HDL (Fig. 4B, lane 4). Western blot analysis of the HDL separated by NDGGE revealed that apoA-I was present on these particles, whereas apoE resided on particles with slightly larger diameters (Fig. 4B, lanes 2 and 4).

The functional properties of the HDL were then tested on ACTH-treated Y1-BS1 cells. Compared with the two mock-treated particles, more HDL-CE cell association and degradation was observed from apoA-I−/− HDL that had been exposed to LCAT (Fig. 4, C and D, bars 3 and 4). SDS-PAGE showed that all the samples contained BSA that had not been completely removed during re-isolation of the HDL by density ultracentrifugation (Fig. 4A). However, NDGGE revealed that LCAT, which has a molecular weight similar to BSA on SDS-PAGE, was also present in the treated samples (Fig. 4B, lanes 3 and 4). Therefore, it was concluded that the Y1-BS1 cells bound and degraded the LCAT-treated apoA-I−/− HDL to a greater extent as a result of the presence of this enzyme.

An enhancement in selective CE uptake was not observed from apoA-I−/− HDL treated with LCAT alone (Fig. 4E, compare bars 2 and 3), even though these particles had a lower FC/PL ratio than apoA-I+/+ HDL and a PL/PT ratio nearly the same as apoA-I−/− HDL (Table II). However, the modifications imparted by incubation with apoA-I plus LCAT resulted in increased CE selective uptake from apoA-I−/− HDL (Fig. 4E, compare bars 2 and 4). Several conclusions can be drawn from these data. First, the failure of LCAT treatment alone to increase HDL CE selective uptake from apoA-I−/− HDL, despite decreasing the FC and PL content and the FC/PL ratio, indicates that these parameters are not key factors in the reduced efficiency of CE transfer from apoA-I−/− HDL. Second, enhanced cell association of apoA-I−/− HDL treated with LCAT alone (Fig. 4C, compare bars 2 and 3) was accompanied by increased HDL processing by the endocytic uptake pathway (Fig. 4D, compare bars 2 and 3), but not by SR-BI-mediated selective uptake (Fig. 4E, compare bars 2 and 3). Third, the increased CE selective uptake resulting from treatment of apoA-I−/− HDL with LCAT plus apoA-I was accompanied by a reorganization of the HDL to yield a more discrete size population similar in diameter to apoA-I+/+ HDL.

Because the prior experiments showed that selective CE uptake was increased from apoA-I−/− HDL treated with apoA-I plus LCAT, we next determined whether apoA-I alone could generate similar changes in the particles. For this study, apoA-I−/− HDL were incubated with lipid-free apoA-I in the absence or presence of LCAT and were subsequently analyzed for changes in apolipoprotein content and size by SDS-PAGE and NDGGE. As seen previously, apoA-I−/− HDL incubated with LCAT and apoA-I had a decreased level of apoE, had acquired apoA-I (Fig. 5A, lane 4), and had formed a distinct particle population with a diameter similar to apoA-I−/− HDL (Fig. 5B, lane 4). Incubation of the apoA-I−/− HDL with apoA-I alone also caused the gain of apoA-I (Fig. 5A, lane 3). However, the size distribution of these particles was not changed (Fig. 5B, lane 2 versus lane 3). Western blot analysis showed that apoA-I was equally distributed among these heterogeneous sized particles, whereas it was concentrated on the smaller distinct particle population formed by apoA-I plus LCAT (Fig. 5B, lane 3 versus lane 4). In addition, the apoA-I−/− HDL incubated with only apoA-I was found to have lost a significant amount of its apoE but not nearly as much as the particles treated with apoA-I plus LCAT.

Testing the functional properties of the HDL on ACTH-treated Y1-BS1 cells showed that addition of apoA-I did not enhance selective CE uptake (Fig. 5E, compare bars 2 and 3). In contrast, selective CE uptake was enhanced following incubation of the apoA-I−/− HDL with apoA-I and LCAT (Fig. 5E, compare bars 2 and 4). Compared with their mock-treated equivalent, the lone addition of apoA-I to the apoA-I−/− HDL caused a slight reduction in HDL-CE cell association and degradation (Fig. 5, C and D). The decrease in these parameters most likely reflects the decreased apoE content (Fig. 5B, compare lanes 2 and 3). From these results it can be concluded that apoA-I is necessary but not sufficient for the enhancement of CE selective uptake and the conversion of the apoA-I−/− HDL into smaller particles of a more discrete size.

In the previous experiments (Figs. 4 and 5), some LCAT was recovered in the LCAT-treated apoA-I−/− HDL after
re-isolation of the particles and, thus, was present during the HDL CE selective uptake measurements with the Y1-BS1 cells. To test whether LCAT activity might contribute to the selective uptake process itself (and not only to the HDL particle reorganization), apoA-I-/- HDL were incubated with apoA-I and LCAT to permit particle reorganization, and then treated with 2 mM DTNB to inactivate LCAT (31). These particles and the mock-treated controls were then re-isolated by centrifugation, and tested for HDL CE selective uptake activity with the Y1-BS1 cells. As shown in Fig. 6, apoA-I-/- HDL incubated with LCAT and apoA-I showed the same increase in HDL CE selective uptake compared with apoA-I-/- HDL whether or not the particles were treated with DTNB (compare bars 3 and 4 with bar 2). Thus, LCAT activity remaining after HDL re-isolation did not contribute to HDL CE selective uptake activity. In other experiments we also have shown that treatment of LCAT with 2 mM DTNB prior to incubation with HDL and apoA-I effectively blocked LCAT activity (data not shown).

**DISCUSSION**

The results from this study indicate that the reduced efficiency of CE selective uptake from apoA-I-/- HDL into adrenal cells is caused by the physical absence of apoA-I. Within the range of properties evaluated, variations in particle size, FC and PL content, the FC/PL ratio, or the apoA-II and apoE contents had little influence on SR-BI-mediated CE selective uptake from apoA-I-/- HDL. Similarly, addition of apoA-I to apoA-I-/- HDL or treatment of the particles with LCAT had little effect. In contrast, addition of apoA-I in combination with LCAT treatment reorganized the apoA-I-/- HDL to a smaller and less heterogeneous particle with increased HDL CE selective uptake activity. The particle size analysis described above indicates that it is not the smaller size per se of the reorganized HDL that is important for enhanced selective uptake activity. These results suggest that not only the presence but also the proper orientation of apoA-I is needed for optimal SR-BI-mediated selective CE uptake from HDL. The inability of LCAT treatment alone to alter the particle size distribution or the CE selective uptake activity is supported by in vivo findings as well. In the apoA-I-/- mouse, HDL is exposed to sufficient LCAT activity to generate CE-rich particles, but the particles remain large and heterogeneous and have reduced CE selective uptake activity (8, 12). That observation as well as the *in vitro* LCAT experiments reported here indicate that other HDL apolipoproteins cannot substitute for apoA-I in these processes, despite the fact that apoCI, apoE, and apoA-IV can activate LCAT (32–34). Thus, the reorganization of apoA-I-/- HDL particles and the enhancement of HDL CE selective uptake appear to be unique properties of apoA-I.

The mechanism by which apoA-I enhances SR-BI-mediated HDL CE selective uptake is unclear but most likely requires apoA-I to assume a specific conformation on the HDL surface in a process requiring LCAT. The presence of apoA-I on the surface of apoA-I-/- HDL had little effect on CE selective uptake until the HDL was reorganized by LCAT. We speculate that, during particle reorganization, apoA-I assumes a specific conformation that is important for efficient lipid transfer but is not important for the initial docking of HDL to SR-BI. Several studies support this dissociation between HDL binding and
The amount of LCAT-mediated HDL CE selective uptake from apoA-I and HDL was analyzed as described in the legend of Fig. 4. Each column represents the mean of four samples (±S.E.) from two experiments. Note that the scale of the y axis for panels C and D is different from that in panel E. Similar results were seen using a separately treated and radiolabeled batch of particles.

In summary, we have determined that the failure of adrenal cells to efficiently internalize CE from apoA-I−/− HDL via selective uptake is directly caused by the absence of apoA-I from the particles. On apoA-I+/− HDL and apoA-I−/− HDL treated with apoA-I and LCAT, apoA-I appears to be organized in such a manner that, through interactions with SR-BI or via effects on HDL lipid organization, or both, HDL CE selective uptake is enhanced.

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