Kinetic characteristics and regulation of HDL cholesteryl ester and apolipoprotein transport in the apoA-I$^{-/-}$ mouse

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Abstract **The concentration dependence and tissue distribution of high density lipoprotein (HDL) cholesteryl ester and apolipoprotein (apo) transport were determined in apoA-I knockout mice (apoA-I$^{-/-}$) that lack normal HDL in plasma. Rates of HDL cholesteryl ester clearance were highly sensitive to plasma HDL cholesteryl ester concentrations with clearance rates falling by 80% in the liver and by 95% in the adrenal glands when plasma HDL cholesteryl ester concentrations were acutely raised to levels normally seen in control mice (~50 mg/dl). With the exception of the brain, saturable HDL cholesteryl ester uptake was demonstrated in all tissues of the body, with the adrenal glands and liver manifesting the highest maximal transport rates (J^m). The plasma concentration of HDL cholesteryl ester necessary to achieve half-maximal transport (K_m) equaled 4 mg/dl in the adrenal glands and liver; as a consequence, HDL cholesteryl ester uptake by these organs is maximal (saturated) at normal plasma HDL concentrations in the mouse. When expressed per whole organ, the liver was the most important site of HDL cholesteryl ester clearance accounting for ~72% of total HDL cholesteryl ester turnover at normal plasma HDL concentrations. HDL cholesteryl ester transporter activity and scavenger receptor type B1 (SR-BI) protein and mRNA levels were not up-regulated in any organ of apoA-I$^{-/-}$ mice even though these animals lack normal HDL.—**Spady, D. K., L. A. Woollett, R. S. Meidell, and H. H. Hobbs. **Kinetic characteristics and regulation of HDL cholesteryl ester and apolipoprotein transport in the apoA-I$^{-/-}$ mouse. *J. Lipid Res.* 1998.** 39: **1483–1492.**

Supplementary key words HDL • cholesteryl ester transport • apoA-I • liver • lipoproteins • reverse cholesterol transport

An inverse relationship exists between the plasma concentration of high density lipoprotein (HDL) cholesterol and the risk of clinical coronary heart disease (1–3). Although the mechanisms responsible for this relationship are poorly understood, the protective effect of HDL is usually attributed to its role in maintaining sterol balance in extrahepatic tissues. Cholesterol that is acquired by most extrahepatic tissues (from de novo synthesis or lipoprotein uptake) must be returned to the liver for excretion in a process that has been termed reverse (4, 5) or centripetal (6) cholesterol transport. Reverse cholesterol transport is initiated in extrahepatic tissues by the transfer of unesterified cholesterol from cell membranes to nascent HDL (4, 5). A portion of this cholesterol is esterified by lecithin:cholesterol acyltransferase and partitions into the hydrophobic core of the HDL particle. HDL cholesteryl esters are cleared from plasma via several pathways including *1*) transfer to lower density lipoproteins with subsequent uptake via the LDL receptor pathway (*7*), *2*) uptake of the whole HDL particle (8), and *3*) selective uptake of HDL cholesteryl esters resulting in an HDL particle of reduced size and cholesteryl ester content (9, 10). This latter process is likely mediated by the scavenger receptor type B1 (SR-BI), which was shown to mediate selective cholesteryl ester uptake when transfected into cells (11).

The kinetic characteristics of HDL cholesteryl ester and apoA-I transport have been analyzed in the hamster in vivo (12). These studies demonstrated saturable HDL cholesteryl ester transport in most tissues of the body with the highest maximal transport rates (J^m) in the adrenal glands and liver. Unexpectedly, the concentration of HDL cholesteryl ester necessary to achieve half-maximal transport (K_m) in these tissues was ~13 mg/dl, well below the normal plasma concentration of HDL cholesteryl ester in the hamster. As a consequence, receptor-dependent HDL cholesteryl ester uptake was maximal (saturated) at normal plasma HDL concentrations. Because HDL concentrations could be acutely raised but not lowered in these in vivo studies, it was not possible to directly determine the kinetic characteristics of the transport process at less than saturating plasma HDL concentrations.

While the hamster is an established model for the study of nutrient and drug effects on cholesterol and bile salt

Abbreviations: HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); J^m, maximal rate of receptor-dependent transport; K_m, concentration necessary to achieve half-maximal transport via receptor-dependent mechanisms; P, proportionality constant for receptor-independent transport; SR-BI, scavenger receptor type B1; FCR, fractional catabolic rate.

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metabolism, the availability of natural and induced mutant strains has made the mouse a valuable model for examining genetic influences on sterol balance and lipoprotein metabolism. A large number of mutant mouse strains exist that lack or overexpress proteins directly involved in HDL metabolism. Of importance with respect to the current studies, apoA-I knockout mice (apoA-$I^{-/-}$) are available that have very low background levels of HDL, making it possible to generate complete kinetic curves defining the concentration dependence of HDL cholesteryl ester transport. The current studies were undertaken to establish the kinetic characteristics and tissue distribution of HDL cholesteryl ester and apoprotein transport in the mouse.

METHODS

Animals and diets

All studies were performed in male C57Bl/6 mice with or without targeted disruption of the apoA-I gene (13). Animals were obtained from Jackson Laboratories and housed in colony cages (5–6 animals/cage) in a room with light cycling (12 h light and 12 h dark) and controlled temperature and humidity. Animals were maintained on a standard cereal-based (low-fat, low-cholesterol) rodent diet (Teklab Premier Laboratory Diets, Madison, WI). All studies were carried out during the mid-dark phase of the light cycle. All experiments were approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center at Dallas.

Determination of HDL cholesteryl ether transport

Human HDL was isolated in the density range of 1.07–1.21 g/ml using sequential preparative ultracentrifugation and standard techniques (14) and labeled with either the intracellularly trapped [1α, 2α(n)-^{3}H]cholesteryl oleyl ether (15–17) or [cholesteryl-4-^{14}C]oleate by exchange from donor liposomes as described (9, 18). The labeled HDL were reisolated by ultracentrifugation, dialyzed against saline, and used within 24 h. The specific activity of the radiolabeled HDL preparations ranged from 20 to 50 dpm/ng cholesteryl ester.

Rates of HDL cholesteryl ether transport were determined using a primed infusion protocol as previously described (12). Polyethylene catheters (ID 0.28 mm, OD 0.61 mm) were placed into the femoral vein under brief (~3 min) methoxyflurane anesthesia. Animals were administered a priming dose of [^{3}H]cholesteryl ether-labeled HDL intravenously followed by a continuous infusion of the same radiolabeled lipoprotein at a rate determined in preliminary studies to maintain a constant plasma specific activity. The primed infusions of [^{3}H]cholesteryl ether-labeled HDL were continued for 4 h at which time each animal was administered [^{14}C]cholesteryl ester-labeled HDL intravenously (as a marker of the volume of plasma within each tissue) and killed 10 min later. Plasma and tissue samples were saponified and the sterols were quantitatively extracted using petroleum ether (12). Exact aliquots of the petroleum ether extracts were dried in scintillation vials and assayed for their ^{3}H and ^{14}C content. The tissue spaces achieved by the labeled HDL at 10 min (^{14}C dpm per gram of tissue divided by the ^{14}C dpm per microliter of plasma) and at 4 h and 10 min (^{3}H dpm per gram of tissue divided by the steady-state ^{3}H dpm per microliter of plasma) were then calculated and have the units of μl/g. The increase in tissue space over the 4-h experimental time period equals the rate of radiolabeled HDL cholesteryl ether movement into each organ and is expressed as the microliters of plasma cleared of its HDL cholesteryl ether content per h per g of tissue or per whole organ (12). Clearance values were multiplied by the plasma HDL cholesteryl ester concentration to obtain the absolute rates of HDL cholesteryl ester uptake expressed as the micrograms of HDL cholesteryl ester taken up per h per g of tissue or per whole organ. The concentration dependency of human HDL cholesteryl ester transport was determined by adding mass amounts of unlabeled human HDL (d 1.07–1.21 g/ml) to the primed infusions of labeled HDL in amounts necessary to achieve new steady-state levels of human HDL cholesteryl ester in plasma that varied from <1 mg/dl to 400 mg/dl.

Preliminary studies were performed to determine how much radiolabeled HDL needed to be infused each hour (relative to the priming dose of ~100,000 dpm) to maintain a constant plasma concentration of radiolabeled HDL in plasma. In apoA-$I^{-/-}$ mice infused with trace-labeled HDL cholesteryl ether, 100% of the priming dose was required per h as a continuous infusion to maintain a constant plasma concentration of radiolabeled HDL. When mass amounts of HDL were added to the trace-labeled HDL cholesteryl ether, the amount of radiolabeled HDL (relative to the priming dose) that needed to be infused per h to maintain a constant plasma specific activity was reduced. In animals receiving the largest mass amounts of HDL, ~5% of the priming dose was required per h to maintain a constant plasma specific activity (and HDL cholesteryl ester concentration).

Determination of HDL apolipoprotein transport

HDL apolipoprotein transport was quantified in vivo using primed infusions of radiolabeled HDL apoprotein as previously described (12). Human HDL (d 1.07-1.21 g/ml) was labeled in situ with the intracellularly trapped marker ^{125}I-labeled tyramine cellobiose (19) or with ^{131}I (20). The final specific activity of the ^{125}I-labeled tyramine cellobiose-labeled HDL was ~175 dpm/ng protein. The final specific activity of the ^{131}I-labeled HDL was ~3000 dpm/ng protein. Animals were administered a priming dose of ^{125}I-labeled tyramine cellobiose-labeled HDL through a femoral vein catheter followed by a continuous infusion of the same radiolabeled lipoprotein at a rate determined in preliminary studies to maintain a constant plasma specific activity; at 4 h each animal was administered ^{131}I-labeled HDL intravenously (as a marker of volume of plasma within each tissue) and killed 10 min later. Plasma and tissue samples were assayed for radioactivity in a gamma counter. Tissue clearance rates were calculated as described above for [^{3}H]cholesteryl ether-labeled HDL. The concentration dependency of human HDL apolipoprotein transport was determined by adding unlabeled human HDL (d 1.07-1.21 g/ml) mass to the primed infusions of labeled HDL in amounts necessary to achieve new steady-state levels of human HDL apolipoprotein in plasma that varied from <1 mg/dl to nearly 600 mg/dl. The concentration of human HDL apolipoprotein achieved in each mouse was calculated from the steady-state level of radioactivity in plasma and the specific activity of the infused HDL.

Preliminary studies were performed to determine how much radiolabeled HDL needed to be infused each hour (relative to the priming dose of ~200,000 dpm) to maintain a constant plasma concentration of radiolabeled HDL in plasma. In apoA-$I^{-/-}$ mice infused with trace-labeled HDL, ~10% of the priming dose was required per h as a continuous infusion to maintain a constant plasma concentration of radiolabeled HDL. In animals receiving the largest mass amounts of HDL, ~5% of the priming dose was required per h to maintain a constant plasma specific activity (and HDL apolipoprotein concentration).

Recombinant adenoviruses

Generation and large scale production of the recombinant adenoviruses AdCMV-apoA-I (21), carrying the cDNA for human apoA-I, and AdCMV-luc (22), carrying the firefly luciferase gene, have been previously described. ApoA-I$^{-/-}$ mice were administered 10^9 pfu recombinant adenovirus by intravenous injection. All experiments were performed 3 days after virus administration.

Immunoblot analyses

Membrane fractions were prepared from pulverized mouse tissue that had been frozen in liquid N_2 immediately after harvesting and stored at −80°C. Immunoblot analysis was performed under reducing conditions using polyclonal antibodies raised against the C-terminal 14 amino acids of the mouse SR-BI (11, 23). An identical protocol was used for immunoblot analysis of the LDL receptor, except that the gels were run under nonreducing conditions, and a polyclonal rabbit anti-bovine LDL receptor antibody was used (24).

Determination of mRNA levels

Hepatic SR-BI and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, used as an invariant control) mRNA levels were determined by nuclease protection (25, 26). Single-stranded ^{32}P-labeled probes for mouse SR-BI and GAPDH were synthesized by oligonucleotide primed synthesis from M13 subclones as previously described (25) using 0.5 μm [^{32}P]dCTP and 1 μm (SR-BI) or 300 μm (GAPDH) unlabeled dCTP. The template cDNA for the SR-BI probe was obtained by reverse transcription-PCR using mouse liver RNA and oligonucleotides that span nucleotides 157–617 of the SR-BI cDNA (11).

Samples of liver were homogenized in guanidinium thiocyanate and the RNA isolated by the method of Chomczynski and Sacchi (27). Total RNA (5–40 μg) was hybridized with the ^{32}P-labeled cDNA probes simultaneously at 42°C overnight. Unhybridized probe, present in excess relative to the amount of specific mRNA, was then digested with mung bean nuclease (GIBCO BRL/Life Technologies, Gaithersburg, MD). The mRNA-protected ^{32}P-labeled probes were separated on 7 m urea, 6% polyacrylamide gels, and the radioactivity in each band, corrected for background radioactivity, was quantified using an isotopic imaging system (Ambis, Inc., San Diego, CA). The level of GAPDH mRNA did not vary among the experimental groups and was used to correct for any procedural losses.

Distribution of cholesterol and radioactivity among plasma lipoproteins

The lipoprotein distribution of plasma cholesterol and apoA-I was determined by FPLC. Equal volumes of plasma from animals within an experimental group were pooled and injected onto a Superose 6 (Sigma Chemical Co., St. Louis, MO) column as previously described (12). Two-ml aliquots were collected and assayed for cholesterol and apoA-I. Cholesterol was assayed using an enzymatic kit (Sigma Chemical Co., catalog #352). Human apoA-I was assayed using a turbidometric assay as described (12, 28).

Calculations and statistical analysis

Rates of human HDL cholesteryl ester transport were quantified in apoA-I$^{-/-}$ mice under conditions in which circulating human HDL concentrations were acutely raised and maintained at levels ranging from <1 mg/dl to 400 mg/dl as described above. Values for tissue HDL cholesteryl ester uptake were plotted as a function of the concentration of HDL cholesteryl ester in the same animal. The kinetic parameters for receptor-dependent (saturable) and receptor-independent (nonsaturable) HDL cholesteryl ester transport were determined by fitting these data to the equation $J_t = (J^m{*}C/K_m + C) + (P{*}C)$, where J_t equals total HDL cholesteryl ester uptake via receptor-dependent and receptor-independent mechanisms, J^m equals the maximal rate of HDL cholesteryl ester uptake via receptor dependent mechanisms, K_m equals the concentration of HDL cholesteryl ester in plasma necessary to achieve half-maximal rates of receptor dependent uptake, C equals the concentration of HDL cholesteryl ester in plasma, and P equals the proportionality constant for receptor-independent uptake (12). The three parameter values (J^m, K_m, and P ± 95% confidence intervals) were calculated with the software package Ultrafit (Biosoft, Cambridge, UK) using the Marquardt algorithm for nonlinear regression and proportional weighting (29). All other data are presented as means ± 1 SD.

RESULTS

Kinetics of HDL cholesteryl ester transport in apoA-I$^{-/-}$ mice

The kinetic characteristics of HDL cholesteryl ester transport were determined in apoA-I$^{-/-}$ mice, which lack normal plasma HDL. Low background levels of circulating HDL were desirable because our previous studies had found the HDL cholesteryl ester transport mechanism to be fully saturated at normal HDL concentrations in the hamster (12). HDL cholesteryl ester transport was traced using human HDL labeled with [^{3}H]cholesteryl oleyl ether, a nondegradable marker that remains intracellularly trapped after uptake. At the end of each experiment, the lipoprotein distribution of radiolabeled HDL was determined by FPLC using a Superose 6 column. No radioactivity was detected in fractions other than those corresponding to authentic human HDL.

Figure 1A shows HDL cholesteryl ether clearance rates in the adrenal gland as a function of the concentration of HDL cholesteryl ester in plasma during the 4-h infusion period. HDL cholesteryl ether clearance by the adrenal glands ranged from 3,000 to 9,000 μl/h per g in apoA-I$^{-/-}$ mice infused with trace amounts of [^{3}H]cholesteryl ether-labeled HDL (shaded circles). When various mass amounts of HDL were added to the primed infusions, clearance rates decreased sharply as a function of the steady-state plasma HDL cholesteryl ester concentration that was maintained during the infusion period (open circles). Clearance rates were reduced by >90% when mass amounts of HDL were added to raise plasma HDL cholesteryl esters to concentrations normally seen in control mice (~50 mg/dl). To obtain the mass amount of HDL cholesteryl ester taken up by the adrenal glands, the clearance rates shown in Fig. 1A were multiplied by the plasma HDL cholesteryl ester concentration in the same animal and these values are plotted in Fig. 1B as a function of the plasma HDL cholesteryl ester concentration in each animal. Absolute rates of HDL cholesteryl ester uptake by the adrenal gland plateaued at a plasma cholesteryl ester concentration of ~50 mg/dl and increased relatively little even when plasma HDL cholesteryl ester concentrations were raised to ~400 mg/dl. HDL cholesteryl ether transport rates were also measured in apoA-I$^{+/+}$ mice (solid circles). HDL cholesteryl ether clearance rates were >20-fold higher in apoA-I$^{-/-}$ animals than in apoA-I$^{+/+}$ ani-

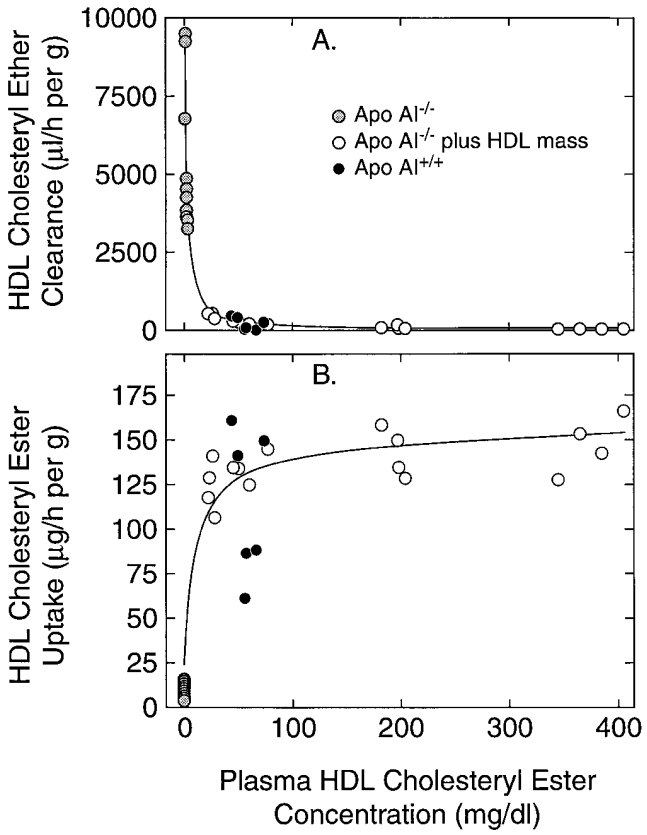

Fig. 1. Rates of HDL cholesteryl ester transport in the adrenal glands as a function of the steady-state concentration of HDL cholesteryl ester in plasma. Rates of HDL cholesteryl ether clearance (A) were quantified in vivo as described in Methods. These clearance values were multiplied by the plasma HDL cholesteryl ester concentration in the same animal to obtain absolute rates of HDL cholesteryl ester uptake (B). Transport rates were measured in apoA-I$^{-/-}$ mice using primed infusions of trace-labeled HDL (shaded circles) or primed infusions of trace-labeled HDL to which HDL mass was added to achieve new steady-state plasma concentrations of HDL cholesteryl ester ranging from 25 to 400 mg/dl (open circles). Transport rates were also measured in apoA-I$^{+/+}$ mice using trace-labeled HDL (solid circles). The solid lines represent the best-fit curves describing the relationship between HDL cholesteryl ester transport and plasma HDL cholesteryl ester concentrations that were determined using curve-fitting software as described in Methods.

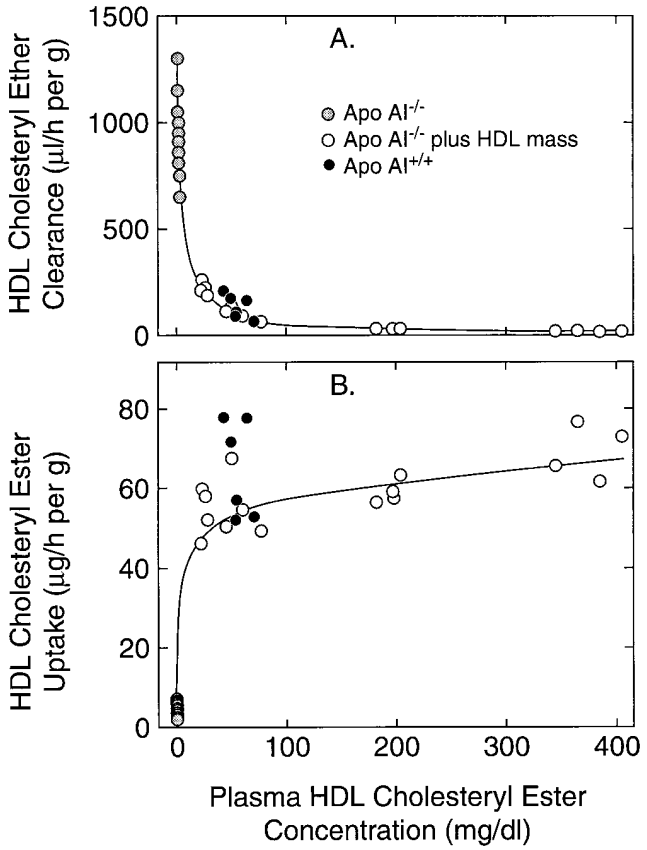

Fig. 2. Rates of HDL cholesteryl ester transport in the liver as a function of the steady-state concentration of HDL cholesteryl ester in plasma. Rates of HDL cholesteryl ether clearance (A) and HDL cholesteryl ester uptake (B) were quantified in vivo under the same conditions as described in the legend to Fig. 1.

mals when trace amounts of radiolabeled HDL were infused (Fig. 1A). However, transport rates were similar in apoA-I$^{-/-}$and apoA-I$^{+/+}$ mice when plasma HDL cholesteryl ester levels in apoA-I$^{-/-}$ mice were acutely raised to match those of apoA-I$^{+/+}$ mice by adding HDL mass to the primed infusions of trace-labeled HDL.

Figure 2 shows the saturation curves for HDL cholesteryl ester transport in the liver. HDL cholesteryl ether clearance by the liver ranged from 700 to 1,400 μl/h per g in apoA-I$^{-/-}$ mice infused with trace amounts of [^{3}H]cholesteryl ether-labeled HDL (shaded circles). As in the adrenal glands, the hepatic clearance of radiolabeled HDL was highly sensitive to the concentration of HDL cholesteryl ester in plasma (Fig. 2A) and absolute rates of HDL cholesteryl ester uptake plateaued at a plasma HDL cholesteryl ester concentration of ~50 mg/dl (Fig. 2B). HDL cholesteryl ether clearance rates were ~5-fold higher in apoA-I$^{-/-}$ animals than in apoA-I$^{+/+}$ animals when trace amounts of radiolabeled HDL were infused. However, as in the adrenal glands, transport rates were similar in apoA-I$^{-/-}$ and apoA-I$^{+/+}$ mice when plasma HDL cholesteryl ester levels in apoA-I$^{-/-}$ mice were acutely raised to match those of apoA-I$^{+/+}$mice by adding HDL mass to the primed infusions of trace-labeled HDL.

While HDL cholesteryl ester transporter activity was highest in the adrenal glands and liver, saturable HDL cholesteryl ester transport could be demonstrated in most tissues as illustrated in **Fig. 3**. HDL cholesteryl ether clearance by the spleen ranged from 80 to 240 μl/h per g in apoA-I$^{-/-}$ mice infused with trace amounts of [^{3}H]cholesteryl ether-labeled HDL and declined steeply when HDL mass was added to the primed infusions. HDL cholesteryl ether clearance in most of the remaining tissues of the body ranged from 20 to 50 μl/h per g and fell significantly as plasma HDL cholesteryl ester concentrations were raised by adding HDL mass to the primed infusions. The brain was the only tissue in which saturable HDL cholesteryl ether clearance could not be detected. To obtain rates of HDL cholesteryl ester uptake by the extrahepatic

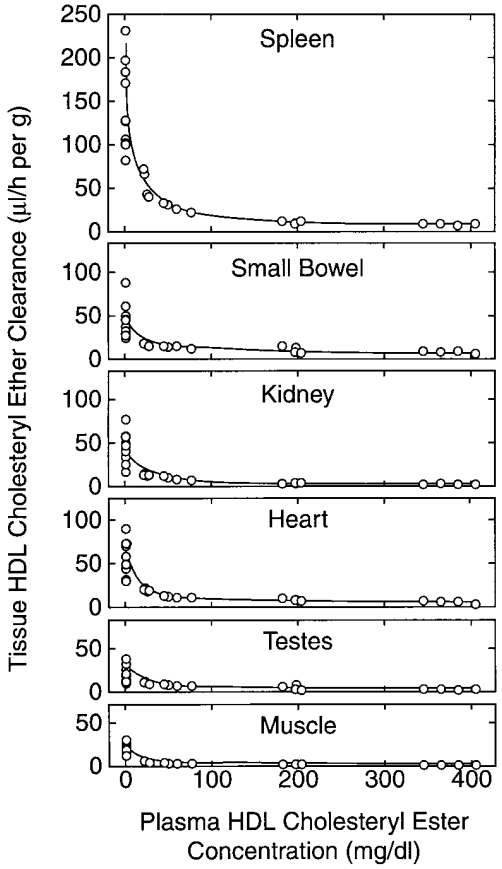

Fig. 3. Rates of HDL cholesteryl ether clearance in selected tissues as a function of the steady-state concentration of HDL cholesteryl ester in plasma. Rates of HDL cholesteryl ether clearance were quantified in apoA-I$^{-/-}$ mice using primed infusions of trace-labeled HDL or primed infusions of trace-labeled HDL to which HDL mass was added to achieve new steady-state plasma concentrations of HDL cholesteryl ester ranging from 25 to 400 mg/dl.

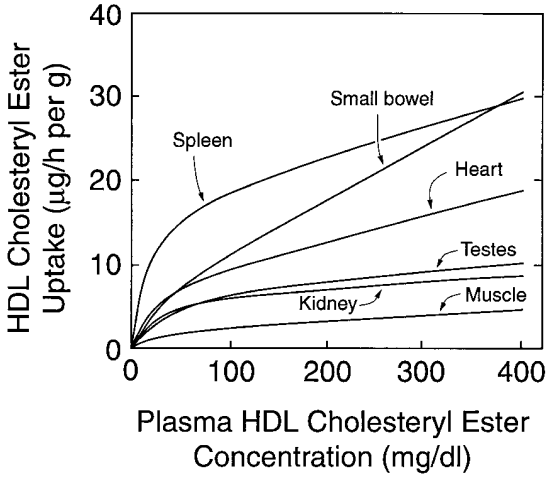

Fig. 4. Concentration dependency of HDL cholesteryl ester uptake in selected tissues. Each of the clearance rates shown in Fig. 3 was multiplied by the plasma HDL cholesteryl ester concentration in the same animal to obtain the absolute rate of HDL cholesteryl ester uptake and these values are plotted as a function of the steady-state concentration of HDL cholesteryl ester in plasma. These data were subjected to nonlinear regression analysis as described in Methods and the solid lines represent the best-fit curves describing the relationship between HDL cholesteryl ester uptake and plasma HDL cholesteryl ester concentrations in these tissues.

tissues, the clearance rates shown in Fig. 3 were multiplied by the plasma HDL cholesteryl ester concentration in each animal. The best-fit curves describing the relationship between HDL cholesteryl ester uptake and plasma HDL cholesteryl ester concentrations were determined as described in Methods and are shown in **Fig. 4**.

The kinetic parameters for HDL cholesteryl ester transport were calculated by subjecting the data illustrated in Figs. 1 and 2 to nonlinear regression analysis using an equation that takes into account receptor-dependent (saturable) and receptor-independent (nonsaturable) transport as described in Methods. The kinetic parameters for receptor-dependent (J^m, K_m) and receptor-independent (P) transport that were generated by this analysis are shown in **Table 1**. Saturable receptor-dependent HDL cholesteryl ester uptake was highest in the adrenal glands and liver with J^m values of 139 μg/h per g and 57 μg/h per g, respectively. The K_m values for both tissues equaled 4 mg/dl, which is well below the plasma concentration of HDL cholesteryl ester in control mice. With the exception of the brain, saturable HDL cholesteryl ester transport was detected in all organs although the J^m values were relatively low when expressed per g of tissue.

Kinetics of HDL apolipoprotein transport

HDL apolipoprotein transport was measured using human HDL labeled in situ with the nondegradable, intracellularly trapped tracer ^{125}I-labeled tyramine cellobiose.

TABLE 1. Transport parameters for receptor-dependent and receptor-independent HDL cholesteryl ester uptake

Tissue	Receptor-dependent J^m	Receptor-dependent K_m	Receptor-independent P
	μg/h per g	*mg/dl*	*μg/h per g per mg/dl*
Adrenal glands	139 ± 31	4 ± 7	0.02 ± 0.06
Liver	57 ± 6	4 ± 1	0.03 ± 0.03
Spleen	18 ± 10	17 ± 12	0.03 ± 0.04
Lungs	17 ± 9	14 ± 39	0.05 ± 0.05
Testes	10 ± 4	24 ± 20	0.01 ± 0.01
Heart	7 ± 4	20 ± 14	0.03 ± 0.02
Small bowel	6 ± 6	27 ± 32	0.06 ± 0.03
Kidneys	6 ± 2	18 ± 19	0.01 ± 0.01
Colon	3 ± 4	18 ± 35	0.02 ± 0.04
Stomach	2 ± 2	12 ± 18	0.02 ± 0.03
Skeletal muscle	2 ± 1	8 ± 18	0.01 ± 0.01
Fat	1 ± 3	19 ± 25	0.01 ± 0.02
Brain	nd*	nd	< 0.01

Rates of HDL cholesteryl ester transport were quantified in apoA-I$^{-/-}$ mice under conditions in which circulating HDL concentrations were acutely raised and maintained at various levels during the 4-h experimental period. Values for tissue HDL cholesteryl ester uptake were plotted as a function of the concentration of HDL cholesteryl ester in the same animal. The kinetic parameters for HDL cholesteryl ester transport (±95% confidence intervals) were calculated by fitting these data to an equation that takes into account both receptor-dependent (saturable) and receptor-independent (nonsaturable transport as described in Methods. *Not detected, In this tissue, the receptor-dependent component was so low that parameter values (J^m, K_m) could not be determined.

About 75% of the label was in apoA-I and most of the rest in apoA-II. At the end of each experiment, the lipoprotein distribution of radiolabeled HDL was determined by FPLC and no radioactivity was detected in fractions other than those corresponding to authentic human HDL. **Figure 5** shows HDL apolipoprotein clearance rates in the adrenal glands (A) and liver (B) as a function of the steady-state level of HDL apolipoprotein in plasma during the 4-h infusion period. In apoA-I$^{-/-}$ mice, HDL apolipoprotein clearance by the adrenal glands and liver decreased significantly when plasma HDL concentrations were acutely raised by adding HDL mass to the primed infusions of ^{125}I-labeled tyramine cellobiose-labeled HDL. In both tissues, rates of HDL apolipoprotein clearance were ~50% higher in apoA-I$^{-/-}$ animals than in apoA-I$^{+/+}$ animals when infused with trace amounts of ^{125}I-labeled tyramine cellobiose-labeled HDL. However, transport rates were similar in the apoA-I$^{-/-}$ and apoA-I$^{+/+}$ mice when plasma HDL apoprotein levels in apoA-I$^{-/-}$ mice were acutely raised to match those of apoA-I$^{+/+}$ mice by adding HDL mass to the primed infusions of trace-labeled HDL.

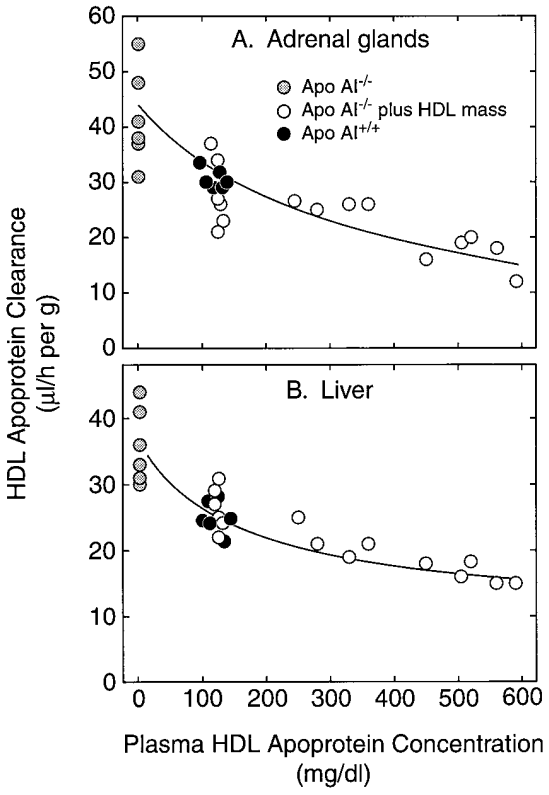

Fig. 5. Rates of HDL apoprotein clearance by the adrenal glands and liver as a function of the plasma HDL apoprotein concentration. Clearance rates were measured in apoA-I$^{-/-}$ mice using primed infusions of trace-labeled HDL (shaded circles) or primed infusions of trace-labeled HDL to which HDL mass was added to achieve new steady-state HDL apolipoprotein concentrations ranging from 100 to 600 mg/dl (open circles). Transport rates were also measured in apoA-I$^{+/+}$ mice using trace-labeled HDL (solid circles). The solid lines represent the best-fit curves describing the relationship between HDL cholesteryl ester transport and plasma HDL cholesteryl ester concentrations that were determined using curve-fitting software as described in Methods.

The kinetic parameters for receptor dependent (J^m, K_m) and receptor independent (P) HDL apolipoprotein transport were calculated as described in Methods. J^m values for receptor-dependent transport in the adrenal glands and liver equaled 129 ± 209 μg/h per g and 71 ± 77 μg/h per g, respectively; the K_m values equaled 365 ± 440 mg/dl and 263 ± 226 mg/dl, respectively. In the remaining tissues, the saturable component of HDL apolipoprotein uptake was so low that parameter values could not be determined.

Tissue distribution of HDL cholesteryl ether and apolipoprotein transport in apoA-I$^{+/+}$ mice

The tissue distribution of HDL cholesteryl ether clearance in control C57Bl/6 mice with normal plasma HDL concentrations is shown in **Fig. 6**. When expressed per g of tissue (Fig. 6A), HDL cholesteryl ether clearance rates were highest in the adrenal glands (211 μl/h per g) and liver (137 μl/h per g). Intermediate clearance rates (10–50 μl/h per g) were observed in the organs of the chest cavity and abdomen. Rates of HDL cholesteryl ether clearance were relatively low in muscle and fat (4 μl/h per g) and undetectable in brain. When expressed per whole organ (Fig. 6B), the liver was the most important site of HDL cholesteryl ether clearance accounting for ~72% of total HDL cholesteryl ether turnover. The tissue distribution of HDL cholesteryl ester and apolipoprotein transport in apoA-I$^{+/+}$mice was indistinguishable from that of apoA-I$^{-/-}$ mice when plasma HDL concentrations in

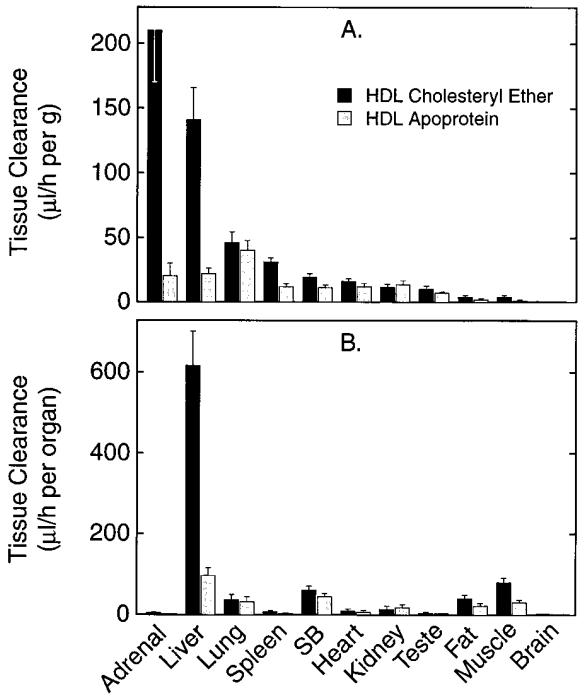

Fig. 6. Tissue distribution of HDL cholesteryl ether and apolipoprotein transport in apoA-I$^{+/+}$ mice. Tissue clearance rates are expressed as the microliters of plasma cleared of HDL cholesteryl ether or apoprotein per h per g of tissue (A) or per whole organ per 100 g body weight (B). Each value represents the mean ± 1 SD for data obtained in 6 animals.

apoA-I$^{-/-}$ mice were raised to the same level as those in apoA-I$^{+/+}$ mice by adding HDL mass to the primed infusions of trace-labeled HDL (data not shown).

Expression of SR-BI in apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice

The transport studies illustrated in Figs. 1 and 2 suggest that HDL cholesteryl ester transporter activity is not different in the tissues of apoA-I$^{-/-}$ mice compared to apoA-I$^{+/+}$ mice. SR-BI is a putative HDL receptor that has been shown to bind HDL and mediate selective HDL cholesteryl ester uptake when transfected into cells (11). We therefore compared SR-BI expression in apoA-I$^{+/+}$ mice, apoA-I$^{-/-}$ mice, and apoA-I$^{-/-}$ mice whose plasma HDL cholesterol concentrations had been returned to normal by the administration of 10^9 pfu of the recombinant adenovirus AdCMV-apoA-I, which carries the cDNA for human apoA-I. SR-BI protein levels were determined by immunoblotting using antiserum raised against the C-terminal 14 amino acids of mouse SR-BI. As illustrated in **Fig. 7A**, SR-BI protein levels in the adrenal glands did not differ among the three experimental groups of mice. In contrast, LDL receptor protein levels were markedly up-regulated in the adrenal glands of apoA-I$^{-/-}$ mice but not in apoA-I$^{-/-}$ mice that had normal HDL cholesterol concentrations as a result of AdCMV-apoA-I administration (Fig. 7B). As illustrated in **Fig. 8**, hepatic SR-BI (A) and LDL receptor (B) protein levels did not differ significantly among the three experimental groups.

SR-BI mRNA levels were measured in the adrenal glands, testes, lung, spleen, liver, and small bowel from the same groups of animals by nuclease protection using a mouse-specific probe. The results of these studies are summarized in **Fig. 9**. In apoA-I$^{+/+}$ mice, mRNA levels for SR-BI were highest in the adrenal glands but were readily detected in most tissues. When the data from 6 animals per group were quantified using an isotopic image analysis system as described in Methods, SR-BI mRNA levels did not differ among the three experimental groups (apoA-I$^{+/+}$

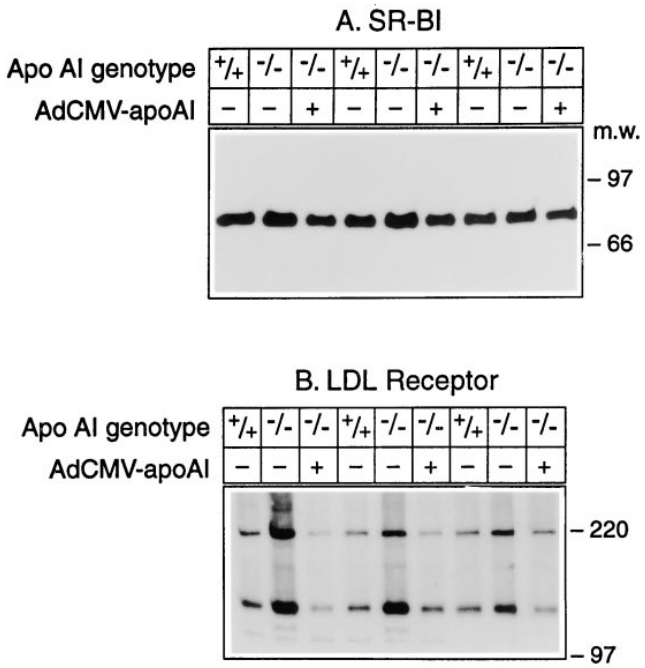

Fig. 7. Immunoblot analysis of SR-BI and LDL receptor protein in the adrenal glands. Membrane fractions were prepared from the adrenal glands of apoA-I$^{-/-}$ mice, apoA-I$^{+/+}$ mice and apoA-I$^{-/-}$ mice that had been administered 10^9 pfu of AdCMV-apoA-I to restore HDL levels to normal values. Immunoblot analysis was performed using antibodies raised against the C-terminal 14 amino acids of mouse SR-BI (A) or bovine LDL receptor (B).

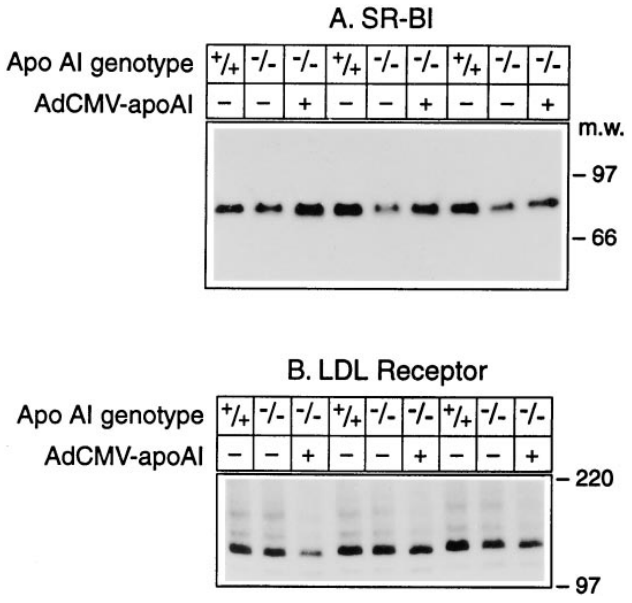

Fig. 8. Immunoblot analysis of SR-BI and LDL receptor protein in the liver. Immunoblot analysis was performed as described in the legend to Fig. 7.

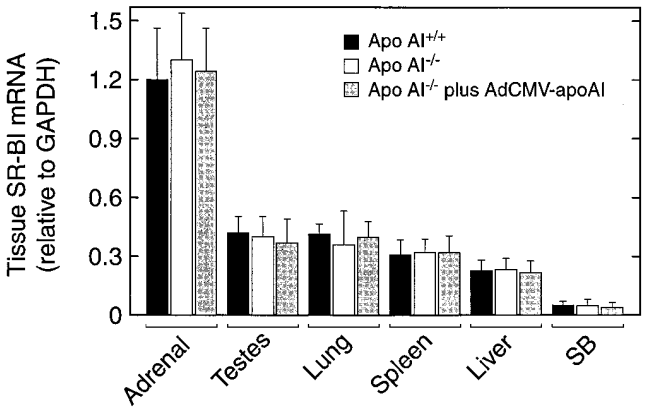

Fig. 9. Tissue SR-BI mRNA levels in apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice. RNA was isolated from apoA-I$^{+/+}$ mice, apoA-I$^{-/-}$ mice, and apoA-I$^{-/-}$ mice that had been administered 10^9 pfu of AdCMV-apoA-I. Total RNA (5 μg for the adrenal glands and 40 μg for the other tissues) was hybridized with ^{32}P-labeled single-stranded cDNA probes encoding mouse SR-BI and mouse GAPDH. Fragments protected from mung bean nuclease digestion were separated by denaturing polyacrylamide gel electrophoresis and the radioactivity in each band, corrected for background radioactivity, was quantified using an isotopic imaging system. Each value represents the mean ± 1 SD for data obtained in 6 animals.

mice, apoA-I$^{-/-}$ mice, and apoA-I$^{-/-}$ mice administered 10^9 pfu of AdCMV-apoA-I) for any tissue.

DISCUSSION

These studies provide a kinetic analysis of HDL cholesteryl ester and apolipoprotein transport in the mouse in vivo. The studies were carried out in apoA-I$^{-/-}$ mice because of their low background levels of plasma HDL, which allowed a more complete description of the relationship between plasma HDL concentrations and rates of HDL cholesteryl ester and apolipoprotein transport. With the exception of the brain, saturable HDL cholesteryl ester uptake was demonstrated in all organs of the body, with the adrenal glands and liver exhibiting the highest J^m values for receptor-dependent uptake when expressed per g of tissue. When expressed per whole organ, the liver was the most important site of HDL cholesteryl ester uptake accounting for ~72% of total HDL cholesteryl ester turnover at plasma HDL cholesterol concentrations normally seen in control C57Bl/6 mice (~50 mg/dl). Notably, the K_m values for receptor-dependent HDL cholesteryl ester uptake equaled 4 mg/dl in the adrenal glands and liver. As a consequence, HDL cholesteryl ester uptake in these organs is maximal (saturated) at normal plasma HDL concentrations. This finding is consistent with previous work demonstrating that HDL cholesteryl ester transport is largely saturated at normal HDL concentrations in the hamster (12) and indicates that an alteration in the plasma concentration of HDL will not result in a change in the absolute flux of HDL cholesteryl ester to the liver unless transporter activity is regulated or plasma HDL concentrations are drastically reduced. In contrast, the K_m for LDL cholesterol transport in vivo is ~100 mg/dl (30), which is much higher than the normal LDL cholesterol concentration in mice (~5 mg/dl) or hamsters (~25 mg/dl). The marked difference in the kinetic characteristics of LDL and HDL transport appears to be due to the fact that other lipoproteins (apoE-containing) compete with LDL for the LDL receptor in vivo thereby increasing the apparent K_m (31). In contrast, lipoproteins other than HDL evidently do not compete for HDL cholesteryl ester transporters in vivo (12) despite the fact that SR-BI binds native LDL with high affinity in transfected cells (32).

Whereas all tissues other than the brain exhibited saturable HDL cholesteryl ester uptake, saturable HDL apolipoprotein transport could be demonstrated only in the adrenal glands and liver. In these organs the K_m values were much higher for the apolipoprotein than for the cholesteryl ester moiety. Consequently, at normal plasma HDL concentrations, the clearance of HDL cholesteryl ether exceeded the clearance of HDL apolipoproteins by ~10-fold in the adrenal glands and ~5-fold in the liver. Whereas previous studies in the hamster demonstrated saturable HDL apoA-I uptake by the kidney (12), no saturable HDL apolipoprotein transport was observed in mouse kidney. In tissues other than the kidney, HDL apoA-I uptake is thought to reflect uptake of the whole HDL particle. In the kidney, filtration and tubular reabsorption of free apoA-I (in equilibrium HDL apoA-I) can occur (19) and is thought to contribute to the high rate of renal apoA-I catabolism in the rat (19) and hamster (12). Additional studies will be required to determine why mouse kidney lacks saturable HDL apoA-I uptake.

The finding that HDL cholesteryl ester clearance rates (equivalent to FCR) are highly sensitive to the concentration of HDL in plasma has implications with respect to the interpretation of HDL turnover studies in animals and humans. In apoA-I$^{-/-}$ mice, the rate of HDL cholesteryl ester clearance by the adrenal glands fell by ~95% when plasma HDL concentrations were raised to levels normally seen in control C57Bl/6 mice and by >99% when HDL concentrations were raised to supernormal levels. In the liver, the major site of HDL cholesteryl ester clearance from plasma, HDL cholesteryl ester clearance rates showed a similar concentration dependency. These marked changes in HDL cholesteryl ester clearance occurred under conditions in which HDL cholesteryl ester transporter activity and SR-BI expression were constant. Numerous HDL turnover studies have been performed in animal models or in humans in an attempt to understand the mechanisms underlying changes in the plasma concentration of HDL. Changes in HDL concentrations have been associated with changes in the FCR of HDL apoA-I (33–36) or cholesteryl ester (37) suggesting regulation of a receptor-dependent clearance mechanism. However, based on the current studies and previous work in the hamster (12), it is likely that these changes in FCR largely reflect the kinetic characteristics of the transport process rather than regulation of transporter activity. Because the clearance of HDL cholesteryl ester is highly sensitive to the plasma concentration of HDL cholesteryl ester, a change in HDL cholesteryl ester clearance (or FCR) can be interpreted only if the relationship between HDL cholesteryl ester transport and plasma HDL concentrations under control conditions is known.

We found that HDL cholesteryl ester transporter activity and SR-BI expression were not altered in the tissues of apoA-I$^{-/-}$ mice (which lack normal HDL) compared to the corresponding tissues of apoA-I$^{+/+}$ mice. The lack of induction of SR-BI expression in the adrenal glands was somewhat surprising. HDL is the major source of cholesterol for steroid hormone production in this organ and the absence of normal HDL in apoA-I$^{-/-}$ mice was associated with up-regulation of de novo cholesterol synthesis and induction of LDL receptor expression in the adrenal glands. These data suggest that SR-BI is not under tight feedback control by cellular cholesterol levels in the adrenal glands. In contrast to the current data, Wang et al. (38) found that SR-BI mRNA and protein were increased in the adrenal glands of apoA-I$^{-/-}$ compared to apoA-I$^{+/+}$ mice. The reason for these discordant results is not clear at present. Recent evidence indicates that there are two isoforms of SR-BI and that the C-terminal 39 amino acids differ in these two isoforms (39). Our immunoblots were performed using antibodies raised against the C-terminal

14 amino acids of SR-BI.1, which is the major form of SR-BI found in the adrenal gland, whereas Wang et al. (38) used antibodies that recognized both forms of SR-BI. On the other hand, both groups used probes that detect both forms of SR-BI mRNA. More recently, using the nuclease protection assay and a cDNA probe that differentiates between SR-BI.1 and SR-BI.2, we have found no difference in the ratio of the two transcripts in the adrenal glands from apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice (D. K. Spady, unpublished observation). SR-BI expression is up-regulated by stress and ACTH in the adrenal glands (23, 38). ApoA-I$^{-/-}$ animals would presumably be more sensitive than apoA-I$^{+/+}$ animals to stress-induced up-regulation of SR-BI. It is possible that differences in the amount of stress prior to harvesting of the adrenal glands account for the conflicting results.

Whereas SR-BI expression in the endocrine organs is coordinately regulated with steroid hormone production (23, 39, 40), there is little evidence that HDL cholesteryl ester transport is regulated in the liver, the major site of HDL cholesteryl ester clearance from plasma. The absence of normal plasma HDL in apoA-I$^{-/-}$ mice did not lead to up-regulation of HDL cholesteryl ester transporter activity or SR-BI expression in the liver indicating that SR-BI expression is not regulated by the amount of HDL cholesteryl taken up by the liver. In previous studies in the hamster we found that HDL cholesteryl ester transporter activity and SR-BI expression were not altered in response to dietary cholesterol in amounts that increased hepatic cholesteryl ester levels 10-fold (41). Thus, HDL cholesteryl ester transport and SR-BI expression in the liver appear not to be regulated by cellular cholesterol levels.

The current studies were performed using human HDL. The limited availability of mouse HDL precluded the performance of kinetic studies using homologous HDL. However, the observation that HDL cholesteryl ether clearance rates in apoA-I$^{+/+}$ mice were similar to those in apoA-I$^{-/-}$ mice infused with sufficient human HDL mass to raise plasma HDL cholesterol concentrations to the same level indicates that mouse and human HDL compete equally well for the HDL cholesteryl ester transport mechanism. In addition, the unfractionated human HDL (d 1.07–1.21 g/ml) used in these studies is clearly not homogenous but rather contains a spectrum of particles differing in size, density and apoprotein composition. HDL can be separated into two major subclasses (HDL_2 and HDL_3) by ultracentrifugation and into 12 distinct subfractions using two-dimensional electrophoresis (42). The physiological relevance of these HDL subfractions is unclear at present and there is little evidence that specific HDL subfractions are a better predictor of coronary heart disease than total HDL (43, 44). Nevertheless, in the future it will be of interest to examine the transport kinetics of specific HDL subfractions, beginning with HDL_2 and HDL_3. The apoA-I$^{-/-}$ mouse with its very low background level of HDL will be an ideal model to determine whether individual HDL subfractions are transported differently in vivo.

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REFERENCES

1. Abbott, R. D., P. W. Wilson, W. B. Kannel, and W. P. Castelli. 1988. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The Framingham study. *Arteriosclerosis.* **8:** 207–211.
2. Miller, G. J., and N. E. Miller. 1975. Plasma high-density-lipoprotein concentration and development of ischaemic heart disease. *Lancet.* **1:** 16–19.
3. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein. The clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
4. Glomset, J. A. 1968. The plasma lecithins:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
5. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
6. Osono, Y., L. A. Woollett, K. R. Marotti, G. W. Melchior, and J. M. Dietschy. 1996. Centripetal cholesterol flux from extrahepatic organs to the liver is independent of the concentration of high density lipoprotein-cholesterol in plasma. *Proc. Natl. Acad. Sci. USA.* **93:** 4114–4119.
7. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
8. Ponsin, G., T. Pulcini, J. T. Sparrow, A. M. Gotto, Jr., and H. J. Pownall. 1993. High density lipoprotein interconversions in rat and man as assessed with a novel nontransferable apolipopeptide. *J. Biol. Chem.* **268:** 3114–3119.
9. Pittman, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor Jr. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262:** 2443–2450.
10. Goldberg, D. I., W. F. Beltz, and R. C. Pittman. 1991. Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. *J. Clin. Invest.* **87:** 331–346.
11. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
12. Woollett, L. A., and D. K. Spady. 1997. Kinetic parameters for HDL apoprotein A-I and cholesteryl ester transport in the hamster. *J. Clin. Invest.* **99:** 1704–1713.
13. Williamson, R., D. Lee, J. Hagaman, and N. Maeda. 1992. Marked reduction of high density lipoprotein cholesterol in mice genetically modified to lack apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA.* **89:** 7134–7138.
14. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
15. Stein, O., G. Halperin, and Y. Stein. 1980. Biological labeling of very low density lioproteins with cholesteryl linoleyl ether and its fate in the intact rat. *Biochim. Biophys. Acta.* **620:** 247-260.
16. Halperin, G., O. Stein, and Y. Stein. 1986. Synthesis of ether analogs of lipoprotein lipids and their biological applications. *Methods Enzymol.* **129:** 816–848.
17. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
18. Hough, J. L., and D. B. Zilversmit. 1984. Comparison of various methods for in vitro cholesteryl ester labeling of lipoproteins from hypercholesterolemic rabbits. *Biochim. Biophys. Acta.* **792:** 338–347.
19. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **258:** 7161–7167.
20. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabo-

lism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
21. Kopfler, W. P., M. N. Willard, T. Betz, J. E. Willard, R. D. Gerard, and R. S. Meidell. 1994. Adenovirus-mediated transfer of a gene encoding human apolipoprotein A-I into normal mice increases circulating high density-lipoprotein cholesterol. *Circulation.* **90:** 1319–1327.
22. Herz, J., and R. D. Gerard. 1993. Adenovirus-mediated transfer of low density lipoprotein receptor gene acutely accelerates cholesterol clearance in normal mice. *Proc. Natl. Acad. Sci. USA.* **90:** 2812–2816.
23. Landschulz, K. T., R. K. Pathak, A. Rigotti, M. Krieger, and H. H. Hobbs. 1996. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* **98:** 984–995.
24. Herz, J., R. C. Kowal, Y. K. Ho, M. S. Brown, and J. L. Goldstein. 1990. Low density lipoprotein receptor-related protein mediates endocytosis of monoclonal antibodies in cultured cells and rabbit liver. *J. Biol. Chem.* **265:** 21355–21362.
25. Horton, J. D., J. A. Cuthbert, and D. K. Spady. 1993. Dietary fatty acids regulate hepatic low density lipoprotein (LDL) transport by altering LDL receptor protein and mRNA levels. *J. Clin. Invest.* **92:** 743–749.
26. Williams, D. L., T. C. Newman, G. S. Shelness, and D. A. Gordon. 1986. Measurement of apolipoprotein mRNA by DNA-excess solution hybridization with single-stranded probes. *Methods Enzymol.* **128:** 671–689.
27. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* **162:** 156–159.
28. Rifai, N., and M. E. King. 1986. Immunoturbidimetric assays of apolipoproteins A, A-I, A-II, and B in serum. *Clin. Chem.* **32:** 957–961.
29. Fitting. 1995. *In* Ultrafit Manual: Numeric Data Processing for Apple Macintosh. Cambridge, UK. Biosoft, 7-1–7-12.
30. Spady, D. K., J. B. Meddings, and J. M. Dietschy. 1986. Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. *J. Clin. Invest.* **77:** 1474–1481.
31. Woollett, L. A., Y. Osono, J. Herz, and J. M. Dietschy. 1995. Apolipoprotein E competitively inhibits receptor-dependent low density lipoprotein uptake by the liver but has no effect on cholesterol absorption or synthesis in the mouse. *Proc. Natl. Acad. Sci. USA.* **92:** 12500–12504.
32. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
33. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1991. Increased apoA-I and apoA-II fractional catabolic rate in patients with low high density lipoprotein–cholesterol levels with or without hypertriglyceridemia. *J. Clin. Invest.* **87:** 536–544.
34. Blum, C. B., R. I. Levy, S. Eisenberg, M. Hall III, R. H. Goebel, and M. Berman. 1977. High density lipoprotein metabolism in man. *J. Clin. Invest.* **60:** 795–807.
35. Chong, K. S., R. J. Nicolosi, R. F. Rodger, D. A. Arrigo, R. W. Yuan, J. J. Mackey, S. Georas, and P. N. Herbert. 1987. Effect of dietary fat saturation on plasma lipoproteins and high density lipoprotein metabolism of the rhesus monkey. *J. Clin. Invest.* **79:** 675–683.
36. Parks, J. S., and L. L. Rudel. 1982. Different kinetic fates of apolipoproteins A-I and A-II from lymph chylomicra of nonhuman primates. Effect of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* **23:** 410–421.
37. Hayek, T., Y. Ito, N. Azrolan, R. B. Verdery, K. Aalto-Setala, A. Walsh, and J. L. Breslow. 1993. Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (apo) A-1. Presentation of a new animal model and mechanistic studies in human apoA-I transgenic and control mice. *J. Clin. Invest.* **91:** 1665–1671.
38. Wang, N., W. Weng, J. L. Breslow, and A. R. Tall. 1996. Scavenger receptor BI (SR-BI) is up-regulated in adrenal gland in apolipoprotein A-I and hepatic lipase knock-out mice as a response to depletion of cholesterol stores. In vivo evidence that SR-BI is a functional high density lipoprotein receptor under feedback control. *J. Biol. Chem.* **271:** 21001–21004.
39. Webb, N. R., W. J. S. de Villiers, P. M. Connell, F. C. de Beer, and D. R. van der Westhuyzen. 1997. Alternative forms of the scavenger receptor BI (SR-BI). *J. Lipid Res.* **38:** 1490–1495.
40. Rigotti, A., E. R. Edelman, P. Seifert, S. N. Iqbal, R. B. DeMattos, R. E. Temel, M. Krieger, and D. L. Williams. 1996. Regulation by adrenocorticotropic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland. *J. Biol. Chem.* **271:** 33545–33549.
41. Woollett L. A., D. M. Kearney, and D. K. Spady. 1997. Diet modification alters plasma HDL cholesterol concentrations but not the transport of HDL cholesteryl esters to the liver in the hamster. *J. Lipid Res.* **38:** 2289–2302.
42. Asztalos, B. F., C. H. Sloop, L. Wong, and P. S. Roheim. 1993. Two-dimensional electrophoresis of plasma lipoproteins: recognition of new apoA-I-containing subpopulations. *Biochim. Biophys. Acta.* **1169:** 291–300.
43. Gofman, J. W., W. Young, and R. Tandy. 1966. Ischemic heart disease, atherosclerosis and longevity. *Circulation.* **34:** 679–697.
44. Stampfer, M. J., F. M. Sacks, S. Salvini, W. C. Willett, and C. H. Hennekens. 1991. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N. Engl. J. Med.* **325:** 373–381.