Mechanisms of HDL deficiency in mice overexpressing human apoA-II

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Abstract **To ascertain the mechanisms underlying the hypoalphalipoproteinemia present in mice overexpressing human apolipoprotein A-II (apoA-II) (line 11.1), radiolabeled HDL or apoA-I were injected into mice. Fractional catabolic rate of [^{3}H]cholesteryl oleoyl ether HDL ([^{3}H]HDL) was 2-fold increased in 11.1 transgenic mice compared with control mice and this was concomitant with increased radioactivity in liver, gonads, and adrenals. However, scavenger receptor class B, type I (SR-BI) was increased only in adrenals. [^{3}H]HDL of 11.1 transgenic mice presented greater binding but decreased uptake compared with control mice when Chinese hamster ovary cells transfected with SR-BI were used, thereby pointing to unknown but SR-BI-independent mechanisms as being responsible for the increased ^{3}H-radioactivity seen in liver and gonads. Synthesis rate (SR) of plasma [^{3}H]HDL was 2-fold decreased in 11.1 transgenic mice. Mouse ^{125}I-apoA-I was 2-fold more rapidly catabolized (mainly by the kidney) in transgenic mice. Mouse apoA-I displacement from HDL by the addition of isolated human apoA-II was reproduced ex vivo; thus, this mechanism may be involved in the increased renal catabolism of apoA-I. ApoA-I SR was 2-fold decreased in 11.1 transgenic mice and this was concomitant with a 2.3-fold decrease in hepatic apoA-I mRNA abundance. Our findings show that multiple mechanisms are involved in the HDL deficiency presented by mice overexpressing human apoA-II.**—Julve, J., J. C. Escolà-Gil, V. Ribas, F. González-Sastre, J. Ordóñez-Llanos, J. L. Sánchez-Quesada, and F. Blanco-Vaca. **Mechanisms of HDL deficiency in mice overexpressing human apoA-II.** ***J. Lipid Res.*** **2002.** 43: **1734–1742.**

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HDLs have received considerable attention owing to their protective role against premature atherosclerosis (1, 2). Significant progress has been made in identifying important factors involved in HDL metabolism. However, the role of apolipoprotein (apo)A-II, the second quantitatively most important HDL protein, is still unclear (3). Unlike apoA-I and mouse apoA-II, human apoA-II is not vital for HDL structure since members of a family with apoA-II deficiency exhibited normal levels of HDL cholesterol (HDL-C) (3–5). However, there is evidence that apoA-II influences several aspects of HDL structure, function, and metabolism, including reverse cholesterol transport and the ability of HDL to serve as substrate or ligand for enzymes and receptors or influence apoA-I conformation (3). Transgenic mice overexpressing human apoA-II (6, 7), but not mouse apoA-II (8), presented HDL deficiency indicating, again, important species-specific differences. During the study of the 11.1 line of human apoA-II transgenic mice conducted in our laboratory, we demonstrated that their HDL present decreased content in cholesterol with an increased percentage of free cholesterol due to a functional lecithin:cholesterol acyltransferase (LCAT) deficiency secondary, at least in part, to decreased mouse apoA-I content (3, 6, 9). This could be due at least in part to displacement of mouse apoA-I from the HDL surface by human apoA-II (10). We also observed a partial mouse apoA-II deficiency in these animals (6), which, at least in part, could be similarly due to displacement of mouse apoA-II by its human counterpart. We have also reported that the HDL of these transgenic mice were smaller in size and presented a reduced capability to efflux cholesterol from cells (3, 6, 9).

The aim of this study was to perform a systematic analysis of the pathophysiological mechanisms involved in the HDL deficiency presented by apoA-II transgenic mice. Because HDL deficiency was greater when the mice were fed a regular chow diet, data presented were obtained when the animals were fed with this diet (3, 6, 9).

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Abbreviations: apo, apolipoprotein; FCR, fractional catabolic rate; LpA-I, high-density lipoprotein with apoA-I without apoA-II; LpA-I/A-II, high-density lipoprotein with apoA-I and apoA-II; SR, synthesis rate; SR-BI, scavenger receptor class B, type I.

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MATERIAL AND METHODS

Mice

Mice were housed in a temperature-controlled (20°C) room with a 12-h light/dark cycle and with free access to food and water. In order to obtain blood from fasted mice, food deprivation usually began at 5 PM and samples were obtained between 8 and 10 AM. All animal procedures were in accordance with published recommendations for the use of laboratory animals (11). Human apoA-II transgenic mice (lines 25.3 and 11.1, which in this study presented, respectively, plasma human apoA-II of 14 ± 6 and 83 ± 31 mg/dl) were created in the C57BL/6 background by injection of a 3-kb pair fragment isolated from human genomic DNA prepared by digestion with *Msp*I that contained the human apoA-II gene (6). Transgenic and control mice used for the studies were fed ad libitum a regular chow diet (ICN Pharmaceuticals Inc., Costa Mesa, CA). All animals were approximately 3-months-old at the beginning of the diet. Studies were performed after 5 months of diet and male and female mice were used in equal proportions.

Biochemical analysis

The methods used for plasma lipid and lipoprotein analyses in these mice have been described in detail elsewhere (6, 9). Protein concentrations were determined by the method of Bradford (12). For quantification of mouse apoA-I-containing HDL subspecies, plasma was electrophoresed using agarose gels (Biomidi, Toulouse, France), transferred to nitrocellulose membranes, and confronted with rabbit polyclonal antibodies against mouse apoA-I, as described (13). Areas containing preβ and α-migrating apoA-I-HDL were scanned and analyzed with the Gel Doc 2000 image analysis program (BioRad, Hercules, CA). Relative concentrations of the HDL subfractions were expressed as percent apoA-I (%). The absolute concentrations for each fraction were calculated by multiplying its percentage by the plasma concentration of apoA-I, which was measured by a radial immunodiffusion assay (9).

Metabolism of [^{3}H]cholesteryl oleoyl ether HDL

[^{3}H]cholesteryl oleoyl ether-radiolabeled HDL ([^{3}H]HDL) were prepared as described (14) and 500,000 cpm of autologous [^{3}H]HDL (15 μg protein of HDL) in 0.1 ml of 0.9% NaCl were injected intravenously into each mouse. In all cases [^{3}H]HDL was <5% of the total mass of plasma cholesterol. Blood was collected into heparinized tubes at the indicated times and the radioactivity contained in 50 μl aliquots determined. The average radioactivity at each point of time was expressed as a fraction of the injected dose; 90–95% of the label was present in the HDL plasma fraction throughout the experiment. At the end of the experiments, organs were extracted, homogenized in chloroform-methanol (1:1, v/v) and ^{3}H counts determined. Computer analysis was used to fit an exponential curve to each set of plasma-decay data. The fractional catabolic rate (FCR) was calculated as the inverse of the area under the decay curves. The synthesis rates (SR) were calculated as plasma cholesteryl ester concentration (μmol/ml) × FCR (pools/h) × 33.3 ml/kg (15).

Scavenger receptor class B, type I expression and HDL binding and uptake to cells transfected with the receptor

Western blot analysis for mouse scavenger receptor class B, type I (SR-BI) was performed using homogenized pieces of liver, ovary, and pools of 3–4 adrenal glands. These were homogenized on ice in 20 mM Tris-HCl buffer (pH 7.5) with proteinase inhibitors [10 μg/ml leupeptin, 20 μg/ml aprotinin, 5 μg/ml pepstatin A, 0.2 mM phenylmethylsulfonylfluoride (PMSF), 5 mM ethylenediamine tetraacetic acid (EDTA)] and centrifuged at 700 *g* for 10 min to remove debris. Protein extracts from the indicated tissues (postnuclear 150,000 *g* membrane pellets) underwent 7.5% SDS-polyacrylamide gel electrophoresis under reducing conditions (25 μg of protein per lane) and were then blotted to nitrocellulose membranes. Immunoreactivity was assessed using specific polyclonal antibodies to mouse SR-BI peptide (rabbit IgG fraction, 1:5000 dilution) (Novus Biologicals, Littleton, CO) (16) and a goat anti-rabbit HRP-conjugated secondary antibody (1:15000 dilution, Sigma, St. Louis, MO) and visualized by enhanced chemiluminescence detection (Pierce, Rockford, IL). Autoradiograms were scanned and analyzed with the Gel Doc 2000 image analysis program (BioRad).

To study the interaction of the HDL of different mouse lines with SR-BI, Chinese hamster ovary (CHO) cells (clone ldlA7) stably transfected with mouse SR-BI (CHO-SR-BI) were used (17). Competitive ligand binding (based on cell-associated radioactivity at 4°C) and binding+uptake (based on cell-associated radioactivity at 37°C) to CHO-SR-BI cells were carried out essentially as described (17). Cells were preincubated in Ham's F12 medium containing 100 units/ml penicillin, 100 mg/ml streptomycin, 2 mM glutamine, 0.5% BSA (BSA) at 37°C for 1 h, and washed twice with a cold solution of 50 mM Tris-HCl, 150 mM NaCl, pH 7.4, containing 0.2% BSA. To test binding of the different HDL, competition experiments were performed with [^{3}H]HDL

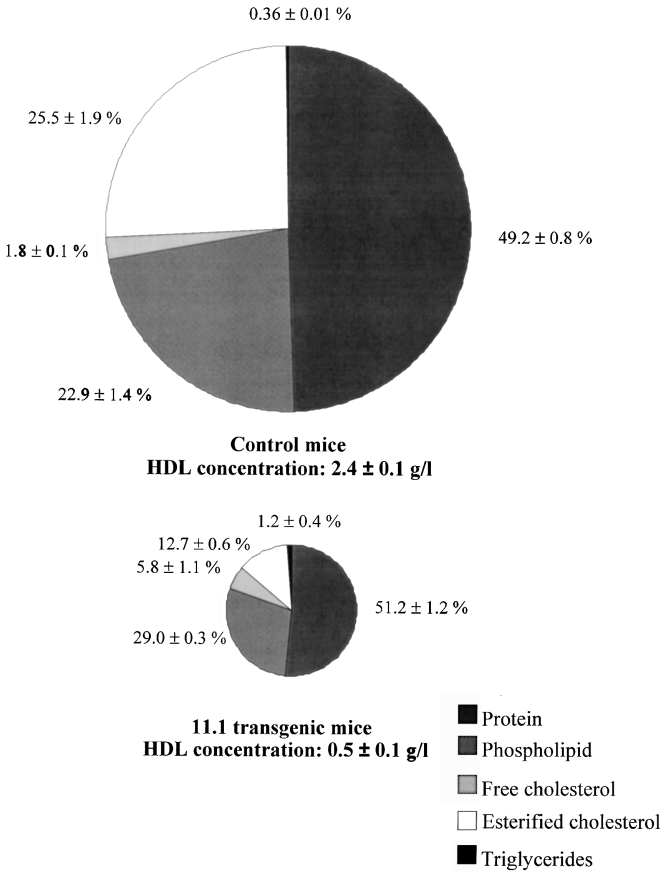


Fig. 1. HDL plasma concentration and relative chemical composition. HDL were isolated by sequential ultracentrifugation and lipids and proteins determined as described in Materials and Methods. Values are expressed as HDL concentration (g/l) and relative (%) chemical composition and correspond to one HDL preparation isolated from 3–4 pooled samples of 8–10 mice in each group. Plasma HDL concentration was 0.5 ± 0.1 g/l in 11.1 transgenic mice and 2.4 ± 0.1 g/l in control mice.

TABLE 1. Distribution of mouse apoA-I among preβ-HDL and α-HDL species in human apoA-II transgenic and in control mice

	Control Mice	25.3 Transgenic Mice	11.1 Transgenic Mice
Number of animals	3	3	3
Mouse apoA-I (mg/dl)			
preβ-HDL	31 ± 1	27 ± 3	22 ± 4[a]
α-HDL	110 ± 10	97 ± 8	9 ± 2[a]
Mouse ApoA-I (%)			
preβ-HDL	21 ± 2	20 ± 3	69 ± 9[a]
α-HDL	79 ± 2	80 ± 3	31 ± 9[a]

Results are expressed as mean ± SEM.

[a] Significantly different ($P < 0.05$) compared with 25.3 transgenic and control mice.

from control mice at 25 μg of protein/ml in the presence of increasing concentrations of unlabeled HDL from 11.1 transgenic or control mice in Ham's F12 buffered with 20 mM Hepes, pH 7.4, and containing 0.5% BSA for 3 h at 4°C. After incubation, cells were washed four times with 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and 0.2% BSA, and twice with 50 mM Tris-HCl, 150 mM NaCl (pH 7.4). Protein concentrations were then determined (12) and [^{3}H]cholesteryl oleoyl ether was extracted with isopropyl alcohol (30 min at room temperature) and quantified by scintillation counting. Results are expressed as a percentage of the binding measured without competitor.

For binding+uptake measurements, cells were preincubated as above and then incubated at 37°C for 3h with 25 μg of protein/ml of [^{3}H]HDL concentration without (total uptake) or with a 40-fold excess of unlabeled HDL (to substract nonspecific uptake) in Ham's F12 buffered with 20 mM Hepes, pH 7.4, and containing 0.5% BSA. Cells were then washed and counted. Results were expressed as nanogram cholesteryl ester incorporated per milligram of cellular protein.

Both SR-BI-specific cell binding and binding+uptake were calculated as the difference between values for CHO-SR-BI cells and non-transfected CHO cells.

ApoA-I metabolism

Mouse apoA-I was purified as described previously (18), dissolved in PBS (pH 6.5) (1 μg of protein/μl), and radiolabeled with Na^{125}I (Amersham Biosciences, Buckinghamshire, UK) using IODO-BEADs (Pierce). Briefly, one IODO-BEAD and 2 μl (200 μCi) of Na^{125}I were added to 0.1 ml of PBS (pH 6.5). The mixture was incubated at room temperature for 5 min, after which 0.1 ml of the apoA-I solution was added. The tube containing this mixture was vortexed and incubated at room temperature for 20 min. The IODO-BEAD was removed and the apoA-I preparation was subjected to chromatography using a PD-10 desalting column (Amersham Biosciences) previously equilibrated with PBS (pH 6.5). The fractions containing the protein-bound radioactivity were identified using a Beckman 5500 γ-counter. These fractions were pooled and dialyzed extensively against PBS (pH 6.5). Analysis of the radiolabeled apoA-I preparations indicated that in every case more than 98% was precipitated by 10% trichloroacetic acid. Specific radioactivity of the final preparation was around 0.43 μCi/μg of protein. ^{125}I-ApoA-I was injected into the mouse tail vein as a bolus of 1 μCi per mouse in a final volume of 0.1 ml of 0.1 M PBS (pH 6.5) (~2.3 μg of protein per mouse; thus, injected ^{125}I-apoA-I mass represented <0.5% that of plasma). Blood samples were taken at the indicated times after isotope injection. The fraction of plasma radioactivity that was protein-bound was determined by trichloroacetic acid precipitation. Computer analysis was used to fit an exponential curve to each set of plasma-decay data. The FCR was calculated from the area under the decay curves. The SR was calculated as plasma mouse apoA-I concentration (mg/ml) × FCR (pools/h) × 33.3 ml/kg (15).

Analysis of ex-vivo remodeling of control HDL by addition of isolated human apoA-II

The size distribution of control mouse HDL (native or modified by incubation with increasing concentrations of human apoA-II) and 11.1 transgenic mouse HDL were analyzed by electrophoresis using non-denaturing conditions on 4–20% precasted polyacrylamide gels (BioRad) (6). In the case of incubation of control HDL with human apoA-II, this lasted 3 h and was performed at 4°C to avoid hepatic lipase-mediated HDL remodeling. Lipoprotein size was estimated by comparison with standard proteins (Amersham Biosciences) and content and size of apoA-I-containing particles were analyzed using specific antibodies to mouse apoA-I.

RNA analyses

Total RNA from tissues was prepared as described (19). RNA samples (50 μg) were separated in 3% formaldehyde-containing agarose gels and transferred to nylon membranes (Hybond N+, Amersham Biosciences). For Northern blot hybridizations, specific apoA-I, apoA-II, and human apoA-II mRNA species were quantified using specific cDNA probes radiolabeled by random priming (Qiagen, Hilden, Germany). A mouse β-actin clone radiolabeled by random priming (Qiagen) was used as an internal standard.

Statistical analyses

Results are expressed as mean ± SEM. Significance of the difference between groups was assessed using the one-way ANOVA test. $P < 0.05$ was considered statistically significant. IC_{50} values were calculated from nonlinear regression analyses using least square algorithms.

RESULTS

Analyses of HDL isolated by ultracentrifugation demonstrated that 11.1 transgenic mice exhibited a 4.9-fold ($P < 0.0001$) decrease in HDL mass and a marked alteration in their chemical composition compared with control HDL (**Fig. 1**). HDL of 11.1 transgenic mice was relatively richer in triglyceride (3.3-fold), free cholesterol (3.2-fold), and phospholipid (1.3-fold), and poorer in cholesteryl esters (2-fold) compared with control HDL. In contrast to HDL lipids, no difference was observed in the percentage of HDL protein. The composition of HDL isolated from plasma of 25.3 transgenic mice did not differ from that presented by control HDL (data not shown).

Plasma from 11.1 transgenic mice displayed a significant reduction in apoA-I-containing preβ and α-HDL (1.4-fold and ~11-fold, respectively; $P < 0.05$) compared with that of control mice (**Table 1**). In order to ascertain the major pathophysiological mechanisms implicated in the HDL deficiency presented by mice overexpressing human apoA-II (line 11.1), we conducted a series of experiments, the results of which are explained below.

Kinetics of HDL lipid moiety

Because HDL-C deficiency could have been due either to reduced HDL synthesis, increased HDL clearance, or both, we examined the fate of autologous [^{3}H]cholesteryl

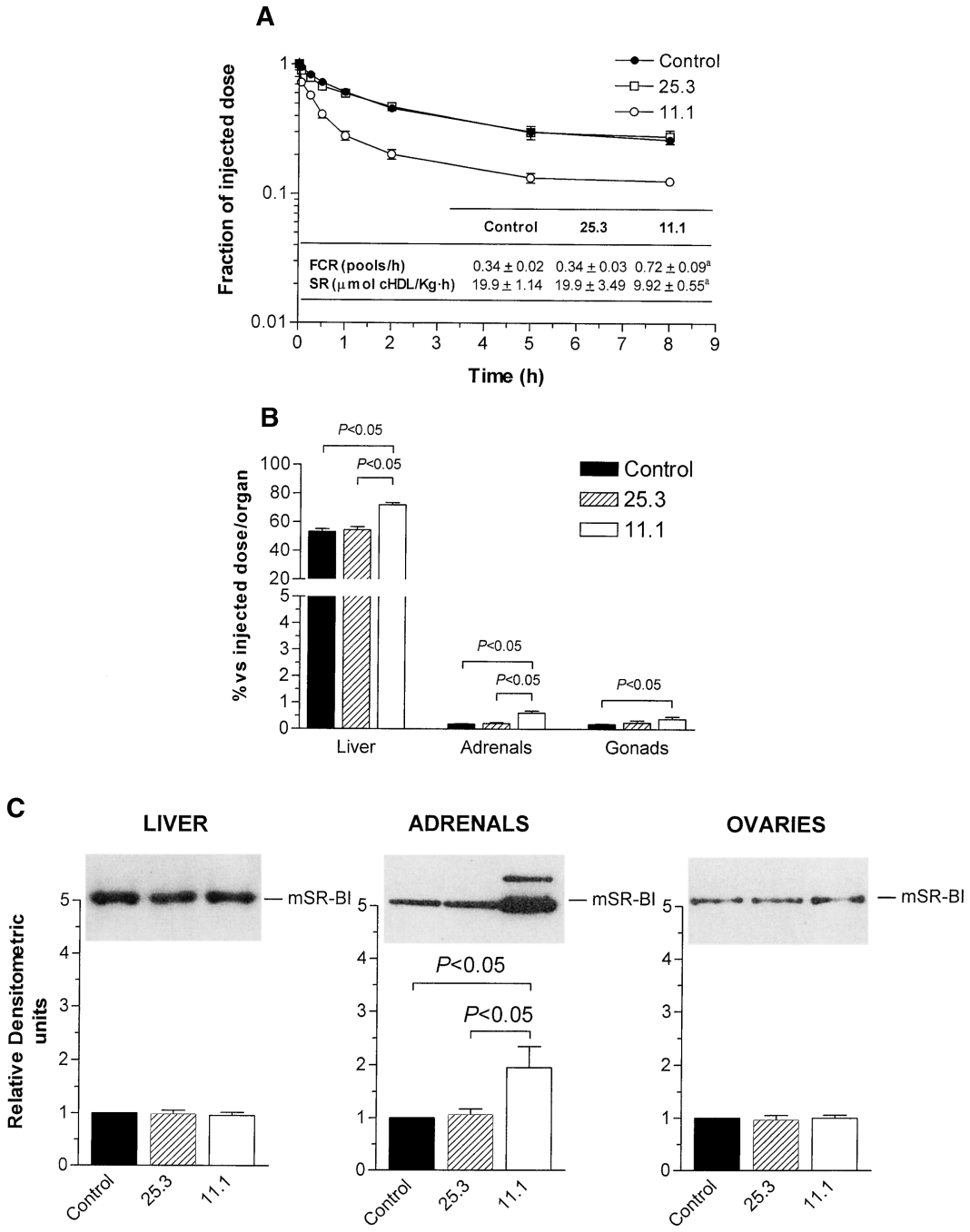


Fig. 2. In vivo metabolism of [^{3}H]cholesteryl oleoyl ether-radiolabeled HDL. Autologous [^{3}H]HDL-cholesteryl oleoyl ether was injected intravenously into mice and radioactivity was measured in plasma, liver, and steroidogenic tissues 8 h after injection. A: Turnover data in plasma are expressed as a fraction of the injected dose. Inset: table showing fractional catabolic rate (FCR) and synthesis rate (SR) values of each genotype. Results shown are mean ± SEM of 6–7 mice. [a] Significantly different ($P < 0.05$) compared with 25.3 transgenic or control mice. B: Organ uptake is expressed as % of injected dose/organ. Results shown are mean ± S.E.M. of 6–7 mice. C: Representative scavenger receptor class B, type I (SR-BI) immunoblot and relative densitometric analyses of four experiments in which 25 μg of protein obtained from liver, adrenal glands and ovaries (per each line of mice) were analyzed.

oleoyl ether ([^{3}H]HDL) injected in each mouse line. Plasma clearance of intravenously injected [^{3}H]HDL was significantly more rapid (2-fold, $P < 0.05$) in 11.1 transgenic mice than in control mice (**Fig. 2A**). In contrast, [^{3}H]HDL FCR in 25.3 transgenic mice did not differ compared with control mice. The synthesis rate of [^{3}H]HDL was significantly decreased in 11.1 transgenic mice (2-fold; $P < 0.05$) compared with either 25.3 transgenic mice or control mice (Fig. 2A).

Organ uptake of [^{3}H]HDL and SR-BI as a potentially involved mechanism

Uptake of [^{3}H]HDL in different tissues and organs was also determined (Fig. 2B), with the major organs involved

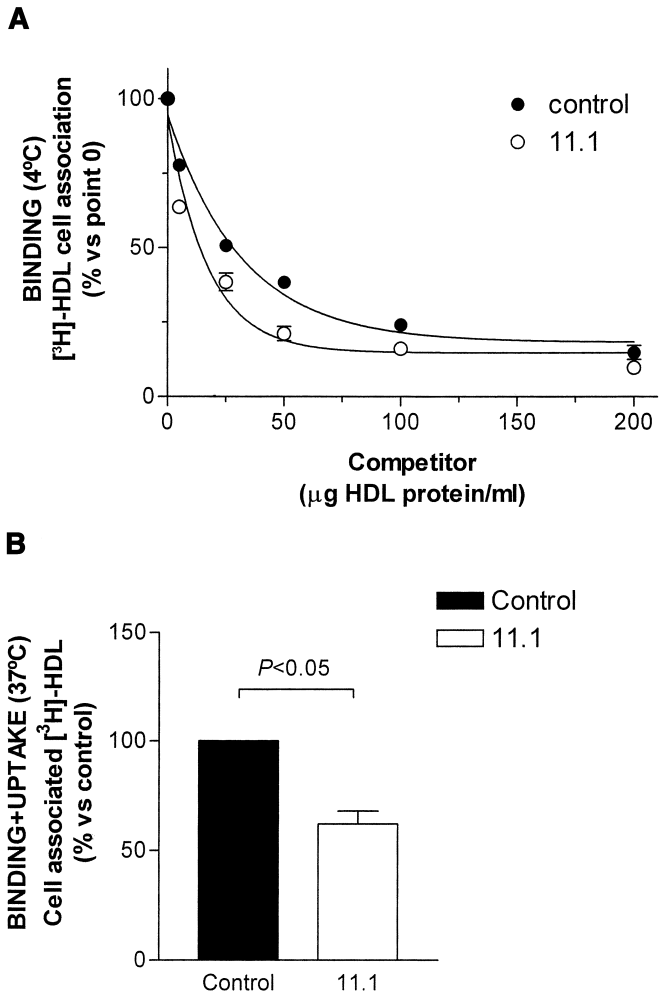


Fig. 3. Specific binding (4°C) and specific binding+uptake (37°C) of [^{3}H]cholesteryl oleoyl ether-labeled HDL to SR-BI transfected Chinese hamster ovary (CHO) cells. A: For binding, CHO-SR-BI cells were incubated with 25 μg/ml protein [^{3}H]cholesteryl oleoyl ether ([^{3}H]HDL) isolated from control mice at 4°C for 3 h in the presence of increasing concentrations of unlabeled HDL (competitors) isolated from 11.1 transgenic or control mice. Specific binding was calculated as the difference between values for CHO-SR-BI cells and non-transfected CHO cells. B: For binding+uptake, CHO-SR-BI cells were incubated with 25 μg/ml HDL protein of ([^{3}H]HDL) prepared from 11.1 transgenic mice and control mice and incubated at 37°C for 3 h without (total) or with a 40-fold excess of autologous unlabeled HDL (non-specific). Relative units of transgenic cholesteryl ester-HDL uptake were calculated taking 100% as the control value in each experiment. Specific binding+uptake was calculated as the difference between total and non-specific values of CHO-SR-BI cells and non-transfected CHO cells. Values of both A and B are the average of three independent experiments.

being liver (72% of the injected dose vs. 53% in controls and 54% in 25.3 transgenic mice), gonads (0.4% vs. 0.2% and 0.3%, respectively), and adrenal glands (0.6% vs. 0.3% and 0.3%, respectively). Only 10% of the injected dose remained in plasma in 11.1 transgenic mice versus 27% and 26% of control mice and 25.3 transgenic mice. HDL uptake in 11.1 transgenic mice was therefore significantly increased in liver (1.4-fold; $P < 0.05$), adrenal glands (3.3-fold; $P < 0.05$), and gonads (2-fold; $P < 0.05$).

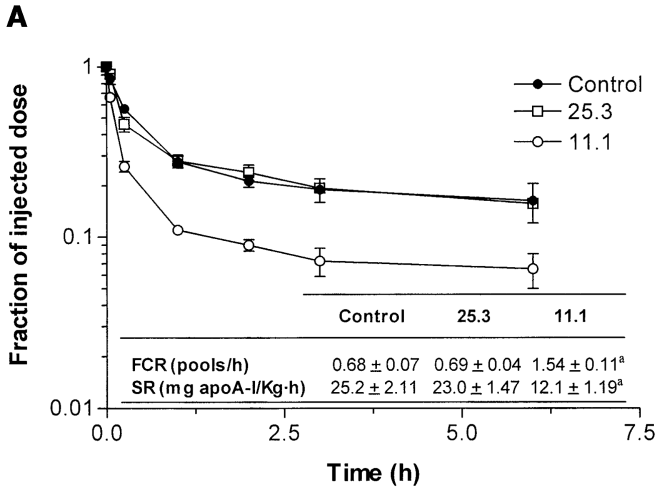
We thus analyzed the involvement of SR-BI in the increased selective uptake of HDL lipid by the liver and steroidogenic tissues. Immunoblots of equal amounts of protein, obtained from organs of all groups of mice, were performed. Figure 2C shows an immunoblot analysis of SR-BI of the liver and steroidogenic tissues of the different mouse lines. The antibody recognized a protein with an apparent molecular mass of ~82 kDa. Expression of mouse SR-BI was significantly increased only in adrenal glands of the 11.1 transgenic mice (~2-fold; $P < 0.05$) compared with that observed in the 25.3 transgenic and control mice. To see whether the higher uptake of [^{3}H]HDL was mediated through an SR-BI-mediated mechanism, CHO cells (clone ldlA7) stably transfected with mouse SR-BI were incubated with HDL of both 11.1 transgenic mice and control mice. SR-BI/HDL interaction was first assessed by competitive ligand binding (at 4°C) using [^{3}H]HDL from control mice as the radiolabeled ligand and unlabeled HDL from 11.1 transgenic or control mice as competitors (**Fig. 3A**). As shown by the competition curves obtained, unlabeled HDL from 11.1 transgenic mice reduced the binding of [^{3}H]HDL obtained from control mice more effectively than unlabeled HDL from control mice. The concentrations of unlabeled HDL required to displace 50% (calculated as the IC-50) of [^{3}H]HDL from control mice were the following: HDL from control mice, 23.2 ± 3.0 μg protein/ml, and HDL from 11.1 transgenic mice, 11.3 ± 1.2 μg protein/ml. The ability of SR-BI to mediate [^{3}H]HDL binding+uptake was also measured (at 37°C) (Fig. 3B). Specific binding+uptake (meaning that obtained by incubating radiolabeled HDL with a 40-fold excess of autologous non-radiolabeled HDL and subtracting the counts obtained in the same conditions in non-transfected cells) of 11.1 transgenic mouse HDL was significantly lower (315.0 ± 8.0 ng cholesteryl ester-HDL/mg cell protein; 1.6-fold; $P < 0.05$) compared with that shown by HDL from control mice (517.3 ± 38.1 ng cholesteryl ester-HDL/mg cell protein).

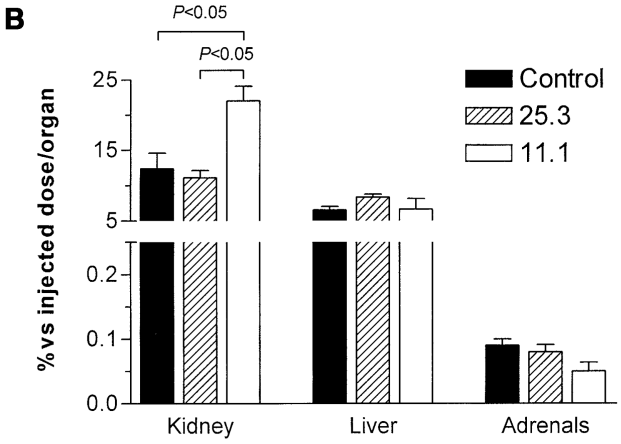
Kinetics of HDL protein moiety

Mouse apoA-I was radiolabeled with ^{125}I to follow its metabolism. As shown in **Fig. 4**, compared with the control mice, apoA-I FCR was significantly higher in the 11.1 transgenic mice (~2-fold; $P < 0.05$) compared with control mice (Fig. 4). ApoA-I FCR in 25.3 transgenic mice did not differ from that of control mice. On the other hand, the synthetic rate of apoA-I was found to be significantly decreased in 11.1 transgenic mice (~2-fold; $P < 0.05$) compared with that of 25.3 transgenic mice and control mice, respectively.

Organ uptake of apoA-I and potentially involved mechanisms

Figure 4B shows the uptake of apoA-I expressed as a percentage of the injected dose per organ, in kidney, liver, and adrenals, organs known to mediate most apoA-I catabolism. In all mouse lines, apoA-I was preferentially cleared by the kidney and 11.1 transgenic mice showed a





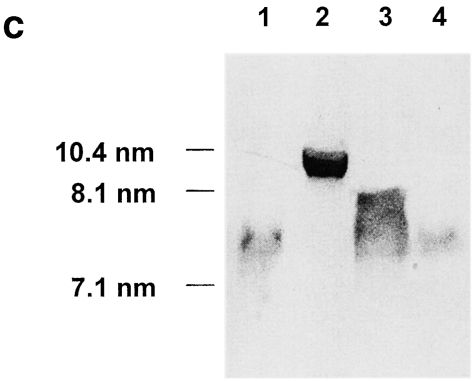


Fig. 4. In vivo metabolism of radiolabeled apoA-I and ex vivo displacement of mouse apoA-I by human apoA-II to control plasma. A: Plasma clearance of radiolabel is expressed as a fraction of the injected dose. Inset, table showing FCR and SR values for each genotype. Results shown are mean ± SEM of 6–7 mice. [a] Significantly different ($P < 0.05$) compared with 25.3 transgenic or control mice. B: Uptake of ^{125}I-apoA-I by target organs (uptake is expressed as % of injected dose/organ) 6 h after an intravenous injection. Results are expressed as mean ± SEM of 3–5 mice. C: Effect of human apoA-II on apoA-I-containing lipoprotein size distribution. Plasma lipoproteins of mice fed a regular diet were separated on a non-denaturing gradient gel electrophoresis and blotted onto a nitrocellulose filter using polyclonal antibodies to mouse apoA-I. Lane 1, plasma from 11.1 transgenic mouse plasma; lane 2, plasma from control mice; lane 3, plasma from control mice with 75 μg of human apoA-II (37.5 mg/dl); lane 4, plasma from control mice with 150 μg of human apoA-II (75 mg/dl).

significantly increased uptake (~1.8-fold; $P < 0.05$) compared with 25.3 transgenic and control mice. No statistical differences could be observed in liver or adrenals among 11.1 transgenic mice, 25.3 transgenic and control mice. Percentage of injected dose/organ in gonads, heart, lung, small intestine, white adipose tissue, spleen, and stomach did not differ among mouse lines, being around 7%.

Non-denaturing gradient gel electrophoresis was used to examine the effect of apoA-II on HDL particle size distribution (Fig. 4C). Control mouse plasma HDL consisted of a single-sized population with a mean diameter around 9.25 nm, whereas HDL of 11.1 transgenic mice consisted of distinct HDL subpopulations of smaller size with a main band of around 7.6 nm. Incubation of control HDL with increasing human apoA-II concentrations (37 mg/dl and 75 mg/dl) caused the appearance of smaller apoA-I-containing HDL particles that were of similar diameter to those of 11.1 transgenic mice (Fig. 4C).

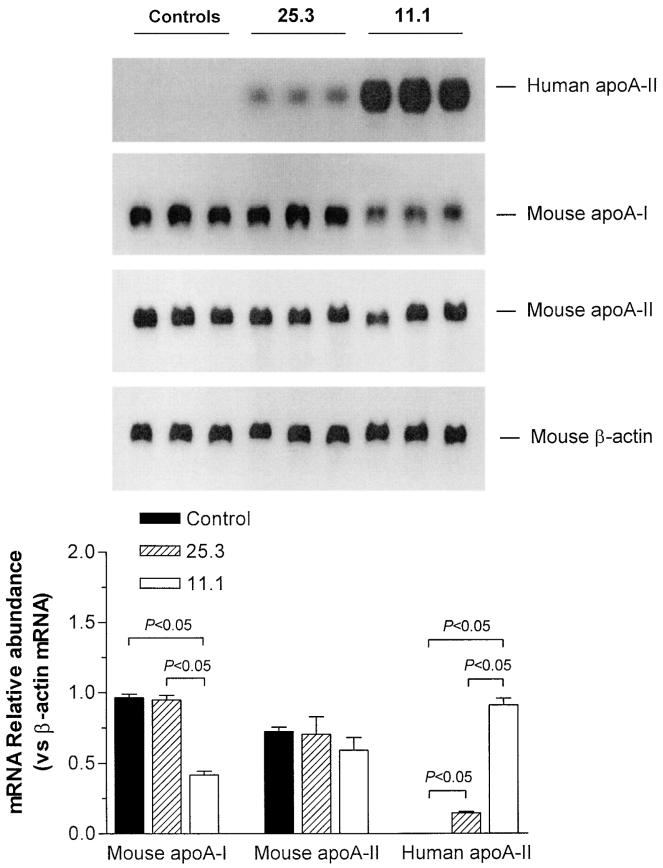


Fig. 5. ApoA-I and apoA-II mRNA liver levels. Relative abundance of hepatic mouse apoA-I and apoA-II and human apoA-II mRNA. Mouse β-actin was used as an internal standard. To permit comparisons, relative units of mouse apolipoprotein mRNA were calculated considering 100% as the highest value of A-I/β-actin in control mice, whereas relative units of human apoA-II were calculated taking 100% as the highest value of human A-II/β-actin in 11.1 transgenic mice. Each point represents the mean ± SEM of three mice.

Relative abundance of apoA-I and apoA-II mRNA

As previously reported, 11.1 transgenic mice expressed mRNA of human apoA-II mainly in the liver (6) (**Fig. 5**). Consistent with plasma human apoA-II protein concentrations, the relative abundance of hepatic human apoA-II mRNA was ~5-fold higher ($P < 0.05$) in 11.1 transgenic mice compared with 25.3 transgenic mice. The relative abundance of mouse apoA-I hepatic mRNA was significantly decreased (2.3-fold; $P < 0.05$) in 11.1 transgenic mice compared with control mice. In contrast, the relative abundance of mouse apoA-II mRNA remained unchanged. No differences in the relative abundance of intestinal mouse apoA-I and apoA-II of mRNA could be observed among the different mouse lines (data not shown).

DISCUSSION

HDL concentration, composition, and size

In accordance with our previous reports on HDL deficiency in mice overexpressing human apoA-II (6, 9), the HDL mass concentration of 11.1 transgenic mice was severely (4.9-fold) decreased compared with that of control mice. Chemical composition was also significantly altered with the ratio between HDL particle surface (protein, phospholipids, and free cholesterol) and hydrophobic core (triglyceride and cholesteryl esters) being significantly elevated (2-fold) in 11.1 transgenic mice compared with control mice. This could be, at least in part, a reflection of the ~9-fold increase in the preβ-HDL/α-HDL ratio of apoA-I-containing particles in 11.1 transgenic mice compared with control mice. Results of a recent study in independently-generated apoA-II transgenic mice showed that human apoA-II can be the sole component of preβ-HDL in these animals (7). The functional LCAT deficiency is likely to be the main mechanism determining HDL particle size in 11.1 transgenic mice (6, 9). Furthermore, hepatic lipase inhibition by free apoA-I (20) could be implicated in the increased percentage of HDL triglyceride seen in 11.1 transgenic mice. Previous studies strongly suggest that apoA-II is a physiological inhibitor of HL and that this action maintains HDL-C levels in the mouse (21–23), although this is a matter of controversy and may not be the case of human apoA-II (3, 24–26).

HDL lipid metabolism

Part of the impairment of HDL-C metabolism in 11.1 transgenic mice is derived from a decreased synthesis rate. This kinetic concept is likely to reflect mainly LCAT impairment (6, 9) and, secondarily, decreased efflux of cholesterol from cells (9). Further, our results show increased catabolism of [^{3}H]HDL in these mice due to increased uptake by liver, adrenal glands, and gonads. This indicates that human apoA-II overexpression acts by modulating, directly or indirectly, the in vivo uptake of HDL lipid by target organs. It is noteworthy that these in vivo studies did not determine SR-BI-specific events and other cellular binding sites and selective uptake processes could have contributed to explaining these observations. To analyze this topic, we investigated the physiological interaction and the expression of SR-BI with the HDL of the different lines of mice. Our results showed that the presence of human apoA-II in HDL increases the binding of this lipoprotein to SR-BI but, in an inverse manner, decreases its binding+uptake. The latter result probably indicates a decreased selective uptake of 11.1 transgenic HDL by SR-BI compared with control HDL, since SR-BI transfected cells show a 100-fold increase in radioactivity compared with non-transfected cells and 84% of this radioactivity corresponds to SR-BI-selective mediated uptake (17, 27). These results concur with a previous report (28) which compared, in an adrenal cell line, the selective uptake from control HDL versus that of apoA-II-enriched HDL and also with another recent report that shows that HDL from apoA-I-deficient mice show similar or increased affinity to SR-BI but decreased V_{max} compared with wild-type HDL (27). They also concur with another report that showed that isolated HDL with apoA-I and apoA-II (LpA-I/A-II) promoted less selective uptake than HDL with apoA-I without apoA-II (LpA-I) in HepG2 and in fibroblasts (29).

In contrast, another study using the same cell line as we used, found reduced association but increased uptake of apoA-I/apoA-II particles compared with those containing apoA-I particles (30). However, the latter used artificial reassembled HDL particles that were homogeneous in apolipoprotein content and particle size (30). It is worthy to note that HDL composition affects selective cholesteryl ester uptake because triglyceride and cholesteryl esters compete for SR-BI-mediated cellular uptake (31). This could be a mechanism that explains, at least in part, the decreased [^{3}H]HDL uptake by the triglyceride enriched, cholesteryl ester-poor HDL of 11.1 transgenic mice.

SR-BI protein expression was increased in the adrenal glands of 11.1 transgenic mice, but not in liver and gonads. The up-regulation of adrenal SR-BI has been previously observed in other mouse models of HDL deficiency (32–34) and is consistent with our observations in 11.1 transgenic mice of selective depletion of adrenal cholesterol and increased plasma ACTH (data not shown). The up-regulation of adrenal SR-BI protein suggests an insufficient compensatory mechanism in response to the HDL deficiency which could be due, at least in part, to the decreased uptake of cholesteryl esters from apoA-II-enriched HDL. Given the low weight of adrenal glands compared with the liver, the increase in adrenal SR-BI mass has little relevance for explaining the increased HDL-lipid catabolism of 11.1 transgenic mice. In consequence, increased HDL lipid liver catabolism seems to be largely due to unknown SR-BI-independent mechanisms.

ApoA-I metabolism

It is noteworthy that apoA-I FCR and SR values obtained in control mice in this study were considerably higher than those of other studies (i.e., reference 15). This is probably due to two factors: one, shorter kinetics (6 h versus 28 h in other studies) that would result in our case in decreased area under the curve and, consequently, in a higher inversion of this parameter which is the FCR; and the other, the fact that in our study, as in another which obtained similar results (18), apoA-I was radiolabeled and injected in free form rather than associated with HDL (15).

A major change in apoA-I metabolism in 11.1 transgenic mice was a ~2-fold decreased synthesis rate compared with control mice. The cause of this may be the 2.3-fold decrease in liver apoA-I mRNA, indicating a change at transcriptional level that was not present in the intestine. In the latter organ, no difference was observed between transgenic and control mice. In the liver, the lower relative abundance of mouse apoA-I mRNA could involve a change in the transcription rate or a diminished proportion of transcript retained at the polysomic fraction leading to enhanced intracellular breakdown of mouse apoA-I mRNA. The molecular mechanisms of decreased apoA-I synthesis at transcriptional level remain to be determined. One possibility is that decreased FXR resulting from increased lipid biliar excretion (which we observed in preliminary experiments in 11.1 transgenic mice) would result in both decreased cholesterol 7α-hydroxylase mRNA and apoA-I mRNA, which are directly correlated between themselves and with HDL-C (35–37).

In contrast to mouse apoA-I, no difference was found in mouse apoA-II mRNA levels either in liver or intestine among the different groups of mice analyzed.

Another major cause of decreased plasma apoA-I in 11.1 transgenic mice was a 2-fold increased catabolism mediated by the kidney, probably through the cubilin-megalin pathway (38). One of the mechanisms implicated in this increased catabolism may be the displacement of mouse apoA-I (and mouse apoA-II) by human apoA-II (10, 39), leading to increased lipid-poor or free apolipoprotein plasma concentration that is rapidly cleared by the kidney (40, 41). In fact, we were able to reproduce the formation of small HDL particles (around 7.6 nm in diameter) by incubating mouse control plasma with isolated, purified human apoA-II in a concentration similar to that present in the plasma of 11.1 transgenic mice. The human apoA-II-remodeling ability elicits generation of smaller HDL particles and free apoA-I, and this is probably related to a higher catabolic rate of apoA-I by the kidney, as has been shown in other models (40–42).

Conclusion

In summary, HDL deficiency in 11.1 transgenic mice is due to complex changes in the metabolism of both their lipid and protein components, at both synthesis and catabolic levels. Further work is required to characterize the molecular basis of the decrease in hepatic apoA-I mRNA and that of the SR-BI-independent increase in lipid catabolism of HDL.

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