The concentration of high density lipoproteins (HDL) is inversely related to the risk of atherosclerosis. The two major protein components of HDL are apolipoprotein (apo) A-I and apoA-II. To study the role of apoA-II in lipoprotein metabolism and atherosclerosis, we have developed three lines of C57BL/6 transgenic mice expressing human apoA-II (lines 25.3, 21.5, and 11.1). Northern blot experiments showed that human apoA-II mRNA was present only in the liver of transgenic mice. SDS-polyacrylamide gel electrophoresis and Western blot analysis demonstrated a 17.4-kDa human apoA-II in the HDL fraction of the plasma of transgenic mice. After 3 months on a regular chow, the plasma concentrations of human apoA-II were 21 ± 4 mg/dl in the 25.3 line, 51 ± 6 mg/dl in the 21.5 line, and 74 ± 4 mg/dl in the 11.1 line. The concentration of cholesterol in plasma was significantly lower in transgenic mice than in control mice because of a decrease in HDL cholesterol that was greatest in the line that expressed the most apoA-II (23 mg/dl in the 11.1 line versus 63 mg/dl in control mice). There was also a reduction in the plasma concentration of mouse apoA-I (32 ± 2, 56 ± 9, 91 ± 7, and 111 ± 2 mg/dl for lines 11.1, 21.5, 25.3, and control mice, respectively) that was inversely correlated with the amount of human apoA-II expressed. Additional changes in plasma lipid/lipoprotein profile noted in line 11.1 that expressed the highest level of human apoA-II include elevated triglyceride, increased proportion of total plasma, and HDL free cholesterol and a marked (>10-fold) reduction in mouse apoA-II. Total endogenous plasma lecithin:cholesterol acyltransferase (LCAT) activity was reduced to a level directly correlated with the degree of increased plasma human apoA-II in the transgenic lines. LCAT activity toward exogenous substrate was, however, only slightly decreased. The biochemical changes in the 11.1 line, which is markedly deficient in plasma apoA-I, an activator for LCAT, are reminiscent of those in patients with partial LCAT deficiency. Feeding the transgenic mice a high fat, high cholesterol diet maintained the mouse apoA-I concentration at a normal level (69 ± 14 mg/dl in line 11.1 compared with 71 ± 6 mg/dl in non-transgenic controls) and prevented the appearance of HDL deficiency. All this happened in the presence of a persistently high plasma human apoA-II (96 ± 14 mg/dl). Paradoxical HDL elevation by high fat diets has been observed in humans and is reproduced in human apoA-II overexpressing transgenic mice but not in control mice. Finally, HDL size and morphology varied substantially in the three transgenic lines, indicating the importance of apoA-II concentration in the modulation of HDL formation. The LCAT and HDL deficiencies observed in this study indicate that apoA-II plays a dynamic role in the regulation of plasma HDL metabolism.

The concentration of high density lipoprotein (HDL) cholesterol in plasma is inversely correlated with the risk of atherosclerosis (1–3). However, the mechanism by which HDL exerts its anti-atherogenic action is poorly understood. Reverse cholesterol transport is one HDL function that may be important in this respect (4). Apolipoprotein (apo) A-I and apoA-II are the main protein components of HDL. ApoA-I plays a structural role in HDL and is a cofactor of the enzyme lecithin:cholesterol acyltransferase (LCAT) (5–7). Human apoA-II has a cysteine in position 6 and is associated with HDL mainly as disulfide-linked homodimers (17.4 kDa) and heterodimers with apoD and apoE (38 and 43 kDa, respectively) (8–10). The function of apoA-II remains poorly defined. ApoA-II was found to activate or inhibit hepatic lipase (11–14), inhibit or not influence the action of the cholesteryl ester transfer protein (15–17), and inhibit the uptake of low density lipoprotein (LDL) by the liver (18–20). The inhibition of LDL uptake by apoA-II is not well understood. The mechanisms by which apoA-II is transferred to and cleared from the plasma are also poorly understood. The presence of apoA-II in human plasma is regulated by the liver and by reticuloendothelial cells (21–23). The hepatic plasma clearance of apoA-II is independent of an increase in apoA-II levels due to transgenic expression, indicating that the liver may not be the source of apoA-II in plasma. The importance of apoA-II concentration in the modulation of HDL formation is indicated by the report that apoA-II is required for the synthesis of HDL precursors (24).
lesteryl ester transfer protein (14, 15), interact with a putative apoA-II in vivo is unclear; one individual with complete apoA-II deficiency did not show any lipoprotein alterations (18). A role for apoA-II in the coagulation pathway has also been proposed (19).

Human HDL particles can be divided into those with apoA-I but without apoA-II (LpA-I) and those with both apoA-I and apoA-II (LpA-I-A-II) (20). Some studies found that cholesterol efflux from cells is induced by LpA-I but not by LpA-I-A-II (for a review see Ref. 21). A third type of HDL that contains apoA-II but not apoA-I has been described recently (22). The concentration of LpA-I and LpA-I-A-II appear to be regulated by different mechanisms; although apoA-I levels are regulated primarily by apoA-I catabolism, those of apoA-II appear to be regulated mainly by apoA-II production (23).

Transgenic mice overexpressing human apoA-II were first reported by Schultz et al. (24). These animals were found to have smaller HDL particles than control mice; otherwise, there were no other significant changes in lipoprotein profile. Transgenic mice overexpressing both human apoA-I and apoA-II showed no difference in lipoprotein plasma concentrations compared with transgenic mice expressing human apoA-I alone. Interestingly, although mice expressing both human apoA-I and apoA-II were more resistant than nontransgenic animals to diet-induced atherosclerosis, they were more susceptible to this process than transgenic animals expressing human apoA-I only (25). In contrast, mice overexpressing a mouse apoA-II transgene showed increased plasma triglycerides and HDL cholesterol (HDLc) in plasma, increased HDL size, and increased susceptibility to atherosclerosis development compared with nontransgenic controls (26, 27). These different effects of mouse and human apoA-II on lipoprotein metabolism and atherosclerosis may be related to their structural differences. Although human apoA-II contains a cysteine residue and exists as a dimer, most mammalian apoA-Is, including mouse apoA-II, have no cysteine and exist as monomers (28). Furthermore, the homodimeric human apoA-II but not monomeric apoA-II is able to induce the efflux of cholesterol from different cell types to the extracellular medium (16, 29, 30). However, recent results from transgenic mice of a mutated form of human apoA-II that cannot form dimers through disulfide linkage are clearly different from those obtained by the overexpression of mouse apoA-II (21). Therefore, dimerization per se is probably not the cause of the different phenotypic effects observed in transgenic mice overexpressing human versus mouse apoA-II.

The role of human apoA-II in lipoprotein metabolism and atherosclerosis is thus of considerable interest. In developing transgenic mice expressing human apoA-II, we noted heretofore unreported phenotypic effects of transgene expression on plasma apoA-I and LCAT activity. The functional LCAT deficiency induced in these animals is associated with a marked HDL deficiency. Furthermore, the phenotypic effects of human apoA-II overexpression are modulated by a high fat, high cholesterol diet in a manner similar to the response of normal human subjects to similar diets.

**MATERIALS AND METHODS**

**Animals and Preparation of Nucleic Acids**—The fertilized eggs used in the microinjection were from the matings of C57BL/6 mice. The microinjected human apoA-II gene was a 3-kilobase pair fragment isolated from human genomic DNA prepared by digestion with the enzyme MspI and was subcloned into the Accl site of Bluescript KS+ phagemid (Stratagene). It contains ~1 kilobase pair in the 5′-flanking region. The human apoA-II gene fragment was removed from the plasmid by double digestion with Clal and Xhol, fractionated on agarose gels, and purified with a Qiagen kit (QIAGEN) before microinjection. The procedure for the identification of transgenic animals consisted of a combination of polymerase chain reaction and Southern blotting of tail DNA. 2 μg of genomic DNA was amplified using primers digonucleotides with the sequences 3′-CCCTCATGGTGTCAAGCAGC-5′ and 3′-TTCCCTGGTACACACTCTTCT-5′. One-fifth of the polymerase chain reaction products (total volume, 50 μl) was electrophoresed on a 2% agarose gel, transferred to a nylon membrane, and hybridized to a 5′-end 32P-labeled digonucleotide with the sequence 3′-GACAGACAG-5′ and 5′-TTTTTT-5′, specific for the human apoA-II gene. From 200 embryos implanted, 145 pups were born. Four of these were transgenic and designated 24.2, 21.5, 25.3, and 11.1. The 24.2 founder died before breeding. Three different lines of transgenic mice were developed from the other 3 founders. The mode of transmission of the transgene in the 11.1 line suggested integration in chromosome X and the other two lines were autosomal. Transgenic and control mice used for the RNA, apolipoprotein, and lipoprotein studies were between 2 and 3 months old at the beginning of the studies. They were fed ad libitum a regular chow diet (5001, The Richmond Standard, PMI Feeds, Inc., Richmond, IN) or a high fat, high cholesterol diet (75% mouse chow 5015, 7.5% cocoa butter, 1.25% cholesterol, 0.5% sodium cholate; TD 88051, Harlan Teklad, Madison WI) and had free access to water. Total RNA was isolated from different tissues as described previously by Chomczynski and Sacchi (32). 5 μg of RNA was electrophoresed in 2% agarose gels, blotted to nylon membranes, and probed with cloned cDNAs labeled with [32P]dCTP by nick translation. Human apoA-II cDNA was used to probe the human apoA-II mRNA, whereas mouse apoA-II and rat apoA-I cDNAs were used to probe the mouse apoA-II and apoA-I mRNAs, respectively. The intensity of the x-ray bands was quantified by an LKB Bromma VitroScan XL enhance laser densitometer with the Gel Scan XL software.

**Lipids, Lipoproteins, and Apolipoproteins**—Blood was obtained from the tail veins of mice and collected in tubes containing EDTA and sodium azide (33). Total cholesterol, free cholesterol, and triglycerides were measured colorimetrically using commercial kits adapted to an autoanalyzer RA-XT (Technicon) (34). Triglyceride determinations were corrected for the free glycerol present in plasma (Sigma). Plasma lipoproteins were fractionated by FPLC; 0.2 ml of plasma was loaded on a Superose 6 HR column (Pharmacia Biotech Inc.) as described previously (35). In some cases, plasma was pooled from two siblings of the same sex that were kept in the same cage before FPLC analysis. Twenty-five 1-ml fractions were collected, and samples corresponding to very low density lipoproteins (VLDL) (3 ml), low density lipoproteins (LDL) (3 ml), and HDL (6 ml) were pooled individually. When large quantities of isolated lipoproteins were required for analysis, sequential ultracentrifugation was used (36).

**HDL size** was analyzed by nondenaturing gradient gel electrophoresis of Sudan Black B prestained plasma in 4–27% polyacrylamide gels (36). Antiserum to mouse apoA-I and apoA-II. Electrophoresis of pooled plasma or isolated lipoproteins prestained with Sudan Black B was also conducted in a 2–3% discontinuous polyacrylamide gels (Lipofilm, Sebia). HDL isolated by preparative ultracentrifugation were dialyzed against ammonium acetate buffer (pH 7.4), and aliquots were stained with 2% sodium phosphotungstate for evaluation by electron microscopy according to previously described methods (38).

**Apolipoprotein Isolation and Generation of Antibodies—ApoHDL** was prepared by delipidation of HDL with diethyl ether-ethanol 3:1 (v/v) (39). Mouse apoA-I was isolated by gel filtration chromatography (Sephacryl S-200, 1200 × 1.5 cm) from apoHDL (40). Antiserum to mouse apoA-I was produced by subcutaneous immunization of a rabbit with the purified apolipoprotein. Antiserum to mouse apoA-II was kindly provided by Drs. R. T. Kitchens and Gustav Schonfeld (Department of Internal Medicine, Washington School of Medicine, St. Louis, MO). Antiserum to human apoA-II was purchased from Boehringer Mannheim. Plasma human apoA-II concentration was measured using a commercial immunoassy kit (Immuno AG). Concentrations of plasma mouse apoA-I and apoA-II were determined by a radial immunodiffusion assay (41).

**LCAT Assays**—Plasma LCAT activity against endogenous substrate was measured by using the lipoproteins of the whole plasma as substrate (42, 43). Results were expressed as cholesterol molar esterification. Plasma LCAT activity against exogenous substrate was determined by using model reassembled HDL that contained 1-palmitoyl-2-oleoyl-sn-3-phosphocholine, [3H]cholesterol and apoA-I as a substrate (44).
Expression of the Human Apolipoprotein A-II in Transgenic Mice—Independent human apoA-II transgenic mouse lines were bred from the three founder animals; these lines were designated 25.3, 21.5, and 11.1 (Fig. 1, A and B). By quantitative dot-blot analysis (data not shown), the 11.1 and 21.5 lines each contained ~20 copies, and the 25.3 line contained ~40 copies of the human apoA-II gene. The tissue distribution of transgene mRNA expression was determined by Northern blot analysis (Fig. 1C). All three lines expressed human apoA-II mRNA in the liver only, with no detectable expression in the small intestine, spleen, kidney, testis, heart, lung, or muscle. Finally, despite the different levels of expression of human apoA-II mRNA, the amount of mouse apoA-II and apoA-I mRNAs only showed minor differences (Fig. 1D).

Because mouse apoA-II exists only as a monomer (28) and human apoA-II exists mainly as a homodimer, the two proteins can be separated on SDS-PAGE. HDL isolated from plasma of transgenic mice showed an additional band with the apparent molecular weight of human apoA-II (17.4 kDa) (Fig. 2A), which was absent in the HDL of control mice. In the two human apoA-II high expression lines (11.1 and 21.5) the electrophoretic analysis revealed a decrease in the amount of mouse apoA-I and other low molecular weight HDL apoproteins, including mouse apoA-II (Fig. 2A). Western blot analysis using an antibody to human apoA-II confirmed that the 17.4-kDa band present in the HDL of the transgenic mice was indeed human apoA-II. The transgene-specific band also showed the expected oxidation reduction pattern characteristic of human apoA-II (Fig. 2B). Immunoreactive human apoA-II was undetectable in VLDL and LDL of transgenic mice (data not shown).

Lipoprotein Profile of Transgenic Mice on a Regular Chow Diet—We first analyzed the plasma and lipoprotein fractions by native polyacrylamide gels. Lipoprotein fractions were isolated by sequential ultracentrifugation. They were prestained with Sudan Black B and fractionated by 2–3% polyacrylamide nondenaturing gradient gel electrophoresis. The electrophoretic pattern of total plasma from the 21.5 and 25.3 lines was similar to that of control mice, with HDL appearing as the main lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice. In contrast, the total plasma of the 11.1 line showed a clearly different pattern. VLDL was the major lipoprotein band, which was slightly less intense in staining than the HDL band from control mice.
The degree of hypocholesterolemia was more severe in almost exclusively by the decreased HDLc in transgenic animals (Table I, B). This was caused by the decreased HDL of 11.1 mice. The migration of the samples as indicated by lane 4, LDL, and HDL from control and transgenic mice were isolated from 1 ml of plasma by sequential ultracentrifugation and diluted to the same final volumes in control and 11.1 transgenic mice (0.45 ml of VLDL, 0.5 ml of LDL, and 0.7 ml of HDL). In all cases, 2.5 liters of sample were applied. Lane 1, plasma of control mice; lane 2, VLDL of control mice; lane 3, LDL of control mice; lane 4, HDL of control mice; lane 5, plasma of 11.1 mice; lane 6, VLDL of 11.1 mice; lane 7, LDL of 11.1 mice; lane 8, HDL of 11.1 mice. The migration of the samples as α, pre-β, or β-lipoproteins is indicated by arrows.

**Fig. 3.** Electrophoresis of Sudan Black B prestained plasma and lipoproteins on a 2.3% discontinuous acrylamide gel. VLDL, LDL, and HDL of 11.1 mice; LDL of 11.1 mice; HDL of 11.1 mice; plasma of 11.1 mice; lane 2, VLDL of control mice; lane 1, plasma of control mice; lane 3, LDL of control mice; lane 4, HDL of control mice; lane 5, plasma of 11.1 mice; lane 6, VLDL of 11.1 mice; lane 7, LDL of 11.1 mice; lane 8, HDL of 11.1 mice. The migration of the samples as α, pre-β, or β-lipoproteins is indicated by arrows.

Total plasma cholesterol was significantly lower in transgenic mice than in control animals (Table I, B). This was caused almost exclusively by the decreased HDLc in transgenic animals. The degree of hypocholesterolemia was more severe in lines 11.1 and 21.5 than in line 25.3. The proportion of free cholesterol in plasma was elevated in line 11.1. Plasma triglycerides were elevated 3-fold in line 11.1 compared with the control group but were unchanged in lines 21.5 and 25.3.

VLDL cholesterol and triglycerides were increased 8.6- and 6.3-fold, respectively, in line 11.1 compared with the control group (Table I, C). The VLDL lipids in the other two lines were similar to control except that line 21.5 showed a 2-fold increase in VLDL triglycerides. LDL lipids were different among the different lines; LDL cholesterol and triglycerides were significantly lower in lines 25.3 and 21.5 than in the controls but were mildly and insignificantly elevated in line 11.1. HDL lipids were decreased in apoA-II transgenic mice compared with control mice, but the decline in HDL triglycerides in lines 11.1 and 25.1 was not significant.

**Table I**

Concentrations of apolipoproteins (A) and lipids (B) in whole plasma and in isolated lipoproteins (C) of control and human apoA-II transgenic mice after 3 months of regular chow diet.

|               | Controls | Line 25.3 | Line 21.5 | Line 11.1 |
|---------------|----------|-----------|-----------|-----------|
| A Number of animals | 16       | 16        | 9         | 16        |
| Murine ApoAI  | 30.1 ± 0.9 | 23.7 ± 3.2 | 22.5 ± 4.6 | 2.7 ± 1.1 |
| Murine ApoAI  | 111 ± 2.2 | 91 ± 7.2a | 55.5 ± 8.5a | 31.8 ± 2.0a |
| Human apoAI   | 0 ± 0     | 21.4 ± 3.7a | 50.8 ± 6.2b | 74.1 ± 4.0a |
| B Number of animals | 16       | 16        | 9         | 16        |
| Cholesterol   | 71.4 ± 12 | 57.0 ± 3.8b | 49.6 ± 6.6a | 42.4 ± 2.7b |
| % Plasma Free cholesterol | 30.4 ± 4.0 | 24.4 ± 2.9 | 29.9 ± 4.0 | 43.3 ± 2.6a |
| Triglycerides | 20.3 ± 4.1 | 18.2 ± 3.9 | 22.2 ± 1.1 | 66.6 ± 18.4a |
| C Number of animals | 16       | 16        | 9         | 16        |
| VLDL cholesterol | 0.86 ± 0.2 | 1.34 ± 0.2 | 1.4 ± 0.5 | 7.4 ± 1.8b |
| VLDL triglycerides | 7.79 ± 1.6 | 8.97 ± 1.6 | 16.5 ± 1.5a | 49.2 ± 15.9b |
| LDL cholesterol | 7.34 ± 0.5 | 5.44 ± 0.4a | 6.28 ± 0.6 | 11.72 ± 2.5 |
| LDL triglycerides | 8.61 ± 0.8 | 5.3 ± 1.1a | 4.57 ± 0.5a | 14.3 ± 3.2 |
| HDL cholesterol | 62.8 ± 1.1 | 44.7 ± 4.1 | 41.4 ± 7.5a | 22.8 ± 2.4b |
| HDL triglycerides | 3.88 ± 1.3 | 0.34 ± 0.1 | 1.15 ± 1.0 | 3.1 ± 1.2 |

a Significantly different (p < 0.05) from control mouse group.

b Significantly different (p < 0.005) from control mouse group.
LCAT Deficiency in Human ApoA-II Transgenic Mice

TABLE II
Concentrations of apolipoproteins (A) and lipids (B) in whole plasma and isolated lipoproteins (C) of control and apoA-II transgenic mice after 3 months of high cholesterol, high fat diet. Lipoproteins were isolated by FPLC. The results are expressed as the averages ± standard error. The units used are mg/dl. ND, not determined.

|                      | Controls | Line 25.3 | Line 11.1 |
|----------------------|----------|-----------|-----------|
| A Number of animals  | 13       | 8         | 9         |
| Murine ApoA-II       | 19.2 ± 2.1 | 19 ± 3.1  | 15.9 ± 3.2 |
| Murine ApoA-I        | 71.4 ± 6.4 | 91 ± 9    | 68.6 ± 14  |
| Human ApoA-II        | 0 ± 0    | 25.2 ± 8.1* | 96 ± 14*  |
| B Number of animals  | 13       | 8         | 9         |
| Plasma Cholesterol   | 190 ± 11 | 230 ± 23  | 153 ± 9*  |
| % Plasma Free cholesterol | 22.6 ± 7.1 | ND       | 25.3 ± 3.7 |
| Plasma Triglycerides | 8 ± 0.6  | 12.3 ± 0.9* | 22.2 ± 2.1 |
| C Number of animals  | 13       | 8         | 9         |
| VLDL cholesterol     | 116 ± 8  | 104 ± 15  | 55.9 ± 8.6* |
| VLDL triglycerides   | 5.2 ± 0.9 | 6.8 ± 1.2  | 12.3 ± 3.4  |
| LDL cholesterol      | 35 ± 5   | 28.9 ± 14.7 | 57.7 ± 9.6* |
| LDL triglycerides    | 0 ± 0.6  | 0.15 ± 0.15 | 0 ± 0     |
| HDL cholesterol      | 38 ± 5   | 96.8 ± 21.5* | 42.9 ± 8.9 |
| HDL triglycerides    | 2 ± 0.4  | 5.4 ± 1.1*  | 7.6 ± 2    |

*Significantly different (p < 0.005) from control mice group.

**Significantly different (p < 0.05) from control mice group.

ol/HDLc was decreased in transgenic mice (especially in line 25.3) compared with controls.

HDL Particle Size in the Plasma of Transgenic Mice—The size of the HDL particles was analyzed by non-denaturing 4-27% gradient polyacrylamide gel electrophoresis of prestained plasma from mice on a regular diet (Fig. 4A) and from those on a high cholesterol, high fat diet (Fig. 4B). In the first immunoblot, human HDL isolated by ultracentrifugation was used as a control. It migrated in a polydisperse pattern with proteins ranging in size from 6.7 to 12.2 nm (Fig. 4A). All three transgenic lines on a regular diet showed substantially reduced lipid staining of their HDL particles compared with controls. In keeping with the results of lipid and lipoprotein composition, this decrease in lipid staining was especially marked in the HDL of line 11.1. There was no difference in the HDL size distribution of the 25.3 line compared with controls, both showing a single peak with a diameter of 10.2 nm. In contrast to the relatively homogenous HDL size found in control mice, HDL particles from the plasma of the control mouse, mostly free cholesterol. This was not due to a delay in preparing the lipoproteins because ultracentrifugation was initiated within a few hours after the collection of plasma. Plasma LCAT activity was measured using the same plasma pools. The assay was performed both against exogenous substrates and against endogenous substrates. The LCAT activity against exogenous substrates was mildly suppressed in the transgenic mouse lines compared with controls, being 10.6 ±

![FIG. 4. Electrophoresis of Sudan Black B prestained plasma (20 liters) in a 4-27% non-denaturing polyacrylamide gel. A, plasma of mice on regular chow diet. Lane 1, human HDL isolated by ultracentrifugation; lanes 2 and 3, plasma from control mice; lane 4, plasma from 21.5 mice; lanes 5 and 6, plasma from 11.1 mice; lane 7, plasma from 25.3 mice. B, plasma of mice on high cholesterol, high fat diet. Lanes 1 and 2, plasma from control mice; lane 3, 4, and 5, plasma from 11.1 mice; lanes 6 and 7, plasma from 25.3 mice. The size of the molecular weight markers in nm is shown at the left.](http://www.jbc.org/)

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0.3, 8.0 ± 0.8, and 9.9 ± 0.3 nmol ml⁻¹ h⁻¹ in lines 25.3, 21.5, and 11.1, respectively, and 14.8 ± 0.3 nmol ml⁻¹ h⁻¹ in controls (Fig. 5A). When the assay was performed against endogenous substrates, there was a substantially greater decrease in LCAT activity in transgenic animals compared with controls. The activities were: 76 ± 0.6, 50 ± 10.2, and 18.4 ± 2.8 nmol ml⁻¹ h⁻¹ in lines 25.3, 21.5, and 11.1, respectively, compared with a value of 92.7 ± 2.0 nmol ml⁻¹ h⁻¹ in controls (Fig. 5A). Therefore, compared with controls, the LCAT activities in transgenics were: for exogenous substrates, 72, 54, and 66.9% and for endogenous substrates, 82, 54, and 20% for lines 25.3, 21.5, and 11.1, respectively. This change in LCAT activity was persistent and reproducible. Similar results were obtained when plasma was obtained from these animals 6 months later. The decrease in the endogenous LCAT activity was in direct proportion to the degree of reduction in plasma apoA-I (Fig. 5B).

Apolipoprotein Composition and Electron Microscopy of HDL of Control and Control Mice—The reduction in LCAT activity in transgenic mice was much more pronounced when endogenous substrates were used instead of exogenous substrates. We decided to examine in greater detail the major endogenous substrate, HDL, in these animals. The relative content of human apoA-II, mouse apoA-II, and mouse apoA-I in HDL samples from transgenic and control mice were investigated by Western blot analysis after fractionation on nondenaturing polyacrylamide gradient gel electrophoresis. No human apoA-II was detected in control HDL (Fig. 6A). It was present only in the 10.2-nm region of line 25.3 mice, the line with the lowest level of expression. In the two high expressor lines, 21.5 and 11.1, it was detected in HDL particles in the 10-, 7.9-, and 7.6-nm regions. The distribution of immunoreactive mouse apoA-II was somewhat different. Both control and 25.3 mouse HDL showed a major band at 10.2 nm. In the 21.5 and 11.1 lines, a weakly staining diffuse pattern spanning from 10 to 7.3 nm was observed (Fig. 6B). Major changes in mouse apoA-I immunoreactivity were also seen (Fig. 6C). In control and the lower expressor 25.3 line, an intensely stained band was detected at 10.2 nm. The immunoreactivity in the two high expressor lines 21.5 and 11.5 was diffuse and spanned from 10 to 7.9 nm. There was also a major difference between these two lines in that the one with the highest human apoA-II expression (line 11.1) had very faint, barely detectable immunoreactive apoA-II in its HDL (Fig. 6C).

In summary, human apoA-II represents ~40% of the apoprotein mass in the HDL of the 21.5 line and accounts for almost the entire complement of apolipoproteins in the HDL of the highest human apoA-II expressor line 11.1. In other words, the vast majority of HDL particles in this line, and to a lower extent in line 21.5, contain only human apolipoprotein A-II and little or no mouse apoA-I or apoA-II.
DISCUSSION

Three lines of transgenic mice overexpressing human apoA-II were generated by microinjection of a 3-kilobase pair fragment of cloned human apoA-II gene. The transgenic mice expressed human apoA-II mRNA only in the liver (Fig. 1), a natural tissue of apoA-II production in humans (24, 46, 47). The human protein was secreted into the plasma compartment and was associated with mouse HDL. The apparent molecular mass of 17.4 kDa, its recognition by antibodies, and the oxidation reduction pattern were those expected for human apoA-II (Fig. 2).

Changes in the Lipoprotein Profile of Transgenic Mice—The concentration of human apoA-II in lines 23.5, 21.5, and 11.1 on a regular chow diet was 62, 147, and 215% of that found in normal human plasma, respectively (30). All transgenic mouse lines showed a significant reduction of plasma cholesterol due to decreased HDLc concentration (Table I). The decrease in these lipid values was correlated with the degree of overexpression of human apoA-II. In the animals (line 11.1) that showed the highest human apoA-II expression, there was also an increased proportion of free cholesterol in both plasma and HDL, an increase in triglycerides and a marked decrease in the concentration of plasma mouse apoA-I and apoA-II. Thus, there were substantial quantitative and qualitative differences in the lipoprotein profiles of the different transgenic lines. The magnitude of the changes was related to the concentration of human apoA-II in plasma (see Table I), and the changes are likely the direct result of human apoA-II overexpression.

When control and transgenic mice were fed a high cholesterol, high fat diet, the lipoprotein profiles between transgenic and control mice became more similar. Interestingly, the HDL deficiency, characterized by low mouse apoA-I and apoA-II and low HDLc with increased percentage of free cholesterol, disappeared when the transgenic mice in the 11.1 line were fed a high cholesterol, high fat diet. Moreover, the 11.1 mice showed twice as much HDLc and HDL triglycerides compared with levels observed with a regular chow diet. A notable increase of HDL, compared with an actual lowering in HDL in control mice, was also observed in 25.3 mice fed a high cholesterol, high fat diet (Tables I and II). These observations are especially interesting because the production of a similar lipoprotein profile in both control and transgenic mice fed a high fat diet occurred in the presence of an equally high, if not higher, plasma human apoA-II, which is 119 and 130% that of control values, respectively, in lines 25.3 and 11.1.

The mechanism for the restoration of the plasma mouse apoA-I and apoA-II and HDL lipids in animals on the high fat, high cholesterol diet is unclear. It is possible that the increased amounts of lipids in HDL provided enough lipoprotein surface to accommodate mouse apoA-I and apoA-II and human apoA-II at the same time. Conversely, the increase in HDL lipids could be the consequence rather than the cause of the more “normal” mouse apoA-I and apoA-II levels.

These human apoA-II transgenic mice represent a useful animal model in which to study the paradoxical HDL-raising effect of a high cholesterol, high fat diet observed in humans. An increase in HDL level in plasma after a high cholesterol, high fat diet was also seen in human apoA-I transgenic mice and found to be a result of increased HDL transport and decreased HDL fractional catabolic rate (48). These data, taken together, strongly suggest that specific structural characteristics of both apoA-I and apoA-II may be involved in the increase of HDL in humans after a high cholesterol, high fat diet.

A significant difference between the HDL of mice that expressed high levels of human apoA-II (line 11.1) and those that expressed lower levels (line 25.3) is the presence of small particles that were not found in the latter or in control mice. The presence of these particles was detected in all cases in mice on a regular chow diet and in most cases in mice on high cholesterol, high fat diet, which suggests that a threshold of human apoA-II may be needed to produce these small HDL particles. The ability of human apoA-II to induce the formation of small HDL particles has been observed previously both in vivo and in vitro (24, 49). Therefore, we can infer from these and other data (reviewed in Ref. 50) that both human apoA-I and apoA-II can contribute to the size heterogeneity of human HDL.

Possible Mechanisms Underlying the Lipoprotein Changes Found in the Transgenic Mice Fed a Regular Chow Diet—There are a number of possible mechanisms that are responsible for the changes found in the lipoprotein profiles in the transgenic mice. Human apoA-II associated with VLDL has been postulated to be an inhibitor of lipoprotein lipase (51). However, no human apoA-II was detected in the triglyceride-rich particles in the transgenic mice when analyzed by SDS-PAGE. Increased apoA-II has also been correlated with elevated plasma free fatty acids (52), which could in turn stimulate VLDL synthesis by the liver. However, we did not find any correlation between free fatty acid and human apoA-II concentrations in plasma in these animals (data not shown). ApoA-II-containing human HDL particles have been found to be better substrates for hepatic lipase than those without apoA-II (11, 12). Hepatic lipase is thought to mediate the conversion of HDL2 to the smaller HDL3 particles, liberating lipids and apoA-I during the process (53). Transgenic mice overexpressing human hepatic lipase displayed HDL particles that are smaller, containing 34% less HDLc than HDL from nontransgenic controls but having normal apoA-I content (54). It is possible that the more effective action of hepatic lipase on the human apoA-II-containing HDL particles contributed to the lower HDLc in the transgenic animals (see Table I). This interpretation is supported by the appearance of smaller human apoA-II-containing HDL particles in transgenic lines 25.1 and 11.1 that expressed high levels of human apoA-II but not in line 25.3, which expressed lower levels of the protein. In contrast, another study using human apoA-II transgenic mice proposed that human apoA-II could inhibit the action of hepatic lipase (14), a conclusion...
corroborated by other studies (13). Any possible interaction between apoA-II and hepatic lipase action must be considered unproven at this point.

Even in the case of a greater susceptibility of apoA-II-containing particles to hepatic lipase action, that should not, in itself, cause a marked reduction in the amount of mouse apoA-I or apoA-II in these particles. An excess of human apoA-II can displace apoA-I from HDL from several species (including humans (15, 17)); it can also displace human apoA-I in artificial vesicles in vitro (55). We speculate that the human apoA-II physically displaced the mouse apoA-I from these HDL particles. The displaced mouse apoA-I would be metabolized rapidly, being cleared mainly by the kidney (56–58). We postulate an increased catabolism instead of decreased synthesis of the mouse apoA-I protein as the cause of the reduction in mouse apoA-I in these animals. This hypothesis is supported by the observation that the level of mouse apoA-I mRNA showed only minor differences between transgenic and nontransgenic animals (Fig. 1D).

The cause of the almost complete disappearance of mouse apoA-II in the high expressor line 11.1 is not known. The mechanism could be similar to that proposed for the decrease of mouse apoA-I, i.e. displacement by human apoA-II. However, to our knowledge, there is no evidence that human apoA-II displaces mouse apoA-II from lipoprotein surfaces, although it is not an unreasonable hypothesis. Interestingly, a similar deficiency of mouse apoA-II in human apoA-II transgenic mice was described, and the mechanism was found to be mediated at a post-transcriptional level (5).

The metabolic effects, if any, of mouse apoA-II deficiency are unclear. In contrast, the displacement of apoA-I by human apoA-II has been shown to sharply decrease LCAT activity (59). Unlike apoA-I, apoA-II lipoproteins cannot serve as substrates for LCAT (60, 61) even though they can bind LCAT (62). Moreover, mice genetically modified to be deficient in apoA-II are also LCAT-deficient (63). Interestingly, compared with controls, mice heterozygous for apoA-I deficiency have similar LCAT activity toward exogenous substrates but only half their endogenous LCAT activity (63). These findings are very similar to those for 11.1 mice (Fig. 5). Thus, it is reasonable to conclude that the low apoA-I concentration of 11.1 mice results in its low endogenous LCAT activity and in an increased proportion of HDL free cholesterol. On the other hand, the much higher LCAT exogenous activity found in 11.1 mice is consistent with the idea that LCAT can associate with apoA-II-containing HDL particles.

The lipoprotein phenotype of 11.1 mice is similar in certain respects (increased free cholesterol in plasma and HDL and hypertriglyceridemia that could be caused by a low LCAT activity) to that of fish eye disease. This disease is characterized by partial LCAT deficiency caused usually by LCAT gene mutations (64, 65) and rarely by apoA-I mutations (66). It is noteworthy that, as in 11.1 mice, a significant improvement of the HDL deficiency also occurs in patients with LCAT deficiency fed a high fat diet (67). However, the 11.1 transgenic mouse HDL did not show the rouleau formation characteristic of the accumulation of discoidal particles in fish eye disease HDL (45). It may be that the concentration of 11.1 HDL on the mesh grid is below the threshold necessary for forming rouleaux, or alternatively, apoA-II-containing particles may have lower tendency to form discs than apoA-I-containing particles (68).

The lipoprotein changes observed in the human apoA-II transgenic mice on a regular chow diet in this study are much more profound than those reported by Schultz et al. (24), who noted mainly the appearance of small HDL particles. The reasons for these differences are unclear, although the substantially higher human apoA-II expressed in this study (about one third higher than the highest overexpressor lines in the previous study) could be a factor. It is noteworthy that the analysis of the progeny of the same human apoA-II transgenic mice generated by Schultz et al. (24) demonstrated lower cholesterol and HDLc concentrations and higher triglycerides in transgenic mice than controls (14). Moreover, a preliminary communication in abstract form from another group also reported the existence of hypocholesterolemia and possible LCAT impairment in human apoA-II transgenic mice (69). Further studies on these genetically modified animals should allow a better understanding of the role of human apoA-II in lipoprotein metabolism and atherosclerosis.

While this manuscript was in preparation, Francone et al. (70) presented evidence that transgenic mice expressing human LCAT showed preference for HDL particles that contain only apoA-I, an observation that complements the findings in this study.

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