Characterization of apoM in normal and genetically modified mice

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Abstract A novel human apolipoprotein [apolipoprotein M (apoM)] was recently described and demonstrated to be a lipocalin. We have now examined apoM in wild-type mice and mice with genetically altered lipoprotein metabolism. Liver and kidney showed high mRNA expression, whereas spleen, heart, brain, and testis demonstrated low expression. ApoM gene expression was initiated on embryonic day 10. Western blot analysis of plasma suggested that mouse apoM, like its human counterpart, is secreted with a retained signal peptide, but unlike human apoM it is not glycosylated. Gel filtration of plasma showed apoM to be associated with HDL-sized particles in wild-type and apoA-I-deficient mice and with HDL- and LDL-sized particles in LDL receptor-deficient mice, whereas apoM was mainly found in VLDL-sized particles in high-fat, high-cholesterol-fed apoE-deficient mice. The plasma concentration of apoM was similar in wild-type, LDL receptor-deficient, and apoE-deficient mice but was reduced to 33% in apoA-I-deficient compared with wild-type mice ($P = 0.007$). These data suggest that apoM mainly associates with HDL in normal mice but also with the pathologically increased lipoprotein fraction in genetically modified mice. The substantially decreased apoM levels in apoA-I-deficient mice suggest a connection between apoM and apoA-I metabolism.—Faber, K., O. Axler, B. Dahlbäck, and L. B. Nielsen. **Characterization of apoM in normal and genetically modified mice. *J. Lipid Res.* 2004.** 45: **1272–1278.**

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Atherosclerosis is the most common cause of mortality and morbidity in affluent societies. It is firmly established that high plasma concentrations of HDL protect against atherosclerosis. Nevertheless, the antiatherogenic mechanisms associated with HDL are not completely understood. Apolipoprotein A-I (apoA-I) is the predominant apolipoprotein in HDL. ApoA-I interaction with ABCA1 mediates cholesterol efflux from peripheral cells (1). Unloading of cholesterol from arterial wall macrophages has been regarded an important mechanism for the antiatherogenic effect of apoA-I (2, 3). Paraoxonase 1 (PON-1), which is associated with an HDL subpopulation in plasma, protects LDL from oxidation (4–7). HDL also has antioxidative effects that are independent from PON-1 (8). Moreover, HDL has immunomodulatory, vasoactive, and anticoagulant effects, although the mechanisms remain to be determined (9).

ApoM is a novel human apolipoprotein of 188 amino acids that is mainly associated with HDL (10). Structural analysis predicts that human apoM belongs to the lipocalin protein family (11). Lipocalins share a common tertiary structure with an eight stranded antiparallel β-barrel surrounding a hydrophobic ligand binding interior (12–14). Lipocalins are extracellular proteins; some interact with specific cell surface receptors and/or are involved in the formation of macromolecular complexes (14). Several lipocalins are antioxidants or modulators of the immune system (12, 14, 15). However, for most lipocalins, including apoM, the biological role has not been determined (12).

Human apoM is secreted with its N-terminal signal peptide being retained in the mature protein (10). This is explained by the lack of a signal peptidase cleavage site after the signal peptide sequence. ApoM shares this unusual feature with two other HDL apolipoproteins, PON-1 and haptoglobin-related protein (16, 17). In the case of PON-1, the hydrophobic signal peptide has been shown to provide an anchor for PON-1 in the HDL particle (11, 16). PON-1 and haptoglobin-related protein are exclusively found in HDL, and HDL deficiency is accompanied by very low PON-1 plasma concentrations in both humans and mice (16, 18). However, although apoM is predominantly found in HDL in human plasma, apoM was initially isolated from chylomicrons, and the apparent content of apoM in chylomicrons was increased after ingestion of a fat-rich meal (10).

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In this study, we demonstrate the presence of an apoM in murine plasma. We have characterized the expression of apoM mRNA during development and in adult mice and the apoM distribution among plasma lipoproteins in wild-type mice and mice with genetically modified plasma lipoprotein distribution.

MATERIALS AND METHODS

Animals

Wild-type mice (C57BL/6) and apoE-deficient mice (C57BL/6JbomApoe^{tm1Unc}) were obtained from M&B (Ry, Denmark) and housed at the Panum Institute, University of Copenhagen, Denmark. ApoA-I-deficient mice (B6.129P2-ApoA-I^{tm1Unc}) were obtained from Jackson Laboratories and housed at the Department of Experimental Pathology, University of Lund, Malmö, Sweden. Blood samples for plasma lipid and lipoprotein analysis were drawn into tubes with Na_2EDTA and centrifuged for 10 min at ~4,000 *g* at 4°C. Plasma was stored at −20°C or −80°C until analysis. The experimental protocols were approved by the local ethics committee in Copenhagen, Denmark, or Lund, Sweden. Plasma from LDL receptor-deficient mice was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). Plasma from hepatic nuclear factor (HNF)-1α-deficent mice and littermate controls was provided my Dr. F. J. Gonzales (National Institutes of Health, Bethesda, MD). Pools of embryonic RNA were from BALB/C mice and were provided by Dr. F. C. Nielsen (Rigshospitalet, Copenhagen, Denmark) (19).

mRNA purification and cDNA amplification

Frozen biopsies of organs from wild-type and apoA-I-deficient mice were homogenized with a Polytron PT 1200CL (Buch and Holm, Herlev, Denmark) in TriZol reagent (Life Technologies, Taastrup, Denmark). Total RNA was isolated according to the manufacturer's manual and suspended in RNase-free water. The RNA concentration was calculated from the absorbance at 260 nm. The RNA integrity was ensured by 1% agarose gel electrophoresis or with an Agilent 2100 Bioanalyzer (Agilent Technologies Denmark A/S, Naerum, Denmark) using a RNA 6000 Nano Assay Kit (Agilent Technologies). First-strand cDNA was synthesized from 1 μg of total RNA with Moloney murine leukemia virus reverse transcriptase (40 units; Roche A/S, Hvidovre, Denmark) and random hexamer primers in 10 μl reactions. Sense and antisense primers for amplification of mouse apoM mRNA were 5′-CCT GGG CCT GTG GTA CTT TA-3′ and 5′-CCA TGT TTC CTT TCC CTT CA-3′ (Sigma-Genosys, Pampisford, UK), the sequences being derived from the available cDNA sequence (accession number AF207820). Primers for β-actin amplification have been reported elsewhere (20). DNA sequencing and agarose gel electrophoresis of apoM and β-actin PCR products confirmed the specificity of the RT-PCR.

mRNA quantification with a real-time PCR assay

Quantitative real-time PCR analysis of apoM mRNA expression was performed with a LightCycler (software version 3.39) and the DNA master SYBR GREEN kit (Roche A/S). Each PCR sample (20 μl) contained 2 μl of SYBR GREEN I mix, 2–2.5 mM $MgCl_2$, 10 pmol of each primer, cDNA synthesized from 20 ng of total RNA, and PCR-grade water. The amount of mRNA transcript in each tissue sample was determined from the time point of the log-linear increase in amplified DNA during the PCR using the fit point option of the LightCycler. To determine the relation between the time point of the log-linear increase of the fluorescence signal and the relative concentration of a mRNA transcript in a sample, a dilution series of a pool of cDNA from mouse liver was analyzed in each run.

Anti-mouse apoM antibodies

A mouse apoM cDNA (National Center for Biotechnology Information GenBank accession number AF207820) encoding the full-length 190 amino acid residue mouse apoM protein was used for the cloning of a truncated cDNA into an expression vector (pET-30 Xa/LIC; Novagen, Sigma-Aldrich, Stockholm, Sweden) and expressed in *Escherichia coli* [strain BL21 (DE3); Stratagene]. The expressed truncated apoM included a His tag, a S tag, and amino acid residues 22–190 of mouse apoM (signal peptide was excluded) and was purified from inclusion bodies and refolded (K. Faber and B. Dahlbäck, unpublished data). The purified truncated recombinant apoM was mixed with complete Freund's adjuvant and used to immunize two female rabbits. The Ig fraction of the apoM antiserum was purified on a protein G column (5 ml; Hi-trap, Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's protocol. Affinity-purified apoM antibodies were further affinity purified on a column coupled with 8.5 mg of recombinant mouse apoM (1 ml; Hi-trap, Amersham Pharmacia Biotech). The apoM column was equilibrated with 50 mM Tris-HCl, 0.15 M NaCl, pH 7.5, and the bound antibodies were eluted with 0.1 M glycine HCl, pH 2.7, and collected in tubes containing one-tenth volume of 1 M Tris-HCl, pH 9.0, to neutralize the pH. The concentration of purified antibodies was determined from the absorbance at 280 nm using an extinction coefficient (1%, 1 cm) of 12.5. The antibodies were stored at 4°C after the addition of 0.02% NaN_3. A similar truncated human apoM was constructed in the pET-30 Xa/LIC vector, and the protein was expressed in *E. coli*, isolated, and refolded as described for mouse apoM. This apoM was used to immunize mice, and three monoclonal antibodies were created with standard techniques. None of these monoclonal antibodies reacted with mouse apoM.

Western blotting

Plasma, gel filtration fractions, or PBS homogenates of liver or kidney were separated on 12% SDS-PAGE gels, and the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Gelman, Lund, Sweden) using semidry electroblotting. The membrane was quenched for 1 h in 50 mM Tris-HCl, 150 mM NaCl, pH 8.0, containing 0.5% (w/v) Tween 20 and 3% fish gelatin (Norland Products, Inc., Cranbury, NY) followed by incubation for 1 h at room temperature with anti-mouse apoM antibodies (30 μg/ml), polyclonal rabbit anti-mouse apoA-I antibodies (1:2,560; BioSite, Täby, Sweden), monoclonal mouse anti-mouse apoB-100 antibodies (1:500; kindly provided by Dr. S. Young, Gladstone Institute of Cardiovascular Disease, San Francisco, CA), or rabbit anti-mouse apoA-II antibodies (Biodesign International) in the same buffer. The membranes were then washed and incubated with swine anti-rabbit or rabbit anti-mouse antibodies coupled with horseradish peroxidase (1:10,000; DAKO, Copenhagen, Denmark) (21). After another round of washes, the membranes were developed with 5-bromo-4-chloro-3-indolylphosphate-*p*-toluidine salt and *p*-nitroblue tetrazolium (Sigma-Aldrich, Stockholm, Sweden). All incubations were done at room temperature.

Endoglycosidase digestion

ApoM-containing HDL was purified from human plasma using a monoclonal antibody against apoM that we recently developed (our unpublished data). This material (10.5 μl with an optical density at 280 nm of 0.07) was mixed with 60 μl of 0.1% sodium dodecyl sulfate containing 0.1 M 2-mercaptoethanol and denatured for 5 min at 90°C. After cooling, 18 μl of 0.5 M so-

dium citrate, pH 5.5, 1.5 μl of water, and peptide:N-glycanase [15 μl of 0.12 mg/ml, kindly provided by Dr. J. Stenflo, Department of Laboratory Medicine, University Hospital (Malmö, Sweden)] were added. After incubation at 37°C overnight, the samples were analyzed by Western blotting using a 12% SDS-PAGE run under reducing conditions. For immunochemical detection, a mixture of polyclonal anti-mouse and anti-human apoM antibody was used to visualize both human and mouse apoM.

Quantification of plasma apoM

A dilution series of a pool of wild-type mouse plasma (corresponding to 0, 0.1, 0.25, 0.5, and 1 μl of plasma) and plasma samples from individual mice (0.5 μl) were analyzed on the same gel by 12% SDS-PAGE. After transfer to a Hybond-P 0.45 μm PVDF membrane (Amersham Biosciences, Copenhagen, Denmark), the blots were incubated at 4°C with anti-apoM antibodies for 14–18 h and a horseradish peroxidase-conjugated goat anti-rabbit antibody for 1 h. Antibody binding was detected with a chemiluminescence reader (Fujifilm LAS-1000 Intelligent Dark Box II, Fujifilms, Trorod, Denmark) after incubation of the blot with SuperSignal West Pico Chemiluminescent Substrate (Pierce Chemicals, Copenhagen, Denmark). Chemiluminescence readings were corrected for background before calculations using the Image Reader LAS-1000 Pro V2.5 ImageGauge 4.0 program (Fujifilms).

Gel filtration of plasma

Lipoproteins in mouse plasma were separated by gel permeation chromatography on a Superose 6 HR 10/30 fast-protein liquid chromatography column (Amersham Pharmacia Biotech). Samples were pooled from 4–11 mice and passed through 0.22 μm filters before loading 0.5 ml on the column. The column was run at room temperature with phosphate-buffered saline, pH 7.4, at a flow rate of 0.1 ml/min. Fractions of 0.5 ml were collected and stored at −20°C until protein and lipid analyses.

Quantification of plasma lipids

Cholesterol concentrations were determined enzymatically as described (20).

RESULTS

Demonstration of apoM in mouse plasma

Murine apoM contains 190 amino acid residues and shares 80% identical residues with the human protein. Analysis of the mouse apoM amino acid sequence with the SignalP signal peptide prediction (22) (www.cbs.dtu.dk/services/SignalP-2.0) suggested that the mouse apoM contains a 21 amino acid signal peptide. As in the case of human apoM, the program predicts low likelihood of signal peptide cleavage, indicating that mouse apoM, like its human counterpart, is secreted without prior cleavage of its N-terminal signal peptide. To examine mouse apoM in vitro, we expressed a truncated murine apoM in *E. coli* and generated an anti-apoM antiserum in rabbits. The affinity-purified antibodies were used in Western blot analysis of mouse plasma (**Fig. 1**). Mouse apoM appeared as a single band on Western blots under both reducing and nonreducing conditions, demonstrating that apoM does not form covalent complexes with other proteins. Human plasma apoM yielded several bands under both reducing and nonreducing conditions (Fig. 1). The major band of human apoM demonstrated an apparent molecular weight that was slightly higher than that of mouse apoM, whereas the migration of the minor human apoM band was similar to that of murine apoM, particularly under reducing conditions. Human apoM has an N-linked carbohydrate side chain that is missing in the murine counterpart (11). To investigate whether the observed molecular weight difference between human and murine apoM was attributable to the carbohydrate side chain, human apoM was subjected to deglycosylation. Deglycosylated human apoM migrated as a single band with an apparent molecular weight similar to both that of the lower band of un-

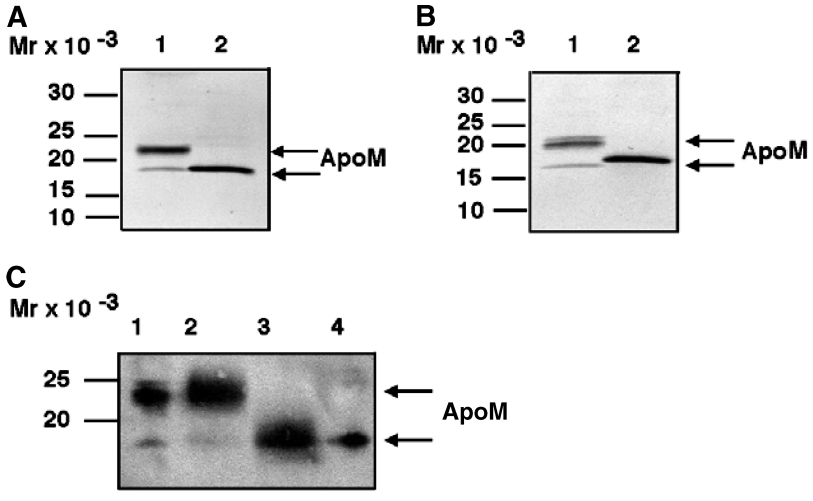

Fig. 1. Western blot analysis of apolipoprotein M (apoM) in mouse and human plasma. Human and mouse plasma were separated by 12% SDS-PAGE under reducing (A) and nonreducing (B) conditions (lane 1, 1 μl of human EDTA-plasma; lane 2, 1 μl of mouse EDTA-plasma). C: Human plasma (lane 1), partial purified human apoM (lane 2), partially purified human apoM after peptide:N-glycanase treatment (lane 3), and mouse plasma (lane 4) were separated under reducing conditions. The proteins were transferred from the gel to a polyvinylidene difluoride membrane by a semidry blotting technique, and apoM was detected by a mixture of polyclonal anti-mouse and anti-human apoM antibodies (30 μg/ml). Mr, molecular weight.

treated human apoM and that of murine apoM. The similar molecular weight of mouse apoM and deglycosylated human apoM suggests that the circulating murine apoM, like its human counterpart, contains a retained signal peptide.

ApoM mRNA expression in adult mice and mouse embryos

In humans, apoM is expressed primarily in liver and kidney (10). To assess the relative levels of expression in those two tissues in mice and to assess other putative sites of apoM mRNA expression, we quantified apoM mRNA expression with a real-time PCR assay, which allowed the measurement of apoM mRNA expression at levels less than 1/1,000th of that in the liver. The tissue distribution of apoM mRNA demonstrated high-level expression in liver and kidney and detectable but low-level expression (less than 5% of that in the liver) in heart, brain, spleen, and testis (**Fig. 2A**). ApoM mRNA was undetectable in muscle, duodenum, and ovaries (Fig. 2A).

To determine whether apoM was expressed during embryonic life, we analyzed 8–17 day old mouse embryos. ApoM mRNA expression increased at day 10 and was detectable throughout embryonic life (Fig. 2B).

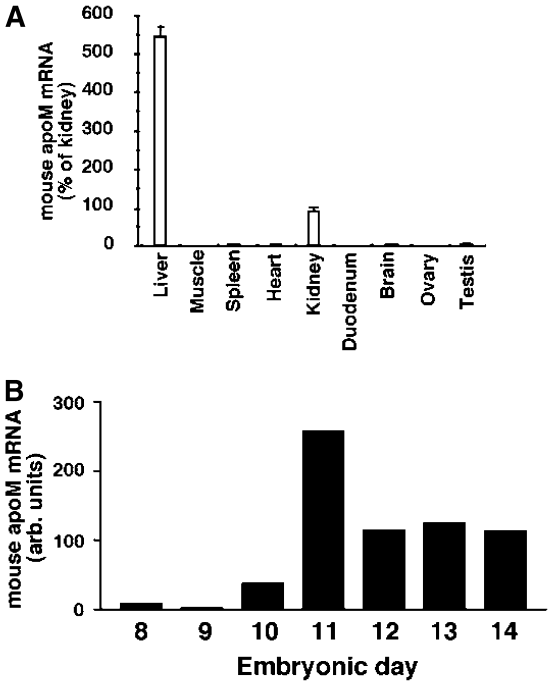

Fig. 2. ApoM mRNA determination. A: Tissue distribution of apoM mRNA in adult mice. ApoM gene expression was measured in nine different tissues from one female and two male mice with a sensitive real-time PCR assay. High-level apoM expression was found in liver and kidney. Mean values for the different organs are expressed as percentages of the kidney value. Normalization of the values for apoM mRNA expression to those of β-actin mRNA gave the same results (not shown). B: ApoM mRNA expression in mouse embryos. ApoM mRNA expression was measured by a sensitive real-time PCR assay. Studies of mRNA from day 8 to day 17 embryos revealed activation of apoM gene expression from ~day 10. Arb. units, arbitrary units. Error bars represent SEM.

Plasma lipid and apolipoprotein profiles

Mean plasma cholesterol concentrations were ~1 mmol/l in wild-type mice, 0.6 mM in apoA-I-deficient mice, 6.5 mmol/l in LDL receptor-deficient mice, 14 mmol/l in chow-fed apoE-deficient mice, and 41 mmol/l in apoE-deficient mice fed a high-fat and high-cholesterol diet. To analyze the distribution of apoM among plasma lipoproteins, we used gel filtration chromatography and Western blot analysis. As expected, in wild-type mouse plasma, cholesterol was predominantly found in HDL and apoM was recovered in the same fractions as HDL, suggesting that murine apoM, like its human counterpart, is associated with the HDL-sized particles (**Fig. 3A**). There was no difference in the size distributions of apoM- and apoA-I-containing particles according to Western blotting. In LDL receptor-deficient mice, the HDL cholesterol level was similar to that in wild-type mice, but the major fraction of the plasma cholesterol in these mice was in LDL (Fig. 3B). Remarkably, apoM was abundant in LDL-sized particles of the LDL receptor-deficient mice, whereas apoA-I was exclusively found in HDL particles (Fig. 3B). ApoE-deficient mice had increased VLDL cholesterol concentrations when fed a normal mouse chow (Fig. 3C). This phenotype was accentuated when the diet was changed to a high-fat/high-cholesterol-enriched diet (Fig. 3D). In chow-fed apoE-deficient mice, apoM was mainly present in the HDL fractions but was also clearly detectable in LDL- and VLDL-sized particles (Fig. 3C). In high-fat- and high-cholesterol-fed apoE-deficient mice, apoM was most prominent in the VLDL fraction and only traces of apoM were found in HDL-sized particles (Fig. 3D). Additional immunoblotting with an antibody against mouse apoB-100 confirmed the presence of apoB in the cholesterol containing VLDL and LDL particles of apoE- and LDL receptor-deficient mice (data not shown).

ApoA-I-deficient mice had a pronounced decrease in HDL cholesterol compared with wild-type mice (Fig. 3E). ApoM was predominantly recovered in HDL-sized particles (Fig. 3E). Additional Western blotting with an anti-apoA-II antibody revealed that the gel filtration distributions of apoM and apoA-II were similar in apoA-I-deficient mice (data not shown).

Plasma concentrations of apoM

The plasma concentrations of apoM in the different types of mice were determined with a Western blotting technique using a chemiluminescence detection system. Plasma apoM was similar in wild-type mice and high-fat- and high-cholesterol-fed apoE-deficient mice (**Fig. 4A**), despite the pronounced difference in the plasma cholesterol concentrations. Normal plasma apoM concentrations were also found in chow-fed apoE-deficient mice and in LDL receptor-deficient mice. In contrast, plasma from apoA-I-deficient mice had significantly lower apoM concentrations than did wild-type mice (Fig. 4A). The apoM concentration in apoA-I-deficient mice was on average 33% of that in wild-type mice (Fig. 4C). A recent study suggested that deficiency of HNF-1α causes low plasma apoM as a result of decreased mRNA expression (23).

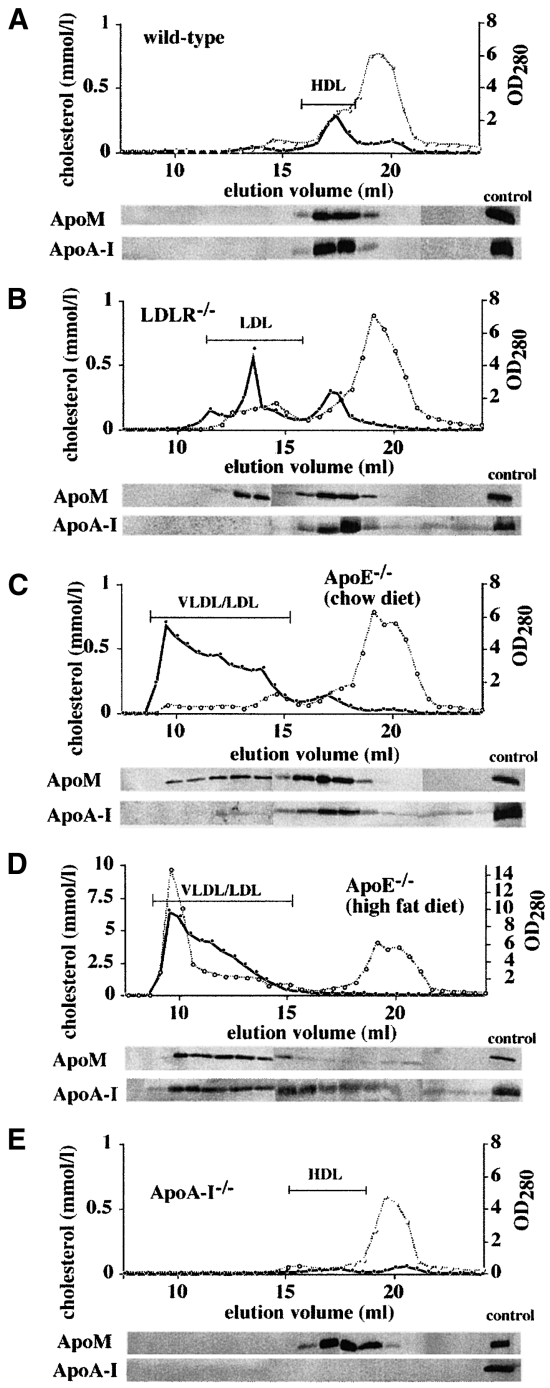

Fig. 3. Size distribution of apoM in plasma of genetically modified mice. Pooled plasma samples (500 μl) from wild-type (A), LDL receptor (LDLR)-deficient (B), apoE-deficient [fed a chow diet (C) or a high-fat/high-cholesterol diet (D)], and apoA-I-deficient (E) mice were subjected to fast-phase liquid chromatography on a Superose 6 HR 10/30 column. The cholesterol content was determined enzymatically. After gel filtration, fractions were analyzed by western blotting with antibodies against mouse apoM or apoA-I. Optical density at 280 nm (OD_{280}) measurements (open symbols and dotted lines) were used to identify the albumin peaks. Cholesterol (closed symbols and solid lines) was measured as well. Wild-type plasma was used as a positive control.

Western blotting of plasma from HNF-1α-deficient mice confirmed this result, as apoM was virtually absent in HNF-1α-deficent mice (Fig. 4D). To determine whether the lower apoM plasma concentration in apoA-I-deficient mice could be attributable to decreased mRNA expression, we analyzed liver and kidney apoM mRNA. There was no statistically significant difference in apoM mRNA expression between wild-type and apoA-I-deficient mice (**Fig. 5**), although there was a trend for liver apoM mRNA to be slightly increased in apoA-I-deficient compared with wild-type mice. We could not detect apoM on Western blots of liver or kidney protein extracts or urine from apoA-I-deficient mice (data not shown).

DISCUSSION

This study documents the presence of a new murine plasma apolipoprotein, apoM, which, like its human counterpart, is normally mainly associated with HDL. Previous studies of human apoM have demonstrated that it is secreted without cleavage of its NH_2-terminal signal peptide (10). Analysis using prediction programs for signal peptidase cleavage and analysis of apoM in mouse plasma with Western blotting suggested that mouse apoM shares this unusual feature of a secreted protein.

Tissue mRNA studies suggested that mouse apoM is produced primarily in liver and kidney. This expression pattern is similar to what was previously observed in a multiple-tissue Northern blot with human mRNA (10). The kidney expresses other apolipoprotein genes, including apoB and apoA-I. However, in rodents and mammals, the kidney expression of apoB and apoA-I is much smaller than the liver expression (24). In contrast, kidney apoM expression was almost ~20% of that in the liver. ApoM is structurally similar to mouse major urinary protein-1, which is secreted into the urine (10). However, we could not detect apoM in either mouse or rat urine, even after concentration of the urine samples (our unpublished data). Thus, the role of kidney-derived apoM remains to be determined.

Like apoM, PON-1 and haptoglobin-related protein circulate in plasma with uncleaved signal peptides that anchor the proteins onto lipoproteins (16). Because PON-1 and haptoglobin-related protein are not associated with lipoproteins other than HDL, we investigated whether apoM would be associated primarily with HDL even in the setting of increased LDL and VLDL concentrations. We compared the lipoprotein distribution and total plasma levels of apoM in wild-type and genetically modified mice. The total concentration of apoM in plasma was similar in wild-type mice and mice with genetically induced hyperlipoproteinemia, despite a striking ~40-fold difference in the total plasma cholesterol concentration between wild-type and high-fat/high-cholesterol-fed apoE-deficient mice. Although apoM was predominantly found in HDL in wild-type mice (as in humans), it tended to associate with the most prominent plasma lipoprotein fraction (i.e., LDL in LDL receptor-deficient mice and VLDL in apoE-

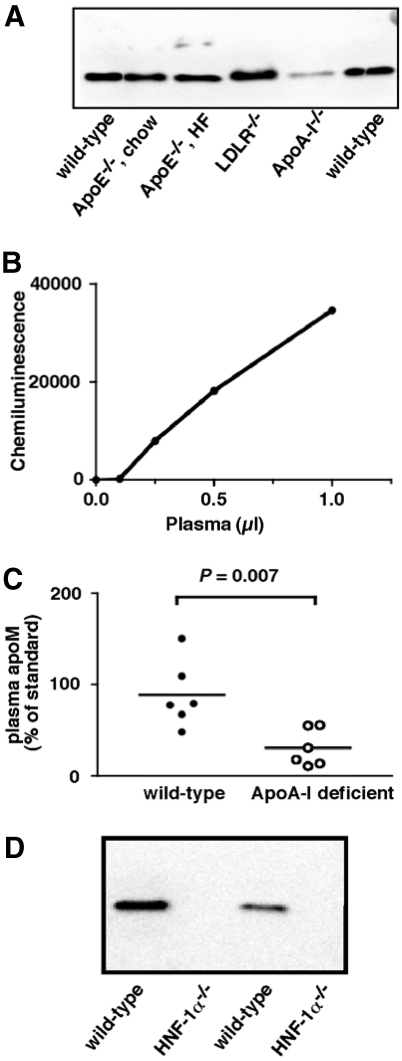

Fig. 4. Quantification of plasma apoM in genetically modified mice. A: Western blot of pools of plasma from wild-type mice (n = 5), apoE-deficient mice fed a normal chow (n = 6), apoE-deficient mice fed a high-fat/high-cholesterol diet (n = 11), LDL receptor (LDLR)-deficient mice (n = 8), and apoA-I-deficient mice (n = 6). Plasma (0.5 μl) was treated with DTT and loaded onto a 12% SDS-PAGE gel. The blot was incubated successively with a polyclonal rabbit anti-mouse apoM antibody, a horseradish peroxidase-conjugated secondary antibody, and SuperSignal (Pierce). ApoM was visualized by chemiluminescence. B: Association between chemiluminescence signal and plasma apoM concentration in a Western blot-based assay. Aliquots (0.1–1 μl) from a pool of wild-type mouse plasma were used in Western blotting. Chemiluminescence was determined with a Fujifilm chemiluminometer. C: Plasma apoM concentrations in wild-type and apoA-I-deficient mice were determined by a Western blot-based assay. Alternating plasma samples from wild-type and apoA-I-deficient mice and a dilution series of wild-type mouse plasma were analyzed on the same gels. Each data point indicates a value from an individual mouse. Lines indicate mean values. The P value is derived from Student's t-test. D: Western blot of 0.5 μl of plasma from two wild-type and two hepatic nuclear factor (HNF)-1α-deficient mice. The experiment was performed as in A.

deficient mice) (10). Notably, apoM was found in apoB-containing LDL-sized particles totally devoid of apoA-I in the plasma of LDL receptor-deficient mice.

Interestingly, in high fat/high cholesterol-fed apoE-deficient mice, apoM was predominantly recovered in intestinally derived VLDL even though apoM mRNA was undetectable in the intestine. This suggests that secretion of apoM from the liver is independent of apoB-containing lipoproteins and that apoM is transferred to these particles in plasma. In contrast, apoA-I-deficient mice had dramatically decreased apoM levels (~33% of those of wild-type mice), suggesting a connection between apoM and apoA-I metabolism in the normal mouse. Although decreased apoM gene expression causes low apoM plasma levels in HNF-1α-deficient mice (23), apoM mRNA expression levels were similar in wild-type and apoA-I-deficient mice. Thus, the lower apoM levels presumably reflect increased clearance. Interestingly, gel filtration studies suggested that apoM in apoA-I-deficient mice is mainly associated with apoA-I-free and apoA-II- and cholesterol-containing HDL-sized particles and not with apoB-containing lipoproteins. Altogether, the present results might indicate that the plasma lipoprotein association of apoM is dependent on lipid interactions that occur after its secretion. Thus, it is conceivable that the decrease of apoM in apoA-I knockout mice simply reflects the presence of fewer HDL particles to interact with. However, it should be kept in mind that normally the plasma concentration of apoM is much lower (~20 mg/l) compared with that of apoA-I (~1 g/l) and that only a subfraction of the HDL particles contain apoM. Although our studies to date have elucidated some basic aspects of apoM biology, the physiological role of apoM remains enigmatic. Like several other apolipoproteins, apoM appeared to be expressed during murine embryonic life. In the mouse embryos, apoM expression was activated from approximately day 10 and throughout embryonic life. DNA sequencing of the apoM gene in more than 200 human subjects (A. Hillarp and B. Dahlbäck, unpublished data) revealed no mutations that would result in the production of a truncated or defect apoM. This may indicate an important physiological role of apoM either before or after birth. The present demonstration of apoM expres-

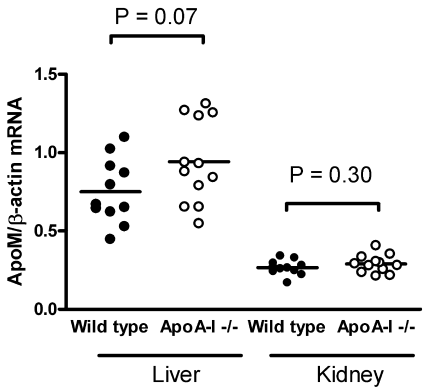

Fig. 5. ApoM mRNA expression in liver and kidney of apoA-I-deficient and wild-type mice. mRNA was quantified with real-time PCR. All data were normalized with the β-actin mRNA content in the same sample. Each data point indicates a value from an individual mouse. Lines indicate mean values. The P values are derived from Student's t-test.

sion in the mouse has paved the way for generating new mouse models with altered apoM expression. Such models should be helpful in providing insights into the physiological role of apoM.

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