Persistence of high density lipoprotein particles in obese mice lacking apolipoprotein A-I

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Abstract **Obese mice without leptin (ob/ob) or the leptin receptor (db/db) have increased plasma HDL levels and accumulate a unique lipoprotein referred to as LDL/HDL1. To determine the role of apolipoprotein A-I (apoA-I) in the formation and accumulation of LDL/HDL1, both ob/ob and db/db mice were crossed onto an apoA-I-deficient (apoA-I$^{-/-}$) background. Even though the obese apoA-I$^{-/-}$ mice had an expected dramatic decrease in HDL levels, the LDL/HDL1 particle persisted. The cholesterol in this lipoprotein range was associated with both α- and β-migrating particles, confirming the presence of small LDLs and large HDLs. Moreover, in the obese apoA-I$^{-/-}$ mice, LDL particles were smaller and HDLs were more negatively charged and enriched in apoE compared with controls. This LDL/HDL1 particle was rapidly remodeled to the size of normal HDL after injection into C57BL/6 mice, but it was not catabolized in obese apoA-I$^{-/-}$ mice even though plasma hepatic lipase (HL) activity was increased significantly. The finding of decreased hepatic scavenger receptor class B type I (SR-BI) protein levels may explain the persistence of LDL/HDL1 in obese apoA-I$^{-/-}$ mice. Our studies suggest that the maturation and removal of large HDLs depends on the integrity of a functional axis of apoA-I, HL, and SR-BI. Moreover, the presence of large HDLs without apoA-I provides evidence for an apoA-I-independent pathway of cholesterol efflux, possibly sustained by apoE.**—Gruen, M. L., M. R. Plummer, W. Zhang, K. A. Posey, M. F. Linton, S. Fazio, and A. H. Hasty. **Persistence of high density lipoprotein particles in obese mice lacking apolipoprotein A-I.** ***J. Lipid Res.*** **2005.** 46: **2007–2014.**

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Unlike apolipoprotein B (apoB)-containing lipoproteins, which are formed by hepatocytes and enterocytes, HDL biogenesis is quite complex and involves mostly extracellular events (1, 2). In the intestine, apoA-I is secreted in association with chylomicrons. Once in the circulation, apoA-I is transferred to HDL in exchange for apoE and the C apolipoproteins. In the liver, apoA-I is secreted as part of nascent cholesterol-poor HDL particles. Additionally, HDL can be formed with excess surface material from remnant lipoproteins during hydrolysis. Circulating HDL is acted upon by many different enzymes, including HL and LCAT, leading to a complex catabolic fate, as its cholesteryl esters can be transferred to triglyceride (TG)-rich lipoproteins via cholesteryl ester transfer protein or be selectively taken up by the hepatic HDL receptor, scavenger receptor class B type I (SR-BI) (3), without internalization of the HDL particle. However, holoparticle uptake of large apoE-enriched HDLs has also been reported (4).

Unlike their human counterparts, mouse models of obesity, such as the leptin-deficient and leptin receptor-deficient mice (ob/ob and db/db, respectively), have increased levels of HDL without an increase in VLDL levels (5, 6). This increase in HDL makes these animals particularly resistant to the development of atherosclerotic lesions (7). Recent work by Tall and colleagues (6, 8) has shown that the increase in HDL is attributable to both typical HDLs and larger HDLs (LDL/HDL1), and is caused by decreased hepatic HDL uptake in these animals. This LDL/HDL1 particle is not unique to ob/ob and db/db mice, however. Stoffel and colleagues (9) recently showed that mice lacking hepatic nuclear factor-1α (HNF-1α) also display the presence of HDL1 particles. More recent studies by this same group elegantly demonstrated that the absence of apoM leads to the formation and accumulation of HDL1 (10). In addition, LCAT trans-

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Abbreviations: apoA-I, apolipoprotein A-I; db/db, leptin receptor-deficient; ERRα, estrogen-related receptor-α; FPLC, fast-protein liquid chromatography; HNF-1α, hepatic nuclear factor-1α; LDLR, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; ob/ob, leptin-deficient; PPARα, peroxisome proliferator-activated receptor α; SR-BI, scavenger receptor class B type I; TC, total cholesterol; TG, triglyceride.

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genic and SR-BI$^{-/-}$ mice also display HDL1-sized particles (11, 12). The HDL1 particles in many of these mouse models contain increased levels of apoE and apoA-I.

In the current study, we sought to delineate the role of apoA-I in the accumulation of HDL1. To this end, we crossed both ob/ob and db/db mice onto the apoA-I$^{-/-}$ background. Even though lean apoA-I$^{-/-}$ mice display a nearly complete absence of HDL (13), obese apoA-I$^{-/-}$ mice had only a slight reduction in total plasma cholesterol levels compared with C57BL/6 controls. Interestingly, the cholesterol in these obese apoA-I$^{-/-}$ mice is carried primarily on HDL1 particles, with an additional small fraction on VLDLs. The HDL1 particles of obese apoA-I$^{-/-}$ mice were large, enriched in apoE, and displayed pre-α mobility. Our data also demonstrate that hepatic SR-BI levels are decreased and HL activity is increased in obese apoA-I$^{-/-}$ mice. We postulate that the normal processing and clearance of HDL1 needs a functional axis involving apoA-I, HL, and SR-BI.

EXPERIMENTAL PROCEDURES

Materials

All chemicals were purchased from Sigma-Aldrich unless indicated otherwise. The primary anti-mouse apoE antibody was produced in the Protein and Immunology Core of the Clinical Research Unit at Vanderbilt University Medical Center. The primary antibody to rat apoB was a gift from Dr. Larry Swift (Vanderbilt University). Primary antibody for mouse apoA-I was purchased from Biodesign International. Antibody to mouse SR-BI was purchased from Novus Biologicals. Antibody to apoA-IV was a kind gift from Dr. Patrick Tso (University of Cincinnati).

Mouse models

C57BL/6, apoA-I$^{-/-}$, db/+, and ob/+ mice were originally purchased from Jackson Laboratories. All mice were on the C57BL/6 background. Obese apoA-I$^{-/-}$ mice were produced by crossing the animals with one mutated leptin or leptin receptor allele (ob/+ or db/+, respectively) with apoA-I$^{-/-}$ mice. The resulting pups heterozygous for the deletion of both apoA-I and leptin or leptin receptor were further crossed to generate mice of all of the genotypes used in the studies described here. Mice were genotyped by PCR analyses as provided by Jackson Laboratories. To avoid repetition, results are shown from either the ob/ob; apoA-I$^{-/-}$ or the db/db;apoA-I$^{-/-}$ for each experiment, as indicated in the text and figures.

Mice were given free access to food and water and were fasted for 5–6 h before all blood collections. Blood collections were performed by retro-orbital venous plexus puncture. Blood samples were kept on ice, centrifuged at 10,000 rpm for 20 min, divided into separate tubes, and frozen at −80°C for future analyses of insulin, total cholesterol (TC), total plasma TGs, and FFA levels. Animal care and experimental procedures were performed according to the regulations of the Institutional Animal Care and Usage Committee of Vanderbilt University.

Blood and plasma analyses

Whole blood was immediately assayed for glucose levels on a Johnson & Johnson LifeScan glucometer. Insulin measurements were performed by the Hormone Assay Core Laboratory of the Diabetes Research and Training Center at Vanderbilt University Medical Center. Plasma TC and TG levels were measured using enzymatic kits from Raichem according to the manufacturer's instructions. FFA measurements were performed using the NEFA C kit from Wako. Fast-protein liquid chromatography (FPLC) analysis of lipoprotein distribution was performed by separating 100 μl of plasma on a Superose 6 column (Amersham Biosciences). Forty 0.5 ml fractions were collected, and cholesterol contents were assayed in fractions 11–40. VLDL is contained in fractions 15–19, LDL in fractions 20–26, and HDL in fractions 27–33. The LDL/HDL1 particles are contained in fractions 24–29.

Two-dimensional gel electrophoresis

Plasma lipoproteins were electrophoresed on 0.7% agarose gels for the first separation by charge. Individual gel strips were cut out and placed along 3–16% nondenaturing polyacrylamide gels and electrophoresed again for separation by size and charge. Gels were transferred to nitrocellulose membranes and then probed first with antibody to mouse apoE and second with antibody to mouse apoA-I (Biodesign). The secondary antibody for each was ^{125}I-labeled anti-rabbit IgG.

Agarose gel electrophoresis

Four microliter plasma samples were electrophoresed on lipoprotein gels (Titan Gel; Helena Laboratories). Gels were dried and stained with Fat Red 7B according to the manufacturer's instructions. For Western blotting, agarose gels were loaded with 2 μl of a 1:10 dilution of plasma, transferred to nitrocellulose membranes, and probed with antibodies for apoA-I and apoB.

TABLE 1. Metabolic parameters in 2-month-old obese apoA-I$^{-/-}$ mice

Mouse Strain	n	Weight	TC	TG	FFA	Glucose	Insulin
		g	*mg/dl*		*mEq/l*	*mg/dl*	*ng/ml*
C57BL/6	11	19 ± 1	85 ± 3	26 ± 5	0.61 ± 0.08	114 ± 6	0.33 ± 0.04
apoA-I$^{-/-}$	15	20 ± 1	45 ± 6	24 ± 5	0.50 ± 0.07	124 ± 6	0.48 ± 0.08
ob/ob[a]	19	34 ± 1	142 ± 4[b]	58 ± 4	0.54 ± 0.06	151 ± 7	7.3 ± 0.6
ob/ob;apoA-I$^{-/-}$[a]	7	36 ± 2	80 ± 11[c]	51 ± 11	0.87 ± 0.16	135 ± 17	6.2 ± 0.9
db/db[a]	8	33 ± 1	123 ± 6[b]	58 ± 5	0.70 ± 0.15	129 ± 4	8.6 ± 3
db/db;apoA-I$^{-/-}$[a]	15	34 ± 2	71 ± 6[c]	64 ± 5	0.66 ± 0.07	150 ± 12	6.0 ± 1

apoA-I$^{-/-}$, apolipoprotein A-I-deficient; db/db, leptin receptor-deficient; FFA, free fatty acid; ob/ob, leptin-deficient; TC, total cholesterol; TG, triglyceride. Plasma samples from 2-month-old mice were analyzed for TC, TG, FFA, and insulin.

[a] Body weight, plasma TGs, and insulin levels were significantly higher in all obese groups compared with lean groups ($P < 0.05$).

[b] $P < 0.01$ compared with all other groups.

[c] $P < 0.01$ compared with lean apoA-I$^{-/-}$ mice.

Real-time quantitative RT-PCR analysis

RNA was collected from homogenized liver samples using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Livers were collected from three 6 month old male mice, so that results were not complicated by potential age or gender differences. The integrity of RNA was determined both by measurement of the optical density at 260 nm-to-optical density at 280 nm ratio and by electrophoresis on formalin-containing agarose gels. Real-time RT-PCR was performed using Assays-on-Demand primer-probe sets from Applied Biosystems. An internal 18S control for RNA concentration was used for each analysis. Quantification of RNA was performed using the deltaCt method described in the ABI User's Bulletin 2. Assays were performed in duplicate. The average gene expression levels from C57BL/6 livers were used as the baseline for comparison with obese db/db, lean apoA-I$^{-/-}$, and obese db/db;apoA-I$^{-/-}$ samples.

PAGE and Western blotting of FPLC fractions and liver lysates

Liver was collected from at least three mice in each group after perfusion with sterile PBS. Pieces weighing 100 mg were homogenized in lysis buffer containing 20 mM Hepes, pH 7.4, 50 mM NaCl, 10% glycerol, 1% Triton X-100, and protease inhibitor cocktail, followed by sonication for 10 s. A modified Lowry protein estimation was performed with modifications to eliminate interference of lipids in the liver from the obese mice. Aliquots were electrophoresed on 4–12% SDS gels (Invitrogen) and stained with Coomassie Blue (Bio-Rad) to verify equal protein concentrations. Separate aliquots were electrophoresed and transferred to nitrocellulose membranes followed by immunoblotting for mouse apoA-IV or SR-BI.

Lipoprotein production studies

Mice were injected with tyloxapol (hereafter referred to as Triton) at a concentration of 500 mg/kg body weight. Blood samples were collected from the retro-orbital venous plexus before injection and at 1 and 2 h after injection for TG analysis.

Lipoprotein clearance studies

Lipoprotein clearance studies were performed using ^{125}I-labeled HDL (d = 1.040–1.21 g/ml) collected from obese apoA-I$^{-/-}$ mice. The lipoproteins were labeled with ^{125}I-NaI (IMS30; Amersham) using Iodogene tubes according to the manufacturer's instructions (Pierce). Labeled lipoproteins were separated by FPLC to confirm the presence of HDL contained in fractions 24–30 and the size of LDL/HDL1 (data not shown). Labeled lipoproteins had a specific activity of 2,000 cpm/ng. C57BL/6, apoA-I$^{-/-}$, db/db, and db/db;apoA-I$^{-/-}$ mice were injected with 3 μg of protein via the tail vein. Immediately after injection, and at 10 min, 1 h, and 3 h after injection, blood was collected from the retro-orbital venous plexus and plasma was isolated after centrifugation. Data represent means ± SEM of counts remaining in plasma as a percentage of the injected counts. Plasma from four mice from each group at the 3 h time point was pooled, and 100,000 cpm was separated on a Superose 6 column. Radioactive counts were taken from each fraction and plotted.

Hepatic lipase assays

Plasma HL activity was measured using the Continuous Fluorometric Lipase Test (Confluolip) specific for HL from Progen according to the manufacturer's instructions. The assay was adapted for 96-well plates using 2 μl of a 1:5 dilution of plasma from 8–10 mice in each group (not heparin treated). The slope of a standard curve with a range of 1.4–22.5 pmol/ml was used to determine the fluorescence emitted over a 10 min period at 37°C.

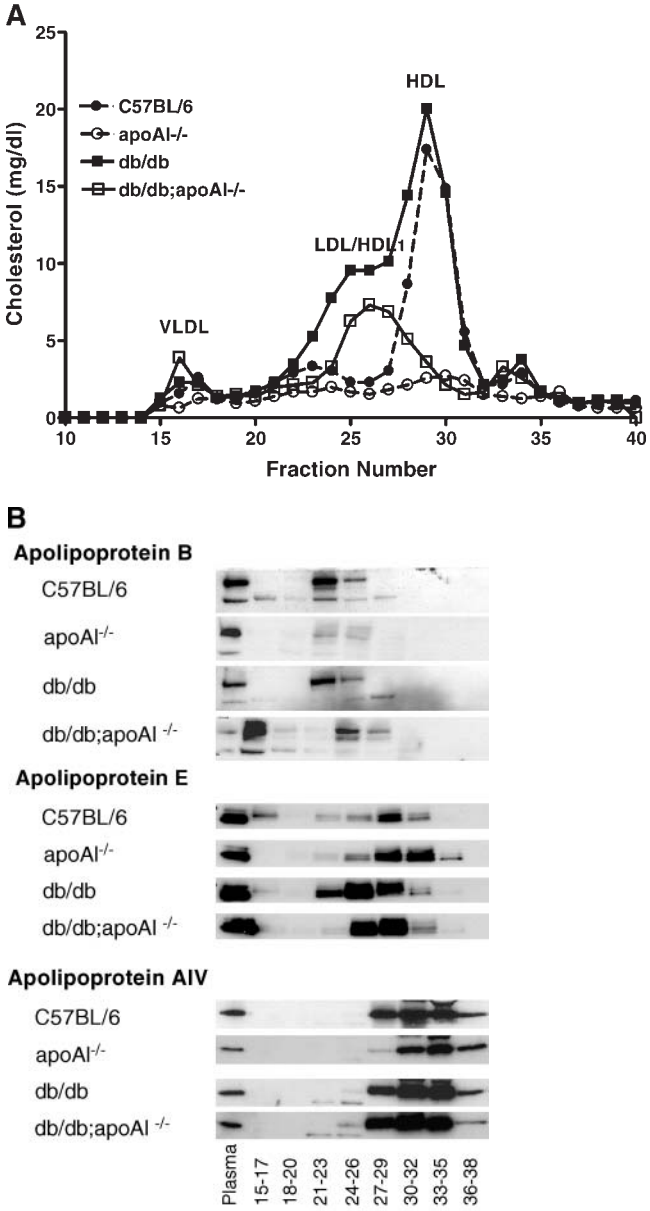

Fig. 1. Lipoprotein profiles of obese apolipoprotein A-I-deficient (apoA-I$^{-/-}$) mice compared with controls. Plasma was collected from C57BL/6 (closed circles), apoA-I$^{-/-}$ (open circles), leptin receptor-deficient (db/db; closed squares), and db/db;apoA-I$^{-/-}$ (open squares) mice. A 100 μl aliquot was separated by gel filtration chromatography according to Experimental Procedures. A: Fractions 11–40 were analyzed for cholesterol content. VLDL elutes in fractions 15–19, LDL in fractions 20–26, and HDL in fractions 27–33. The unique LDL/HDL particles elute in a size range between LDL and HDL from fractions 24–29. B: A pool of 10 μl from each of three samples from fast-protein liquid chromatography (FPLC) fractions shown in A was electrophoresed by 4–12% SDS-PAGE. Gels were transferred to nitrocellulose membranes and probed for apoB, apoE, and apoA-IV as described in Experimental Procedures. In the top panel, the higher molecular weight band corresponds to apoB-100 and the lower molecular weight band corresponds to apoB-48. The first column contains a 1:10 dilution of plasma. Remaining columns are from pooled fractions as indicated.

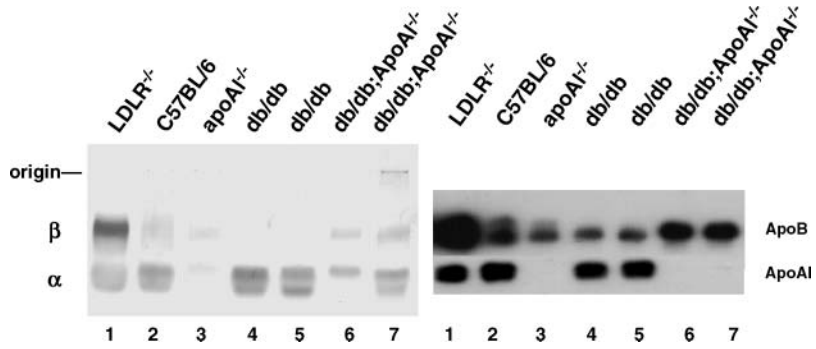

Fig. 2. Lipoprotein agarose gel electrophoresis. Plasma was collected from fasted low density lipoprotein receptor-deficient (LDLR$^{-/-}$), C57BL/6, apoA-I$^{-/-}$, db/db, and db/db;apoA-I$^{-/-}$ mice. Four microliters of each sample was electrophoresed on Titan agarose gels according to standard procedures. A: Gels were stained with Fat Red 7B for lipids. B: Lipoprotein gels were transferred to nitrocellulose membranes and probed for apoB and apoA-I, as indicated. Lane 1, LDLR$^{-/-}$ used as a reference for β-migrating particles; lane 2, C57BL/6; lane 3, apoA-I$^{-/-}$; lanes 4 and 5, db/db; lanes 6 and 7, db/db;apoA-I$^{-/-}$.

Statistical analyses

Data for lipoprotein production and clearance were analyzed by one-way ANOVA with a Bonferroni posthoc test. No significant differences were found between groups. Differences in expression levels of hepatic genes and plasma parameters in mice were analyzed using one-way ANOVA followed by the Bonferroni posthoc test. Significance was attributed for $P < 0.05$.

RESULTS

Plasma lipoproteins and lipoprotein profiles in obese apoA-I$^{-/-}$ mice

Glucose, insulin, TC, TG, and FFA were measured in ob/ob;apoA-I$^{-/-}$ and db/db;apoA-I$^{-/-}$ mice and littermate controls at 2 months of age (**Table 1**). The absence of apoA-I did not influence body weight in lean or obese animals. Insulin levels were increased in all obese animals compared with their lean littermates but were not affected by apoA-I deficiency. Both ob/ob;apoA-I$^{-/-}$ and db/db;apoA-I$^{-/-}$ mice had higher TC levels compared with lean apoA-I$^{-/-}$ mice ($P < 0.01$). The cholesterol in these animals was carried on VLDL and on lipoprotein particles in the size range of small LDLs/large HDLs (**Fig. 1A**).

Characterization of lipoproteins from obese apoA-I$^{-/-}$ mice

To determine the apolipoprotein content of the LDL/HDL1, FPLC fractions were pooled and immunoblotted for apoB, apoE, and apoA-IV (Fig. 1B). The distribution of apoB-100 shifted from larger LDLs (fractions 21–23) in all control groups to smaller LDLs (fractions 24–26) in the db/db;apoA-I$^{-/-}$ mice. Particles in the LDL/HDL1 lipoprotein fraction (24–26 and 27–29) displayed increased levels of apoE in both db/db and db/db;apoA-I$^{-/-}$ mice. ApoA-IV content was slightly lower in the lean apoA-I$^{-/-}$

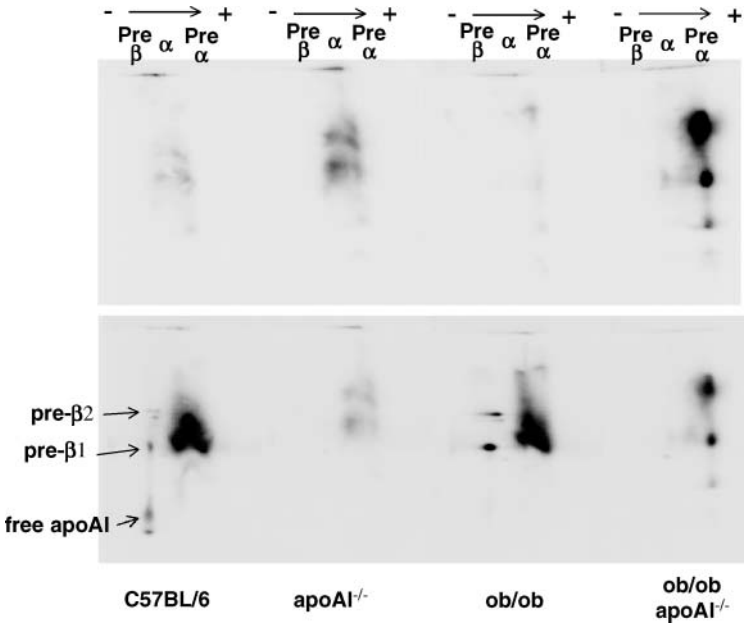

Fig. 3. Two-dimensional gel electrophoresis of HDL from leptin-deficient (ob/ob;apoA-I$^{-/-}$) mice. Plasma from fasted C57BL/6, apoA-I$^{-/-}$, ob/ob, and ob/ob;apoA-I$^{-/-}$ mice was pooled (three mice in each group) and used for two-dimensional gel electrophoresis as described in Experimental Procedures. The origin of the sample loading is marked by the minus sign, with the charge of the particles being progressively negative as the samples move to the right of the gel (symbols indicate the charge of gel). After transfer to nitrocellulose membranes, antibodies against mouse apoE were applied to detect the location of the HDL (top panel). After the first apoE blotting, the same membranes were probed with apoA-I antibody to establish the recognized HDL subpopulations, such as pre-β1, pre-β2, and free apoA-I, as indicated (bottom panel). The majority of HDL in the ob/ob;apoA-I$^{-/-}$ mice migrate with an extreme negative charge and are defined to be pre-α in nature. Residual labeled apoE probe can be seen in the apoA-I$^{-/-}$ and ob/ob;apoA-I$^{-/-}$ lanes probed with apoA-I antibody.

mice; however, there were no differences in the distribution of apoA-IV between the other three groups.

Apolipoprotein content of VLDL (d < 1.019 g/l), LDL (d = 1.019–1.040 g/l), HDL1/2 (d = 1.040–1.100 g/l), and HDL3 (d = 1.100–1.210 g/l) from pooled plasma samples was analyzed using SDS-PAGE followed by Coomassie staining. The primary difference in apolipoprotein content between the lean and obese apoA-I$^{-/-}$ mice was an increase in apoE in the HDL fractions (data not shown), confirming the results of the FPLC Western blots shown in Fig. 1B. ApoA-II levels were not different between groups.

Plasma from apoA-I$^{-/-}$ mice contained almost no α-migrating particles, whereas plasma from db/db mice contained increased levels of α-migrating particles (**Fig. 2**, left panel, lanes 4 and 5). The obese apoA-I$^{-/-}$ mice displayed reduced levels of α-migrating particles, and this was noted as a reduction in the more negatively charged particles (Fig. 2, lanes 6 and 7). In addition, the db/db;apoA-I$^{-/-}$ mice demonstrated an increase in apoB-containing particles of β-mobility (Fig. 2, right panel, lanes 6 and 7).

Lipoproteins from obese apoA-I$^{-/-}$ mice were also analyzed by two-dimensional gel electrophoresis (**Fig. 3**). A comparison of the electrophoretic mobility pattern for the four groups demonstrated that the ob/ob;apoA-I$^{-/-}$ mice had mostly HDL of pre-α mobility.

Lipoprotein production and clearance

In Triton studies, there was no difference in the production of hepatic VLDL between C57BL/6, apoA-I$^{-/-}$, db/db, and db/db;apoA-I$^{-/-}$ mice (140 ± 16, 163 ± 14, 163 ± 26, and 152 ± 32 mmol TG/h, respectively) (**Fig. 4A**).

Lipoprotein clearance studies were performed using ^{125}I-labeled HDL (d = 1.040–1.21 g/ml) from obese apoA-I$^{-/-}$ mice. Labeled lipoproteins were separated by FPLC to confirm the presence of HDL1 (data not shown). These labeled lipoproteins were injected into C57BL/6, apoA-I$^{-/-}$, db/db, or db/db;apoA-I$^{-/-}$ mice. There was no significant difference in the clearance of this unique lipoprotein particle between these groups of mice (Fig. 4B). However, FPLC analysis of 3 h postinjection plasma revealed a complete remodeling of LDL/HDL1 into smaller, "normal"-sized HDL particles in the C57BL/6 animals. A partial remodeling of LDL/HDL1 was detected in both db/db and apoA-I$^{-/-}$ mice; however, no remodeling was detected in the db/db;apoA-I$^{-/-}$ mice (Fig. 4C).

Hepatic gene and protein expression

To further analyze the roles of apolipoproteins, lipoprotein clearance, and processing in the accumulation of VLDL and LDL/HDL1, livers from db/db;apoA-I$^{-/-}$ mice were analyzed for the expression of genes related to these processes. Levels of low density lipoprotein receptor (LDLR), low density lipoprotein receptor-related protein (LRP), apoA-II, and apoE were not different between groups as assessed by real-time RT-PCR analysis (**Table 2**), but SR-BI mRNA was reduced by 50% in db/db and db/db;apoA-I$^{-/-}$ mice. Based upon a recent report demonstrating significant posttranscriptional regulation of SR-BI in hepatocytes

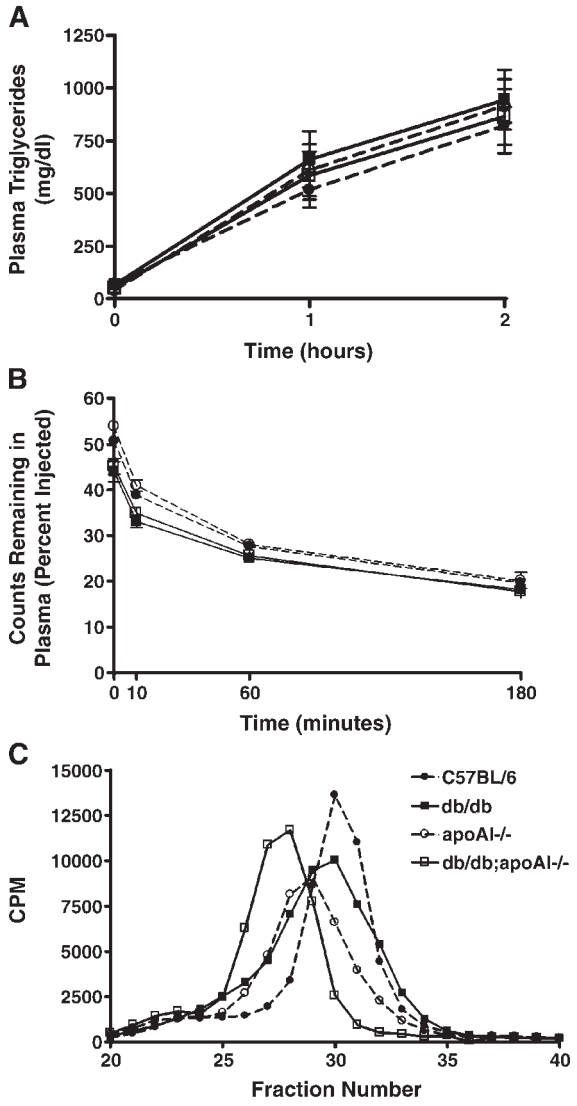

Fig. 4. Lipoprotein production and clearance in db/db;apoA-I$^{-/-}$ mice. A: Mice were injected with 500 mg/kg tyloxapol. Blood was collected before injection and at 1 and 2 h after injection for analysis of triglyceride (TG) accumulation. Plasma levels of TGs accumulated in a linear manner over the 2 h period in all groups. Symbols represent means ± SEM for each group (n = 8–12 mice). B: LDL/HDL1 (d = 1.04–1.21) was collected from obese apoA-I$^{-/-}$ mice by ultracentrifugation. Lipoproteins were iodinated, and the presence of LDL/HDL1 was confirmed by FPLC. Iodinated LDL/HDL1 was injected into C57Bl/6, apoA-I$^{-/-}$, db/db, and db/db;apoA-I$^{-/-}$ mice (n = 4 for each group). Mice were bled at 10 min, 1 h, and 3 h after injection, and percentage counts remaining in plasma were calculated. C: Plasma from the 3 h time point was pooled for each group, and the equivalent of 100,000 cpm was separated by FPLC. Total counts were taken for each fraction. Closed circles, C57BL/6; open circles, apoA-I$^{-/-}$; closed squares, db/db; open squares, db/db;apoA-I$^{-/-}$.

(14), we also analyzed liver cell lysates for SR-BI protein levels. The db/db and db/db;apoA-I$^{-/-}$ mice had significant reductions in SR-BI protein levels, with only 34% and 17% as much SR-BI protein, respectively, as the lean controls (**Fig. 5**). ApoA-IV mRNA levels were increased by 18- and 26-fold in db/db and db/db;apoA-I$^{-/-}$ mice, respectively, compared with lean controls ($P < 0.001$). Hepatic protein levels

TABLE 2. mRNA levels of hepatic genes in db/db;apoA-I$^{-/-}$ mice

Variable	C57BL/6	apoA-I$^{-/-}$	db/db	db/db;apoA-I$^{-/-}$
Lipoprotein receptors				
Low density lipoprotein receptor	1.38 ± 0.35	0.92 ± 0.16	1.83 ± 0.37	1.51 ± 0.33
Low density lipoprotein receptor-related protein	1.23 ± 0.11	1.3 ± 0.12	0.85 ± 0.04	0.87 ± 0.11
SR-BI	1.02 ± 0.06	0.75 ± 0.07	0.67 ± 0.01	0.56 ± 0.05
Apolipoproteins				
ApoE	0.91 ± 0.14	0.72 ± 0.14	1.0 ± 1.05	0.96 ± 0.17
ApoA-II	1.04 ± 0.14	1.57 ± 0.23	1.04 ± 0.04	1.0 ± 0.2
ApoA-IV	0.96 ± 0.04	1.50 ± 0.26	26 ± 9.1[a]	18.5 ± 5.7[a]
ApoM	1.0 ± 0.02	1.85 ± 0.28	0.30 ± 0.04	0.38 ± 0.03
Transcription factors				
Peroxisome proliferator-activated receptor γ	1.17 ± 0.30	1.30 ± 0.45	5.49 ± 0.40	5.58 ± 0.23
Peroxisome proliferator-activated receptor α	1.08 ± 0.19	1.66 ± 0.92	2.87 ± 0.79	1.44 ± 0.40
Estrogen-related receptor-α	1.09 ± 0.20	1.71 ± 0.11	2.02 ± 0.32	1.60 ± 0.23

SR-BI, scavenger receptor class B type I. Total RNA was isolated and used for real-time quantitative PCR analysis using Assays-on-Demand primer-probe sets from Applied Biosystems, with the exception of SR-BI, which was assayed as described in Experimental Procedures. A VIC-labeled 18S RNA control was used as a control for each reaction. Data represent means ± SEM of three to four different liver samples per group, with real-time RT-PCR performed in duplicate. Data are in arbitrary units compared with C57BL/6 levels.

[a] $P < 0.001$ compared with C57BL/6.

of apoA-IV were also increased by 3.2- and 4.9-fold in db/db and db/db;apoA-I$^{-/-}$ mice compared with controls (Fig. 5).

Estrogen-related receptor-α (ERRα) is a transcription factor critical for apoA-IV expression (15). Despite the increase in apoA-IV levels in db/db and db/db;apoA-I$^{-/-}$ mice, there was only a 2-fold, nonsignificant increase in ERRα levels in these animals compared with C57BL/6 mice (Table 2). A near absence of apoM in obese mice reported previously (16) was confirmed in the db/db and db/db;apoA-I$^{-/-}$ mice (Table 2). In addition, peroxisome proliferator-activated receptor α (PPARα) and PPARγ were increased by 2.9- and 5.5-fold, respectively, in the db/db mice, similar to what was reported previously for ob/ob and db/db animals (17).

Hepatic lipase activity

Plasma HL activity was increased by 1.8- to 2.8-fold in db/db;apoA-I$^{-/-}$ mice compared with control animals (**Fig. 6**).

DISCUSSION

The most important finding of this study is the persistence of a unique pool of lipoprotein particles, including large HDLs, in obese mice deficient in apoA-I. Several different mouse models have been reported to contain HDL1 particles in the presence of apoA-I. Silver et al. (6, 8) described a unique HDL particle in ob/ob and db/db mice. They referred to this shoulder on the traditional HDL peak as "LDL/HDL1." Stoffel and colleagues also detected HDL1 particles in mice lacking HNF-1α, which appears to be related to the absence of apoM (9, 10). In addition, LCAT transgenic and SR-BI$^{-/-}$ mice display increased HDL1 (11, 12). In previous HDL1 models described, apoA-I levels were increased. This suggests that apoA-I is critical for HDL1 formation; however, our data now show that HDL1, but not traditional HDL, persists even in the absence of apoA-I.

There are some similarities in the HDL1 of obese apoA-I$^{-/-}$ mice and the HDL1 of apoA-I-sufficient ob/ob, db/db, HNF-1α$^{-/-}$, SR-BI$^{-/-}$, and LCAT transgenic mice. First, the size of the lipoprotein is the same in all cases. Second, in all cases, the HDL1 fraction contains increased levels of apoE. This might be attributable to the ability of apoE to provide the structure for HDL formation and maturation. Third, as in the LCAT transgenic mice (11), the HDL1 in obese apoA-I$^{-/-}$ mice was also highly negatively charged (Fig. 3).

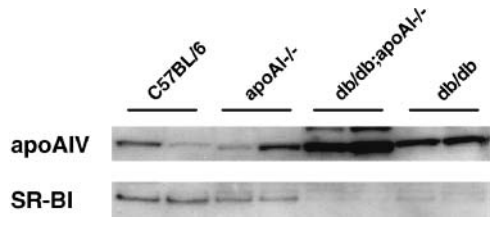

Fig. 5. ApoA-IV and scavenger receptor class B type I (SR-BI) protein levels in livers from db/db;apoA-I$^{-/-}$ mice. Protein from cell lysates of 6 month old mice was prepared and electrophoresed on 4–12% SDS gels. Gels were transferred to nitrocellulose and immunoblotted for SR-BI and apoA-IV. Images are of duplicates from at least three different mouse liver preparations in each group.

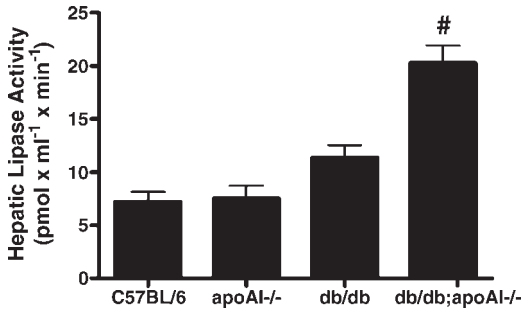

Fig. 6. Hepatic lipase assays. Hepatic lipase activity was measured in nonheparin plasma. A 1:5 dilution of plasma was assayed using the Confluolip assay adapted for microtiter plates as described in Experimental Procedures. Data are expressed as means ± SEM from 8–10 mice per group. # $P < 0.001$ compared with all other groups.

Despite these similarities, there are also differences between the HDL1 present in obese apoA-I$^{-/-}$ mice and those described in ob/ob, HNF-1α$^{-/-}$, and LCAT transgenic mice. First, the HDL1 in LCAT transgenic mice was reported to contain small amounts of apoB-48 and apoB-100, and HDL1 in ob/ob and HNF-1α$^{-/-}$ mice were reported to be nearly pure HDL. In contrast, we demonstrate a clear presence of apoB by Western blotting of both FPLC fractions and lipoprotein agarose gels (Figs. 1B, 2). Thus, our data indicate the presence of a heterogeneous population of particles containing both LDL and HDL1 when these particles accumulate in the absence of apoA-I. Second, we were able to show that the HDL1 particles can be remodeled into traditional HDL when injected into C57BL/6 mice (Fig. 4C).

We are tempted to speculate that the large HDL observed in these apoA-I-free mice represents a transition lipoprotein bridging the metabolism of apoB-containing lipoproteins and that of α-migrating particles. This hypothesis is partly corroborated by our turnover studies, which showed no differences in clearance of LDL/HDL1 between groups but proved that this particle had been remodeled into a smaller HDL only in the wild-type mice (Fig. 4C). Interestingly, the obese db/db mice and the lean apoA-I$^{-/-}$ mice had partial remodeling of the LDL/HDL1 particles. Taken together, these data indicate that both obesity and apoA-I deficiency contribute to the absence of remodeling of large HDLs and that this lack of remodeling is responsible for the accumulation of LDL/HDL1 in these animals.

Changes in the expression of apoA-II and apoE do not appear to contribute to the presence of LDL/HDL1; however, apoA-IV may be critical to the formation of LDL/HDL1. Both mRNA and protein levels of apoA-IV were found to be dramatically increased in the db/db and db/db;apoA-I$^{-/-}$ mice (Table 2, Fig. 5). The presence of increased levels of apoA-IV in obese ob/ob mice has been reported previously (18). Despite the increase in hepatic mRNA and protein levels of apoA-IV, the absence of a dramatic increase in plasma lipoprotein levels of apoA-IV provides an indication that it may not be relevant to the formation of HDL1.

The unaltered mRNA expression levels of lipoprotein receptors in the liver (LDLR and LRP; Table 2) provide evidence that apoB/apoE receptors are not involved in the increased VLDL or LDL/HDL1 levels seen in obese apoA-I$^{-/-}$ mice. More interesting is our observation that SR-BI protein levels are significantly reduced in db/db and db/db;apoA-I$^{-/-}$ mice (Fig. 5). As SR-BI$^{-/-}$ mice are known to accumulate HDL1 because of an absence of selective lipid uptake (12), a reduction of SR-BI in our studies implicates a contribution of SR-BI in the clearance of HDL in obese mice. It is important to note that Silver, Jiang, and Tall (8) did not detect reductions in SR-BI in ob/ob mice, whereas Lundasen et al. showed similar results to our current study, i.e. a 30% reduction in SR-BI mRNA and a 70% reduction in SR-BI protein levels in ob/ob mice (19).Thus, the contribution of SR-BI to the clearance of HDL1 in ob/ob and db/db mice remains inconclusive.

Based upon the HDL1 particles present in HL$^{-/-}$ mice (20) and the reduced HL activity in HNF-1α$^{-/-}$ mice with HDL1 (9), we anticipated that HL activity would be decreased in db/db;apoA-I$^{-/-}$ mice. In fact, we observed the opposite: HL activity was 1.8- to 2.8-fold increased in db/db;apoA-I$^{-/-}$ mice compared with controls ($P < 0.001$). This activity is as high as the levels detected in post-heparin plasma of human HL-overexpressing mice (21). These results indicate that the processing and clearance of HDL1 by HL and SR-BI requires the presence of apoA-I.

The increase in VLDL levels in obese apoA-I$^{-/-}$ mice is also of interest. Our lipoprotein production studies demonstrate that VLDL accumulation is not attributable to increased production. Also, the turnover studies did not show differences in the clearance of LDL/HDL1 in the different animal groups. It is possible that apoA-I$^{-/-}$ HDL1 has a reduced ability to remodel apoB lipoproteins, leading to an accumulation of VLDL, as was proposed to be responsible for the proatherogenic lipoprotein profile of apoA-II transgenic mice (22).

It is well established that HDL and apoA-I levels are inversely correlated with the risk of cardiovascular disease (23, 24). Animal models have also been used to demonstrate the atheroprotective effects of HDL and apoA-I (25–32). In each of these studies, the presence of apoA-I also increased total HDL levels, making it difficult to distinguish between the effects of apoA-I versus HDL. More recently, we have shown that even low-level apoA-I expression in macrophages induced by retroviral vectors reduce lesion area in hyperlipidemic mice (33, 34), providing evidence that apoA-I may be protective independent of plasma HDL concentrations. The presence of apoA-I$^{-/-}$ HDL1 suggests the intriguing possibility that there is a physiologically viable apoA-I-independent component of cholesterol efflux. However, it remains to be determined whether the HDL lacking apoA-I is sufficiently cardioprotective.

In conclusion, our data demonstrate that apoA-I is not required for the accumulation of HDL1 in plasma of ob/ob and db/db mice. These data suggest the presence of a functional axis based on the presence of apoA-I, efficient processing by HL, and cholesterol uptake via SR-BI in the processing and clearance of HDL1.

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