Hepatic Hepatocyte Nuclear Factor 4α Is Essential for Maintaining Triglyceride and Cholesterol Homeostasis

Liya Yin, Huiyan Ma, Xuemei Ge, Peter A. Edwards, Yanqiao Zhang

Objective—Loss-of-function mutations in human hepatocyte nuclear factor 4α (HNF4α) are associated with maturity-onset diabetes of the young and lipid disorders. However, the mechanisms underlying the lipid disorders are poorly understood. In this study, we determined the effect of acute loss or augmentation of hepatic HNF4α function on lipid homeostasis.

Methods and Results—We generated an adenovirus expressing LacZ (Ad-shLacZ) or short hairpin RNA of Hnf4 (Ad-shHnf4). Tail vain injection of C57BL/6J mice with Ad-shHnf4α reduced hepatic Hnf4α expression and resulted in striking phenotypes, including the development of fatty liver and a >80% decrease in plasma levels of triglycerides, total cholesterol, and high-density lipoprotein cholesterol. These latter changes were associated with reduced hepatic lipogenesis and impaired very-low-density lipoprotein secretion. Deficiency in hepatic Hnf4α did not affect intestinal cholesterol absorption despite decreased expression of genes involved in bile acid synthesis. Consistent with the loss-of-function data, overexpression of Hnf4α induced numerous genes involved in lipid metabolism in isolated primary hepatocytes. Interestingly, many of these Hnf4α-regulated genes were not induced in wild-type mice that overexpressed hepatic Hnf4α. Because of selective gene regulation, mice overexpressing hepatic Hnf4α had unchanged plasma triglyceride levels and decreased plasma cholesterol levels.

Conclusion—Loss of hepatic HNF4α results in severe lipid disorder as a result of dysregulation of multiple genes involved in lipid metabolism. In contrast, augmentation of hepatic HNF4α activity lowers plasma cholesterol levels but has no effect on plasma triglyceride levels because of selective gene regulation. Our data indicate that hepatic HNF4α is essential for controlling the basal expression of numerous genes involved in lipid metabolism and is indispensable for maintaining normal lipid homeostasis. (Arterioscler Thromb Vasc Biol. 2011;31:328-336.)

Key Words: diabetes mellitus ■ gene expression ■ lipids ■ lipoproteins ■ metabolism ■ receptors ■ HNF4α

Nuclear receptors are ligand-activated transcription factors that regulate diverse physiological processes such as reproduction, development and metabolism. Hepatocyte nuclear factor 4α (HNF4α, NR2A1) is a member of the nuclear receptor superfamily. It is highly expressed in the liver, with lower levels in the kidney, intestine, and pancreatic β cells. Like other members of the nuclear receptor superfamily, HNF4α has a highly conserved DNA-binding domain and a variable ligand-binding domain. However, HNF4α is known to be constitutively active. Structural analysis of the ligand-binding domain of HNF4α indicates the C14 to C18 long-chain fatty acids are tightly bound to the hydrophobic pocket and cannot be dissociated from the receptor under non-denaturing conditions. Recent data also show that linoleic acid selectively occupies the binding pocket of ligand-binding domain but does not affect the transcriptional activity of HNF4α.

Loss-of-function mutations in human HNF4α are associated with maturity-onset diabetes of the young (MODY1), characterized by autosomal dominant inheritance, early-onset diabetes, and pancreatic β-cell dysfunction. The diabetes phenotype appears to result from reduced insulin secretion in response to glucose stimulation. In addition, patients with MODY1 also have reduced levels of plasma triglycerides and cholesterol. However, the mechanism underlying hypolipidemia remains poorly understood.

Homozygous mutations in whole body Hnf4α result in early embryonic lethality in mice, consistent with the central role of HNF4α in development. Liver-specific Hnf4α−/− (L-Hnf4α−/−) mice have increased plasma bile acid levels and reduced plasma levels of triglycerides and cholesterol. The disorder in bile acid homeostasis is a result of reduced hepatic expression of genes involved in bile acid biosynthesis and bile acid uptake from the blood, whereas hypotriglyceridermia may result from reduced hepatic expression of microsomal transport protein (MTP) and apolipoprotein...
tein B (ApoB), 2 proteins that play a critical role in very-low-density lipoprotein (VLDL) secretion. Hayhurst et al suggested that the increased expression of scavenger receptor class B type I (SR-BI), a high-density lipoprotein (HDL) receptor that selectively uptakes cholesteryl esters from plasma HDL, is responsible for hypercholesterolemia observed in L-Hnf4α-/- mice. It is unknown whether other genes or pathways are also involved in HNF4α-regulated lipid homeostasis.

Both the liver and the intestine play an important role in maintaining cholesterol homeostasis. Plasma cholesterol levels may be affected by intestinal cholesterol absorption, hepatic de novo cholesterol biosynthesis, VLDL secretion, plasma cholesterol uptake by the liver, and subsequent hepatobiliary cholesterol secretion. HMG-coenzyme A (CoA) reductase is the rate-limiting enzyme in the cholesterol biosynthetic pathway. Once synthesized, cholesterol and long-chain fatty acids are esterified to form cholesteryl esters by acyl-CoA:cholesterol acyltransferase 2 (ACAT2; SOAT2) for storage or secretion in the form of VLDL from the liver or chylomicrons from the intestine. Plasma HDL levels may be affected by many factors. The data from human Tangier disease and genetically engineered mice have clearly demonstrated that ATP-binding cassette (ABC) transporter A1 (ABCA1) is the major determinant of plasma HDL levels.16–20 Hepatic ABCA1 has been shown to be responsible for ~80% of plasma total HDL. In addition, ApoA-I, the major component of HDL, is also required for maintaining plasma HDL cholesterol (HDL-C) levels.21

Liver takes up plasma HDL-C or non-HDL-C via SR-BI, low-density lipoprotein receptor (LDLR) or LDLR-related protein (LRP). In the liver, cholesterol may be converted to bile acids by cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1), 2 key enzymes that control bile acid biosynthesis. Bile acids are subsequently secreted to the bile via transporters, such as bile acid export protein (BSEP). Hepatic cholesterol may also be secreted directly to the bile via ABC transporters G5 (ABCG5) and G8 (ABCG8)22,23 or SR-BI.24

In this report, we demonstrate that acute loss of hepatic Hnf4α causes hypotriglyceridemia and the development of fatty liver via reducing VLDL secretion. We also demonstrate that hepatic Hnf4α deficiency results in hypercholesterolemia, likely by reducing de novo cholesterol biosynthesis, VLDL secretion, and HDL biogenesis. In contrast, hepatic overexpression of HNF4α reduces the levels of hepatic triglycerides and plasma cholesterol but has no effect on plasma triglyceride levels. Together, these data demonstrate that hepatic HNF4α is essential for maintaining triglyceride and cholesterol homeostasis.

Materials and Methods

Mice
C57BL/6J mice and db/db mice were purchased from the Jackson Laboratory (Bar Harbor, Maine) and fed a standard chow diet. All experiments were approved by the Institutional Animal Care and Use Committee at the Northeastern Ohio Universities College of Medicine. All mice were euthanized during the light cycle.

Adenovirus
Ad-Hnf4α was generated by cloning mouse Hnf4α cDNA to adenoviral vector pShuttle-ires-hGFP (Stratagene) as previously described. To generate adenovirus expressing small hairpin RNA (shRNA) corresponding to β-galactosidase (Ad-shLacZ, control) or Hnf4α (Ad-shHnf4α), oligonucleotides were designed using BLOCK-iT RNAi Designer (Invitrogen), annealed and ligated to pEnter/U6 vector (Invitrogen). Adenovirus was then generated following the instructions provided by Invitrogen. Three different shRNA oligonucleotides against murine Hnf4α were designed. The sequences that produced the most inhibitory effect on endogenous Hnf4α expression were 5’-CACCGGTTGCAAATTCTATTCCGAGGGATGATATGG-3’ (top strand) and 5’-AAAAGTGGCAACCCTGATGCTACCTACCTTGGAATGGG-3’ (bottom strand). Adenovirus was grown in 293A cells and purified using a BD Adeno-X Virus Purification Kit (BD Biosciences) or by cesium chloride density gradient centrifugation. To infect primary hepatocytes, adenovirus was added at a multiplicity of infection of 10, and cells were harvested after 48 hours. To overexpress genes in mice, 10⁸ to 10⁹ plaque formation units of adenovirus was transfused into each mouse via tail vein injection. To knock down hepatic genes in mice, 10¹⁰ plaque formation units of Ad-shLacZ or Ad-shHnf4α was transfused to each mouse via tail vein injection. In general, 6 to 7 days postinfection, mice were fasted for 6 hours and then euthanized.

Primary Hepatocytes
Mouse primary hepatocytes were isolated and cultured as described. Three days after isolation, hepatocytes were infected with adenovirus for 48 hours before RNA extraction.

Real-Time Polymerase Chain Reaction
RNA was isolated using TRIzol Reagent (Invitrogen), and mRNA levels were determined by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) using SYBR Green Supermix and a 7500 real-time PCR machine from Applied Biosystems (Foster City, Calif). The primer sequences for qRT-PCR are provided in Supplemental Table I, available online at http://atvb.ahajournals.org. Results were normalized to 36B4 mRNA.

Western Blot Assay
Whole liver lysates were prepared and Western blot assays were performed as described previously. β-Actin antibody was from Novus Biologicals. HNF4α and MTP antibodies were from Santa Cruz Biotechnology. ApoB48/100 and ApoA-I antibodies were from Biodesigen. Cyp7a1 antibody was a kind gift from David Russell at the University of Texas Southwestern Medical Center.

Chromatin Immunoprecipitation Assay
Adenovirus expressing green fluorescent protein (Ad-GFP) or Ad-HNF4α was used to infect primary hepatocytes for 48 hours, or C57BL/6 mice via intravenous injection. The protein lysates from the primary hepatocytes or the liver (6 days postinfection) were incubated with IgG or HNF4α antibody, and chromatin immunoprecipitation assays were performed as described previously (n = 3 per treatment).

Glucose and Insulin Tolerance Test
Nine-week-old db/db mice were injected intravenously with Ad-shLacZ or Ad-shHnf4α. On day 7, a glucose tolerance test was performed. Briefly, mice were fasted for 6 hours, followed by intraperitoneal injection of glucose (2 g/kg). Blood glucose levels were determined at indicated time points using an AlphaTrak glucometer (Abbott Laboratories). On day 9, an insulin tolerance test was performed. Mice were injected intraperitoneally with insulin (Humulin, 0.85 units/kg) after a 6-hour fast, and blood glucose levels were determined at the indicated time points.
Lipid Disorder

VLDL Secretion

De Novo Lipogenesis

Intestinal Cholesterol Absorption

Statistical Significance was analyzed using the unpaired Student t test, 1-way ANOVA, or Mann-Whitney test (GraphPad InStat3 software). All values are expressed as mean±SEM. Differences were considered statistically significant at P<0.05.

Results

Acute Loss of Hepatic Hnf4α Results in Severe Lipid Disorder

To determine the effect of acute loss of hepatic HNF4α on lipid homeostasis, we generated 3 adenoviruses expressing shRNA corresponding to Hnf4α (Ad-shHnf4α) and 1 to galactosidase Z (Ad-LacZ, control). One of 3 Hnf4α shRNAs was chosen for further studies, as it was particularly efficient in knocking down endogenous Hnf4α mRNA (Supplemental Figure 1A) and protein (Supplemental Figure 1B).

To test the physiological consequences of endogenous Hnf4α deficiency, we transfused Ad-shLacZ or Ad-shHnf4α to C57BL/6 mice via tail vein injection. Tail vein injection essentially limits adenovirus to the liver. As shown in Figure 1A, expression of Hnf4α shRNA significantly reduced hepatic Hnf4α mRNA levels by 67% but had no effect on the mRNA levels of Bsep and many other genes (data not shown), indicating that Hnf4α shRNA specifically and efficiently targets Hnf4α in vivo.

The livers of mice receiving Ad-shHnf4α were enlarged and white in color (Figure 1B). Analysis of hepatic mRNA levels indicated that Hnf4α-deficient mice had ~4-fold increase in triglyceride levels (Figure 1C) and unchanged cholesterol levels in the liver (Figure 1D). These data demonstrate that hepatic Hnf4α deficiency leads to the development of fatty liver as a result of increased triglyceride accumulation.

In addition, Hnf4α-deficient mice had strikingly low levels of plasma lipids; plasma triglyceride levels decreased by 84% (Figure 1E) and plasma total cholesterol and HDL-C levels decreased by ~87% (Figure 1F). Together, the data shown in Figure 1 demonstrate that hepatic Hnf4α deficiency results in severe lipid disorder in both the liver and plasma.

Hepatic Hnf4α Deficiency Results in Dysregulation of Multiple Genes Involved in Lipid Metabolism

To understand the mechanism by which hepatic Hnf4α deficiency results in lipid disorder, we analyzed hepatic gene expression by qRT-PCR. The data show that the mRNA levels of genes involved in VLDL secretion (Mtp, Apob), de novo cholesterol biosynthesis (Hmgr, Hmgs, Srebp-2), cholesterol catabolism (Cyp7a1, Cyp8b1), cholesterol esterification (Aca2, Lcat), and cholesterol uptake (Ldlr, SR-BI) were all significantly reduced in Hnf4α-deficient mice (Figure 2A). In addition, hepatic mRNA levels of genes encoding transporters (Abca1, Abcg5, Abcg8, Mdr2) (Figure 2B), apolipoproteins (Apoa1, Apo2, Apoc2, Apoc3, and Apoe) (Figure 2C), or nuclear receptors (Ppara, Pparg, Pparb, Ppargc1a) were also significantly reduced in Hnf4α-deficient mice (Figure 2D). These data suggest that hepatic Hnf4α deficiency leads to dysregulation of multiple genes involved in lipid metabolism.

Figure 1. Hepatic HNF4α deficiency causes severe lipid disorder. C57BL/6 mice were transfused with Ad-shLacZ or Ad-shHnf4α via tail vein injection (n=7 mice per group). After 6 days, mice were euthanized after a 5-hour fast. A, Hepatic mRNA levels were determined by qRT-PCR. B, Representative livers from both genotypes are shown. C to F, Hepatic triglycerides (C), hepatic total cholesterol (D), plasma triglycerides (E), and plasma total cholesterol (TC) and HDL-C (F) were determined. *P<0.05, **P<0.01 versus Ad-shLacZ treatment.
Pparγ) (Figure 2D) were also significantly reduced in Hnf4α-deficient mice. The finding that VLDL receptor (Vldr) (Figure 2A) and Abcg1 (Figure 2B) were induced in Hnf4α-deficient mice suggests that Hnf4α deficiency does not cause a global repression of genes involved in lipid metabolism. In addition, hepatic protein levels of Hnf4α, Mtp (Figure 2E) and ApoB48/100 (Figure 2F) were also significantly reduced in Hnf4α-deficient mice. Interestingly, hepatic Hnf4α deficiency did not significantly alter Cyp7a1 protein levels (Figure 2E), consistent with a previous report that Cyp7a1 protein levels are unchanged in L-Hnf4α/−/− mice during the light cycle. Together, these data indicate that HNF4α is important for normal expression of multiple genes that are involved in lipid metabolism.

**Hnf4α-Deficient Mice Have Impaired VLDL Secretion**

The significant reduction in hepatic Mtp and Apob in Hnf4α-deficient mice (Figure 2) suggested that these mice likely had reduced VLDL secretion. We tested this hypothesis by injecting Tyloxapol, an inhibitor for lipoprotein lipase, to mice that had been infected with Ad-shLacZ or Ad-shHnf4α for 6 days. Hnf4α-deficient mice had markedly reduced levels of plasma triglycerides (Figure 3A) and ApoB48/100 secretion (Figure 3B) at 30, 60, and 90 minutes after injection of Tyloxapol, which corresponded to a 3.5-fold reduction in VLDL triglyceride production rate (Figure 3C). Thus, the data shown in Figure 3A to 3C demonstrate that hepatic HNF4α deficiency results in a significant reduction in lipogenesis and de novo cholesterol synthesis.

**Hepatic Hnf4α Deficiency Does Not Affect Intestinal Cholesterol Absorption**

Bile acids are involved in emulsification and absorption of dietary lipids. Loss of Cyp7a1 or Cyp8b1 has been shown to reduce intestinal cholesterol absorption. Interestingly, hepatic Hnf4α deficiency reduced Cyp7a1 mRNA but not protein levels (Figure 2). Previous data have shown that the ratio of cholic acid-derived metabolites to muricholic acid is similar between L-Hnf4α/−/− mice and the control mice, indicating that hepatic Hnf4α deficiency does not alter the hydrophobicity index of the bile acid pool and thus may not affect cholesterol absorption. Consistent with this latter finding, intestinal cholesterol absorption, determined by the plasma dual-isotope ratio method, was unchanged between Hnf4α-deficient mice and the control mice (P=0.23) (Figure 3G). Thus, intestinal cholesterol absorption does not contribute to the reduced plasma cholesterol levels in hepatic Hnf4α-deficient mice.
Effect of Hepatic HNF4α Deficiency on Body Weight and Insulin Sensitivity

The striking phenotypes of fatty liver and hypolipidemia (Figure 1) led us to investigate the role of hepatic Hnf4α deficiency in glucose homeostasis. In wild-type mice, hepatic Hnf4α deficiency had no effect on food intake, body weight, glucose tolerance, or insulin sensitivity (Supplemental Figure II and data not shown). Interestingly, in 9-week-old db/db mice, infection with Ad-shHnf4α for 9 days caused a significant reduction in body weight (Figure 3H) but had no effect on food intake (data not shown). In addition, db/db mice receiving Ad-shHnf4α had increased glucose intolerance (Figure 3I) and increased insulin insensitivity (Figure 3J). These data indicate that hepatic HNF4α deficiency may be sufficient to cause insulin insensitivity.

Ex Vivo Expression of HNF4α Induces Many Genes Involved in Lipid Metabolism

Our loss-of-function studies have demonstrated that hepatic HNF4α is required for maintaining the expression of numerous genes involved in lipid metabolism (Figures 2 and 3). To determine whether overexpression of HNF4α also alters these genes, we generated adenovirus expressing Hnf4α (Ad-Hnf4α). Ad-Hnf4α or Ad-GFP (control) was subsequently used to infect primary hepatocytes isolated from wild-type mice. Consistent with the loss-of-function data, overexpression of Hnf4α significantly induced the mRNA levels of Mtp, Apob (VLDL secretion), Cyp8b1 (bile acid synthesis), Lrp, Ldlr, SR-BI (cholesterol uptake), Acat2, Lcat (cholesterol esterification), Abca1, Abcg5, Abcg8 (transporters), Apoa1, Apoa2, and Apoc2 (apolipoproteins) (Table). However, overexpression of Hnf4α had no significant effect on Srebp-2, Hmgcr, Srebp-1c, or Fas (Table), suggesting that augmentation of hepatic HNF4α activity may not affect lipogenesis.

Hepatic Overexpression of HNF4α Lowers Plasma Cholesterol Levels But Not Triglyceride Levels

To determine the physiological consequences of overexpression of hepatic HNF4α in vivo, we transfused Ad-GFP or Ad-Hnf4α to C57BL/6 mice via tail vein injection. Overexpression of hepatic Hnf4α significantly increased hepatic Hnf4α protein levels (Figure 4A) and modestly reduced plasma total cholesterol levels but had no effect on plasma triglyceride levels (Figure 4B). Interestingly, overexpression of hepatic Hnf4α also reduced hepatic triglyceride levels but had no effect on hepatic total cholesterol levels (Figure 4C).

Figure 3. Effect of hepatic HNF4α deficiency on VLDL secretion, lipogenesis, intestinal cholesterol absorption, and insulin sensitivity. A to D, C57BL/6 mice were injected with Ad-shLacZ or Ad-shHnf4α via tail vein injection (n = 5 mice per group). After 6 days, mice were fasted overnight, followed by intravenous injection of Tyloxapol (500 mg/kg). Blood was taken at the indicated time points, and plasma triglyceride levels were determined (A). Plasma ApoB48/100 levels were determined (A). Plasma ApoB48/100 levels were determined by Western blot assays (B). The relative VLDL triglyceride (TG) production rate was determined by the slopes of linear increases of plasma TG (C). Hepatic mRNA levels were determined by qRT-PCR (D). E and F, C57BL/6 mice were infected with Ad-shLacZ or Ad-shHnf4α (n = 5 to 6). After 8 days, mice were injected intraperitoneally with 2H2O, and hepatic palmitate (E) and cholesterol synthesis (F) were determined. G, Intestinal cholesterol absorption was determined using plasma dual-isotope ratio method (n = 5). H to J, Nine-week-old db/db mice were injected intravenously with Ad-shLacZ or Ad-shHnf4α (n = 6 mice per group). Body weight (H) was determined, and the glucose tolerance test (I) and insulin tolerance test (J) were performed. *P < 0.05, **P < 0.01 versus Ad-shLacZ treatment.
Table. HNF4α Regulates the Expression of Numerous Genes Involved in Lipid Metabolism in Primary Hepatocytes

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Primary hepatocytes were infected with Ad-GFP or Ad-HNF4α for 48 hours. mRNA levels were quantified by qRT-PCR. Fold changes indicate the relative mRNA levels induced by HNF4α vs GFP (control).

In addition, overexpression of hepatic Hnf4α had no effect on intestinal cholesterol absorption (data not shown). Analysis of plasma by fast protein liquid chromatography indicated that overexpression of Hnf4α in the liver lowered plasma HDL-C and reduced hepatic triglyceride levels (Figure 4E). In addition, overexpression of hepatic Hnf4α had no effect on intestinal cholesterol absorption (data not shown). Together, the data shown in Figure 4 indicate that overexpression of hepatic Hnf4α lowers plasma cholesterol levels, primarily by lowering HDL-C, and reduces hepatic triglyceride levels.

Overexpression of Hepatic HNF4α Selectively Regulates Gene Expression

The data in Figure 5A show that overexpression of hepatic Hnf4α increases the mRNA levels of Apob, Cyp8b1, SR-BI, and Apoa1 (Figure 5A) but did not affect the mRNA levels of Hmgcr, Hmgcs, Cyp7a1, Abca1, Abcg5, Abcg8, Apoa1, Acat2, Lcat, or Srebp-1c (Figure 5A). The levels of hepatic Mtp were highly variable; hepatic Mtp mRNA (Figure 5A; P=0.06) and protein levels (Figure 5B) tended to increase following overexpression of Hnf4α in the liver. Overexpression of Hnf4α in the liver lowered HDL-C, and reduces hepatic triglyceride levels (Figure 4). In addition, the induction of hepatic SR-BI following overexpression of Hnf4α in the liver (Figure 5A) is consistent with reduced plasma triglyceride levels (Figure 4).

The finding that HNF4α overexpression induces many genes in isolated primary hepatocytes (Table) but not in the liver (Figure 5A) is intriguing. Compared with the liver, primary hepatocytes had much lower levels of Hnf4α mRNA (<6%; data not shown) and protein (Figure 5D). We hypothesized that many genes were inducible in primary hepatocytes by exogenous HNF4α because endogenous HNF4α expression was low in these cells; we also hypothesized that many of these genes were not inducible in the liver by exogenous HNF4α because endogenous HNF4α expression was already high in vivo. We used Abcg5, which is a known HNF4α target gene34 and is highly inducible by HNF4α in isolated primary hepatocytes (Table) but not in the liver (Figure 5A), as an example to test our hypothesis.

HNF4α regulates Abcg5 expression through binding to a specific direct repeat (DR)-1 element in the Abcg5 promoter.34 We infected primary hepatocytes with Ad-GFP (control) or Ad-HNF4α, followed by chromatin immunoprecipitation assays; the data indicated that HNF4α protein bound to the

Figure 4. Overexpression of HNF4α in the liver lowers plasma cholesterol levels but has no effect on plasma triglyceride levels. C57BL/6 mice were injected intravenously with adenovirus expressing GFP (Ad-GFP) or HNF4α (Ad-HNF4α) (n=5 mice per group). After 6 days, mice were fasted for 5 hours before euthanization. Hepatic protein levels were determined by Western blot assays (A). Triglyceride (TG) and total cholesterol (TC) levels in the plasma (B) and liver (C) were determined. Plasma cholesterol lipoprotein profile (D) and triglyceride lipoprotein profile (E) were determined by fast protein liquid chromatography analysis. *P<0.05 versus Ad-GFP treatment.
known DR-1 element only when HNF4α was overexpressed in isolated primary hepatocytes (Figure 5E, left). In contrast, HNF4α protein bound to the known DR-1 element in both the absence and the presence of exogenous HNF4α protein, and the expression of exogenous HNF4α protein did not further increase the recruitment of HNF4α protein to the DR-1 element in Abcg5 promoter (Figure 5E, right). Thus, the data shown in Figure 5D and 5E suggest that the difference in the abundance of endogenous HNF4α between the liver and primary hepatocytes is responsible for the differential regulation of certain genes by exogenous HNF4α.

Discussion

In this study, we used loss-of-function and overexpression approaches to determine the role of hepatic HNF4α in lipid homeostasis. Acute loss of hepatic HNF4α resulted in striking phenotypes, including low blood triglyceride and cholesterol levels, fatty liver, and hepatomegaly (Figure 1). These changes were associated with reduced lipogenesis, de novo cholesterol synthesis, and VLDL secretion. Consistent with changes, the expression of numerous genes that are involved in lipid metabolism was significantly altered. In addition, acute loss of hepatic HNF4α was also associated with increased glucose intolerance (Figure 3). Interestingly, overexpression of HNF4α in the liver also moderately lowered plasma cholesterol levels but had no effect on plasma triglyceride levels (Figure 4). Together, these data demonstrate that hepatic HNF4α is indispensable for maintaining normal triglyceride and cholesterol homeostasis.

The hypolipidemic effect of acute loss of hepatic Hnf4α reported in the current study is consistent with a previous study that used L-Hnf4a−/− mice. In the latter report, however, the investigators attributed the hypocholesterolemia to increased hepatic SR-BI expression. In the current study, we demonstrated that acute loss of hepatic Hnf4a reduces SR-BI expression (Figure 2A). We noted the methodological difference between our studies and the studies by Hayhurst et al., which includes knockdown methods (shRNA versus Cre-loxP), time (6 to 7 days versus 45 days), and the degree of Hnf4a deletion (67% versus >90%). Importantly, our loss-of-function data are supported by the finding that overexpression of HNF4α increases SR-BI expression in both isolated primary hepatocytes (Table) and the liver (Figure 5A). We also demonstrated that hepatic Hnf4a deficiency results in reduced expression of genes involved in de novo cholesterol biosynthesis (Hmgcs, Hmgcr), VLDL secretion (Mtp, Apob), and HDL biogenesis (Abca1, ApoA1) (Figure 2). We further demonstrated that such changes in gene expression have functional consequences; hepatic HNF4α deficiency reduces de novo cholesterol synthesis and VLDL secretion (Figure 3). In addition, we demonstrated that hepatic HNF4α deficiency has no effect on intestinal cholesterol absorption (Figure 3G). Thus, our data show that the reduced plasma cholesterol levels in Hnf4α-deficient mice may result from reduced de novo cholesterol synthesis and VLDL secretion.

Both the current study and a previous study have demonstrated that loss of hepatic Hnf4a decreases hepatic Mtp and Apob expression. However, the functional significance of such changes has not previously been documented. In this study, we showed that hepatic VLDL secretion is severely impaired in Hnf4α-deficient mice (Figure 3A to 3C). Consistent with reduced levels of Fas, Dgat1, and Dgat2 (Figure 3D), hepatic HNF4α deficiency also significantly reduced lipogenesis (Figure 3E). Consequently, the current data demonstrate that the development of fatty liver and hypotriglyc-
plasma cholesterol levels,24 our data suggest that the reduced expression of Hnf4α, but not SR-BI have 3 DR-1 elements and 1 DR-1 element, respectively; SR-BI, but not Abcg5, can be induced by exogenous HNF4α in the liver (Figure 5A). Consistent with the selective induction of certain genes in vivo (Figure 5), hepatic overexpression of Hnf4α does not affect plasma triglyceride levels but modestly reduces plasma cholesterol levels (Figure 4). Because hepatic overexpression of SR-BI is known to reduce plasma cholesterol levels,24 our data suggest that the reduced plasma cholesterol levels in Hnf4α-overexpressing mice may result from increased hepatic SR-BI expression (Figure 5). Interestingly, overexpression of HNF4α in the liver also reduces hepatic triglyceride levels, which may be accounted for in part by increased hepatic ApoB expression (Figure 5A).

In summary, we have used loss-of-function and overexpression approaches to demonstrate that hepatic HNF4α is required for maintaining basal expression of many genes involved in lipid metabolism and is indispensable for maintaining normal lipoprotein homeostasis. Our data indicate that augmentation of hepatic HNF4α activity has only modest effects on lipid homeostasis.

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Disclosures

None.

References


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Supplementary Material

Figure legends

**Figure I.** Expression of Hnf4α shRNA in primary hepatocytes reduces endogenous HNF4α expression. Mouse primary hepatocytes were infected with Ad-shLacZ or Ad-shHnf4α (n=3 per treatment). After 48 h, mRNA levels were determined by qRT-PCR and protein levels determined by Western blot assays. **P<0.01.

**Figure II.** Hepatic HNF4α deficiency has no effect on body weight or glucose tolerance in wild-type mice. C57BL/6 mice were injected intravenously with Ad-shLacZ or Ad-shHNF4α (n=6 mice per group). Body weight was collected on day 0 and day 9. Glucose tolerance test (B) and insulin tolerance test (C) were performed on day 9 and day 11, respectively.

**Table I.** Mouse primer sequences for qRT-PCR.
Figure I

A

B

shLacZ  shHnf4α

Hnf4α

β-actin

**
Figure II

A. Bar graph showing body weight (g) over days post infection.

B. Line graph showing glucose (mg/dL) over minutes.

C. Line graph showing glucose (mg/dL) over minutes.
<table>
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<th>Name</th>
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