Transgenic Expression of Dominant-Active IDOL in Liver Causes Diet-Induced Hypercholesterolemia and Atherosclerosis in Mice

Anna C. Calkin, Stephen D. Lee, Jason Kim, Caroline M.W. Van Stijn, Xiao-Hui Wu, Aldons J. Lusis, Cynthia Hong, Rajendra I. Tangirala, Peter Tontonoz

Rationale: The E3 ubiquitin ligase inducible degrader of the low-density lipoprotein receptor (IDOL) triggers lysosomal degradation of the low-density lipoprotein receptor. The tissue-specific effects of the IDOL pathway on plasma cholesterol and atherosclerosis have not been examined.

Objective: Given that the liver is the primary determinant of plasma cholesterol levels, we sought to examine the consequence of effect of chronic liver-specific expression of a dominant-active form of IDOL in mice.

Methods and Results: We expressed a degradation-resistant, dominant-active form of IDOL (super IDOL [sIDOL]) in C57Bl/6J mice from the liver-specific albumin promoter (L-sIDOL transgenics). L-sIDOL mice were fed a Western diet for 20 or 30 weeks and then analyzed for plasma lipid levels and atherosclerotic lesion formation. L-sIDOL mice showed dramatic reductions in hepatic low-density lipoprotein receptor protein and increased plasma low-density lipoprotein cholesterol levels on both chow and Western diets. Moreover, L-sIDOL mice developed marked atherosclerotic lesions when fed a Western diet. Lesion formation in L-sIDOL mice was more robust than in apolipoprotein E*3 Leiden mice and did not require the addition of cholate to the diet. Western diet–fed L-sIDOL mice had elevated expression of liver X receptor target genes and proinflammatory genes in their aortas.

Conclusions: Liver-specific expression of dominant-active IDOL is associated with hypercholesterolemia and a marked elevation in atherosclerotic lesions. Our results show that increased activity of the IDOL pathway in the liver can override other low-density lipoprotein receptor regulatory pathways leading to cardiovascular disease. L-sIDOL mice are a robust, dominantly inherited, diet-inducible model for the study of atherosclerosis. (Circ Res. 2014;115:442-449.)

Key Words: atherosclerosis • cholesterol, LDL • ubiquitin-protein ligases

The E3 ligase inducible degrader of the low-density lipoprotein receptor (IDOL) ubiquinates and facilitates lysosomal degradation of the low-density lipoprotein receptor (LDLR).1 IDOL expression is controlled by the sterol-responsive liver X receptor (LXR) nuclear receptors. In response to elevated cellular cholesterol levels, LXRs induce IDOL expression, thereby causing degradation of the LDLR and inhibiting additional cholesterol uptake by the cell. The LXR–IDOL pathway provides a complementary mechanism to the sterol regulatory element–binding proteins for feedback regulation of LDL uptake by sterols. We have identified the molecular basis for IDOL target recognition and shown that loss of IDOL expression in cells alters LDLR protein levels and cholesterol uptake.2–3 However, the tissue-specific effects of the IDOL pathway on plasma cholesterol levels and cardiovascular disease are unknown. The ability of IDOL to affect atherosclerosis development in mice has not been addressed previously.

Human genetic studies support a link between the activity of the LXR–IDOL pathway and plasma lipid levels. Genome-wide association studies have identified noncoding variants within IDOL that are associated with LDL cholesterol levels.4 In addition, a coding single nucleotide polymorphism in IDOL, N342S, was associated with lower plasma cholesterol levels in Mexicans.5 Most recently, a complete loss-of-function IDOL allele was reported to be associated with low LDL levels.6

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The importance of the LDLR in the regulation of plasma cholesterol levels is well established. Loss-of-function LDLR mutations in humans reduce hepatic LDL clearance, elevate plasma LDL levels, and accelerate atherosclerosis. Moreover, the LDLR knockdown mouse is one of the most widely used models of hypercholesterolemia and atherosclerosis. The relative contribution of LDLR deletion in specific tissues to hypercholesterolemia and atherosclerosis in these mice has not been defined. Because the liver is the key organ for the uptake of LDL cholesterol, we sought to examine the consequence of chronic liver-specific expression of a dominant-active form of IDOL in mice. Our results show that chronic stable expression of IDOL effectively reduces hepatic LDLR protein levels and raises plasma LDL cholesterol levels and that this effect is durable over the life of the animal. Liver-selective IDOL transgenic mice are a robust, dominantly inherited, diet-inducible model for the study of atherosclerosis.

Methods

An expanded methods section is available in the online-only Data Supplement.

Mice

Briefly, mice were generated expressing a dominant-active form of IDOL (K293R, K309R, K310R, K320R) known as super IDOL (sIDOL) under the direction of the albumin enhancer/promoter. At 6 weeks of age, liver-specific sIDOL transgene (L-sIDOL) mice and their wild-type (WT) littersmates were fed a Western diet (WD; 21% fat 0.21% cholesterol; Research Diets) or maintained on a chow diet for 20 or 30 weeks as indicated. LDLR knockout mice and apolipoprotein E (ApoE)*3 Leiden mice were placed on a WD from 6 weeks of age for 20 weeks. All mice were maintained on a 12-hour light/dark cycle and had access to food and water ad libitum. Mice were fasted for 6 hours before euthanization. Animal studies were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the University of California, Los Angeles Animal Research Committee guidelines. Lesion area was quantified by en face and aortic root analysis.

Results

Generation of L-sIDOL Transgenic Mice

The E3 ubiquitin ligase IDOL is a potent post-transcriptional modifier of LDLR protein levels. In addition to targeting the LDLR for lysosomal degradation, IDOL also targets itself for proteasomal degradation through autoubiquitination. We previously identified lysine residues in the IDOL FERM domain critical for IDOL autoubiquitination and stability. An IDOL mutant lacking these residues (K293R, K309R, K310R, K320R) is unable to undergo autoubiquitination but maintains its ability to degrade the LDLR. We generated transgenic mice expressing this degradation-resistant, dominant-active form of human IDOL (sIDOL) in C57Bl/6J mice from the liver-specific albumin promoter (L-sIDOL transgenic mice; Figure 1A). Real-time polymerase chain reaction analysis confirmed that transgene expression was selective for liver (Figure 1B). Two independent lines of L-sIDOL mice exhibited a marked

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**Nonstandard Abbreviations and Acronyms**

ApoE: apolipoprotein E

IDOL: inducible degrader of the low-density lipoprotein receptor

LDLR: low-density lipoprotein receptor

L-sIDOL: liver-specific super inducible degrader of the low-density lipoprotein receptor transgene

LXR: liver X receptor

sIDOL: super inducible degrader of the low-density lipoprotein receptor

WD: Western diet

**Figure 1.** Generation of liver-specific super inducible degrader of the low-density lipoprotein receptor transgenic (L-sIDOL) mice. A, Schematic of the construct used to generate L-sIDOL mice demonstrating albumin (Alb) promoter, super IDOL (sIDOL), and bovine growth hormone poly A tail (bGH poly A). B, Expression of human IDOL mRNA in tissues normalized to Rplp0. C, Immunoblot of hepatic low-density lipoprotein receptor (LDLR) and actin expression in wild-type (WT) and L-sIDOL mice at 6 weeks of age on chow diet. D, Plasma cholesterol levels of mice on chow diet at 6 weeks of age. E, Hepatic mRNA expression of mouse LDLR normalized to Rplp0. Data are expressed as mean±SEM. ***P<0.001 vs matched wild type. Tg indicates transgenic.
reduction in hepatic LDLR protein expression, confirming increased IDOL activity (Figure 1C). We chose to perform subsequent studies on line no. 1 because this line was associated with slightly more hepatic LDLR degradation and higher plasma cholesterol levels than line no. 2 (Figure 1C and 1D).

As expected, there was no change in LDLR mRNA, consistent with the role of IDOL as a post-translational modifier of LDLR protein levels (Figure 1E).

L-sIDOL mice and their littermate controls were fed chow or WD for 20 or 30 weeks. There was no difference in body or liver weight between WT mice and L-sIDOL mice within their respective age or diet groups (Table; Online Table I). WD feeding was associated with increased weight gain across all groups at both time points. Male L-sIDOL mice showed a small reduction in hepatic fat mass as assessed by ex vivo MRI, consistent with reduced hepatic lipid uptake (P<0.05; Figure 2A). L-sIDOL expression did not cause overt hepatotoxicity because plasma alanine aminotransferase was not different from WT controls on chow or WD (Figure 2B). There was no significant difference in mouse IDOL or ATP-binding cassette transporter AI mRNA expression in the liver between WT and L-sIDOL transgenic mice across all groups (Figure 2C). Thus, the sIDOL transgene does not seem to provoke a compensatory response in the activity of the endogenous LXR pathway in the liver.

Stable Expression of IDOL in Mouse Liver Raises Plasma LDL Cholesterol Levels

Plasma cholesterol levels were markedly elevated in L-sIDOL mice not only in association with a WD but even on a chow diet (Figure 3A and 3B). This effect was even more profound after 30 weeks (P<0.001). However, L-sIDOL mice did not develop hypercholesterolemia to the level seen in LDLR knockout mice on WD, likely reflecting either residual hepatic LDLR expression or preserved peripheral LDLR expression, in L-sIDOL mice (P<0.001; Figure 3C). High-performance liquid chromatography profiling of plasma lipid distribution revealed that the increase in cholesterol could be attributed primarily to an increase in the LDL fraction (Figure 3D and 3G). Consistent with the high-performance liquid chromatographic data, we observed increased expression of ApoB protein levels in both chow and WD-fed L-sIDOL mice by immunoblotting.

### Table. Male Mouse Metabolic Data

<table>
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<td>TGs, mmol/L</td>
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<td>0.46±0.04</td>
<td>0.67±0.04</td>
<td>1.00±0.06</td>
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</tr>
</tbody>
</table>

BW indicates body weight; KO, knockout; LDLR, low-density lipoprotein receptor; L-sIDOL, albumin driven super inducible degrader of the low-density lipoprotein receptor transgenic mice; LW, liver weight; TGs, triglycerides; WD, Western diet; and WT, wild type.

*Not included in statistical analysis.

†P<0.001 vs matched wild type.

‡P<0.01.
Liver IDOL Expression Induces Atherosclerosis

Stable Expression of IDOL in Mouse Liver Promotes Atherosclerosis

To determine the impact of chronic expression of IDOL and the consequent downregulation of hepatic LDLR protein on the development of cardiovascular disease, we analyzed atherosclerosis with both en face and aortic root section approaches. En face analysis after 20 weeks on WD revealed substantial lesion development in the aortas of L-sIDOL mice, whereas WT controls developed no detectable lesions at this time point (Figure 4A and 4B). The extent of lesions in L-sIDOL mice was further enhanced when they were maintained on WD for 30 weeks. Interestingly, lesion formation in L-sIDOL mice was highly diet dependent because only minimal lesions were seen on chow diet (Figure 4A and 4B).

We observed similar effects of L-sIDOL expression on atherosclerosis development in the aortic root (Figure 5A). L-sIDOL mice had a marked increased in lesion area compared with the negligible lesion development in WT mice (P<0.001). Hematoxylin and eosin suggested that some lesions were beginning to develop an acellular core (Figure 5B, top). Trichrome staining further revealed collagen deposition in lesions of L-sIDOL mice (Figure 5B, bottom). Immunohistochemical staining for CD68 confirmed that the lesions formed in L-sIDOL aortas were macrophage rich (Figure 5B, middle bottom). Overall, the architecture of the L-sIDOL lesions was consistent with early lesions observed in LDLR knockout mice.

We next assessed gene expression in the aortas of WT and L-sIDOL mice maintained on WD. Real-time polymerase chain reaction analysis of mRNA expression in whole aorta revealed an upregulation of the LXR target genes IDOL and...
apoptosis inhibitor expressed in macrophages, consistent with increased sterols within the vessel wall of the lesion-rich transgenic mice (Figure 6). We also observed an upregulation in inflammatory genes, including the p65 subunit of nuclear factor-κB, relA (v-rel avian reticuloendotheliosis viral oncogene homolog A [p65]), as well as the proatherogenic vascular cell adhesion molecule-1 (Figure 6). Increased scavenger receptor A was also observed, consistent with increased macrophage content (Figure 6).

Because the L-sIDOL mice represent a new monoallelic mouse model of atherogenesis, we compared plasma lipid levels and lesion development between L-sIDOL mice and 2 widely used mouse models, LDLR knockout mice and ApoE*3 Leiden mice. In comparison with LDLR knockout mice, the phenotype of L-sIDOL mice is less severe, both in terms of the degree of plasma LDL cholesterol elevation and the extent of lesion development by en face and root section analysis (Figure 7A–7C). The higher LDL cholesterol levels...

Figure 4. En face atherosclerosis analysis in liver-specific super inducible degrader of the low-density lipoprotein receptor transgenic (L-sIDOL) mice. A, Representative pictures of en face assessment of atherosclerotic lesions in chow and Western diet–fed male wild-type (WT) and L-sIDOL mice after 20 weeks and 30 weeks of diet. B, Quantification of en face aortic lesions area in mice as indicated. **P<0.01 vs matched WT. Tg indicates transgenic.

Figure 5. Liver-specific super inducible degrader of the low-density lipoprotein receptor transgenic (L-sIDOL) mice develop atherosclerosis in the aortic root. A, Quantification of aortic root lesions in male mice after 30 weeks of Western diet. B, Representative pictures of aortic root lesions in wild-type (WT) and L-sIDOL mice stained with hematoxylin and eosin (H&E; top), Oil red O (top middle), CD68 (bottom middle), and trichrome (bottom) at magnifications as indicated. Tg indicates transgenic.
in LDLR knockout mice, despite the severely reduced hepatic LDLR protein levels in L-sIDOL mice, are suggestive of a significant role for extrahepatic LDLR in LDL clearance in this setting. We also compared L-sIDOL mice with female ApoE*3 Leiden mice because lesion development is reported to be greatest in females in this strain as a result of higher cholesterol and triglyceride levels than in males.8 When both L-sIDOL and ApoE*3 Leiden mice were fed WD for 20 weeks, L-sIDOL transgenic mice developed substantially more lesions as assessed by both en face and aortic root section analysis (Figure 7D and 7E). Plasma total and LDL cholesterol levels were comparable between L-sIDOL and ApoE*3 Leiden mice on WD (L-sIDOL: 11.87±1.16 mmol/L [n=10]; E3L: 13.28±0.63 mmol/L [n=8]; Figure 7F). Thus, L-sIDOL mice fed WD developed more severe atherosclerosis, despite similar plasma cholesterol levels. Consistent with the ApoE mutation, however, ApoE*3 Leiden mice had higher levels of very-low-density lipoprotein cholesterol compared with L-sIDOL mice.

In summary, chronic stable expression of IDOL in mouse liver is sufficient to durably increase plasma LDL cholesterol levels and promote substantial atherosclerotic lesion formation. Atherosclerosis development in L-sIDOL mice is robust for a transgenic mouse on the C57BL/6J background in the absence of a concurrent genetic lesion in LDLR or ApoE. Furthermore, lesion formation in this model does not require the addition of cholate to the diet.

**Discussion**

We have shown here that chronically increased hepatic IDOL activity results not only in elevated plasma cholesterol levels but also marked atherosclerotic lesion deposition on a WD. Our data indicate that dominant activation of the IDOL pathway in the mouse liver can override other LDLR regulatory pathways leading to cardiovascular disease.

Prior work has established that susceptibility to atherosclerosis differs markedly between mouse strains.9 In C57Bl/6J mice, there seems to be a threshold above which atherosclerosis development is observed. In our study, feeding C57Bl/6J mice WD raised plasma cholesterol levels to a measureable degree,
but we observed virtually no lesion development. Introduction of the L-sIDOL transgene further increased plasma cholesterol levels in response to WD feeding and this resulted in substantial lesion formation. Interestingly, although the plasma cholesterol levels in L-sIDOL mice on WD are below those of LDLR knockout mice, they are clearly above the threshold for lesion development because we observe robust atherosclerosis in the aortic arch. The lower cholesterol levels seen in L-sIDOL mice compared with LDLR knockout mice may be because of low level residual hepatic LDLR expression in L-sIDOL transgenic mice; however, it is also possible that extrahepatic LDLR expression contributes meaningfully to LDL clearance in a setting in which hepatic LDLR expression is lost. No tissue-specific knockout models of the LDLR exist, so this has not been directly addressed; however, some studies have suggested a role for intestinal LDLR in systemic cholesterol regulation.10

There are several advantages to a monoallelic transgenic mouse model for the study of atherosclerosis. Most importantly, the breeding strategy required to generate a compound mutant is greatly simplified, saving both time and money. L-sIDOL mice represent an attractive new model to test the effect of other transgenic or knockout alleles on atherosclerosis. Lesion deposition in L-sIDOL mice is highly dependent on diet, with few lesions being observed in chow-fed mice. In this respect, L-sIDOL mice are similar to LDLR knockout mice but different from ApoE knockout mice.11 The fact that lesion deposition is driven by diet makes L-sIDOL mice an attractive model system. It is also notable that L-sIDOL mice develop substantial atherosclerotic lesion formation on standard WD formulation (41% calories from fat, 0.2% cholesterol). Unlike what has been reported for some other monoallelic atherosclerosis models, L-sIDOL mice do not require high levels of dietary cholesterol or the addition of cholate to induce lesion formation.12 Also, because the LDLR is still genetically present in L-sIDOL mice, pathways that affect LDLR expression or function could potentially still be studied with this model.

A further advantage of the L-sIDOL mice is the greater similarity of the lipid profile to humans compared with other mouse models. In contrast to some other transgenic models of hypercholesterolemia, L-sIDOL mice carry the majority of their cholesterol in the LDL fraction and thus represent a more humanized model. L-sIDOL mice are distinct from ApoE knockout mice and ApoE*3 Leiden mice, which carry much of their cholesterol in the very-low-density lipoprotein fraction, a profile that is not seen in humans.8,13 ApoE*3 Leiden mice have been bred with CETP (cholesteryl ester transfer protein) transgenic mice to exhibit or a more humanized lipid profile;14 however, a complex breeding strategy is required to introduce the transgenic alleles into a transgenic or knockout line of interest. We observed substantially greater lesion formation in L-sIDOL mice in a direct comparison to ApoE*3 Leiden mice after 20 weeks of WD feeding. Most published studies using ApoE*3 Leiden mice have used more extreme diets, typically containing 0.5% to 1.0% wt/wt cholesterol. Many studies also included 0.1% to 0.5% cholate to increase the absorption of fat and cholesterol.15 A smaller number of studies have used diets comparable to the WD used here.14,16

The majority of lesions observed in the aortic root of L-sIDOL mice are early lesions comprised primarily of foam cells and smooth muscle cells, with some, such as that shown in Figure 5, beginning to develop an acellular core. We have not observed the highly advanced lesions that can be observed with the LDLR knockout or ApoE knockout models. Thus, at the present time, our model, like the ApoE*3 Leiden model, seems best suited to the study of early lesion formation. It remains possible that more severe feeding protocols might further enhance lesion severity and complexity in L-sIDOL mice, and we are actively testing alternate regimens. Westerterp et al16 demonstrated that although ApoE*3 Leiden mice can develop complex lesions, the vast majority of lesions observed were early lesions comprised fatty streaks and foam cells.16 ApoE*3 Leiden mice must be crossed with hCETP (human cholesteryl ester transfer protein) transgenic mice to develop severe lesions on a WD.14,16 Interestingly, although it is difficult to compare lesion quantifications performed in different laboratories, prior studies have estimated the total atherosclerotic burden in the aortic root of ApoE*3 Leiden mice to be 50,000 μm² after 19 weeks on a WD.14,16 These findings are in excellent agreement with our quantification of the ApoE*3 Leiden mice on 20 weeks of WD, which showed a mean total lesion area of 47,000 μm².

In summary, hepatic expression of dominant-active IDOL results in diet-induced hypercholesterolemia and atherosclerotic lesion deposition. These data establish that increased activity of the IDOL pathway in the liver can override other LDLR regulatory mechanisms and result in cardiovascular disease. In addition, we anticipate that L-sIDOL mice will prove to be a valuable tool for the study of atherosclerosis development in transgenic and knockout models.

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**Disclosures**
None.

**References**
Novelty and Significance

What Is Known?

- The low density lipoprotein receptor (LDLR) plays an important role in the regulation of plasma cholesterol levels, and the LDLR null mouse is a common model of atherosclerosis.
- Inducible degrader of the low-density lipoprotein receptor (IDOL), is an E3 ligase that targets the LDLR for ubiquitination and subsequent degradation and can modulate plasma cholesterol levels in humans.

What New Information Does This Article Contribute?

- Chronic hepatic expression of a dominant-active form of IDOL, (liver-specific super IDOL transgene) is associated with marked hypercholesterolemia and atherosclerosis in a diet-inducible manner.
- Liver-specific super IDOL transgenic mice are a novel mouse model to study atherosclerosis, with potential advantages compared with other models related to their lipid profile, breeding strategy, and susceptibility to lesions on diet.

Current mouse models of atherosclerosis have limitations, including the use of extreme diets to generate atherosclerotic lesions, nonhuman-like lipid profiles, or the need for complex breeding strategies to study a gene of interest. The LDLR is well established to play a role in hypercholesterolaemia, and the LDLR null mouse is one of the most common models of atherosclerosis, but the relative contribution of specific tissues to this phenotype is currently unknown. We aimed to determine the effect of chronic hepatic LDLR degradation by a dominant-active form of the E3 ligase IDOL in mice. Liver-specific super IDOL transgenic mice exhibited degradation of hepatic LDLR protein and marked hypercholesterolaemia. Lesion deposition was observed in the aortic arch and aortic root in association with a Western diet. Our data demonstrate that the hepatic IDOL–LDLR pathway can override other LDLR pathways, leading to vascular disease. In summary, liver-specific super IDOL transgenic mice are a robust, dominantly inherited, diet-inducible model of atherosclerosis. Advantages over other models include their human-like lipid profile, their susceptibility to develop lesions without being placed on an extreme dietary regimen, and a simplified breeding strategy to test a transgenic or knockout alleles of interest, saving both time and money.
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Online Methods

Mice
Transgenic mice were generated at the UCLA core facility. A dominant active form of IDOL (K293R, K309R, K310R, K320R) known as super IDOL (sIDOL) was expressed in mice under the direction of the albumin enhancer/promoter. A pBluescript vector containing the albumin enhancer/promoter followed by sIDOL and bovine growth hormone polyA was generated. The linearized construct was gel purified and microinjected into C57B/6J mice fertilised zygotes. Founders were identified using the following PCR primers (For-GCCAGGGGCTCTGTACA; Rev-GAATGAATGACACCTACTCAGACAAT). At 6 weeks of age L-sIDOL mice and their wild type littermates were fed a western diet (WD; 21% fat 0.21% cholesterol; Research Diets; n=69) or maintained on a chow diet (n=46) for 20 or 30 weeks as indicated. LDLR KO mice (n=7) were purchased from Jackson Laboratories at 5 weeks of age and placed on a WD from 6 weeks of age for 20 weeks. The ApoE*3 Leiden mice were generously provided to Jake Lusis by Louis M. Havekes in 2008. The strain designation is E3L. Since being received at UCLA they have been crossed to the C57BL/6J background. ApoE*3 Leiden mice (n=9) were placed on a WD from 6 weeks of age for 20 weeks. All mice were maintained on a 12-hour light/dark cycle and had access to food and water ad libitum. Mice were fasted for 6 hours prior to sacrifice. Animal studies were carried out in accordance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals and the UCLA Animal Research Committee guidelines.

Hepatic Fat Mass
Hepatic fat mass was assessed using a 3-in-1 magnetic resonance imager (EchoMRI). A lobe of the liver was excised from mice and placed in ice cold PBS until the time of analysis. Data was expressed as tissue fat mass as a percentage of total tissue mass (fat and lean mass).

Plasma Metabolites
Plasma cholesterol (Wako) and triglyceride (Wako) levels were measured by colourimetric assay according to the manufacturers instructions. Alanine aminotransferase (ALT) levels were measured by enzymatic assay according to the manufacturers instructions (Thermo Fisher).

Lipid Profiles
Cholesterol and triglyceride concentrations were determined by size-exclusion chromatography as previously described. An aliquot of plasma containing 25µg of
cholesterol or triglyceride was diluted with buffer (0.9% saline with 0.01% sodium azide/EDTA) to a final volume of 0.2mL. 15µg of cholesterol or triglyceride was then loaded onto the column (Superose 6 100/300 GL; GE Healthcare). The detector response was converted to concentration (mmol/L) to allow for comparison between groups.

**En face Lesion Analysis**

Mice were euthanized and perfused with 7.5% sucrose in paraformaldehyde. Aortas were subsequently excised, cleaned of adventitial fat, pinned and stained with Sudan IV. Images were taken with a CCD camera. Computer assisted image analysis of the aortic arch was performed as described previously. Lesions were quantitated as percentage surface area covered.

**Aortic Root Analysis**

Excised aortas were embedded in OCT (Sakura Finetech) and stored at -80°C. Aortic root sections were quantified as previously reported. Briefly, serial 10 µM thick cryosections were cut from the middle portion of the left ventricle of the aortic root. Alternate sections obtained from the region spanning the appearance to the disappearance of the aortic valves were placed on poly-L-lysine coated slides (n=10 sections/mouse). Sections were stained with oil red O (Sigma) and counter-stained with haematoxylin. The mean cross-sectional lesion area was determined for each mouse as square micrometers per section in a blinded fashion. Sections were stained to assess lesion architecture with haematoxylin and eosin using standard methods. Sections were stained for collagen using the standard Masson’s Trichrome method. Immunohistochemical studies were performed as previously described. Briefly, cryosections were mounted on poly-D-lysine coated plates, fixed using dry acetone and blocked with normal rabbit serum (Vector Laboratories). Macrophages were detected using an anti-CD68 antibody (AbD Serotec). Sections were incubated with biotinylated secondary antibody followed by avidin/biotinylated enzyme complex-alkaline phophatase (Vector Laboratories) and vector red substrate (Vector Laboratories) then counterstained with haematoxylin. Sections were visualised using a Zeiss upright microscope equipped with an Axiocam 506 colour camera.

**Western Blotting**

Liver tissue was solubilised in RIPA buffer (Boston Bioproducts; Tris-HCl 50mM, pH 7.4, NaCl 150mM, NP-40 1%, Sodium deoxycholate 0.5%, SDS 0.1%) supplemented with protease inhibitors (Roche Diagnostics) using a Dounce homogeniser. Lysates were clarified by centrifugation then quantified using the Bradford assay (Biorad) with BSA as a reference.
For apolipoprotein distribution, 50µL of plasma was brought to 0.4mL above buffer (0.9% saline with 0.01% sodium azide/EDTA) and 0.2mL injected into an HPLC column (Superose 6 100/300 GL; GE Healthcare) and fractions were collected. Samples were separated on Nupage Bis-Tris gels then transferred to polyvinylidene difluoride membranes (GE Osmonics). Membranes were probed with antibodies against LDLR (Cayman Chemical Company), β-actin (Sigma), apolipoprotein A-I (Meridian Life Sciences) or apolipoprotein B (Abcam). Appropriate horseradish peroxidase-conjugated secondary antibodies were used (Invitrogen, Biorad) and blots visualized with chemiluminescence (Amersham).

**RNA isolation and quantitative PCR**

Excised aorta was cleaned of adventitial fat and stored in RNA later at -20°C until RNA extraction. An RNA isolation kit (Qiagen) was used to extract RNA. For liver, muscle and adipose samples, RNA was extracted using Trizol (Invitrogen) according to the manufacturer’s instructions. RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-rad). Quantitative PCR was performed using SYBR green (Diagenode) on an Applied Biosystems 7900HT sequence detector. Gene expression was normalised to rplp0. For primer sequences see Online Table II.

**Statistical Analysis**

An unpaired students t-test with or without Welch’s correction, a Mann Whitney test or analysis of variance (ANOVA) with Tukey’s multiple comparison test were performed where appropriate. Statistical significance was determined as p<0.05. Data is expressed as mean ± standard error of the mean (SEM).
<table>
<thead>
<tr>
<th></th>
<th>Chow 20 weeks</th>
<th>WD 20 weeks</th>
<th>Chow 30 weeks</th>
<th>WD 30 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>L-sIDOL</td>
<td>WT</td>
<td>L-sIDOL</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>4</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Body weight</td>
<td>26.5±1.1</td>
<td>23.3±0.7</td>
<td>37.6±1.0</td>
<td>36.5±2.1</td>
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<tr>
<td>Liver Weight</td>
<td>1.0±0.0</td>
<td>1.0±0.0</td>
<td>1.8±0.1</td>
<td>2.1±0.3</td>
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<tr>
<td>LW/BW (%)</td>
<td>3.8±0.2</td>
<td>4.1±0.1</td>
<td>5.0±0.3</td>
<td>5.5±0.4</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.46±0.05</td>
<td>0.46±0.02</td>
<td>0.29±0.02</td>
<td>0.45±0.05**</td>
</tr>
</tbody>
</table>

**Online Table I. Female mouse metabolic data.** BW – body weight; LW – liver weight; TGs - triglycerides; L-sIDOL – Albumin driven super IDOL transgenic mice; WT – wild type. * p<0.05, **p<0.01 vs matched wild type.
<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward primer 5’-3’</th>
<th>Reverse primer 5’-3’</th>
</tr>
</thead>
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<tr>
<td>mRplp0</td>
<td>AGATGCAGCAGATCCGCA</td>
<td>GTTCTTGCCCATCACGCACC</td>
</tr>
<tr>
<td>mABCAI</td>
<td>GGGTTGGAGATGGTATACAAATAGTTGT</td>
<td>CCCGGAAACGCAAATCC</td>
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<td>CTTCCACAGCGGTGGGCA</td>
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<tr>
<td>mICAM</td>
<td>CCCACGCTACCTCTGCCT</td>
<td>GATGGATACCTGACCATCACC</td>
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<td>mIDOL</td>
<td>TTGGAGGCCTACCTCTCCTGTCTT</td>
<td>AGGGACTCTTTTAATGTGGAAG</td>
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<tr>
<td>mLDR</td>
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<td>TGCGGTCCAGGGTCTCCT</td>
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<td>mRelA</td>
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<td>GCTCCAGGTCTCGCTTCTTT</td>
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<td>mSra</td>
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<td>mSTAT6</td>
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<td>hIDOL</td>
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**Online Table II. Primer sequence.** m = mouse; h = human.
REFERENCES


