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Permalink

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Journal

Circulation Research, 115(4)

ISSN

0009-7330

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Publication Date

2014-08-01

DOI

10.1161/circresaha.115.304440

Peer reviewed

Published in final edited form as:

Circ Res. 2014 August 1; 115(4): 442–449. doi:10.1161/CIRCRESAHA.115.304440.

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

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Abstract

Rationale—The E3 ubiquitin ligase IDOL triggers lysosomal degradation of the LDL 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 (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 LDLR protein and increased plasma LDL 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 ApoE*3 Leiden mice and did not require the

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In May 2014, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 14.87 days.

DISCLOSURES

None.

addition of cholate to the diet. Western diet-fed L-sIDOL mice had elevated expression of LXR target genes and pro-inflammatory 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 LDLR regulatory pathways leading to cardiovascular disease. L-sIDOL mice are a robust, dominantly-inherited, diet-inducible model for the study of atherosclerosis.

Keywords

Atherosclerosis; lipoprotein metabolism; mouse model; E3 ubiquitin ligase; LDL cholesterol; nuclear receptor

INTRODUCTION

The E3 ligase inducible degrader of the LDL receptor (IDOL) ubiquitinates and facilitates lysosomal degradation of the LDL receptor (LDLR).¹ IDOL expression is controlled by the sterol-responsive 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 SREBPs 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 non-coding variants within IDOL that are associated with LDL cholesterol levels.⁴ In addition, a coding single nucleotide polymorphism (SNP) 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

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.⁷ Moreover, the LDLR KO 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. As 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 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 L-sIDOL mice and their wild type littermates 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 KO mice and 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 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. Lesion area was quantitated 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.² 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; Fig. 1A). Realtime PCR analysis confirmed that transgene expression was selective for liver (Fig. 1B). Two independent lines of L-sIDOL mice exhibited a marked reduction in hepatic LDLR protein expression, confirming increased IDOL activity (Fig. 1C). We chose to perform subsequent studies on line #1, as this line was associated with slightly more hepatic LDLR degradation and higher plasma cholesterol levels than line #2 (Fig. 1C, D). As expected, there was no change in LDLR mRNA, consistent with the role of IDOL as a post-translational modifier of LDLR protein levels (Fig. 1E).

L-sIDOL mice and their littermate controls were fed chow or Western diet (WD) for 20 or 30 weeks. There was no difference in body or liver weight between wild-type (WT) mice and L-sIDOL mice within their respective age or diet groups (Table 1 and 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* magnetic resonance imaging, consistent with reduced hepatic lipid uptake ($p < 0.05$; Fig. 2A). L-sIDOL expression did not cause overt hepatotoxicity, as plasma ALT was not different from WT controls on chow or WD (Fig. 2B). There was no significant difference in mouse IDOL or ABCA1 mRNA expression in the liver between WT and L-sIDOL transgenic mice

across all groups (Fig. 2C). Thus, the sIDOL transgene does not appear 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 (Fig. 3A, B). 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 KO mice on WD, likely reflecting either residual hepatic LDLR expression or preserved peripheral LDLR expression, in L-sIDOL mice ($p < 0.001$; Fig. 7C). HPLC profiling of plasma lipid distribution revealed that the increase in cholesterol could be attributed primarily to an increase in the LDL fraction (Fig. 3D, G). Consistent with the HPLC data, we observed increased expression of ApoB protein levels in both chow and WD-fed L-sIDOL mice by immunoblotting (Fig. 3C, F). In contrast to LDL cholesterol, we observed little difference in HDL cholesterol fractions as assessed by HPLC profiling (Fig. 3D, G), and there was no difference ApoA-I protein expression (Fig. 3C, F). Interestingly, chow-fed male L-sIDOL mice showed a reduction in the VLDL triglyceride levels compared to WT, but this difference was not observed in females or when the mice were fed WD (Fig. 3E, H; Table 1, Supplemental Table 1). Similar to LDLR KO mice, L-sIDOL mice exhibited elevated plasma triglyceride levels in the VLDL and LDL fractions compared to WT littermates when maintained on WD (Table 1, Online Table I, Fig. 3H), although these values were not elevated to the levels seen in LDLR KO mice (Table 1).

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 analysed 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 (Fig. 4A, B). 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, as only minimal lesions were seen on chow diet (Fig. 4A, B).

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

We next assessed gene expression in the aortas of WT and L-sIDOL mice maintained on WD. Real-time PCR analysis of mRNA expression in whole aorta revealed an upregulation of the LXR target genes IDOL and AIM, consistent with increased sterols within the vessel wall of the lesion-rich transgenic mice (Fig. 6). We also observed an upregulation in

inflammatory genes, including the p65 subunit of NK- κ B, relA, as well as the proatherogenic vascular cell adhesion marker 1, VCAM-1 (Fig. 6). Increased scavenger receptor A (SR-A) was also observed, consistent with increased macrophage content (Fig. 6).

As 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 two widely-used mouse models, LDLR KO mice and ApoE*3 Leiden mice. In comparison to LDLR KO 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 (Fig. 7A–C). The higher LDL cholesterol levels in LDLR KO mice, despite the severely reduced hepatic LDLR protein levels in L-sIDOL mice, are suggestive of a significant role for extra-hepatic LDLR in LDL clearance in this setting. We also compared L-sIDOL mice to female ApoE*3 Leiden mice, as lesion development is reported to be greatest in females in this strain due to higher cholesterol and triglyceride levels than in males.¹⁹ 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 (Fig. 7D, E). Plasma total and LDL cholesterol levels were comparable between L-sIDOL and ApoE*3 Leiden mice on WD (L-sIDOL: 11.87 \pm 1.16 mmol/L (n=10), E3L: 13.28 \pm 0.63 mmol/L (n=8); Fig. 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 VLDL cholesterol compared to 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.²⁰ In C57BL/6 mice, there appears 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 KO mice, they are clearly above the threshold for lesion development, since

we observe robust atherosclerosis in the aortic arch. The lower cholesterol levels seen in L-sIDOL mice compared to LDLR KO mice may be due to 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 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.²¹

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 upon diet, with few lesions being observed in chow-fed mice. In this respect, L-sIDOL mice are similar to LDLR KO mice but different from ApoE KO mice.²² 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.²³ Also, since 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 as compared to 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 KO mice and ApoE*3 Leiden mice, which carry much of their cholesterol in the VLDL fraction, a profile that is not seen in humans.^{19, 24} ApoE*3 Leiden mice have been bred with CETP transgenic mice to exhibit a more “humanized” lipid profile;²⁵ 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 employed more extreme diets, typically containing 0.5–1.0% w/w cholesterol. Many studies also included 0.1–0.5% cholate to increase the absorption of fat and cholesterol.²⁶ A smaller number of studies have utilized diets comparable to the WD used here.^{25, 27}

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 Fig. 5, beginning to develop an acellular core. We have not observed the highly advanced lesions that can be observed with the LDLR KO or ApoE KO models. Thus, at the present time our model, like the ApoE*3 Leiden model, appears best suited to the study of early lesion formation. It remains possible that more extreme dietary feeding protocols might further enhance lesion severity and complexity in L-sIDOL mice, and we are actively testing alternate regimens. Westerterp et al. demonstrated that while ApoE*3 Leiden mice can

develop complex lesions, the vast majority of lesions observed were early lesions comprised of fatty streaks and foam cells.^{25, 27} ApoE*3 Leiden mice must be crossed with hCETP transgenic mice in order to develop severe lesions on a WD.^{25, 27} Interestingly, although it is difficult to compare lesion quantitations 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 μm^2 after 19 weeks on a WD.^{25, 27} 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 μm^2 .

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

SOURCES OF FUNDING

ACC was funded by a National Heart Foundation of Australia Overseas Fellowship (O 08M 3934). P.T. is an Investigator of the Howard Hughes Medical Institute. This work was supported by NIH grant R01 HL066088.

Nonstandard Abbreviations and Acronyms

AIM	Apoptosis inhibitor expressed in macrophages
ABCAI	ATP-binding cassette transporter AI
ALT	Alanine aminotransferase
ApoE	Apolipoprotein E
BSA	Bovine serum albumin
CCD	Couple-charged device
CD	Cluster of differentiation
EDTA	Ethylenediaminetetraacetic acid
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
ICAM	Intracellular adhesion molecule
IDOL	Inducible degrader of the LDLR
KO	Knockout

LDLR	Low-density lipoprotein receptor
L-sIDOL	Liver-specific super IDOL transgene
LXR	Liver X receptor
OCT	Optimal cutting temperature
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RelA	v-rel avian reticuloendotheliosis viral oncogene homolog A (p65)
SNP	Single nucleotide polymorphism
SR-A	Scavenger receptor A
SREBP	Sterol regulatory element-binding protein
STAT	Signal transducer and activator of transcription
Tg	Transgenic
VCAM	Vascular cell adhesion molecule
VLDL	Very low-density lipoprotein
VEGF	Vascular endothelial growth factor
WD	Western Diet

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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 LDLR, 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, (L-sIDOL) is associated with marked hypercholesterolemia and atherosclerosis in a diet-inducible manner.
- L-sIDOL mice are a novel mouse model to study atherosclerosis with potential advantages over 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, non-“human-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 are 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. L-sIDOL 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, L-sIDOL 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.

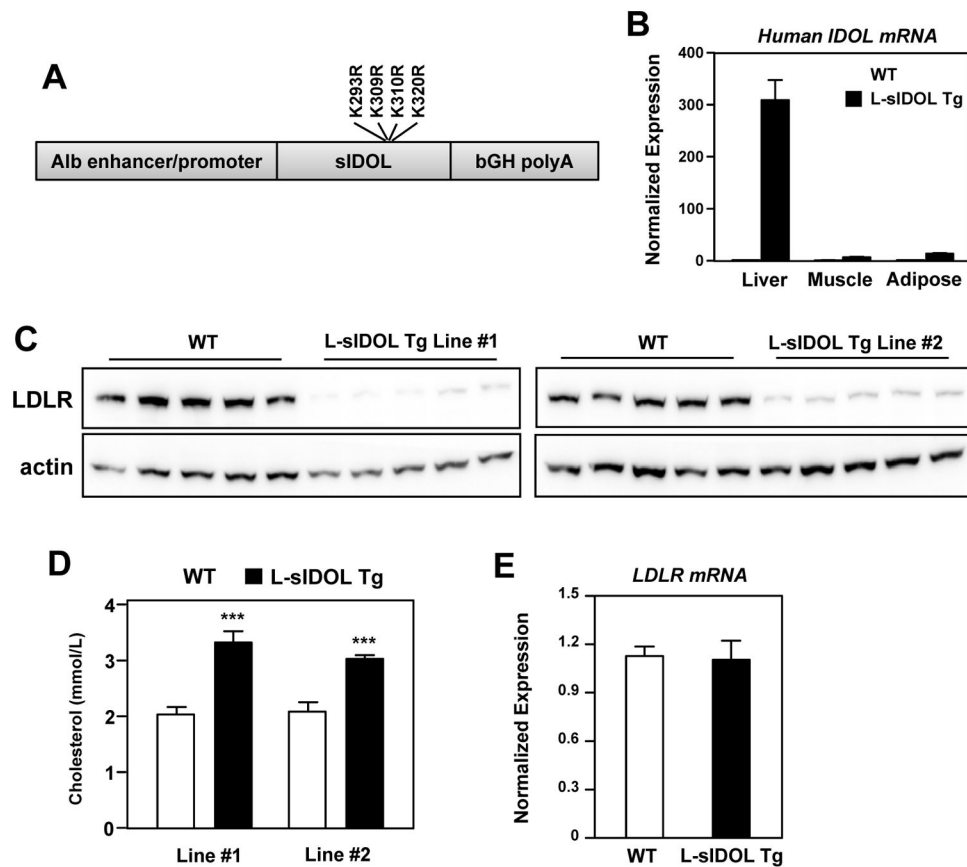


Figure 1. Generation of L-sIDOL transgenic mice

(A) Schematic of the construct used to generate L-sIDOL mice demonstrating albumin (Alb) promoter, super IDOL and bovine growth hormone poly A tail (bGH polyA); (B) Expression of human IDOL mRNA in tissues normalised to *Rplp0*; (C) Immunoblot of hepatic 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 normalised to *Rplp0*; Data is expressed as mean \pm SEM. *** $p < 0.001$ vs matched wild-type.

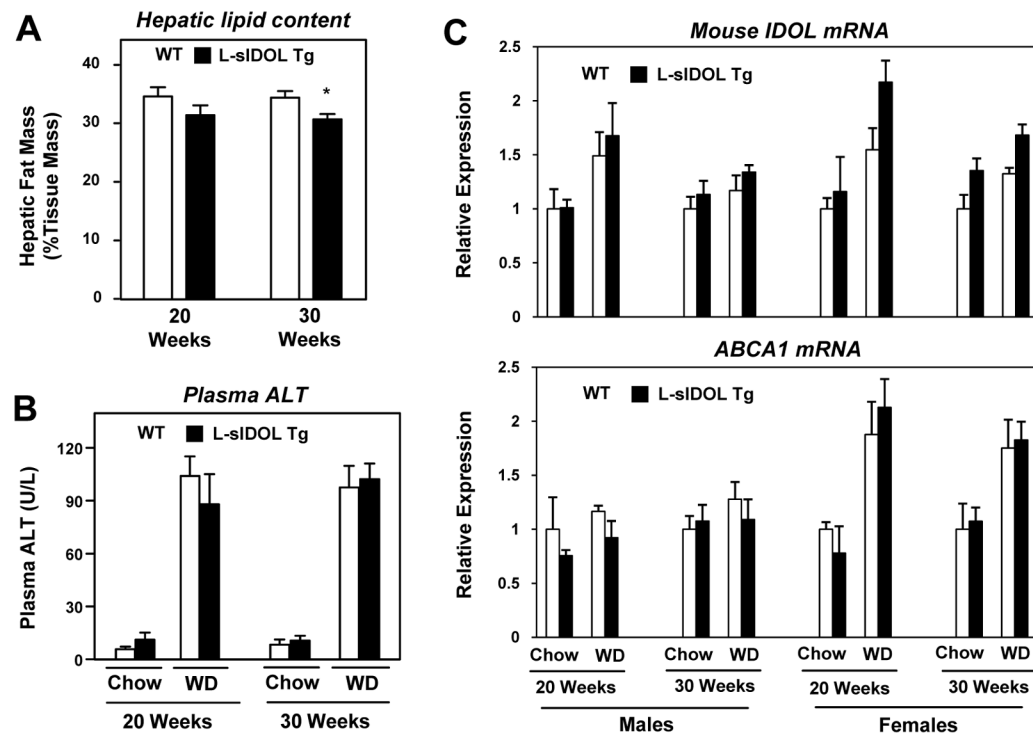


Figure 2. Effect of L-sIDOL expression on hepatic function and gene expression

(A) Hepatic fat mass (as assessed by magnetic resonance imaging) in male mice fed a western diet for duration as indicated; (B) Plasma alanine aminotransferase (ALT) levels in male mice on diets for durations as indicated; (C) Hepatic mRNA expression of mouse IDOL and ABCA1 in mice on diet as indicated. Data normalised to *Rplp0* and to chow fed WT mice at each time point for males and females; Data is expressed as mean \pm SEM.

* $p < 0.05$ vs matched wild-type.

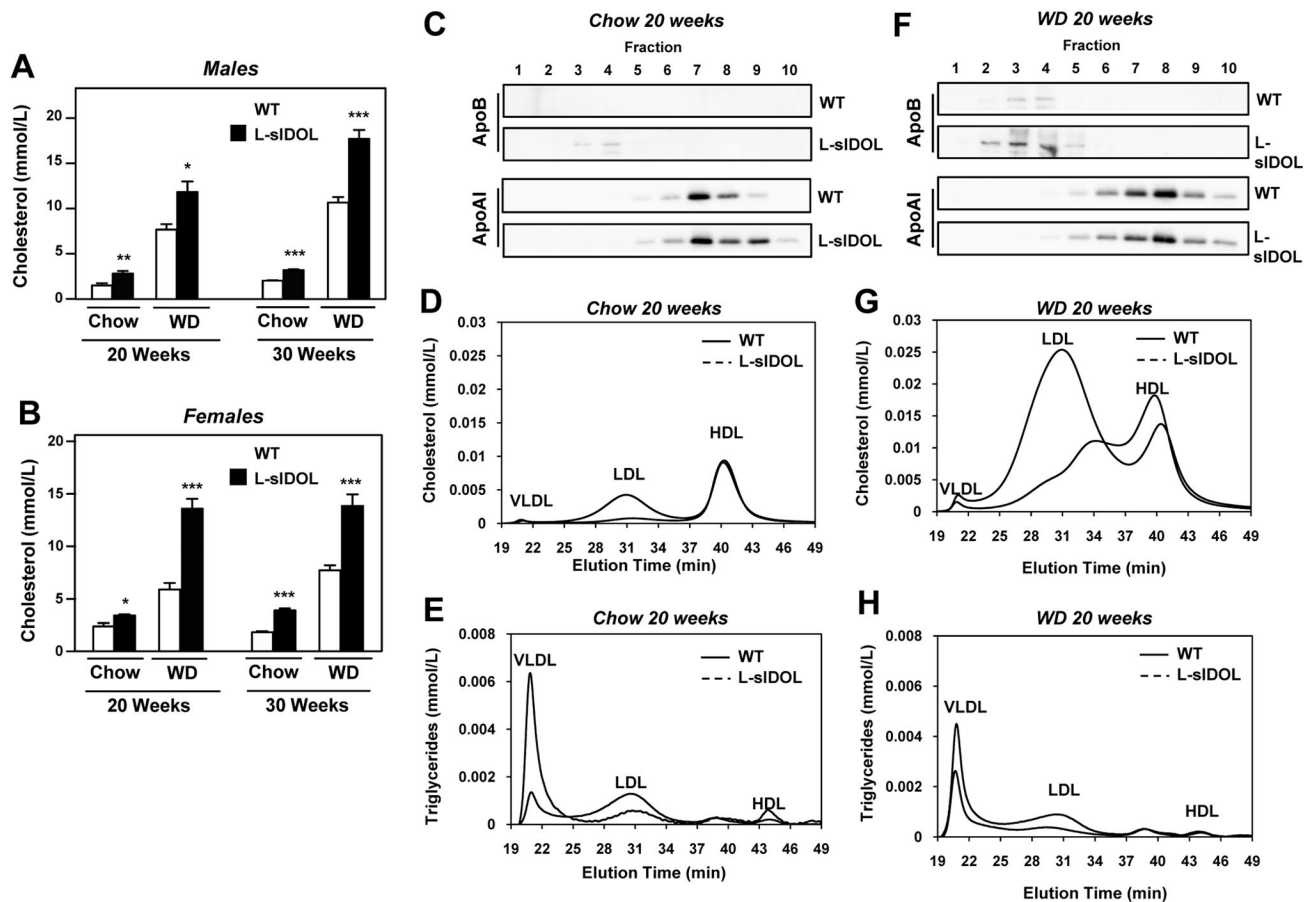


Figure 3. Altered plasma lipid and apolipoprotein profiles in L-sIDOL mice

(A, B) Plasma cholesterol levels in male and female mice on diets for durations as indicated; (C, F) Immunoblot of apolipoprotein B (ApoB) and apolipoprotein A-I (ApoA-I) in fractionated plasma for mice on 20 weeks diet as indicated; (D, G) HPLC chromatogram of plasma cholesterol after 20 weeks on chow or western diet (WD); (E, H) HPLC chromatogram of plasma triglycerides after 20 weeks on chow or western diet (WD); Data is expressed as mean \pm SEM (A, B). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs matched wild-type.

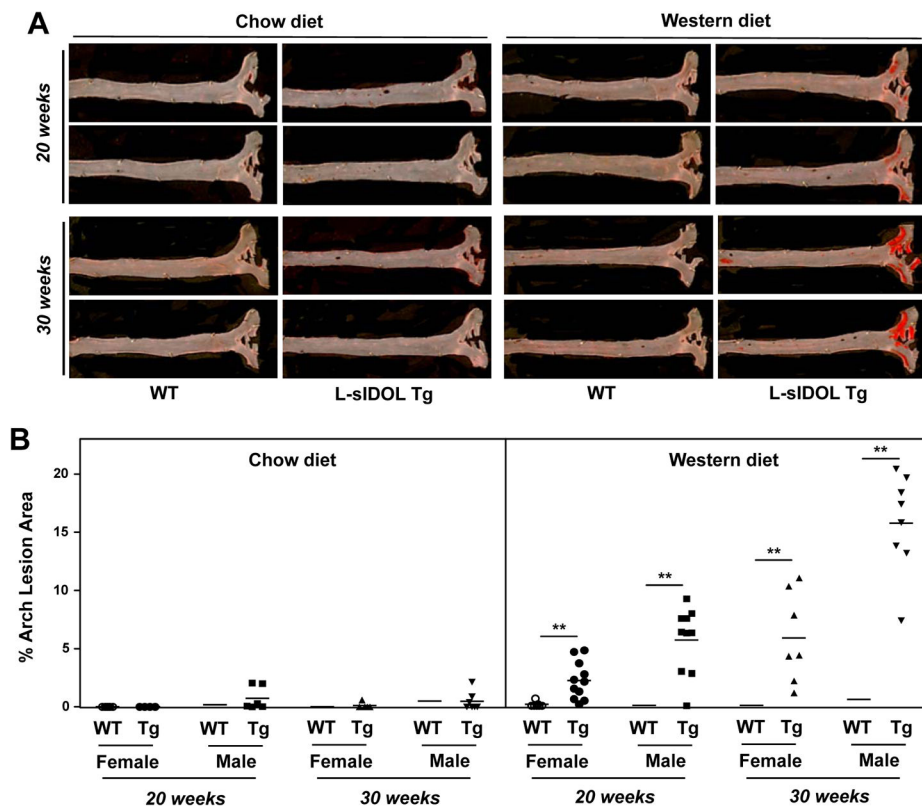


Figure 4. *En face* atherosclerosis analysis in L-sIDOL mice

(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) Quantitation of *en face* aortic lesions area in mice as indicated; ** $p < 0.01$ vs matched wild-type.

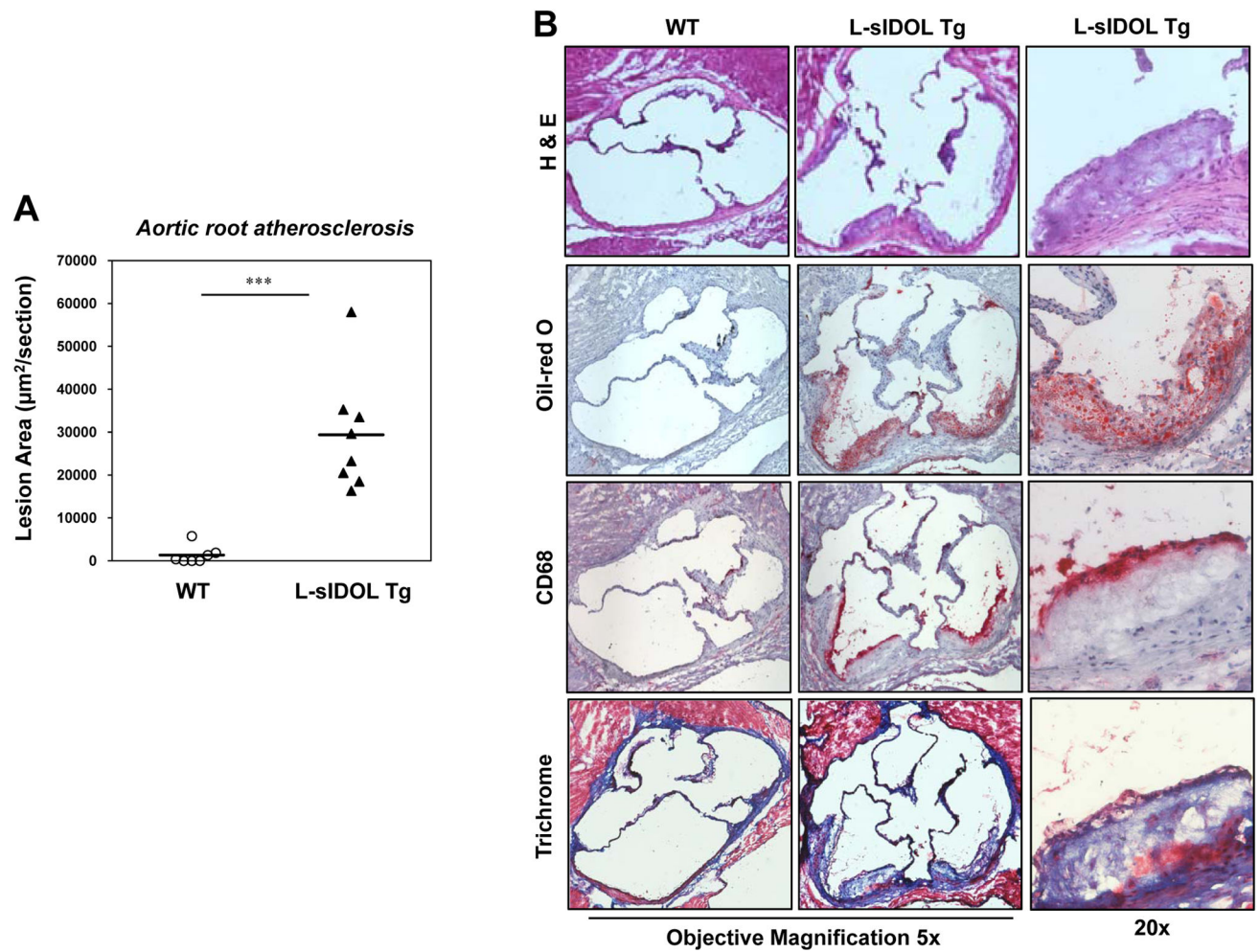


Figure 5. L-sIDOL mice develop atherosclerosis in the aortic root

(A) Quantitation of aortic root lesions in male mice following 30 weeks of western diet; (B) Representative pictures of aortic root lesions in WT and L-sIDOL mice stained with haematoxylin and eosin (H&E; top), Oil red O (top middle), CD68 (bottom middle) and trichrome (bottom) at magnifications as indicated.

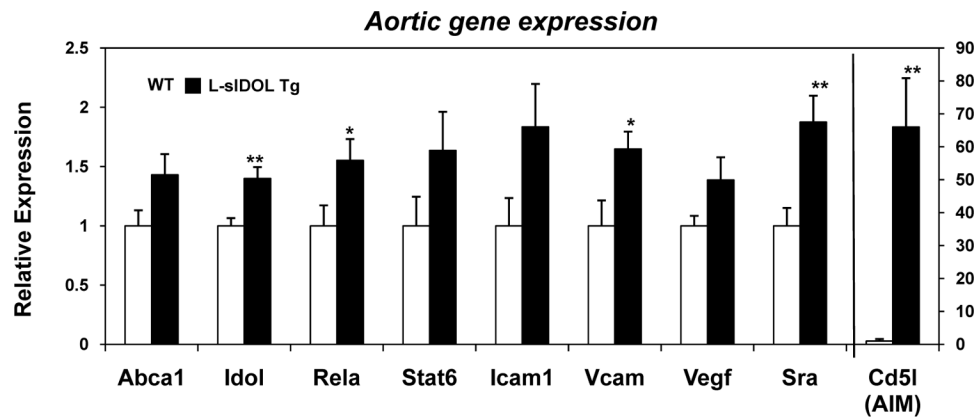


Figure 6. L-sIDOL mice aortic mRNA expression

(A) mRNA expression of genes in aortas of male mice after 20 weeks WD, normalised to *Rplp0*; Data expressed as fold increase over wild type and represented as mean \pm SEM.

* $p < 0.05$, ** $p < 0.01$ vs wild-type.

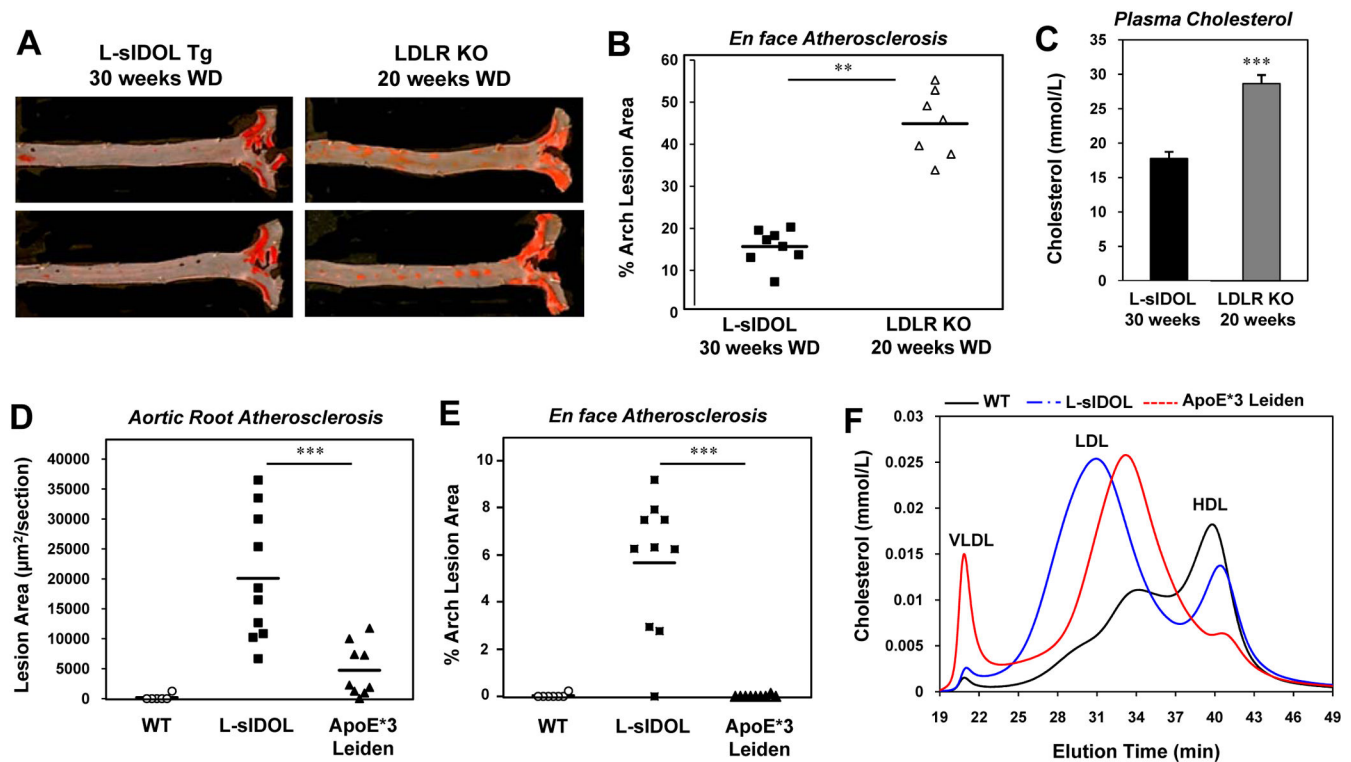


Figure 7. Comparison of atherosclerotic mouse models

(A) Representative pictures of *en face* assessment of atherosclerotic lesions L-sIDOL and LDLR KO mice fed a western diet; (B) Quantitation of *en face* aortic lesions in L-sIDOL and LDLR KO mice; (C) Plasma cholesterol levels of L-sIDOL and LDLR KO mice; Quantitation of (D) aortic root lesions and (E) *en face* aortic root lesions, in WT, L-sIDOL and ApoE*3 Leiden mice following 20 weeks of western diet; data for WT and L-sIDOL mice are the same data shown in Figure 4B; (F) HPLC chromatogram of plasma cholesterol following 20 weeks on western diet; data for WT and L-sIDOL mice are the same data shown in Figure 3G. ** $p < 0.01$; *** $p < 0.001$.

Table 1

Male mouse metabolic data.

	Chow 20 weeks		WD 20 weeks		Chow 30 weeks		WD 30 weeks		WD 20 weeks LDLR KO [†]
	WT	L-sIDOL	WT	L-sIDOL	WT	L-sIDOL	WT	L-sIDOL	
n	5	6	7	10	5	7	7	8	7
Body Weight (g)	28.6±1.4	28.1±0.8	47.1±0.7	45.8±1.8	31.6±1.0	31.5±1.3	48.1±1.2	47.2±1.1	44.2±1.9
Liver Weight (g)	1.1±0.0	1.0±0.1	4.3±0.3	3.9±0.5	1.4±0.1	1.3±0.1	4.0±0.2	3.7±0.2	2.7±0.4
LW/BW (%)	3.9±0.1	3.6±0.2	9.2±0.5	8.3±0.7	4.4±0.1	4.2±0.1	8.3±0.4	7.8±0.4	6.0±0.5
TGs (mmol/L)	0.79±0.04	0.46±0.04***	0.67±0.04	1.00±0.06**	0.60±0.05	0.50±0.03	0.29±0.03	0.54±0.04***	6.35±1.09

BW – body weight; LW – liver weight; TGs – triglycerides; L-sIDOL – Albumin driven super IDOL transgenic mice; WT – wild type.

**
p<0.01,***
p<0.001 vs matched wild type.[†]
not included in statistical analysis.