SUPPLEMENTAL MATERIAL

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.[1] The linearized construct was gel purified and microinjected into C57B/6J mice fertilised zygotes. Founders were identified using the following PCR primers (For-GCCAGGAGGGCTCTGTACA; Rev-GAATAGAATGACACCTACTCAGACAAT). 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.[2] 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.[3] 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.[4] 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.[5-9] 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 haemotoxylin. 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.[10] Immunohistochemical studies were performed as previously described.[11] 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 haemotoxylin. 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 manufacturers 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).

	Chow 20 weeks		WD 20 weeks		Chow 30 weeks		WD 30 weeks	
	WT	L-sIDOL	WT	L-sIDOL	WT	L-sIDOL	WT	L-sIDOL
N	7	4	11	11	7	5	6	7
Body weight	26.5±1.1	23.3±0.7	37.6±1.0	36.5±2.1	26.4±1.6	28.0±1.9	39.1±1.5	36.0±1.9
Liver Weight	1.0±0.0	1.0±0.0	1.8±0.1	2.1±0.3	1.1±0.1	1.0±0.1	2.1±1.6	2.2±1.7
LW/BW (%)	3.8±0.2	4.1±0.1	5.0±0.3	5.5±0.4	4.0±0.2	3.5±0.1	4.9±0.3	6.2±0.4*
Triglycerides	0.46±0.05	0.46±0.02	0.29±0.02	0.45±0.05**	0.56±0.08	0.45±0.06	0.13±0.03	0.25±0.04**

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.

mRNA	Forward primer 5'- 3'	Reverse primer 5' - 3'
mRplp0	AGATGCAGCAGATCCGCA	GTTCTTGCCCATCAGCACC
mABCAI	GGTTTGGAGATGGTTATACAATAGTTGT	CCCGGAAACGCAAGTCC
mAIM	TTTGTTGGATCGTGTTTTTCAGA	CTTCACAGCGGTGGGCA
mICAM	CCCACGCTACCTCTGCTC	GATGGATACCTGAGCATCACC
mIDOL	TGTGGAGCCTCATCTCATCTT	AGGGACTCTTTAATGTGCAAG
mLDLR	AGGCTGTGGGCTCCATAGG	TGCGGTCCAGGGTCATCT
mRelA	CCCAGACCGCAGTATCCAT	GCTCCAGGTCTCGCTTCTT
mSra	CTTTACCAGCAATGACAAAAGAGA	ATTTCACGGATTCTGAACTGC
mSTAT6	CCTTTGAGGAGAGCCTAGCA	CTGCTGCAGCTGGGAATAA
mVCAM	TTCACGTGGGGCACAAAGAA	AGCTTGAGAGACTGCAAACAGTA
mVEGF	AATGCTTTCTCCGCTCTGAA	CTCACCAAAGCCAGCACATA
hIDOL	CGAGGACTGCCTCAACCA	TGCAGTCCAAAATAGTCAACTTCT

Online Table II. Primer sequence. m = mouse; h = human.

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