Supplementary Data

METHODS

Chemicals

All polymerase chain reaction (PCR) primers and the Trizol™ reagent were purchased from Invitrogen (Carlsbad, CA, USA). Human leukocyte myeloperoxidase (MPO) and other routine chemicals were purchased from Sigma (St. Louis, MO, USA).

Animals

Sixty-six 4-week-old female LDLR$^{-/-}$ mice weighing 18–20 g were obtained from Jackson Laboratory (Bar Harbor, ME, USA). After 2 weeks of acclimatization, eighteen animals ($n = 5 + 3 + 10$) were sacrificed to obtain baseline parameters. The remaining animals were divided into two groups: group 1 was fed an atherogenic diet ($n = 7 + 6 + 10$), and group 2 ($n = 8 + 7 + 10$) was fed an atherogenic diet reformulated with sesame oil (sesame oil diet). The animals were regularly monitored and a weekly record of body weight was maintained up to 90 days. All procedures were performed according to the protocol approved by The Institutional Animal Care and Use Committee. Three separate and independent studies were performed.

Supplementary Table S1. Fatty Acid and Lignan Composition of Sesame Oil

Component	*Percentage*
Fatty acids	
Palmitic acid C16	11.46%
Stearic acid C18	3.64%
Oleic acid	40.12%
Linoleic acid	44.47%
Lignans[4]	
Sesamin	0.63%
Sesamolin	0.39%

Diet

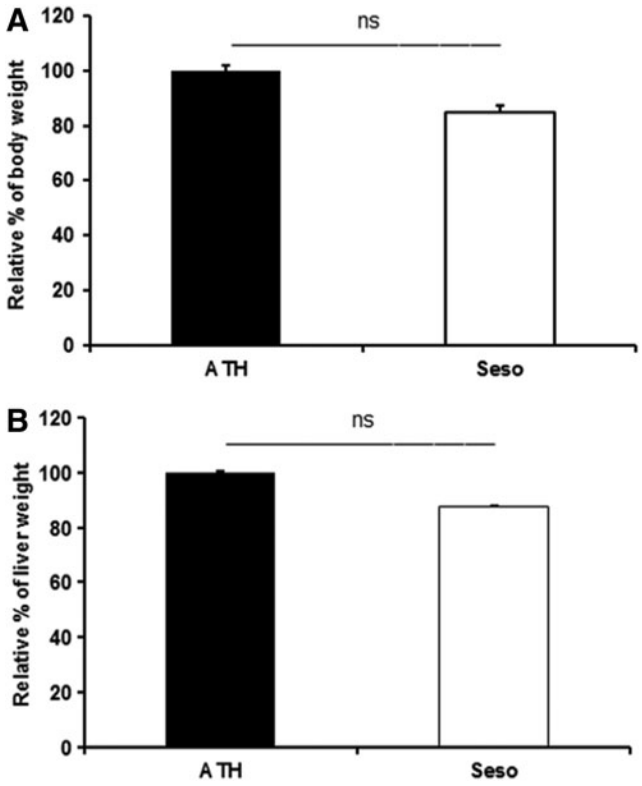
For our studies, 17% AMF (anhydrous milk fat) diet TD.04287 with 0.2% cholesterol as the atherogenic diet and the atherogenic diet reformulated with sesame oil (TD .04288) were purchased from Harlan Teklad (Madison, WI, USA). The composition of the diet was identical to that described previously.[1] Diets were stored at 4°C to avoid oxidation. The fatty acid composition of sesame oils was analyzed as methyl esters by Varian CP-3380 Gas Chromatography (Varian, Inc., Palo Alto, CA, USA).[2,3] Fatty acid and Lignan[4] composition of sesame oil used is represented in the Supplementary Table S1.

Collection of plasma and organs

After 15 weeks, mice were fasted overnight and anesthetized with 1–2% isoflurane. Fasting blood samples

Supplementary Table S2. List of Oligonucleotide Primers Used for the Real Time-Polymerase Chain Reaction

Target	*Forward primer*	*Reverse primer*
ABCA1	5′-GGGAATTGAACCTGAGTCCT-3′	5′-AGTCATTCCTCCTCCCATTC-3′
ABCG1	5′-CCAGACAGTTGTGGATGTGG-3′	5′-GACCTCGCTCTTCCTTCCTT-3′
APOA1	5′-CCAATGGGACAAGTGAAGG-3′	5′-ACGGTTGAACCCAGAGTGTC-3′
Catalase	5′-ACGCTTCAACAGTGCTAATG-3′	5′-GTTCTCACAGGGGTTTC-3′
CD36	5′-TGCTGGAGCTGTTATTGGTG-3′	5′-TGGGTTTTGCACATCAAAGA-3′
CD68	5′-TAGCCCAAGGAACAGAGGAA-3′	5′-TGGCAGGGTTATGAGTGACA-3′
CYP7A1	5′-ACATGGAGAAGGCTAAGACG-3′	5′-CTTCTTCAGAGGCTGCTTTC-3′
FXR	5′-ACATCCCCATCTCTCTGC-3′	5′-TGTGAGGGCTGCAAAGGTT-3′
GAPDH	5′-ACCCAGAAGACTGTGGATGG-3′	5′-CACATTGGGGGTAGGAACAC-3′
IL-1 α	5′-GCAACGGGAAGATTCTGAAG-3′	5′-TGACAAACTTCTGCCTGACG-3′
IL-1 β	5′-AACCTGCTGGTGTGTGACTTC-3′	5′-CAGCACGAGGCTTTTTTGT-3′
IL-4	5′-GCGACAAAAATCACTTGAGAG-3′	5′-CCTTGGAAGCCCTACAGAC-3′
IL-6	5′-AGTTGCCTTCTTGGGACTGA-3′	5′-TCCACGATTTCCCAGAGAAC-3′
IL-10	5′-AACCTGCTGGTGTGATTC-3′	5′-CAGCACGAGGCTTTTTTGT-3′
LXR	5′-ACCTCTGCAATCGAGGTC-3′	5′-GGTTGATGAACTCCACCTG-3′
MCP-1	5′-CAGCAAGATGATCCCAATGA-3′	5′-TGGTTCCGATCCAGGTTTT-3′
MMP-9	5′-CGACGACGACGAGTTGTG-3′	5′-CTGTGGTGCAGGCCGAATAG-3′
MnSOD	5′-CTAAGGGTGGTGGAGAACC-3′	5′-ACCTTGGACTCCCACAGAC-3′
MPO	5′-CCACTCCTGCCAAAACTGAAT-3′	5′-CACTGTGCTAGGCTGTGGAA-3′
NPC1L1	5′-GTCTGTCCCCGCCTATACAAA-3′	5′-GGTCAGGGCTGCAGGTATTA-3′
PON1	5′-AGAGGTGCTTCGAATCCAGA-3′	5′-AACACTGTGCCAATCAGCAG-3′
PXR	5′-GACCTGCCTATTGAGGACCA-3′	5′-TTCTGGAAGCCACGATTAGG-3′
SRA1	5′-AAAGGTGATCGGGGACAAA-3′	5′-TTGCCCCAATATGATCAGG-3′
SRB1	5′-GGGCTCGATATTGATGGAGA-3′	5′-GGAAGCATGTCTGGGAGGTA-3′
TNF-α	5′-CACACTCAGATCATCTTCCAAAA-3′	5′-GCAATGACTCTAAGTAGACCTGC-3′
PPARα	5′-AAGAGGGCTGAGCGTAGGT-3′	5′-GGCCGGTTAAGACCAGACT-3′



SUPPLEMENTARY FIG. S1. Reduced body weight **(A)** and liver weight **(B)** of sesame oil diet-fed animals. ATH, atherogenic chow; Seso, sesame oil diet; ns, not significant.

were collected into EDTA tubes by heart puncture. Plasma was separated as described previously[1] and stored at −80°C. The liver was perfused with PBS, weighed, and the tissue used for RNA isolation.

Isolation and quantification of aortic lesions

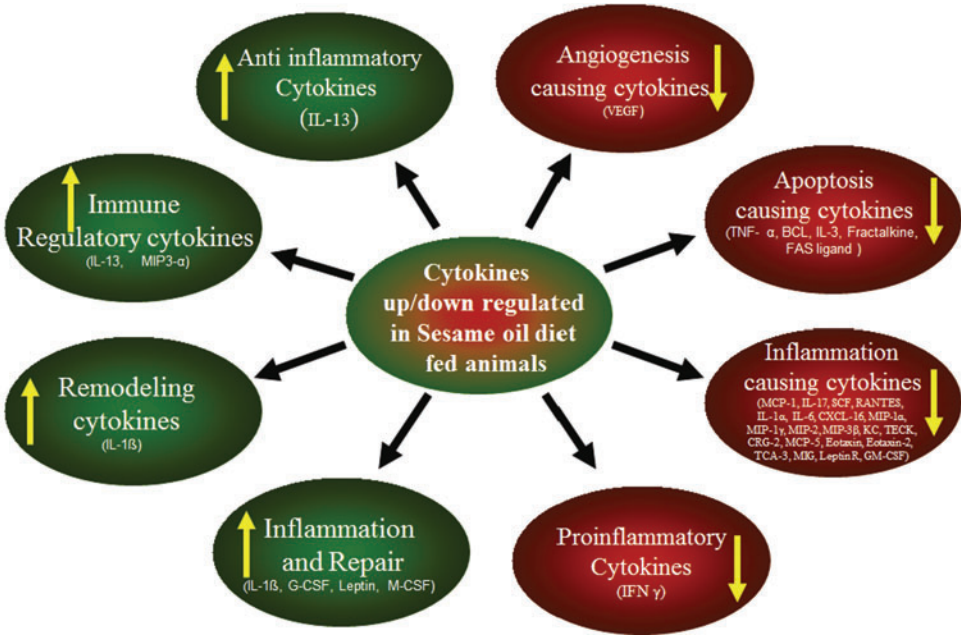
Isolation of the aorta and quantification of aortic lesions was performed as described previously.[1] Lesion areas were marked on photographs. The lesion area was quantified using ImageJ software.[5] After imaging, aortas were saved for RNA extraction.

Plasma lipid analysis

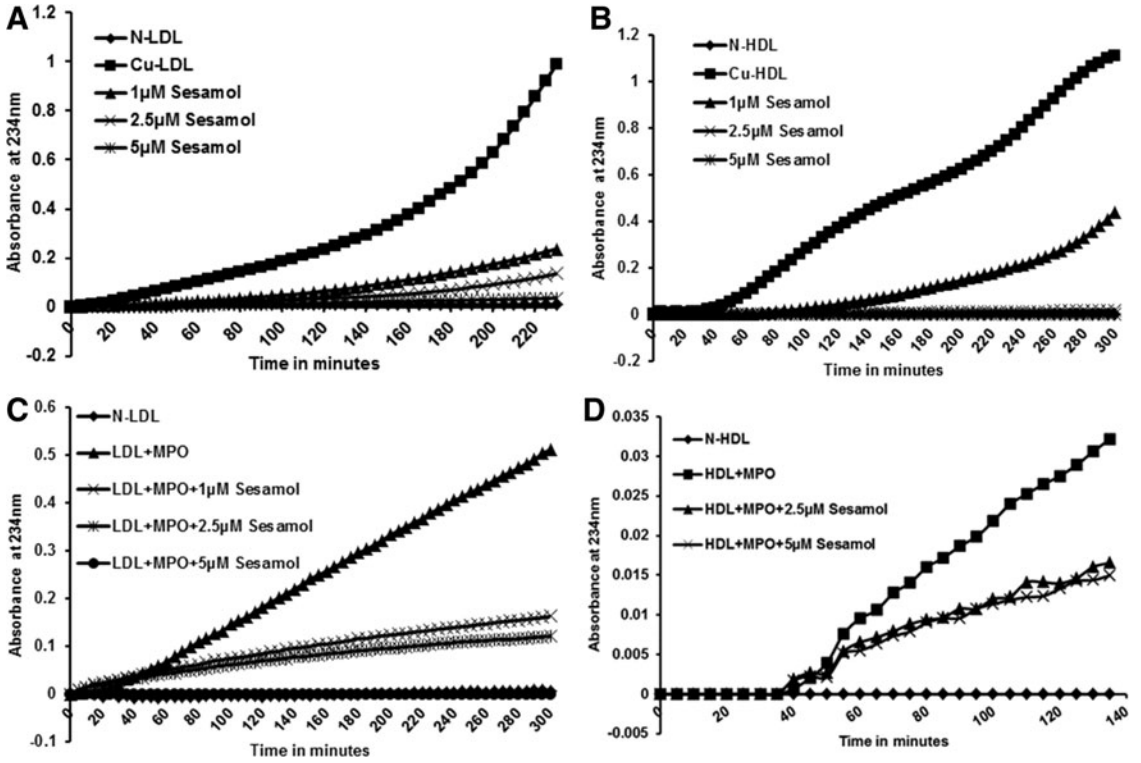
Plasma lipid profiles of total cholesterol (TC), triglyceride (TRG), HDL cholesterol (HDLc), and LDL cholesterol (LDLc) were determined by using a Cholestech L*D*X analyzer (Cholestech Corp, Hayaward, CA, USA).

cDNA synthesis and real time-PCR

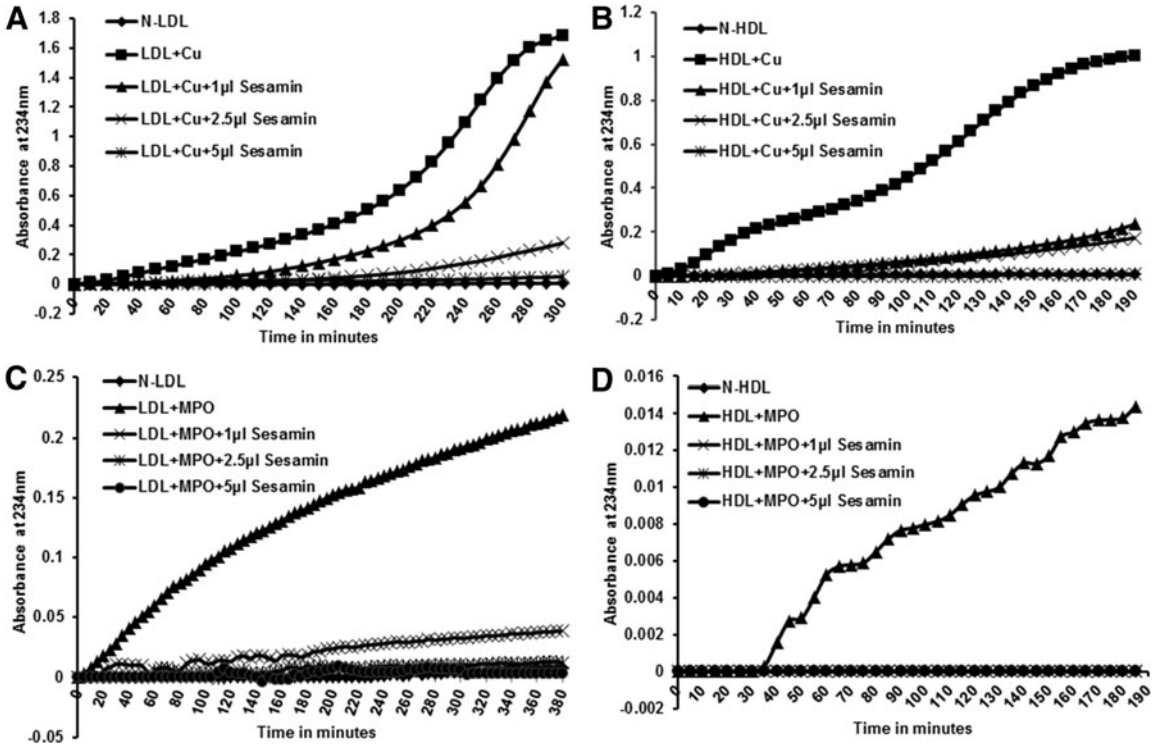
Total RNA from the liver and aortic tissue was isolated by using Trizol reagent. Total RNA of 1 μg was then reverse transcribed into cDNA using the Superscript™ III First-Strand Synthesis System (Invitrogen). cDNA (50 ng) samples were used to perform quantitative real-time PCR by the iQTM5 iCycler Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with SYBR Green (Invitrogen). Mouse Oligonucleotide primers for real time-PCR were purchased from Invitrogen. PCR was carried out with ABCA1, ABCG1, SRB1, Cyp7a1, NPC1L1, MCP-1, IL-1α, IL-1β, IL-6, IL-4, IL-10, IL-13, Catalase, MnSOD,



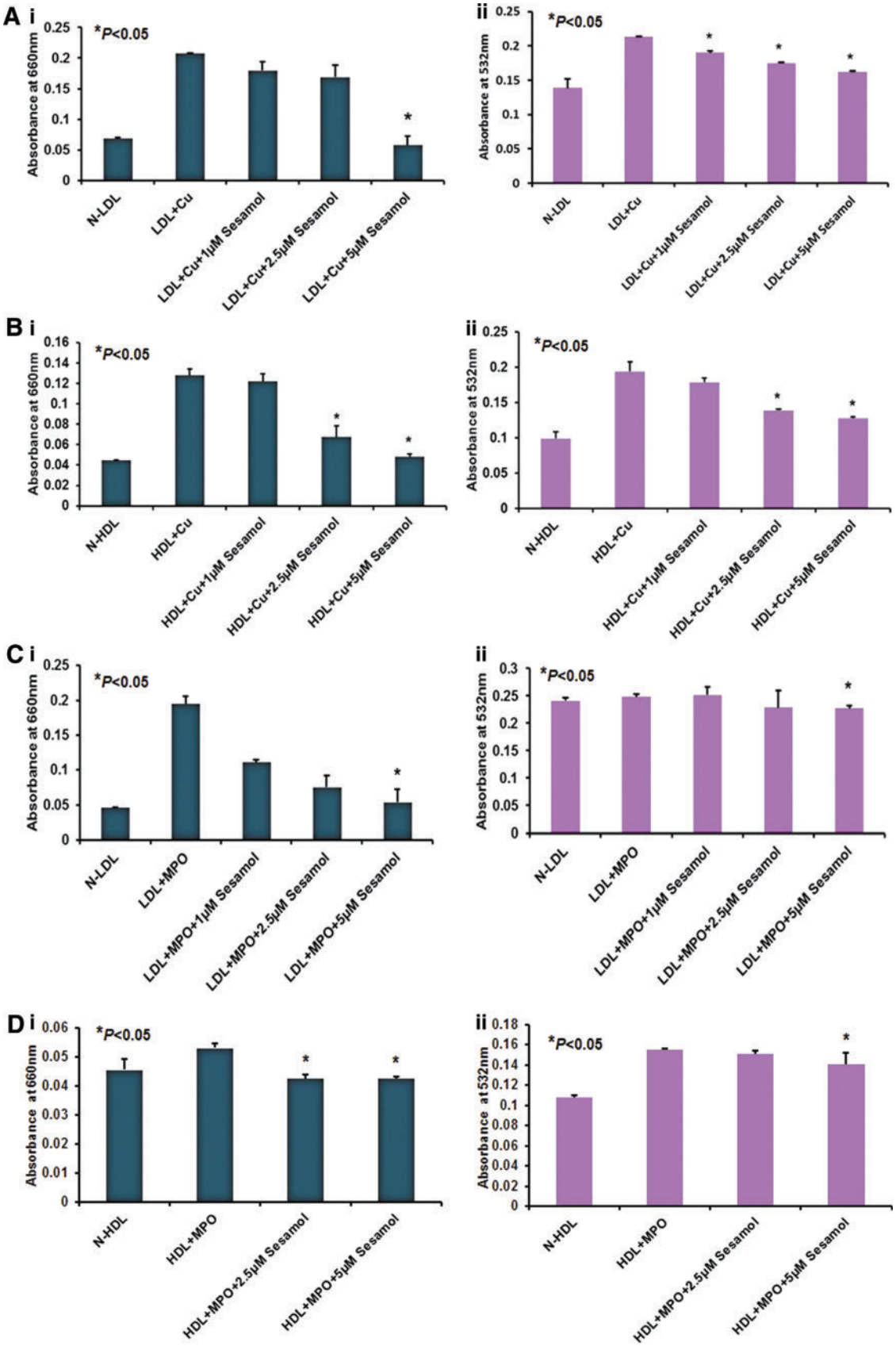
SUPPLEMENTARY FIG. S2. Groups of cytokines that are upregulated/downregulated in Sesame oil diet-fed animals.



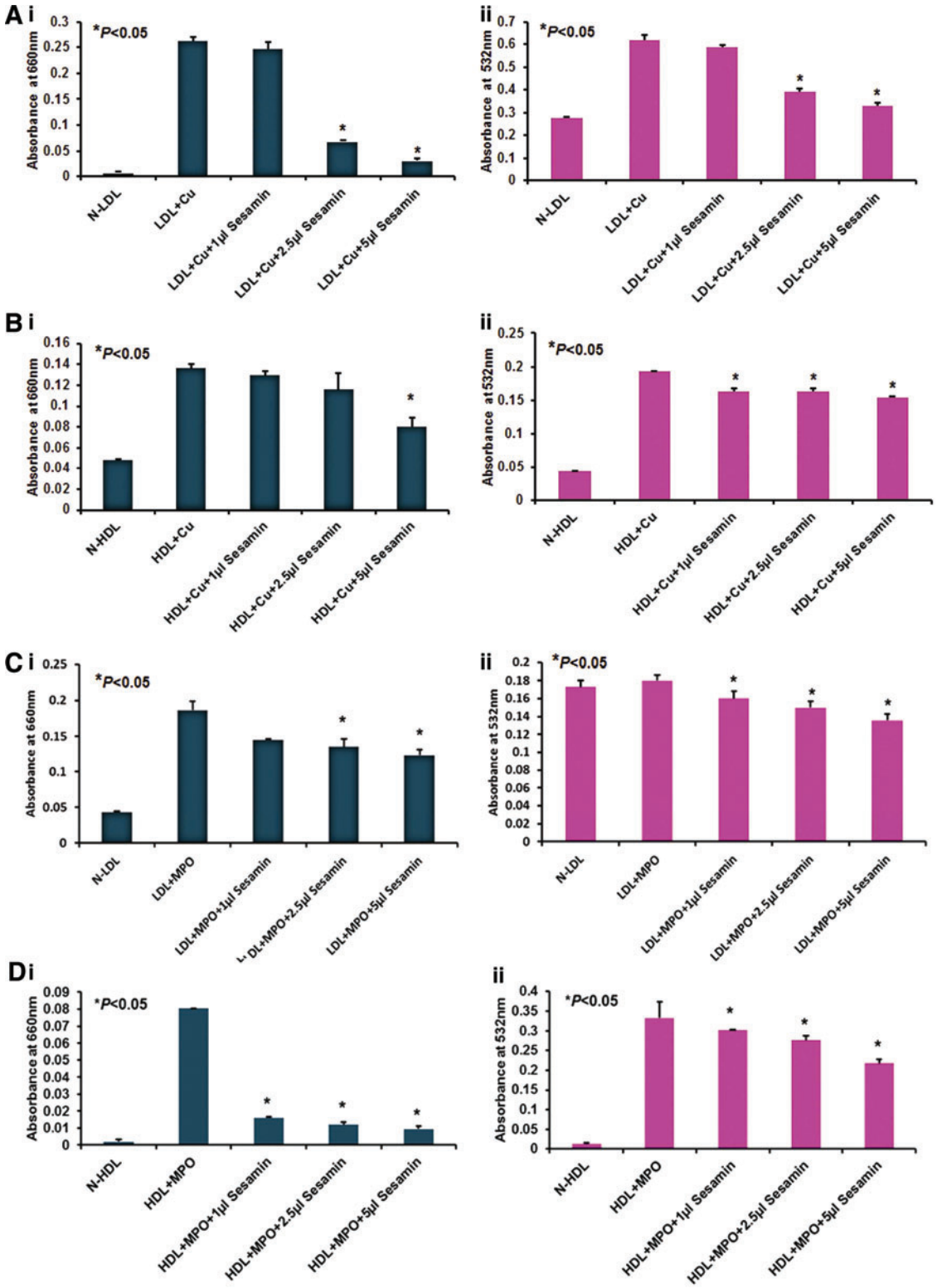
SUPPLEMENTARY FIG. S3. Sesamol inhibits oxidation of lipoproteins. Lipoproteins were isolated from the plasma of consented subjects and used for oxidation with Cu and myeloperoxidase (MPO). One hundred micro grams of LDL/HDL was treated with sesamol of different concentrations (1, 2.5, and 5 μM) incubated for 1 h at room temperature, and then oxidation was performed either with Cu or 0.2 U MPO in 1 mL of PBS and OD was measured at 234 nm. As shown in figures **(A)** LDL with Cu, **(B)** HDL with Cu, **(C)** LDL with MPO, and **(D)** HDL with MPO, as the concentration of sesamol increased, lipoprotein oxidation was inhibited.



SUPPLEMENTARY FIG. S4. Sesamin inhibits oxidation of lipoproteins. Lipoproteins were isolated from the plasma of consented subjects and used for oxidation with Cu and MPO. One hundred micro grams of LDL/HDL was treated with sesamin of different concentrations (1, 2.5, and 5 μL) incubated for 1 h at room temperature, and then oxidation was performed either with Cu or 0.2 U MPO in 1 mL of PBS and OD was measured at 234 nm. As shown in figures **(A)** LDL with Cu, **(B)** HDL with Cu, **(C)** LDL with MPO, and **(D)** HDL with MPO, as the concentration of sesamin increased, lipoprotein oxidation was inhibited.



SUPPLEMENTARY FIG. S5. Leucomethylene blue (LMB) and thiobarbituric acid reactive substances (TBARS) test for Cu and MPO oxidized LDL/HDL samples in the presence and absence of sesamol. Lipoproteins were isolated from the plasma of consented subjects and used for oxidation with copper. One hundred micro grams of LDL/HDL was treated with sesamol of different concentrations (1, 2.5, and 5 μM) incubated for 1 h at room temperature, and then oxidation was performed either with Cu or 0.2 U MPO in 1 mL of PBS and OD was measured at 234 nm. After oxidation, the samples were used for LMB assay **(Ai, Bi, Ci, Di)** and TBARS assay **(Aii, Bii, Cii, Dii)** also showed dose-dependent inhibition.



SUPPLEMENTARY FIG. S6. LMB and TBARS Assay for Cu and MPO oxidized LDL/HDL samples in the presence and absence of sesamin. Lipoproteins were isolated from the plasma of consented subjects and used for oxidation with copper. One hundred micro grams of LDL/HDL was treated with sesamin of different concentrations (1, 2.5, and 5 μL) incubated for 1 h at room temperature, and then oxidation was performed either with Cu or 0.2 U MPO in 1 mL of PBS and OD was measured at 234 nm. After oxidation, the samples were used for LMB assay **(Ai, Bi, Ci, Di)** and TBARS assay **(Aii, Bii, Cii, Dii)** also showed dose-dependent inhibition.

SRA1, CD36, LXR, pregnane X receptor (PXR), farnesoid X receptor (FXR), MPO, and CD68 with mouse-specific primers (Supplementary Table S1), resulting in a 200 bp fragment each. As a reference gene, we used GAPDH primers, resulting in a 200 bp fragment. PCR was performed with an initial step of denaturation at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 20 s and 60°C for 20 s. Melt curves were established for the reactions. Normalized fold expression was calculated by using the $2^{-\Delta\Delta Ct}$ method.

Global cytokine and gene array

Plasma samples were analyzed by the global cytokine array by Ray Biotech, Inc., (Norcross, GA, USA) using the RayBio®Mouse G Series Array three and four glass chip. Liver tissue RNA samples were used for the mouse gene array PAMM-080 (Qiagen, Valencia, CA, USA) analysis.

In vitro *oxidation of LDL in the presence of sesamol and sesamin*

Lipoproteins from human plasma were isolated as described previously.[6] Oxidation of LDL and HDL was performed with 5 μM copper or 0.2 U MPO both in the presence and absence of different concentrations of sesamol and sesamin. Formation of conjugated dienes was monitored at 234 nm in a UVIKON XL spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) equipped with a 12-chamber cuvette changer. Samples were monitored continuously for periods of up to 8 h. The degree of lipoprotein oxidation was assessed by determination of the peroxide content using the leucomethylene blue (LMB) assay[7] and thiobarbituric acid reactive substances (TBARS).

Statistical analyses

Values are presented as mean ± standard deviation (SD), and statistical analyses were performed by using Student's *t*-test, with $P < .05$ as the level of significance. Significance between groups was calculated by using two-tailed Student's *t*-test and the Wilcoxon matched paired test using Prism Pad software, with $P < .05$ considered significant.

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