FULL COMMUNICATIONS

Anti-Atherosclerotic and Anti-Inflammatory Actions of Sesame Oil

Chandrakala Aluganti Narasimhulu, Krithika Selvarajan, Dmitry Litvinov, and Sampath Parthasarathy

Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida, USA.

ABSTRACT   Atherosclerosis, a major form of cardiovascular disease, has now been recognized as a chronic inflammatory disease. Nonpharmacological means of treating chronic diseases have gained attention recently. We previously reported that sesame oil has anti-atherosclerotic properties. In this study, we have determined the mechanisms by which sesame oil might modulate atherosclerosis by identifying genes and inflammatory markers. Low-density lipoprotein receptor knockout (LDLR$^{-/-}$) female mice were fed with either an atherogenic diet or an atherogenic diet reformulated with sesame oil (sesame oil diet). Plasma lipids and atherosclerotic lesions were quantified after 3 months of feeding. Plasma samples were used for cytokine analysis. RNA was extracted from the liver tissue and used for global gene arrays. The sesame oil diet significantly reduced atherosclerotic lesions, plasma cholesterol, triglyceride, and LDL cholesterol levels in LDLR$^{-/-}$ mice. Plasma inflammatory cytokines, such as MCP-1, RANTES, IL-1α, IL-6, and CXCL-16, were significantly reduced, demonstrating an anti-inflammatory property of sesame oil. Gene array analysis showed that sesame oil induced many genes, including ABCA1, ABCA2, APOE, LCAT, and CYP7A1, which are involved in cholesterol metabolism and reverse cholesterol transport. In conclusion, our studies suggest that a sesame oil-enriched diet could be an effective nonpharmacological treatment for atherosclerosis by controlling inflammation and regulating lipid metabolism.

KEY WORDS: ● *cholesterol transport* ● *inflammation* ● *lipid metabolism* ● *sesamin* ● *sesamol*

INTRODUCTION

ATHEROSCLEROSIS IS A chronic inflammatory disease characterized by the accumulation of lipids and infiltration of leukocytes into the artery wall.[1,2] Oxidative stress and inflammation play a pivotal role in every stage of the disease[3–6] and both conditions represent targets for intervention. Current therapies for the prevention and treatment of atherosclerosis are unable to target the inflammatory mechanisms involved in the initiation and progression of the disease.[7,8]

A cardioprotective diet and exercise are important in the prevention and treatment of atherosclerosis. The influence of cooking oil on plasma lipids and atherosclerosis has been one of the most studied topics in cardiovascular nutrition.[9–11] Both poly- and monounsaturated fatty acids (PUFAs and MUFAs) have been reported to lower cholesterol,[9–11] but the mechanisms by which dietary fat desaturation affects atherosclerosis are unknown. Sesame oil (*Sesamum indicum*) is rich in both MUFAs and PUFAs. Many studies have identified that sesame oil contains lignans, such as sesamin, sesamolin, and several antioxidant compounds such as sesa-

minol.[12–14] It has been observed that sesamol is able to reduce lipopolysaccharide-induced oxidative stress[15] and upregulate phosphatidylinositol 3-kinase/Akt/endothelial nitric oxide synthase pathways.[16] Sesame oil plays an important role in controlling hypertension and stress.[17,18] Recently, we revealed the close relationship between inflammation and oxidative stress.[19] Thus, it would be extremely important and beneficial to determine the effects of sesame oil feeding on inflammation and atherosclerosis *in vivo*.

Inflammation has been postulated to play a major role in atherosclerosis development.[20] Over the past two decades, research has been focused on the role of modified lipoproteins, primarily oxidized LDL (Ox-LDL). Evidence supports the concept that Ox-LDL may be a key antigen in atherosclerosis.[21] Sesamin feeding is associated with a reduction of serum lipid levels in rodents, concomitant with an increased fatty acid oxidation[22–25] perhaps attributable to activation of peroxisome proliferator-activating receptor (PPAR) pathways. Activation of PPARα has been demonstrated to modulate many aspects of lipoprotein metabolism and inflammation *in vitro* as well as in animal and human studies.[26–29] PPAR-mediated effects include a decrease in lipid accumulation in macrophages by downregulating the SR-A1 receptor and promoting cholesterol efflux by increasing hepatic LDL and SR-B1 receptors. Similarly, activation of the liver X receptor (LXR) pathway would enhance ABCA1, SR-B1, and SREBP, thus enhancing

reverse cholesterol transport (RCT) and catabolism.[30–31] We previously reported that sesame oil has anti-atherosclerotic properties.[32] In this study, we evaluated the effect of sesame oil on inflammation, RCT, and lipid metabolism beyond its ability to reduce lesion formation in low-density lipoprotein receptor (LDLR) knockout mice.

METHODS

A detailed description of methods is available in Supplementary Data (Supplementary Data are available online at www.liebertpub.com/jmf).

Animals

Sixty-six 4-week-old female LDLR$^{-/-}$ mice weighing 18–20 g were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and used for the study.

Diet

An atherogenic diet (TD.04287) and 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.[32] The fatty acid composition of sesame oils was analyzed as methyl esters by Varian CP-3380 Gas Chromatography (Varian, Inc., Palo Alto, CA, USA).[33,34] The fatty acid and lignan[35] composition of the sesame oil used is represented in the Supplementary Table S1.

Collection of plasma and organs

After 15 weeks, mice were fasted overnight and blood, plasma, and tissue samples were collected as described previously[32] and stored at −80°C.

Isolation and quantification of aortic lesions

Isolation of the aorta and quantification of aortic lesions were performed as described previously.[32,36]

Plasma lipid analysis

Plasma lipid profiles were determined by using a Cholestech L*D*X analyzer (Cholestech Corp, Hayaward, CA, USA).

cDNA synthesis and real time-polymerase chain reaction

Total RNA from the liver and aortic tissue was isolated by using Trizol™ reagent. One micro gram of RNA was then reverse transcribed into cDNA using the Superscript™ III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). cDNA (50 ng) samples were used to perform the quantitative real-time polymerase chain reaction (PCR) by the iQTM5 iCycler Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with SYBR Green (Invitrogen). mRNA expression of ABCA1, ABCG1, SRB1, Cyp7a1, NPC1L1, MCP-1, IL-1α, IL-1β, IL-6, IL-4, IL-10, IL-13, Catalase, MnSOD, SRA1, CD36, LXR, pregnane X receptor (PXR), farnesoid X receptor (FXR), MPO, and CD68 with mouse-specific primers was analyzed. The primers for the genes are provided in the Supplementary Table S2.

Global cytokine and gene array

Plasma samples were analyzed by the global cytokine array (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.[37] 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. The degree of LDL oxidation was assessed by conjugated diene measurement, while the peroxide content was determined by using the leucomethylene blue assay[38] and thiobarbituric acid reactive substances.

Statistical analyses

Values are presented as mean ± standard deviation (SD), and statistical analyses were performed using Student's *t*-test and the Wilcoxon matched paired test using Prism Pad software, with $P < .05$ considered significant.

RESULTS

Body weight analysis in mice

The atherogenic diet contained 17% saturated fat provided by milk, and in the reformulated diet, milk fat was replaced with an equal amount (17%) of sesame oil. A slight, but insignificant, decrease in body weight and liver weight (approximately 15% and 13%, respectively) was observed in sesame oil diet-fed animals (Supplementary Fig. S1A, B).

Reduced plasma lipid levels in sesame oil diet-fed animals

Visual observation of plasma following centrifugation revealed a clear plasma in sesame oil diet-fed animals compared with control animals. This probably reflected a decrease in plasma lipids of sesame oil diet-fed animals. Plasma lipid profile analysis revealed a significant decrease in TRG, TC, LDL cholesterol, and very low-density lipoprotein (VLDL) cholesterol in sesame oil diet-fed animals. A slight increase in HDL cholesterol levels was observed. Figure 1A–C represents the plasma lipid profile of control and sesame oil diet-fed animals from three independent studies. The total cholesterol was significantly reduced in the sesame oil diet-fed animals ($P < .05$). In animals fed with an atherogenic diet, the cholesterol level was 1158.13 ± 4.37 mg/dL, whereas in the animals fed with a sesame oil diet, the cholesterol level was
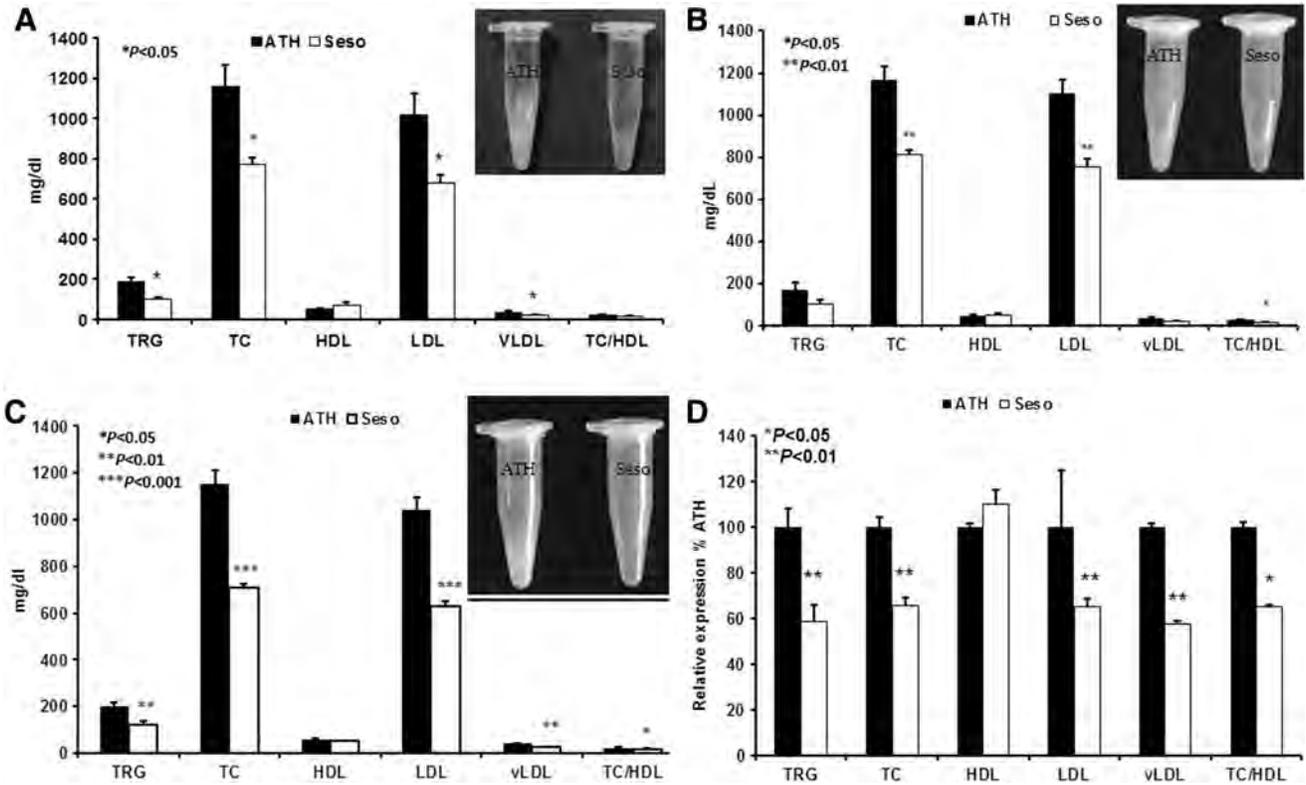
FIG. 1. Reduced plasma lipid levels in sesame oil diet-fed animals. Plasma lipid levels (**A**-Study I; **B**-Study II, **C**-Study III, and **D**-Aggregated relative fold) in mice fed an atherogenic diet ($n = 7 + 6 + 10$) and sesame oil diet ($n = 8 + 7 + 10$). Values are represented as mean $\pm$ SD. *$P < .05$.

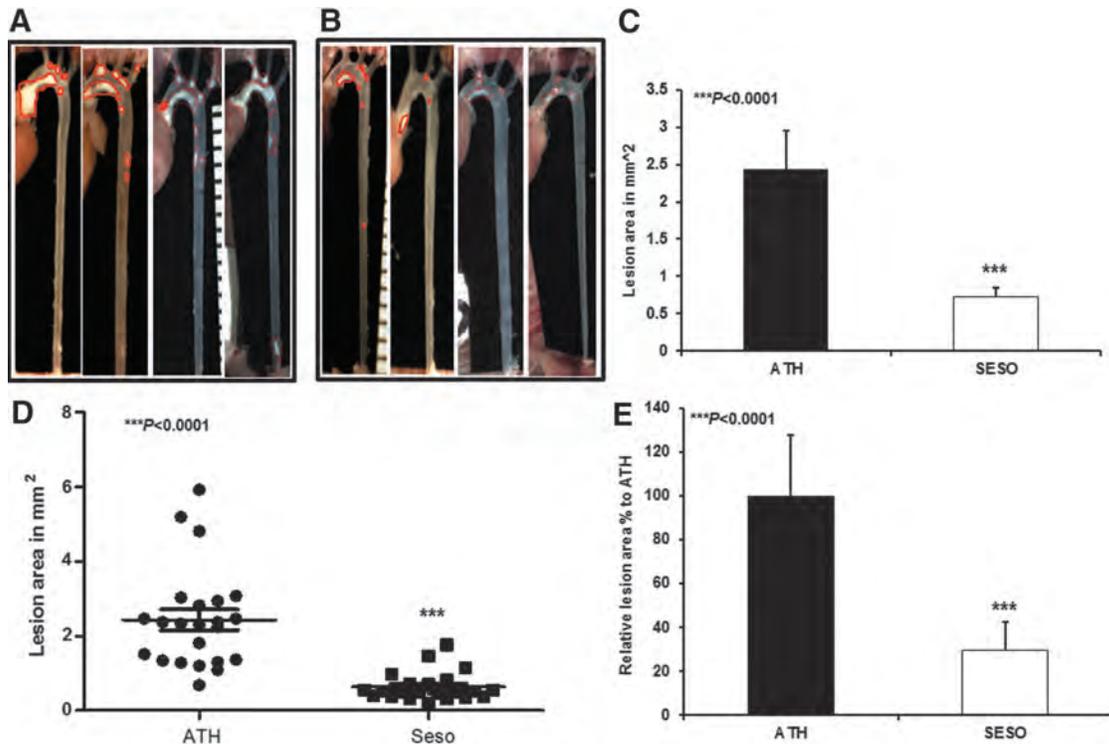**FIG. 2.** Reduced atherosclerotic lesions in sesame oil diet-fed animals. Representative images of atherosclerotic lesions in (**A**) high-fat diet-fed animals, (**B**) sesame oil diet-fed LDLR$^{-/-}$ mice, (**C**) average lesion area of three independent studies, (**D**) lesion area of all the animals, and (**E**) relative lesion area of animals. The values are expressed as mean $\pm$ SD (mm^2). ***$P < .0001$.

TABLE 1. CHANGES IN THE LEVELS OF GENE EXPRESSION IN MOUSE LIVERS BETWEEN HIGH-FAT DIET
AND SESAME OIL DIET GROUPS WERE ANALYZED BY THE GLOBAL GENE ARRAY

Gene name	Symbol	ATH/High-fat diet	Seso diet	P value
(A) Genes upregulated in cholesterol transport of sesame oil diet-fed animals				
HDL-associated proteins				
Apolipoprotein A-I	Apoa1	1	1.285	.242
Apolipoprotein D	Apod	1	2.003	.292
Apolipoprotein F	Apof	1	1.598	.068
Apolipoprotein L8	Apol8	1	1.697	.110
Cholesterol transporters				
ATP-binding cassette subfamily A member 1	Abca1	1	1.244	.282
Apolipoprotein A-I	Apoa1	1	1.285	.242
Apolipoprotein E	Apoe	1	1.270	.113
START domain containing 3	Stard3	1	1.295	.126
Cholesterol efflux				
ATP-binding cassette subfamily A member 1	Abca1	1	1.244	.282
Apolipoprotein E	Apoe	1	1.270	.113
Reverse cholesterol transport				
ATP-binding cassette subfamily A member 1	Abca1	1	1.244	.282
Apolipoprotein A-I	Apoa1	1	1.285	.242
Apolipoprotein A-II	Apoa2	1	1.317	*.041
Apolipoprotein E	Apoe	1	1.270	.113
Lecithin–cholesterol acyltransferase	Lcat	1	1.225	.125
Other genes involved in cholesterol transport				
NPC1-like1	Npc1l1	1	1.280	.567
Oxysterol-binding protein-like 5	Osbp15	1	1.605	.441
(B) Genes upregulated in cholesterol catabolism of sesame oil diet-fed animals				
Cholesterol catabolism				
Aldo–Keto reductase family1, member D1	Akr1d1	1	1.270	.318
Apolipoprotein E	Apoe	1	1.270	.113
Cytochrome P450, family 46, subfamily a, polypeptide 1	Cyp46a1	1	1.058	.926
Cytochrome P450, family 47, subfamily a, polypeptide 1	Cyp7a1	1	2.731	.444
Scavenger receptor class F, member 1	Scarf1	1	1.096	.569
Sorting nexin 17	Snx 17	1	1.810	.422
Cholesterol homeostasis				
ATP-binding cassette subfamily A member 1	Abca1	1	1.244	.282
Angioprotein-like 3	Angptl3	1	1.666	.130
Apolipoprotein A-I	Apoa1	1	1.285	.242
Apolipoprotein A-II	Apoa2	1	1.317	*.041
Apolipoprotein E	Apoe	1	1.270	.113
Lecithin–cholesterol acyltransferase	Lcat	1	1.225	.125
Low-density lipoprotein receptor	Ldlr	1	1.423	.360
Ldlr-associated protein 1	Ldlrap1	1	1.051	.588
Proprotein convertase subtilisin/kexin type 9	Pcsk9	1	1.064	.799
Cholesterol biosynthesis				
Acetyl-Coenzyme A acyltransferase 2 (mitochondrial3-oxoacyl-Coenzyme A thiolase)	Acaa2	1	1.476	.059
Cellular nucleic acid-binding protein	Cnbp	1	1.297	.230
Cytochrome b5 reductase 3	Cyb5r3	1	1.537	*.037
Cytochrome P450, family 51	Cyp51	1	1.218	.212
24-dehydrocholesterol reductase	Dhcr24	1	1.212	.102
Phenylalkylamine Ca+2 antagonist-binding protein	Ebp	1	1.221	*.014
Farnesyl diphosphate farnesyl transferase 1	Fdft1	1	1.419	*.041
Farnesyl diphosphate synthetase	Fdps	1	1.204	.712
3-hydroxy-3-methylgutaryl-Coenzyme A reductase	Hmgcr	1	1.811	.241
3-hydroxy-3-methylgutaryl-Coenzyme A synthase 1	Hmgcs1	1	1.031	.778
3-hydroxy-3-methylgutaryl-Coenzyme A synthase 2	Hmgcs2	1	1.235	.357
Isopenenyl-diphosphate delta isomerase	ldi1	1	1.287	.185
Isopenenyl-diphosphate delta isomerase 2	ldi2	1	2.988	.266
Mevalonate decarboxylase	Mvd	1	1.057	.983

(continued)

TABLE 1. (CONTINUED)

Gene name	Symbol	ATH/High-fat diet	Seso diet	P value
NPC1-like 1	Npc1l1	1	1.280	.567
NAS(P)-dependent steroid dehydrogenase-like	Nsdhl	1	1.936	.230
Phospho mevalonate kinase	Pmvk	1	1.357	*.025
Protein kinase, AMP-activated, alpha 2 catalytic subunit	Prkaa1	1	1.914	*.031
Transmembrane 7 superfamily member 2	Tm7sf2	1	1.519	.295
Other genes involved in cholesterol metabolism				
ATP-binding cassette subfamily A member 2	Abca2	1	1.036	.762
Apolipoprotein B	Apob	1	1.053	.990
Apolipoprotein c3	Apoc3	1	1.568	*.014
Apolipoprotein f	Apof	1	1.598	.068
Apolipoprotein L8	Apol8	1	1.697	.110
Cytochrome P450, family 7, subfamily b polypeptide 1	Cyp7b1	1	2.731	.444
Chymotrypsin-like elastase family, member 3B	Cela3b	1	1.774	.525
High-density lipoprotein (HDL)-binding protein	Hdlbp	1	1.041	.577
Interleukin 4	Il4	1	1.674	.322
Leptin	Lep	1	1.529	.315
Lipase, hormone sensitive	Lipe	1	1.008	.966
Membrane-bound transcription factor peptidase, site1	Mbtps1	1	1.702	.354
Nuclear receptor subfamily o, group B, member 2	Nrob2	1	1.157	.597
Nuclear receptor subfamily1, group H, member 4	Nr1h4	1	1.728	*.041
Oxysterol-binding protein-like 1A	Osbpl1a	1	1.181	.300
Oxysterol-binding protein-like 5	Osbpl5	1	1.605	.441
SRBEF Chaperone	Scap	1	1.293	*.024
Sterol O-acyl transferase2	Soat2	1	1.105	.418
Sterol regulatory element-binding transcription factor 1	Srebf1	1	1.020	.962
START domain containing 3	Stard3	1	1.295	.126
Very low-density lipoprotein receptor	Vldlr	1	1.180	.594

The results are expressed as fold change between the groups. *$P < 0.05$.

763.73 ± 30.7 mg/dL. Triglyceride levels were 184.85 ± 8.35 and 109.3 ± 7.29 mg/dL in control and sesame oil diets, respectively. The results of our study once again confirm that not only plasma cholesterol but also TRG is lowered by sesame oil feeding. There was also a significant reduction in LDL and VLDL cholesterol. On the other hand, HDL cholesterol was elevated in sesame oil-fed animals, suggesting that the effects cannot be attributed to fatty acid composition alone, as none of the PUFA/MUFA-rich dietary oils are known to have such a pronounced effect on HDL. As shown in Figure 1D, a 30–40% reduction in plasma lipid levels (TC, TRG, LDL, and VLDL) was observed in sesame oil diet-fed animals compared with atherogenic diet-fed animals.

Inhibition of atherosclerotic lesions in sesame oil diet-fed animals

The atherosclerotic lesion formation was analyzed in experimental animals and the extent of lesion formation was quantified by measuring the lesion surface area. We observed a significant reduction in lesion formation in sesame oil diet-fed animals compared with control animals. Figure 2A shows that control animals had prominent lesions in the aortic arch and in some animals it extended up to the abdominal aorta, whereas the sesame oil diet-fed animals had lesser and smaller lesions (Fig. 2B). Quantitation of the lesions also showed that the lesion areas of sesame oil-fed animals were significantly reduced (Fig. 2C). In most of the

sesame oil-fed animals (with the exception of three to four animals), the artery was clear compared with control animals. A scatter plot represents the aggregate of the three studies (Fig. 2D). The sesame oil diet reduced lesions by 70% compared with atherogenic diet-fed animals (Fig. 2E; mean ± SD (mm^2), ***$P < .0001$).

Gene array of mouse livers

The gene array results of our studies for lipoprotein signaling and cholesterol metabolism showed that sesame oil-treated animals exhibited several changes not only in antiatherosclerotic genes but also in genes involved in cholesterol transport and metabolism. As shown in Table 1 (A and B), there was an increase in genes related to RCT and lipid metabolism that included ABCA1, ABCA2, APOE, LCAT, CYP7a1, SR-B1, and LXR. The activation of LXR would have the additional benefit of suppressing tissue factor expression, which plays an important role in thrombus formation after plaque rupture. In contrast, many of the proatherogenic genes were suppressed in sesame oil-fed animals.

In addition to the global gene array, many of the proinflammatory genes (Fig. 3A; MCP-1, IL-1α, IL-1β, IL-6, TNF-α, and MPO), anti-inflammatory genes (Fig. 3B; IL-4 and IL-10), lipid loading genes (Fig. 3C; CD36 and SRA1), antioxidant genes (Fig. 3D; Catalase and MnSOD), genes involved in RCT and lipid metabolism (Fig. 3E; ABCA1, ABCG1, SRB1, NPC1L1, Cyp7A1), nuclear receptor
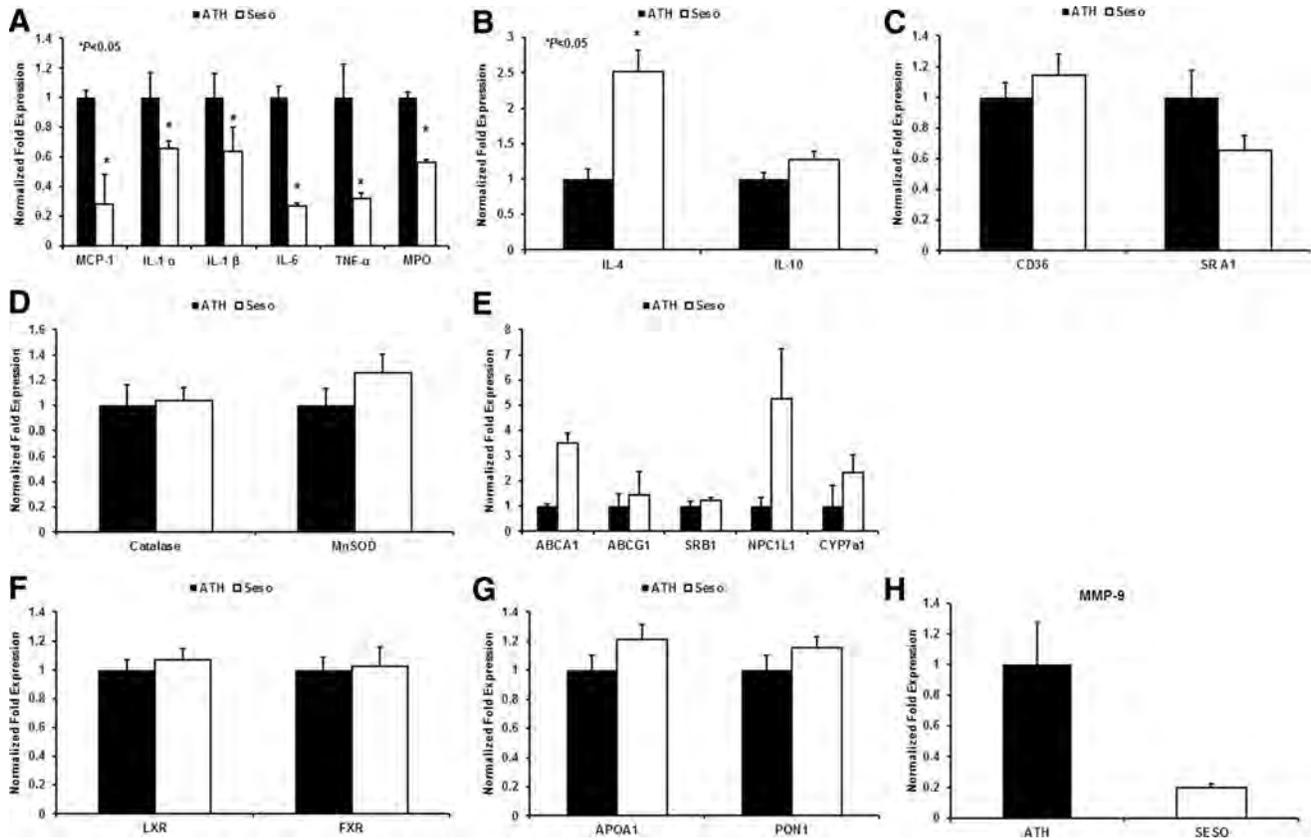
FIG. 3. Gene analysis of mice liver. The mRNA level of several genes was analyzed in the liver tissue and aorta of LDLr$^{-/-}$ mice after 3 months of feeding with a high-fat diet and the sesame oil diet. Bar diagrams that represent **(A)** proinflammatory genes, **(B)** anti-inflammatory genes, **(C)** scavenger receptors, **(D)** antioxidant genes, **(E)** genes involved in RCT and lipid metabolism, **(F)** nuclear transcription factors, **(G)** genes associated with HDL, and **(H)** matrix metalloproteinase were analyzed from the liver tissue. Values are represented as mean $\pm$ SD. *$P < .05$.

transcription factors (Fig. 3F; LXR and FXR), genes associated with HDL (Fig. 3G; ApoA1 and PON1), and the matrix metalloproteinase gene (Fig. 3H; MMP9) were analyzed independently by real-time PCR analysis. The pattern of regulation of pro- and anti-inflammatory genes was consistent with the cytokine array analysis. Similarly, expression of genes involved in RCT and lipid metabolism was also consistent with gene array studies. No difference between PXR and PPARα expression was identified (data not shown).

Gene expression in mouse aorta

Aortic gene expressions were also analyzed using real-time PCR. The results showed that sesame oil diet-fed

animals had increased mRNA levels of the RCT gene ABCA1, but reduced levels of ABCG1 (Fig. 4A) were observed. Expression of monocyte/macrophage markers and scavenger receptors CD68, SRA1, and CD36 was decreased (Fig. 4B).

Cytokine array

The sesame oil diet caused minimal changes in the profile of 96 inflammation-related proteins as measured by the cytokine array. An analysis of over 96 serum cytokines showed an up- or downregulation of cytokines related to (1) angiogenesis, (2) apoptosis, (3) matrix remodeling, (4) inflammation and immune response, (5) growth promotion,
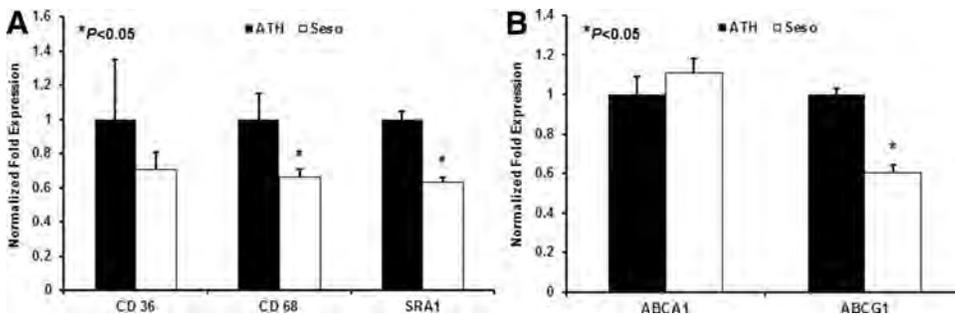

FIG. 4. Gene analysis from mice aorta. The mRNA level of several genes was analyzed in aorta of LDLr$^{-/-}$ mice after 3 months of feeding with a high-fat diet and the sesame oil diet. Bar diagrams that represent **(A)** scavenger receptors and **(B)** RCT genes were analyzed from aorta. Values are represented as mean $\pm$ SD.*$P < .05$.

and (6) scavenger function in sesame oil diet-fed animals (Supplementary Fig. S2). Upregulation of proteins involved in the removal of dead cells and growth promotion was observed in sesame oil diet-fed animals. In addition, many of the proinflammatory cytokines were downregulated.

Significant changes in the levels of inflammatory mediators, such as MCP-1, IL-1α, IL-6, and RANTES, were noted in sesame oil-fed animals compared with atherogenic diet-fed animals (Table 2), whereas the anti-inflammatory gene such as IL-13 was upregulated.

TABLE 2. CHANGES IN THE LEVELS OF INFLAMMATORY MEDIATORS IN MOUSE PLASMA
BETWEEN HIGH-FAT DIET AND SESAME OIL DIET GROUPS

Protein name	Symbol	Relative protein levels compared to ATH, fold difference		
		ATH/HF diet	Sesame oil diet	P values
Downregulated				
Tyrosine protein kinase	Axl	1	0.548	*.018
B-lymphocyte chemoattractant	BLC	1	0.759	*.036
Eotaxin	Eotaxin	1	0.871	.69
Eotaxin2	Eotaxin-2	1	0.818	.167
TNF superfamily, member 6	FAS ligand	1	0.975	.909
Fractalkine	Fractalkine	1	0.886	.19
Granulocyte macrophage colony-stimulating factor	GM-CSF	1	0.823	.618
Interferon gamma	IFN-γ	1	0.728	.348
Insulin growth factor I	IGF-I	1	0.856	.11
Insulin growth factor II	IGF-II	1	0.965	.851
Interleukin 1 alpha	IL1-α	1	0.664	.055
Interleukin 2	IL2	1	0.476	*.034
Interleukin 3	IL3	1	0.746	.364
Interleukin 3 receptor b	IL3 Rb	1	0.918	.541
Interleukin 6	IL6	1	0.894	.749
Interleukin 7	IL-7	1	0.862	.435
Interleukin 15	IL-15	1	0.984	.904
Interleukin 17B receptor	IL-17B R	1	0.934	.602
Keratinocyte-derived chemokine	KC	1	0.693	.323
Monocyte chemotactic protein 1	MCP-1	1	0.780	.21
Monokine induced by gamma interferon	MIG	1	0.951	.714
Macrophage inflammatory protein 1 alpha	MIP-1-α	1	0.930	.607
Macrophage inflammatory protein 1 gamma	MIP-1-γ	1	0.955	.422
Macrophage inflammatory protein 2	MIP-2	1	0.706	.505
Matrix metalloproteinase 2	MMP-2	1	0.919	.595
Matrix metalloproteinase 3	MMP-3	1	0.972	.659
Osteroprotegerin	Osteoprotegerin	1	0.859	.015
Regulation upon activation normal T cell expressed and presumably secreted	RANTES	1	0.757	.23
Stem cell factor	SCF	1	0.824	.435
Stromal cell-derived factor 1 alpha	SDF-1 α	1	0.804	.459
Thymus and activation-regulated chemokine	TARC	1	0.680	.228
Thymus-expressed chemokine	TECK	1	0.869	.416
Tissue inhibitor of metalloproteinase-1	TIMP-1	1	0.791	.154
Tissue inhibitor of metalloproteinase-2	TIMP-2	1	0.998	.972
Tumor necrotic factor alpha	TNF-α	1	0.909	.774
Soluble tumor necrotic factor receptor 1	sTNF RI	1	0.698	.238
Soluble tumor necrotic factor receptor 2	sTNF RII	1	0.700	.303
Thrombopoetin	TPO	1	0.881	*.009
Vascular cell adhesion molecule-1	VCAM-1	1	0.953	.84
Vascular endothelial growth factor	VEGF	1	0.957	.646
Vascular endothelial growth factor D	VEGF D	1	0.732	.078
Upregulated				
Granulocyte colony-stimulating factor	G-CSF	1	1.011	.933
Macrophage colony-stimulating factor	M-CSF	1	1.049	.078
Interleukin-1 beta	IL1-beta	1	1.461	.679
Interleukin 13	IL13	1	1.195	.679
Leptin	Leptin	1	30.348	.419
Macrophage inflammatory protein 3 beta	MIP-3β	1	1.163	.707
Macrophage inflammatory protein 3 alpha	MIP-3α	1	1.236	.203

Five samples from each group were analyzed by the Ray Bio cytokine array analysis. The protein levels are expressed as fold change between the groups. *$P < 0.05$.

Antioxidant properties of sesamol and sesamin

Sesame components are suggested to have antioxidant properties, and numerous *in vitro* systems have documented the ability of sesamol to inhibit oxidative processes. It has been identified that many antioxidants have the capacity to inhibit atherosclerosis, although to our knowledge, there is no evidence to suggest that antioxidants alone can modulate lipid levels. *In vitro* antioxidant effects of sesamol and sesamin were measured by their ability to inhibit the oxidation of lipoproteins (LDL and HDL) both enzymatically and nonenzymatically. As shown in Supplementary Figures S3 and S4, an increase in lag time of oxidation was observed with increasing concentrations/volume of sesamol and sesamin. Reduced peroxide levels and thiobarbituric reactive substances were observed in the presence of sesamol/sesamin compared with Ox-LDL, as shown in Supplementary Figures S5 and S6. The results also suggest that sesamol and sesamin could delay the oxidation of lipoproteins, but may not change the propagation or total oxidation. However, the ability of sesamol, sesamin, or other nonsaponifiables associated with sesame oil to inhibit lipoprotein oxidation *in vivo* and hence be antiatherogenic requires further investigation.

DISCUSSION

In the present study, we observed that a sesame oil-containing diet effectively reduced atherosclerotic lesion formation in LDLR$^{-/-}$ female mice as seen in our previous studies with male mice. The plasma levels of TC, TRG, VLDLc, and LDLc were decreased in these animals compared with atherosclerotic diet-fed animals. The plasma HDL level was increased in sesame diet-fed animals. It has been reported that sesame oil can lower lipid levels in serum as well as in the liver of rodents.[39] Multiple components of sesame oil, such as MUFA, PUFA, and PPAR ligands, could be responsible for lowering plasma lipids.[9,40] Our results corroborate these previous studies. However, the mechanism involved in the effects observed with sesame oil and atherosclerosis is unknown.

The present study has multiple findings. (1) Dietary supplementation with sesame oil for a 15-week period significantly decreases atherosclerotic plaque burden, (2) reduces levels of inflammatory cytokines and gene expressions, and (3) increases expression of genes involved in cholesterol transport and lipid metabolism.

There are at least three major mechanisms by which sesame oil could inhibit atherosclerosis: (1) lowering plasma cholesterol by accelerated catabolism through the oxidation of cholesterol, mediated by cholesterol 7α-hydroxylase or CYP7A1, (2) enhancing RCT mediated by SR-B1 and ABC transporters, such as ABCA1 and ABCG1, and (3) controlling mediators of inflammation. It is likely that all three play a vital role in the effects seen in animals fed with sesame oil.

Several anti-atherogenic genes were upregulated in sesame oil diet-fed animals, including genes in cholesterol transport and metabolism, matrix degrading enzymes, anti-inflammatory chemokines and cytokines, and scavenger receptors (Fig. 3). Thus, the data support our basic contention that sesame oil may have multiple components that could affect the atherogenic process in a number of ways. In peripheral cells, including vascular macrophages, ABCA1 regulates the energy-dependent transport of cholesterol and phospholipids to ApoA-I, the major protein in HDL. Similarly, PPARs are involved in the expression of several inflammatory cytokines and matrix metalloproteinases.

Increased mRNA levels of CD36 in the liver tissue were observed in sesame oil diet-fed animals, whereas no effect was seen with SR-A1. It has been identified that CD36 is activated by PPARs.[41] Although the activation of CD36 might be construed as a negative effect, this protein also is involved in fatty acid metabolism,[42] and thus, its increase might signify a general increase in fatty acid utilization. Similarly, expression of antioxidant genes such as catalase and Mn-SOD was increased, suggesting that they may be induced because of the generation of oxidized lipids[43] or because of PPAR induction.[28,44] Reduced mRNA levels of CD36 and SR-A1 were observed in aortic lesions of sesame oil diet-fed animals, suggesting the ability of sesame oil to prevent foam cell formation.

PUFAs are known to increase the conversion of cholesterol to bile acids by way of CYP7A1. Recent studies have shown that the orphan nuclear receptors, FXR and LXR, are negative and positive regulators of CYP7A1 transcription, respectively. Since we observed a trend toward activation of LXR pathways in sesame oil diet-fed animals, it is likely that the conversion of cholesterol to bile acids is enhanced in sesame oil-treated animals.

It has been identified that PUFAs promote RCT through PPARs. Potent ligands for FXR activation are the bile acids, chenodeoxycholic, deoxycholic, and lithocholic acid, while the ligands for LXR activation are oxysterols. We recently reported that oxidized fatty acids mimic bile acids in their properties and could potentially affect the FXR, thus reducing the conversion of cholesterol to bile acids.[45] Conversely, the components of sesame oil could activate the LXR and promote RCT. The induction of catalase by sesame oil nonsaponifiables, combined with their direct antioxidant effects, would suppress the negative regulation of the FXR by preventing the formation of oxidized fatty acids (which mimic bile acids), while at the same time promoting the induction of the LXR. Although sesame oil components such as sesamol have not been tested for their ability to induce CYP7A1, such methylene dioxyphenols readily interact with other cytochromes and enhance their synthesis. Thus, a pathway consisting of increased conversion of cholesterol into bile acids would be expected to increase, as well as an increase in RCT mediated by the LXR. The bile acid receptor FXR plays a major role in lipid metabolism, perhaps acting through FGF19 and FGFR4.

MMP9 plays a major role in SMC migration and proliferation during plaque formation. MMP overexpression has been implicated in pathological processes, including atherosclerosis, tumor invasion, and rheumatoid arthritis. Unstable atherosclerotic plaques show a reduced extracellular matrix due to increased MMP secretion mainly from local

inflammatory cells. Excessive production of collagenases (MMP-1) and stromelysins (MMP-3) is probably key in plaque rupture. The MMP-1, -3, and -9 promoters contain activator protein-1 (AP-1) sites and an NF-kB-binding site. Sesame oil feeding reduced the expression of MMPs.

The formation of oxidized LDL, adherence of leukocytes (VCAM-1), recruitment of monocytes (MCP-1), smooth muscle cell proliferation, disruption of the plaque (MMPs), and several other steps are influenced by oxidative stress. Hence, each step could be targeted by antioxidants. For example, TNF-α-induced expression of VCAM-1 on endothelial cells is inhibited by the presence of antioxidants. Many inflammatory cytokine genes (IL-1α, IL-6, CSF, and others) are influenced and induced by oxidative stress. Overall, many antioxidants have been shown to affect experimental atherosclerosis. Both sesamol and sesamin have phenolic hydroxyl groups and such compounds usually have potent antioxidant activities. Supplementary Figures S3 and S4 show the *in vitro* antioxidant effect of sesamol and sesamin as measured by their ability to inhibit the oxidation of LDL both enzymatically and nonenzymatically. As we go from the left to the right of the figure (with increasing concentration of sesamol or sesamin) there is a shift of the curve, suggesting an increase in lag time of oxidation. The top of the curve remains the same showing that propagation, and not the total oxidation, is affected. Thus, sesamol and sesamin could delay the oxidation of lipoproteins. However, these *in vitro* studies do not reveal whether sesamol or sesamin or other nonsaponifiables associated with sesame oil could be carried in LDL *in vivo* and whether such antioxidant effects play any role in the oil's *in vivo* anti-atherogenic effects.

In our study, we also observed reduced body and liver weights of sesame oil diet-fed animals, but they are not significantly different. This might be due to the sesame oil component lignans, which have the ability to activate the body to oxidize more fat and decrease the storage of fat. Yet another possibility is decreased lipogenesis caused by decreasing lipogenic enzymes in the liver metabolism. Sesame seeds and oil are known to benefit the digestive system, relieve constipation, and cause detoxification, which might pave the way for a healthier digestive system and a healthy colon. In addition, increased leptin levels might direct the central nervous system for lessened food intake due to high-energy stores in the adipose. Leptin is a hormone secreted from fat cells, which circulates in the bloodstream and goes to the brain. Increased levels of leptin suggest a satisfactory level of energy storage. The circulating leptin levels reflect the amount of energy stored in adipose tissue and direct the central nervous system to regulate energy homeostasis (either up or down, depending upon the status of storage depots), neuroendocrine function, and metabolism.[46] Additional mechanistic insights are needed to determine the role of sesame oil in leptin synthesis as human and animal studies seem to differ, as noted from literature.

Our observations shed light on some of the mechanisms by which a sesame oil-rich diet could influence both the progression and regression of atherosclerotic lesions in a positive manner. Understanding the molecular mechanisms by which sesamol and other similar nonsaponifiables in sesame oil could be anti-atherogenic would open up enormous new areas of research in the dietary prevention of cardiovascular disease.

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AUTHOR DISCLOSURE STATEMENT

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