Water-Soluble Components of Sesame Oil Reduce Inflammation and Atherosclerosis

Chandrakala Aluganti Narasimhulu, Krithika Selvarajan, Kathryn Young Burge,
Dmitry Litvinov, Bhaswati Sengupta, 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, is now recognized as a chronic inflammatory disease. Nonpharmacological means of treating chronic diseases have gained attention recently. We previously reported that sesame oil aqueous extract (SOAE) has anti-inflammatory properties, both *in vitro* and *in vivo*. In this study, we have investigated the antiatherosclerotic properties of SOAE, and the mechanisms, through genes and inflammatory markers, by which SOAE might modulate atherosclerosis. Low-density lipoprotein receptor (LDL-R) knockout female mice were fed with either a high-fat (HF) diet or an HF diet supplemented with SOAE. Plasma lipids and atherosclerotic lesions were quantified after 3 months of feeding. Plasma samples were used for global cytokine array. RNA was extracted from both liver tissue and the aorta, and used for gene analysis. The high-fat diet supplemented with SOAE significantly reduced atherosclerotic lesions, plasma cholesterol, and LDL cholesterol levels in LDL-R^{-/-} mice. Plasma inflammatory cytokines were reduced in the SOAE diet-fed animals, but not significantly, demonstrating potential anti-inflammatory properties of SOAE. Gene analysis showed the HF diet supplemented with SOAE reduced gene expression involved in inflammation and induced genes involved in cholesterol metabolism and reverse cholesterol transport, an anti-inflammatory process. Our studies suggest that a SOAE-enriched diet could be an effective nonpharmacological treatment for atherosclerosis by controlling inflammation and regulating lipid metabolism.

KEY WORDS: cholesterol transport • inflammation • lipid metabolism • sesame oil

INTRODUCTION

ATHEROSCLEROSIS IS A COMPLEX DISEASE involving several stages, including the development of macrophage-rich early fatty streak lesions, smooth muscle cell proliferative lesions, and advanced calcified lesions.^{1,2} Inflammation and oxidative stress are crucial in every stage of atherosclerosis, and both conditions suggest targets for therapeutic intervention.^{3–6} Current therapies for the prevention and treatment of atherosclerosis are unable to effectively target the inflammatory mechanisms involved in the initiation and progression of the disease.^{7,8}

Diet and exercise have profound preventative effects on atherosclerosis and are also effective in the treatment of preexisting atherosclerosis. Despite the lack of a clear mechanism, a diet high in polyunsaturated fat has repeatedly been shown to be a deterrent of experimental atherosclerosis. The influence of various cooking oils 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 unsaturated fats affect atherosclerosis is unknown.

Sesame (*Sesamum indicum*) oil (SO) is rich in both MUFA and PUFA. Many of the *in vivo* studies have identified that sesame oil contains lignans^{12–14} and is able to reduce oxidative stress and inflammation.¹⁵ Recently, we revealed a close relationship of inflammation with oxidative stress,¹⁶ as well as the effects of sesame oil feeding on inflammation and atherosclerosis *in vivo*.¹⁷

Sesame oil has been shown to reduce high blood pressure and lower the amount of medication needed to control hypertension.^{18–19} The oil is also capable of reducing plasma cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels.²⁰ Our earlier studies of SO diet-fed LDL receptor (LDL-R)^{-/-} mice showed that the plasma levels of total cholesterol, TGs, VLDLcholesterol, and LDL cholesterol were decreased, while HDL was significantly increased in these animals compared to high-fat (HF) diet-fed control animals. The SO diet effectively prevented inflammation and atherosclerotic lesion formation in LDL-R^{-/-} male mice.^{17,21} The ratio of saturated to unsaturated fatty acid composition in SO is less²² compared with many other oils, but the observed level of inhibition of atherosclerosis is remarkable. This extraordinary finding prompted us to question whether components in the oil beyond simply the fatty acid composition could be responsible for the

Manuscript received 16 December 2015. Revision accepted 12 May 2016.

Address correspondence to: Sampath Parthasarathy, PhD, MBA, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 6900 Lake Nona Blvd, Orlando, FL 32827, USA, E-mail: sparth@ucf.edu

observed level of atherosclerotic inhibition. Hence, we tested whether nonlipid components associated with sesame oil have anti-inflammatory and antioxidant properties, and whether the sesame oil aqueous extract (SOAE) might be effective in inhibiting inflammation both *in vitro* and *in vivo*. Interestingly, SOAE appears to have all the properties reported in our earlier studies,²² then attributed to the unsaturated quality of sesame oil. In this study, we tested whether SOAE alone might be effective in inhibiting atherosclerosis. In addition, we also tested the effect of SOAE on regulation of lipid metabolism by studying expression of reverse cholesterol transport (RCT) genes, as well as investigating macrophage scavenger receptors, as a complex interplay of all the factors involved in atherosclerosis.

MATERIALS AND METHODS

Detailed description of methods is available in Supplementary Data.

Preparation and analysis of aqueous extract

SOAE was prepared and used as described previously.²² Briefly, SOAE was prepared by using sesame oil and distilled water. The aqueous portion was separated by filtration and lyophilized. The lyophilized sample was reconstituted with pyrogen-free water. Absence of lipid portion and residual protein was confirmed by TLC and SDS-PAGE analysis. In addition, absence of possible endotoxin contamination of SOAE was confirmed using the limulus assay.

Animals

Fifty-seven 4-week-old female LDL-R^{-/-} mice on a C57BL/6J background (B6.129S7-Ldlr^{tm1Her/J} strain), weighing 18–20 g, were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and used for the study.

Diet

The atherogenic diet (TD.04287) was purchased from Harlan Teklad (Madison, WI, USA) and the SOAE diet was prepared by supplementing the above-mentioned atherogenic diet with SOAE (340 mg/kg). The composition of the HF diet was identical to that described previously,²³ and the composition of both diets is delineated in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/jmf).

Isolation of mouse peritoneal macrophages

Macrophages from the peritoneal cavity of HF diet and SOAE diet-fed animals were isolated by peritoneal lavage using saline.²⁴ Cells were utilized for RNA isolation.

Collection of plasma and organs

After 15 weeks, mice were fasted overnight and blood, plasma, and tissue samples were collected as described previously and stored at -80°C .²³

Isolation and quantification of aortic lesions

Isolation of the aorta and quantification of aortic lesions were performed as described previously.^{17,23} Lesion areas were marked on photographs by direct microscopic observations. The lesion area was quantified using ImageJ software.²³ After imaging, aortas were stored at -80°C for RNA extraction.

Plasma lipid analysis

Plasma lipid profiles were determined by using a Cholestech LDX analyzer (Cholestech Corp, Hayward, CA, USA).

cDNA synthesis and real-time polymerase chain reaction

Total RNA from peritoneal macrophages, liver, and aortic tissue was isolated by using TRIzolTM reagent. Total RNA of 1 μg was then reverse transcribed into cDNA using the SuperscriptTM III First-Strand Synthesis system (Invitrogen, Carlsbad, CA, USA). cDNA (50 ng) samples were used to perform quantitative real-time polymerase chain reaction (RT-PCR) by CFX iCycler Multicolor RT-PCR Detection System (Bio-Rad, Hercules, CA, USA) with SYBR Green (Invitrogen). Mouse oligonucleotide primers for RT-PCR were purchased from Invitrogen. PCR was carried out with ABCA1, ABCG1, SRB1, Cyp7a1, NPC1L1, TNF α , MCP-1, IL-1 α , IL-1 β , IL-6, IL-4, IL-10, catalase, MnSOD, SRA1, CD36, LXR, pregnane X receptor, farnesoid X receptor (FXR), PPAR α , ApoA1, PON1, MMP-9, MPO, CD4, P-selectin, and CD68, represented in Supplementary Table S2.

Global cytokine array

Plasma samples ($n=3$ from each group) were analyzed by global cytokine array by Ray Biotech, Inc. (Norcross, GA, USA) using RayBio[®] Mouse G Series Array 3 and 4 glass chip.

Cytochrome C reduction assay

Cytochrome C reduction was run in a quartz cuvette in 1 mL PBS containing 100 μM xanthine and 10 μM cytochrome C from bovine heart, both in the presence and absence of SOAE. Reduction of cytochrome C was initiated by adding 0.02 units of xanthine oxidase and was followed by monitoring the optical density at 550 nm for 30 min in a UVIKON XL spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA).

Statistical analyses

Values are presented as mean $\pm$ 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 Wilcoxon matched pairs test using GraphPad Prism software, with $P < .05$ considered to be significant.

RESULTS

Body weight analysis in mice

The atherogenic diet contains 17% saturated fat provided by milk, while the SOAE diet, an identical preparation plus a small supplement (*i.e.*, 340 mg of SOAE per kg of HF diet), roughly translates to 1 mg per mouse per day. Even though there was no significant difference in food consumption between the two groups, a slight but insignificant decrease in body weight ($\sim 3\%$) and liver weight (6%) was observed in SOAE diet-fed animals compared to the control animals (Supplementary Figs. S1A and S1B).

Reduced plasma lipid levels in SOAE diet-fed animals

Visual observation of plasma following centrifugation revealed a clear plasma in SOAE diet-fed animals compared to control animals. This probably reflects a decrease in plasma lipids in SOAE diet-fed animals. As shown in Figure 1A–C, the plasma lipid profile analysis showed a decrease in total cholesterol and LDL cholesterol in SOAE diet-fed animals compared to controls even though the SOAE is supplementary to HF diet. A slight increase in HDL cholesterol levels was observed in SOAE diet animals (Fig. 1D). Elevated levels of HDL cholesterol in SOAE diet-

fed animals in the presence of HF diet suggest that the components of SOAE have a pronounced effect on HDL.

Inhibition of atherosclerotic lesions in SOAE diet-fed animals

The atherosclerotic lesion formation was analyzed and the extent of lesion formation was quantified by measuring the lesion surface area. We observed a significant reduction in lesion formation in SOAE diet-fed animals compared to control animals. Figure 2A shows that control animals had prominent lesions in the aortic arch and in some animals, these lesions extended down to the abdominal aorta. Comparatively, the SOAE diet-fed animals had smaller lesions (Fig. 2B). Quantitation of the lesions also showed that the lesion areas of SOAE diet-fed animals were significantly reduced (Fig. 2C). Scatter plot represents the aggregate of the three studies (Fig. 2D). The SOAE diet reduced lesions by 40% compared to atherogenic diet-fed animals (Fig. 2E; mean $\pm$ SD [mm^2], *** $P < .0001$).

Liver gene expressions

Many of the proinflammatory genes (Fig. 3A; MCP-1, IL-1 α , IL-1 β , IL-6, TNF- α and MPO), anti-inflammatory genes (Fig. 3B; IL-4 and IL-10), lipid-loading genes (Fig. 3C; CD36

FIG. 3. Gene analysis from mice liver mRNA level of several genes was analyzed in liver tissue and aorta of $\text{LDLr}^{-/-}$ mice after 3 months of feeding with high-fat diet, and the SOAE diet. Bar diagrams 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, (H) Matrix metalloproteinases analyzed from the liver tissue ($n=7$ from each group). Values are represented as mean $\pm$ SD, * $P < .05$. ns, nonsignificant; RCT, reverse cholesterol transport.

FIG. 4. Gene analysis from mice aorta. mRNA level of several genes was analyzed in aorta of $LDLR^{-/-}$ mice after 3 months of feeding with high-fat diet, and the SOAE diet. Bar diagrams represent (A) RCT genes, (B) Scavenger receptors, and (C) inflammatory genes analyzed from aorta. (D) Relative level of mRNA of CD68 in aortic arch and abdominal aorta ($n=7$ from each group). Values are represented as mean $\pm$ SD, $*P < .05$. ns, nonsignificant.

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 transcription factors (Fig. 3F; LXR, FXR and PPAR α), genes associated with HDL (Fig. 3G; ApoA1 and PON1), and the matrix metalloproteinase gene (Fig. 3H; MMP9) were analyzed independently by RT-PCR analysis. Increased gene expression of antioxidant, nuclear receptor

transcription factors, and HDL-associated genes was observed, whereas reduced expression was observed in inflammatory, lipid loading, as well as matrix metalloproteinases.

Aorta gene expressions

Aortic gene expressions were also analyzed using RT-PCR. The results showed that SOAE animals had increased

FIG. 5. Gene analysis from mice peritoneal macrophages. mRNA level of several genes was analyzed in peritoneal macrophages of $LDLR^{-/-}$ mice after 3 months of feeding with HF diet, and the SOAE diet. Bar diagrams represent (A) RCT genes and (B) scavenger receptors analyzed from peritoneal macrophages ($n=7$ from each group). Values are represented as mean $\pm$ SD, $*P < .05$. ns, nonsignificant.

mRNA levels of the RCT gene ABCA1, but reduced levels of ABCG1 (Fig. 4A). Expression of monocyte/macrophage markers and scavenger receptors SRA1 and CD36 were decreased in SOAE diet-fed animals (Fig. 4B). Similarly, genes involved in inflammation (MCP-1, CD4, and P-selectin) were reduced in experimental animals compared to those of the controls (Fig. 4C). In addition, there was a difference in the level of CD68 between HF and SOAE diet-fed animals in the aortic arch segments and lesion-free abdominal aorta segments (Fig. 4D). SOAE supplementation decreased CD68 levels in the aortic arch by 20% and by 80% ($P < .05$) in abdominal segments of the aorta, both relative to identical segments in the controls.

Gene expression in mouse peritoneal macrophages

In addition to the liver and aortic gene expressions, peritoneal macrophage gene expressions were also analyzed using RT-PCR. The results showed that SOAE diet-fed animals had slight increase in mRNA levels of the RCT gene SRB1, but reduced levels of ABCG1 (Fig. 5A) were ob-

served. Reduced expressions of scavenger receptors SRA1 and CD36 were also identified (Fig. 5B).

Cytokine array

SOAE induced minimal changes in the profile of 96 inflammation-related proteins as measured by the cytokine array. Cytokines were grouped and analyzed as described previously.¹⁷ Upregulation of proteins involved in the removal of dead cells and growth promotion were observed in SOAE diet-fed animals. In addition, many of the proinflammatory cytokines were downregulated in SOAE animals. Reduced levels of inflammatory mediators such as IL-1 α , TNFR2, MCSF, P-selectin, and ICAM1 were noted in SOAE diet-fed animals compared to atherogenic diet-fed animals (Table 1), whereas anti-inflammatory cytokines such as IL-4 and IL-10 were upregulated in SOAE animals.

Evaluation of antioxidant properties of SOAE

In our earlier studies, we provided evidence that SOAE is able to inhibit copper-induced, as well as myeloperoxidase-

TABLE 1. CHANGES IN THE LEVEL OF INFLAMMATORY MEDIATORS IN MOUSE PLASMA BETWEEN HF DIET AND SOAE DIET GROUPS

Protein name	Protein symbol	Relative protein levels compared to ATH, fold difference		P
		ATH/HF diet	SOAE diet	
Cutaneous T cell-attracting chemokine	CTACK	1	0.99	.92
Insulin-growth factor binding protein 6	IGF-BP-6	1	0.99	.98
Macrophage inflammatory protein 1 gamma	MIP-1-gamma	1	0.98	.32
Intercellular adhesion molecule 1	ICAM-1	1	0.98	.93
Basic fibroblast growth factor	bFGF	1	0.97	.86
Platelet selectin	P-Selectin	1	0.97	.85
Osteoprotegerin	Osteoprotegerin	1	0.95	.88
Sonic hedgehog N-terminal domain	Shh-N	1	0.93	.76
Stem cell factor	SCF	1	0.92	.81
Lymphocyte selectin	L-Selectin	1	0.92	.90
Interleukin 3 receptor b	IL3 Rb	1	0.91	.55
Platelet factor 4	PF4	1	0.91	.48
Matrix metalloproteinase 3	MMP-3	1	0.90	.53
Interleukin 17B receptor	IL-17B R	1	0.89	.63
Low-affinity immunoglobulin gamma Fc region receptor II-b	Fc γ RIIB	1	0.89	.51
Insulin growth factor 1	IGF-I	1	0.88	.78
Matrix metalloproteinase 2	MMP-2	1	0.86	.57
Tyrosine protein kinase	Axl	1	0.86	.67
Macrophage inflammatory protein 1 alpha	MIP-1-alpha	1	0.86	.70
Interleukin 1 alpha	IL1-alpha	1	0.84	.49
Vascular endothelial growth factor receptor 2	VEGF R2	1	0.83	.09
Hepatocyte growth factor receptor	HGF R	1	0.82	.19
Interleukin 15	IL-15	1	0.78	.41
Tissue inhibitor of metalloproteinase-1	TIMP-1	1	0.75	.14
Macrophage colony-stimulating factor	M-CSF	1	0.73	.40
Soluble tumor necrotic factor receptor 2	sTNF RII	1	0.15	.45
<i>Upregulated</i>				
Interleukin 4	IL-4	1	1.11	.33
Interleukin 10	IL-10	1	1.58	.03*
Insulin growth factor binding protein-3	IGFBP-3	1	1.20	.17

Three samples from each group were analyzed by Ray-Bio[®] cytokine array analysis. The protein levels are expressed as fold change between the groups.

* $P < .05$.

HF, high-fat; SOAE, sesame oil aqueous extract.

induced LDL/HDL oxidation. In this study, we further tested whether SOAE is capable of inhibiting cytochrome C reduction by xanthine oxidase generation of superoxide, a reaction playing a major role in atherosclerosis and ischemia/reperfusion injury. Increasing concentrations of SOAE resulted in a decrease in the reduction of cytochrome C by xanthine oxidase-generated superoxide (Supplementary Fig. S2). Of course, the possibility remains that SOAE reacted only with the superoxide radical, thereby preventing the latter's interaction with cytochrome C.

Statistical analysis

Values are presented as mean $\pm$ 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 Wilcoxon matched pairs test using Graph Pad Prism software, with $P < .05$ considered to be significant.

DISCUSSION

Oxidized LDL is a huge complex providing a large number of lipid peroxides and degradative products in atherosclerotic plaques. In our previous study, we established that SOAE has anti-inflammatory effects both *in vitro* and *in vivo*.²⁴ The effect of SOAE on development of atherosclerosis has not yet been elucidated. To evaluate the effect of SOAE on the development of atherosclerotic plaques, we utilized female LDL-R^{-/-} mice fed HF, high-cholesterol ("Western") diet, a well-known animal model of atherosclerosis.

A cardioprotective diet regime and use are often recommended along with drug therapy as an effective treatment for cardiovascular diseases. This study provides numerous insights into potential dietary prevention and regulation of atherosclerosis: (1) Dietary supplementation with SOAE for a 15-week period significantly decreases atherosclerotic plaque burden, (2) attenuates the levels of inflammatory cytokines, and (3) increases expression of genes involved in cholesterol transport and lipid metabolism, similar to the effects of sesame oil.

The plasma levels of TC and LDL-c were significantly decreased in SOAE animals compared to atherosclerotic diet-fed animals. An increase in plasma HDL-c levels was observed in SOAE diet-fed animals as shown in Figure 1, suggesting that SOAE has a substantial influence even in the presence of an HF diet. Reduced mRNA levels of CD36 and SR-A1 in the liver tissue were observed in SOAE diet-fed animals. Increased expression of antioxidant genes such as catalase and Mn-SOD was observed, suggesting that these genes may be induced due to the generation of oxidized lipids²⁵ or as a result of PPAR induction.²⁶ PUFAs and sesame oil (our recent report) are known to increase the conversion of cholesterol to bile acids by way of CYP7A1 through nuclear receptors FXR and LXR. A similar trend was observed toward activation of LXR pathways in SOAE diet-fed animals, suggesting the conversion of cholesterol to bile acids, as well as enhanced RCT with increased ex-

pressions of ABCA1, ABCG1, and SRB1 in SOAE-treated animals. The bile acid receptor FXR plays a major role in lipid metabolism, perhaps acting through FGF19 and FGFR4. Growing evidence indicates that the tissue-specific action of these receptors is also essential for the proper functioning of the cardiovascular, endocrine, immune, pancreas, renal, reproductive, and central nervous systems. Taken together, LXRs and FXRs have been considered as potential therapeutic targets for the treatment and prevention of several metabolic and lipid-related diseases.

The induction of PON1 and ApoA1 in SOAE diet animals suggests protection against oxidative stress-induced atherosclerosis as well as enhanced functionality of HDL. PON1 is synthesized mainly by the liver and circulates in association with ApoA-I and HDL.²⁷ PON1 is known to inactivate lipid peroxides and hydrogen peroxide, and therefore is thought to offer protection against oxidative stress.²⁸⁻³⁰ PON1-deficient mice have been found to be more susceptible to lipoprotein oxidation and atherosclerosis,^{31,32} and transgenic mice overexpressing PON1 have been found to have decreased atherosclerotic lesions.³³ ApoA1 is the primary protein associated with HDL, and mice overexpressing ApoA1 show reduced atherosclerosis.³⁴

Decreased MMP9 expression was observed in SOAE diet-fed animals. MMP9 plays a key role in smooth muscle cell (SMC) migration and proliferation during plaque formation and is considered a major pathological factor in atherosclerosis. In addition, reduced plasma levels of MMP2 and MMP3 were also observed in SOAE diet-fed animals.

Reduced mRNA levels of CD36 and SRA1 were observed in aortic lesions of SOAE diet-fed animals, suggesting the ability of SOAE to prevent foam cell formation. Sesame oil contains lignans, which are known to complex cholesterol from the gut and prevent cholesterol absorption. The contribution of these lignans to the observed drop in plasma cholesterol cannot be ignored. Similarly, nonsaponifiable components of sesame oil (SOAE) might be contributing to the prevention of cholesterol absorption. We observed a decrease in the mRNA level of CD68, a marker for monocyte/macrophages, in SOAE diet-fed animals compared to HF diet-fed animals. The decrease of CD68 in the aortic arch area containing lesions is a confirmation that the presence of foam cell-forming macrophages in SOAE animals was reduced.

The decrease of CD68 mRNA was also observed in the abdominal aorta section, which did not contain any visible lesions, potentially suggesting SOAE may have reduced the number of resident macrophages inside the arterial wall or on its surface. There was a difference in MCP-1 gene expression between HF and SOAE groups, denoting the possibility that reduced expression of other chemotactic factors may have contributed to the reduced presence of macrophages.

We observed an increased level of plasma IGFBP-3 in animals with reduced atherosclerosis in SOAE diet-fed animals compared to HF diet-fed animals, yet the changes in plasma IGF-I or IGF-II were not statistically significant (data not shown). It has been identified that IGFBP-3 is

anti-inflammatory in nature, having the ability to inhibit TNF alpha, CRP, and further NFkB activity.³⁵ IGFBP-3 also inhibits the proliferation of SMCs. Conflicts exist in the discussion of plasma IGFBP3 levels, as some studies noted that an increased level of IGFBP-3 is associated with a higher degree of atherosclerosis in humans.³⁶ However, in most human studies, the opposite association has been observed: a lower level of IGFBP-3 is associated with an increased extent of atherosclerosis, a higher risk of nonfatal myocardial infarction, and ischemic stroke, and decreased plaque stability.^{37–40} Based on our studies, we suggest that an increase in IGFBP-3 is antiatherogenic itself.

There is a slight discrepancy of gene expression versus protein analysis of pro- and anti-inflammatory cytokines (IL1 β , TNF- α , IL-10) due to the short half-life of cytokines and also due to the number of samples used. Yet another possibility might be the posttranslational modifications, which might be a reason for the difference in mRNA and protein levels.

Atherosclerosis has been linked to the oxidation of lipoproteins, mainly LDL, in the vascular wall⁴¹; however, the mechanisms by which LDL may be oxidized *in vivo* have not been fully revealed.⁴² Involvement of superoxide in the modification of LDL might be due to the reactive peroxynitrite formed through the reaction between NO and O₂^{•−},⁴³ generated due to impaired vascular reactivity. Several sources of O₂^{•−} in the vascular wall have been reported, including NAD(P)H oxidases in endothelial cells, fibroblasts, macrophages, and SMCs.^{44–47} Other potential sources of O₂^{•−} are lipoxygenases, NO synthases, and xanthine oxidase.^{48–50} Furthermore, increased production of O₂^{•−} has been reported in a variety of vascular pathologies. Hence, we tested the ability of SOAE to inhibit the production of superoxide radical using xanthine oxidase and cytochrome C oxidations. Indeed, SOAE is able to inhibit the cytochrome C reduction by superoxide radical generated by xanthine oxidase.

Our data suggest that SOAE is able to inhibit atherosclerosis in animals. While identifying the specific components in SOAE will shed light on the nature of compounds, one has to keep in mind that the specific mix might be a more powerful nonpharmacological agent due to potential synergistic interaction among the components. Dietary approaches to prevent, treat, or manage atherosclerosis are more desirable than pharmacological control as these are considered to be safer. An in-depth understanding of the nature of SOAE components and their mechanism of action could lead to the development of inexpensive and powerful “adjunctive therapy” to existing medicines.

ACKNOWLEDGMENT

This study was supported by National Institutes of Health Grant 5R01AT004106-05.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Hansson GK: Inflammation, atherosclerosis, and coronary artery disease *N Eng J Med* 2005;352:1685–1695.
2. Libby P: Inflammation in atherosclerosis. *Nature* 2002;420:868–874.
3. Parthasarathy S, Steinberg D, Witztum JL: The role of oxidized low density lipoproteins in the pathogenesis of atherosclerosis. *Annu Rev Med* 1992;43:219–225.
4. Steinberg D, Lewis A: Conner memorial lecture: Oxidative modification of LDL and atherogenesis. *Circulation* 1997;95:1062–1071.
5. Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D: Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 1989;84:1086–1095.
6. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL: Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915–924.
7. Rader DJ, Daugherty A: Translating molecular discoveries into new therapies for atherosclerosis. *Nature* 2008;451:904–913.
8. Nissen SE, Tardif JC, Nicholls SJ, Revkin JH, Shear CL, Duggan WT, Ruzyllo W, Bachinsky WB, Lasala GP, Tuzcu EM: Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med* 2007;356:1304–1316.
9. Mensink RP, Katan MB: Effect of a diet enriched with mono-unsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *N Engl J Med* 1989;321:436–441.
10. Parks JS, Rudel LL: Effect of fish oil on atherosclerosis and lipoprotein metabolism. *Atherosclerosis* 1990;84:83–94.
11. Sanders TA: Olive oil and the mediterranean diet. *Int J Vitam Nutr Res* 2001;71:179–184.
12. Grishina NL, Kuznetsov DI: Fatty acid composition of oils from different varieties of sunflower, peanut and sesame. *Vopr Pita* 1970;29:81–88.
13. Sengupta A, Roychoudhury SK: Triglyceride composition of sesamum indicum seed oil. *J Sci Food Agric* 1976;27:165–169.
14. Egbekun MK, Ehieze MU: Proximate composition and functional properties of full fat and defatted beniseed (*Sesamum indicum* L.) flour. *Plant Foods Hum Nutr* 1997;51:35–41.
15. Hou RC, Chen HL, Tzen JT, Jeng KC: Effect of sesame anti-oxidants on LPS-induced NO production by BV2 microglial cells. *Neuroreport* 2003;14:1815–1819.
16. Aluganti Narasimhulu C, Jiang X, Yang Z, Selvarajan K, and Parthasarathy S: Is there a connection between oxidative stress and inflammation? *Chronic Inflammation Molecular Pathophysiology, Nutritional and Therapeutic Interventions*. (Roy S, Bagchi D, Raichaudhury S, eds.) CRC Press, Taylor and Francis, Boca Raton, FL, 2012, pp.138–152.
17. Aluganti Narasimhulu C, Selvarajan K, Litvinov D, Parthasarathy S: Anti-atherosclerotic and anti-inflammatory actions of sesame oil. *J Med Food* 2015;18:11–20.
18. Ross R: The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* 1993;362:801–809.
19. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK: T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci U S A* 1995;92:3893–3897.
20. Kok T, et al.: Induction of hepatic ABC transporter expression is part of the PPARalpha-mediated fasting response in the mouse. *Gastroenterology* 2003;124:160–171.

21. Vosper H, Patel L: The peroxisome proliferator-activated receptor delta promotes lipid accumulation in human macrophages. *J Biol Chem* 2001;276:44258–44265.
22. Selvarajan K, Aluganti Narasimhulu C, Bapputty R, Parthasarathy S: Anti-inflammatory and antioxidant activities of the non-lipid (aqueous) components of sesame oil: Potential use in atherosclerosis. *J Med Food* 2015;18:393–402.
23. Bhaskaran S, Santanam N, Penumetcha M, Parthasarathy S: Inhibition of atherosclerosis in low-density lipoprotein receptor-negative mice by sesame oil. *J Med Food* 2006;9:487–490.
24. Selvarajan K, Moldovan L, Chandrakala AN, Litvinov D, Parthasarathy S: Peritoneal macrophages are distinct from monocytes and adherent macrophages. *Atherosclerosis* 2011;219:475–483.
25. Meilhac O, Zhou M, Santanam N, Parthasarathy S: Lipid peroxides induce expression of catalase in cultured vascular cells. *J Lipid Res* 2000;41:1205–1213.
26. Sulzle A, Hirche F, Eder K: Thermally oxidized dietary fat up-regulates the expression of target genes of PPAR alpha in rat liver. *J Nutr* 2004;134:1375–1383.
27. Teiber JF, Draganov DI, La Du BN: Purified human serum PON1 does not protect LDL against oxidation in the in vitro assays initiated with copper or AAPH. *J Lipid Res* 2004;45:2260–2268.
28. Shamir R, Hartman C, Karry R, Pavlotzky E, Eliakim R, Lachter J, Suissa A, Aviram M: Paraoxonases (PONs) 1, 2, and 3 are expressed in human and mouse gastrointestinal tract and in Caco-2 cell line: Selective secretion of PON1 and PON2. *Free Radic Biol Med* 2005;39:336–344.
29. Deakin S, Moren X, James RW: HDL oxidation compromises its influence on paraoxonase-1 secretion and its capacity to modulate enzyme activity. *Arterioscler Thromb Vasc Biol* 2007;27:1146–1152.
30. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN: Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest* 1998;101:1581–1590.
31. Karabina SA, Lehner AN, Frank E, Parthasarathy S, Santanam N: Oxidative inactivation of paraoxonase—implications in diabetes mellitus and atherosclerosis. *Biochim Biophys Acta* 2005;1725:213–221.
32. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, et al.: Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 2000;394:284–287.
33. Tward A, Xia YR, Wang XP, Shi YS, Park C, Castellani LW, Lusis AJ, Shih DM: Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation* 2002;106:484–490.
34. Rader DJ: High-density lipoproteins and atherosclerosis. *Am J Cardiol* 2002;90:62–70.
35. Mohanraj L, Kim H, Li W, Cai Q, Kim KE, Shin H, Lee Y, Lee WJ, Kim J, Oh Y: IGFBP-3 inhibits cytokine-induced insulin resistance and early manifestations of atherosclerosis. *PLoS One* 2013;8:e55084.
36. Kawachi S, Takeda N, Sasaki A, Kokubo Y, Takami K, Sarui H, Hayashi M, Yamakita N, Yasuda K: Circulating insulin-like growth factor-I and insulin-like growth factor binding protein-3 are associated with early carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005;25:617–621.
37. Schuler-Luttman S, Monnig G, Enbergs A, Schulte H, Breithardt G, Assmann G, Kerber S, vonEckardstein A: Insulin-like growth factor-binding protein-3 is associated with the presence and extent of coronary arteriosclerosis. *Arterioscler Thromb Vasc Biol* 2000;20:E10–E15.
38. Kaplan RC, McGinn AP, Pollak MN, Kuller LH, Strickler HD, Rohan TE, Cappola AR, Xue X, Psaty BM: Association of total insulin-like growth factor-I, insulin-like growth factor binding protein-1 (IGFBP-1), and IGFBP-3 levels with incident coronary events and ischemic stroke. *J Clin Endocrinol Metab* 2007;92:1319–1325.
39. Johnsen SP, Hundborg HH, Sørensen HT, Orskov H, Tjønneland A, Overvad K, Jørgensen JO: Insulin-like growth factor (IGF) I, -II, and IGF binding protein-3 and risk of ischemic stroke. *J Clin Endocrinol Metab* 2005;90:5937–5941.
40. Martin RM, Gunnell D, Whitley E, Nicolaides A, Griffin M, Georgiou N, Davey Smith G, Ebrahim S, Holly JM: Associations of insulin-like growth factor (IGF)-I, IGF-II, IGF binding protein (IGFBP)-2 and IGFBP-3 with ultrasound measures of atherosclerosis and plaque stability in an older adult population. *J Clin Endocrinol Metab* 2008;93:1331–1338.
41. Steinberg D: Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997;272:20963–20966.
42. Heinecke JW, Baker L, Rosen H, Chait A: Superoxide-mediated modification of low density lipoprotein by arterial smooth muscle cells. *J Clin Invest* 1986;77:757–761.
43. Darley-USmar VM, Hogg N, O'Leary VJ, Wilson MT, Moncada S: The simultaneous generation of superoxide and nitric oxide can initiate lipid peroxidation in human low density lipoprotein. *Free Radic Res Commun* 1992;17:9–20.
44. Gorlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R: A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res* 2000;87:26–32.
45. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW: Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994; 74:1141–1148.
46. Johnston RBJ, Lehmeyer JE, Guthrie LA: Generation of superoxide anion and chemiluminescence by human monocytes during phagocytosis and on contact with surface-bound immunoglobulin G. *J Exp Med* 1976;143:1551–1556.
47. Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, Quinn MT: Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: Enhancement by angiotensin II. *Proc Natl Acad Sci U S A* 1997; 94:14483–14488.
48. Kukreja RC, Kontos HA, Hess ML, Ellis EF: PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. *Circ Res* 1986;59:612–619.
49. White CR, Darley-USmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, Parks DA, Tarpey MM, Freeman BA: Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. *Proc Natl Acad Sci U S A* 1996;93:8745–8749.
50. Pou S, Pou WS, Bredt DS, Snyder SH, Rosen GM: Generation of superoxide by purified brain nitric oxide synthase. *J Biol Chem* 1992;267:24173–24176.