Dietary α-Lipoic Acid Supplementation Inhibits Atherosclerotic Lesion Development in Apolipoprotein E–Deficient and Apolipoprotein E/Low-Density Lipoprotein Receptor–Deficient Mice

Wei-Jian Zhang, MD, PhD; Karyn E. Bird, DVM, PhD; Timothy S. McMillen, PhD; Renee C. LeBoeuf, PhD; Tory M. Hagen, PhD; Balz Frei, PhD

Background—Vascular inflammation and lipid deposition are prominent features of atherosclerotic lesion formation. We have shown previously that the dithiol compound α-lipoic acid (LA) exerts antiinflammatory effects by inhibiting tumor necrosis factor-α– and lipopolysaccharide-induced endothelial and monocyte activation in vitro and lipopolysaccharide-induced acute inflammatory responses in vivo. Here, we investigated whether LA inhibits atherosclerosis in apolipoprotein E–deficient (apoE−/−) and apoE/low-density lipoprotein receptor–deficient mice, 2 well-established animal models of human atherosclerosis.

Methods and Results—Four-week–old female apoE−/− mice (n=20 per group) or apoE/low-density lipoprotein receptor–deficient mice (n=21 per group) were fed for 10 weeks a Western-type chow diet containing 15% fat and 0.125% cholesterol without or with 0.2% (wt/wt) R,S-LA or a normal chow diet containing 4% fat without or with 0.2% (wt/wt) R-LA, respectively. Supplementation with LA significantly reduced atherosclerotic lesion formation in the aortic sinus of both mouse models by ≈20% and in the aortic arch and thoracic aorta of apoE−/− and apoE/low-density lipoprotein receptor–deficient mice by ≈55% and 40%, respectively. This strong antiatherogenic effect of LA was associated with almost 40% less body weight gain and lower serum and very low-density lipoprotein levels of triglycerides but not cholesterol. In addition, LA supplementation reduced aortic expression of adhesion molecules and proinflammatory cytokines and aortic macrophage accumulation. These antiinflammatory effects of LA were more pronounced in the aortic arch and the thoracic aorta than in the aortic sinus, reflecting the corresponding reductions in atherosclerosis.

Conclusions—Our study shows that dietary LA supplementation inhibits atherosclerotic lesion formation in 2 mouse models of human atherosclerosis, an inhibition that appears to be due to the “antiobesity,” antihypertriglyceridemic, and antiinflammatory effects of LA. LA may be a useful adjunct in the prevention and treatment of atherosclerotic vascular diseases. (Circulation. 2008;117:421-428.)

Key Words: atherosclerosis ■ cell adhesion molecules ■ inflammation ■ obesity ■ triglycerides

Clinical Perspective p 428

The initial monocyte-endothelial interactions are triggered by local expression of cellular adhesion molecules by the endothelium, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), and proinflammatory cytokines and chemokines such as tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). Studies in...
experimental animals and humans have shown increased expression of adhesion molecules and MCP-1 in developing and established atherosclerotic lesions.4–6 Conversely, genetic deficiencies of adhesion molecules, MCP-1, or TNFα in mice are associated with decreased atherosclerosis.7–10 The early atherosclerotic events and the initiation of lesion formation appear particularly dependent on VCAM-1.11 Human studies also demonstrated that increased plasma levels of soluble VCAM-1 and ICAM-1 are correlated with clinical manifestations of coronary atherosclerosis.12 Thus, inhibition of monocyte-endothelial interactions may be an effective strategy to prevent or treat atherosclerosis and cardiovascular disease.

Recent data from experimental and clinical studies indicate that α-lipoic acid (LA; 1,2-dithiolane-3-pentanoic acid) acts as an antiinflammatory agent that may help prevent cardiovascular disease.12–17 LA is found in the human diet and is available in the United States as a dietary supplement in its natural R (D) form or racemic R,S (D,L) mixture. In addition to its antiinflammatory effects, LA plays an important role in mitochondrial energy metabolism, stimulates insulin signaling and glutathione synthesis, and has antioxidant properties.18–21 LA has been used safely in patients with diabetes mellitus and has been shown to significantly improve diabetic neurovascular and metabolic complications.22 We have shown that LA exerts antiinflammatory effects by inhibiting TNFα-induced expression of adhesion molecules and MCP-1 and adherence of monocytes to human aortic endothelial cells.16 Furthermore, we have recently found that LA inhibits lipopolysaccharide-induced synthesis of MCP-1 and TNFα in monocytes in vitro and acute inflammatory responses in vivo by activating the phosphoinositide 3-kinase/Akt signaling pathway, thereby inhibiting activation of nuclear factor-κB, the central transcription factor orchestrating the inflammatory response.17 However, the potential effect of LA on atherosclerosis is unknown. Therefore, the present study was undertaken to determine whether dietary supplementation with LA inhibits atherosclerotic lesion development in apolipoprotein E-deficient (apoE−/−) and apoE/LDL receptor−deficient (apoE/LDLR−/−) mice, 2 well-established animal models of human atherosclerosis.

Methods
A full description of all Methods can be found in the online-only Data Supplement.

Animals and Experimental Procedures
Female C57BL/6, apoE−/−, and apoE/LDLR−/− mice on a C57BL/6 background at 4 to 5 weeks of age and weighing 12 to 15 g were purchased from Jackson Laboratory (Bar Harbor, Me). All animal procedures were reviewed and approved by the Oregon State University Institute Animal Care and Use Committee. For experiments, apoE−/− mice were fed ad libitum a Western-type chow diet (No. 311372, Dyets Inc, Bethlehem, Pa) containing 15% hydrogenated coconut oil and 0.125% cholesterol without (control) or with 0.2% (wt/wt) R,S-LA (Sigma-Aldrich, St Louis, Mo). ApoE/LDLR−/− mice were fed ad libitum Purina 5001 chow diet (Dyets No. 610000) containing 4% hydrogenated coconut oil without (control) or with 0.2% (wt/wt) R-LA (a gift from Dr Hans Tritschler, Vitris Inc, Frankfurt, Germany). Pair feeding of apoE/LDLR−/− mice was accomplished by measuring food intake of the LA-treated mice daily and then providing the same amount of food without LA to pair-fed control mice. For comparison, a group of C57BL/6 mice was fed ad libitum Purina 5001 chow diet containing 4% hydrogenated coconut oil without LA. At the end of the 10-week treatment period, the animals were killed, and heart and aorta were collected and processed as described below. Blood was collected and serum samples were prepared and stored at −80°C until analysis.

Serum Lipids and Lipoproteins
Serum total cholesterol was determined with a colorimetric kit (Diagnostic Chemicals Ltd, Oxford, Conn). Serum triglycerides were determined colorimetrically (Roche Diagnostics, Indianapolis, Ind). Serum lipoproteins were separated by high-resolution size exclusion/fast protein liquid chromatography (Amersham Pharmacia Biotech AB, Piscataway, NJ).

Analysis of Atherosclerotic Lesions
Aortic Sinus Lesions
Atherosclerotic lesions were quantified at the aortic sinus as described.23 The aortic sinus sections were prepared and stained with Movat’s stain.24 Quantification of atherosclerotic lesion areas was performed with a Leica microscope (Leica Microsystems GmbH, Wetzlar, Germany) and computer-assisted image analysis program (Image Pro Plus, Media Cybernetics, Bethesda, Md).

Aortic Arch and Thoracic Aorta Lesions
Whole aortic atherosclerotic lesions were quantified as described.25,26 The aorta was opened in situ longitudinally along the ventral midline and pinned flat on a black wax surface. The images of the aorta were captured with a Nikon digital camera (Coolpix 990; Nikon Instruments Inc, Tokyo, Japan) mounted on a Nikon stereo microscope. The total aortic surface and atherosclerotic lesion areas were analyzed en face by computerized quantitative morphometry (Image Pro Plus).

Immunohistochemistry
Aortic sinus sections were processed with the Cell and Tissue Staining Kit (R&D Systems, Minneapolis, Minn) and the Universal LSAB+ Kit (Dako Inc, Carpinteria, Calif). Primary antibodies were as follows: goat polyclonal anti-mouse VCAM-1 (R&D Systems), goat polyclonal anti-mouse ICAM-1 (R&D Systems), rat monoclonal anti-mouse Mac-2 (Cedarlane Laboratories, Ontario, Canada), rabbit polyclonal anti-human von Willebrand factor (Dako), and mouse monoclonal anti-chicken smooth muscle α-actin (Santa Cruz Biotecnotogy, Inc, Santa Cruz, Calif).

Real-Time Polymerase Chain Reaction for Analysis of Aortic mRNA Levels
Total RNA was isolated from aortas with TRIzol Reagent (Invitrogen, Carlsbad, Calif). cDNA synthesis was performed with the high-capacity cDNA archive kit (Applied Biosystems, Foster City, Calif). All TaqMan primers and probes for mouse VCAM-1, ICAM-1, MCP-1, TNFα, IL-6, CD68, and GAPDH were purchased as Assays on Demand from Applied Biosystems.

Statistical Analysis
Data were calculated as mean±SEM and analyzed by unpaired Student t test. Statistical significance was accepted at P<0.05.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
LA Lowers Body Weight Gain in ApoE−/− and ApoE/LDLR−/− Mice
ApoE−/− mice were fed ad libitum a Western-type, high-fat/high-cholesterol diet without (control) or with 0.2% (wt/wt) R,S-LA for 10 weeks. ApoE/LDLR−/− mice were fed ad
libitum a normal chow diet supplemented with 0.2% (w/w) R-LA, and control apoE/LDLR−/− mice were pair fed the same daily amounts of chow without LA. Body weight was assessed before and at the end of the treatment period. Initial body weights did not differ between control and LA-treated animals, and all animals gained weight during the 10-week period. However, final body weights and hence body weight gain were significantly lower in the LA-supplemented mice than the corresponding non–LA-supplemented control animals (Figure 1). Body weight gain of apoE−/− mice was 8.84±0.46 g in controls and 5.35±0.29 g in LA-treated animals (P<0.05; n=20 animals per group) (Figure 1). For apoE/LDLR−/− mice, body weight gain was 5.87±0.14 g in the control group and 3.63±0.21 g in the LA-treated group (P<0.05; n=21 per group). This result is particularly remarkable because the apoE/LDLR−/− control mice were pair fed with the corresponding LA-supplemented animals, yet the latter still gained significantly less weight.

LA Lowers Serum Triglycerides but Not Cholesterol in ApoE−/− and ApoE/LDLR−/− Mice

At the end of the treatment period, serum triglycerides were much higher in apoE−/− and apoE/LDLR−/− mice than in wild-type C57BL/6 mice (P<0.001) (Figure 2A). However, serum triglycerides were significantly lower in apoE−/− mice fed 0.2% LA (132±13 mg/dL) than non–LA-fed apoE−/− mice (219±21 mg/dL; P<0.05; n=20 per group) (Figure 2A). Serum triglycerides also were lower in apoE/LDLR−/− mice fed 0.2% LA (95±8 mg/dL) compared with control animals (116±7 mg/dL), although this difference did not quite reach statistical significance (P=0.058; n=21 per group) (Figure 2A).

Serum total cholesterol in control apoE−/− and apoE/LDLR−/− mice was increased ~9- and 7-fold, respectively, compared with wild-type C57BL/6 mice (P<0.001) (Figure 2B). LA treatment did not affect cholesterol levels in apoE−/− mice (Figure 2B). A significant increase in serum total cholesterol was observed in apoE/LDLR−/− mice fed LA (800±43 mg/dL) compared with non–LA-fed controls (668±26 mg/dL; P<0.05; n=21 per group) (Figure 2B).

Serum lipoprotein distribution was almost identical in control and LA-fed apoE/LDLR−/− mice (Figure 3). The LA-treated group had lower total serum triglycerides, as also suggested by the data in Figure 2A, and fast protein liquid chromatography analysis indicated that this difference was due mainly to lower triglyceride levels in very LDL (VLDL) and to some degree LDL (Figure 3A). Conversely, the LA-treated group exhibited higher total serum cholesterol levels, confirming the data in Figure 2B, and this excess cholesterol was contained in the VLDL fraction (Figure 3B). These data indicate that LA treatment of apoE/LDLR−/− mice increased cholesterol and decreased triglyceride content of VLDL.

LA Inhibits Atherosclerotic Lesion Formation in ApoE−/− and ApoE/LDLR−/− Mice

By the end of the 10-week treatment period, apoE−/− mice fed the high-fat/high-cholesterol diet and apoE/LDLR−/− mice fed the normal chow diet had developed widespread atherosclerotic lesions in the aortic sinus, aortic arch, and thoracic aorta (Figure 4A and 4C). Fatty streaks and more advanced lesions that covered a significant portion of the luminal surface and contained extracellular cholesterol deposits were observed in the aortic sinus of both mouse models (Figure 4A). LA supplementation significantly reduced aortic sinus lesion formation by 22% in both apoE−/− and apoE/LDLR−/− mice (Figure 4B).

Figure 1. LA lowers body weight gain in apoE−/− and apoE/LDLR−/− mice. ApoE−/− mice fed a Western-type chow diet containing 15% hydrogenated coconut oil and 0.125% cholesterol and apoE/LDLR−/− mice fed a normal chow diet containing 4% hydrogenated coconut oil were supplemented without (black bars) or with (gray bars) 0.2% (w/w) LA for 10 weeks as described in Methods. The animals were weighed before and at the end of the treatment period, and weight gain was determined. Data shown are mean±SEM of 20 apoE−/− and 21 apoE/LDLR−/− mice in each group. *P<0.05 vs control animals.

Figure 2. Effects of LA on serum triglycerides (A) and cholesterol (B) in apoE−/− and apoE/LDLR−/− mice. ApoE−/− and apoE/LDLR−/− mice were treated without (black bars) or with (gray bars) LA as described for Figure 1. Wild-type (WT) C57BL/6 mice were fed a normal chow diet containing 4% hydrogenated coconut oil. Serum concentrations of triglycerides and cholesterol were determined as described in Methods. Data shown are mean±SEM of 5 wild-type, 20 apoE−/−, and 21 apoE/LDLR−/− mice in each group. *P<0.05 vs control animals.
mice (Figure 4A and 4B). The lesion areas in apoE−/− mice were 175.8±11.2 µm²x10³ in the control group and 138.0±14.1 µm²x10³ in the LA-supplemented group (P<0.05; n=20 per group). In apoE/LDLR−/− mice, the aortic sinus lesions were 124.4±11.5 µm²x10³ in the control group and 96.2±7.5 µm²x10³ in the LA-treated group (P<0.05; n=21 per group). These data are similar to those of a recent study showing that 0.165% (wt/wt) LA fed to apoE−/− mice for 16 weeks reduced aortic sinus lesions by ≈25%.27

The extent of atherosclerosis was further determined using en face analysis of the aorta. LA supplementation significantly reduced atherosclerotic lesion development in the aortic arch and thoracic aorta by 54% in apoE−/− mice and 39% in apoE/LDLR−/− mice compared with non–LA-treated control animals (P<0.05; n=9 to 10 per group) (Figure 4C and 4D). The results for the LA-fed apoE/LDLR−/− mice are remarkable because the control animals had lower serum cholesterol levels (Figure 2B) and were pair fed to eliminate possible differences in food intake as a confounding factor.

**LA Reduces Aortic Expression of Adhesion Molecules and Proinflammatory Cytokines in ApoE/LDLR−/− Mice**

Different cell types in the lesion areas of the aortic sinus were first identified by immunohistochemical staining for endothelial cells (von Willebrand factor), smooth muscle cells

![Image](https://example.com/image1.png)

**Figure 3.** Effects of LA on serum lipoprotein profile in apoE/LDLR−/− mice. ApoE/LDLR−/− mice were treated without (●) or with (○) LA as described for Figure 1. Serum was collected and lipoproteins were separated by fast protein liquid chromatography with a Superose 6 column as described in Methods. Concentrations of triglycerides (A) and cholesterol (B) were determined colorimetrically and normalized to total serum levels. Fractions 12 to 15 contain VLDL; fractions 16 to 24, LDL; and fractions 25 to 30, high-density lipoprotein.

![Image](https://example.com/image2.png)

**Figure 4.** LA inhibits atherosclerotic lesion formation in the aortic sinus (A and B) and the aortic arch and thoracic aorta (C and D) of apoE−/− and apoE/LDLR−/− mice. ApoE−/− and apoE/LDLR−/− mice were treated without or with LA as described for Figure 1. Aortas and hearts were prepared as described in Methods. A, Cross sections of the aortic sinus stained with Movat’s stain (muscle fibers are stained red; ground substance and mucin, blue; collagen and reticular fibers, yellow; elastic fibers, black; and proteoglycans, gray-blue). Images a, b, and c are representative of 3 control apoE−/− mice; images d, e, and f are of 3 LA-treated apoE−/− mice. Magnification ×50. B, Total sinus lesion areas in control (black bars) and LA-treated (gray bars) mice. Data shown are mean±SEM of 20 apoE−/− and 21 apoE/LDLR−/− mice in each group. C, Pinned-out whole aortas from 1 representative animal of each group showing surface lesions (white areas). D, Total aortic surface lesion areas in control (black bars) and LA-treated (gray bars) mice. Data shown are mean±SEM of 10 apoE−/− and 9 apoE/LDLR−/− mice in each group. *P<0.05 vs control animals.
(smooth muscle α-actin), and macrophages (Mac-2) (Figure I of the online-only Data Supplement). To investigate whether LA reduced vascular inflammation, endothelial VCAM-1 and ICAM-1 protein expression was assessed. LA supplementation slightly but significantly reduced VCAM-1 and ICAM-1 expression by 17% and 12%, respectively, in the aortic sinus of apoE/LDLR mice compared with non–LA-treated control animals (P<0.05; n=6 per group) (Figure 5A and 5B).

Furthermore, we assessed inflammatory gene expression in whole aortas of apoE/LDLR mice. Aortic mRNA levels of VCAM-1, ICAM-1, TNFα, and IL-6 were increased 8.7±0.9-, 4.1±0.5-, 5.3±0.4-, and 8.4±2.3-fold, respectively, in control apoE/LDLR mice compared with wild-type C57BL/6 mice (P<0.01; n=6 per group). LA supplementation strongly reduced these mRNA levels by 44% (VCAM-1), 65% (ICAM-1), 63% (TNFα), and 100% (IL-6) (P<0.05 versus non–LA-treated apoE/LDLR mice; n=6 per group) (Figure 6). MCP-1 mRNA levels were increased 3.1-fold in apoE/LDLR mice compared with wild-type animals and reduced nonsignificantly by ~40% with LA treatment (data not shown).

**LA Reduces Macrophage Accumulation in Whole Aorta of ApoE/LDLR Mice**

Extensive staining for the macrophage marker Mac-2 was observed in aortic sinus lesions of control apoE/LDLR mice, indicating that most of these lesions comprised macrophage-derived foam cells (Figure 5A). However, no significant difference was present in sinus lesion macrophage content of control and LA-treated animals (33.1±3.5% and 29.8±2.2%, respectively; P>0.05; n=6 per group) (Figure 5C).

mRNA levels of CD68, a macrophage-specific marker, were increased 14.4±2.6-fold in whole aortas of control apoE/LDLR mice compared with wild-type C57BL/6 mice (Figure 6). LA supplementation significantly reduced aortic CD68 mRNA levels by 48% (P<0.05 versus non–LA-treated
The antiobesity effect of LA is consistent with previous findings in diabetic rat models. For example, Segermann et al reported that intraperitoneal injections of LA caused a 45% decrease in serum triglycerides, and Ford and colleagues found that dietary supplementation with LA lowered plasma triglycerides by >50% in streptozotocin-diabetic rats without changing plasma total or high-density lipoprotein cholesterol. Dietary LA also was found to reduce accumulation of triglycerides in nonadipose tissue of OLETF rats such as skeletal muscle and pancreatic islets. Interestingly, Lee et al observed that LA treatment of OLETF rats completely reversed excessive accumulation of triglycerides in aortic endothelial cells and hence endothelial dysfunction.

The triglyceride-lowering effect of LA could be related to both reduced food consumption and increased catabolic activity mediated by AMP-activated protein kinase, as explained above. In addition, we recently observed that dietary LA supplementation prevented hypertriglyceridemia in Zucker diabetic fatty rats by downregulating gene expression of several hepatic enzymes involved in the de novo synthesis of fatty acids and triglycerides (R. Moreau, PhD, J.A. Butler, MS, and T.M.H., unpublished observation, 2007). The finding that LA supplementation lowers serum triglycerides has important implications for the prevention and treatment of cardiovascular disease because hypertriglyceridemia is an independent predictor of myocardial infarction and stroke.

Strategies to reduce hypertriglyceridemia in patients with metabolic syndrome, type 2 diabetes mellitus, or atherosclerotic vascular diseases have used pharmacological agents and dietary approaches, and LA may be added to that repertoire.

Interestingly, LA exerted different effects on serum total cholesterol levels in the 2 murine models in our study. LA treatment did not affect serum cholesterol levels in apoE−/− mice fed a high-fat/high-cholesterol diet but increased total cholesterol by ~20% in apoE/LDLR−/− mice fed a normal chow diet. Lipoprotein distribution showed nearly identical patterns in control and LA-fed apoE/LDLR−/− mice. Our data indicate that alterations in serum total cholesterol did not contribute to the reduction of atherosclerosis in LA-treated animals and suggest that LA may increase cholesterol and decrease triglycerides in VLDL.

Our observation that LA increased serum total cholesterol in apoE/LDLR−/− mice is inconsistent with previous findings. Angelucci and Masciulli-Coriandol reported that LA decreased plasma cholesterol by 50% in rabbits. Similarly, Ivanov observed that dietary supplementation with LA reduced cholesterol and β-lipoproteins in serum and aortic tissue of rabbits with atherosclerosis, and Shih showed that LA decreased both serum total cholesterol and β-lipoproteins by ~40% in a Japanese quail model. In contrast, Kritchevsky found no cholesterol-lowering effect of LA supplementation. These observed discrepancies might be due to different animal models and LA doses used.
Inflammation and lipid accumulation have been recognized as prominent features of atherosclerosis.\(^1\)\(^,\)\(^3\) Ross\(^3\) observed that feeding an atherogenic diet to experimental animals induced adhesion of monocytes and other inflammatory cells to the arterial wall in a matter of days. Recruitment of monocytes to the arterial intima is dependent on the expression of endothelial adhesion molecules, monocyte chemotactants, and proinflammatory cytokines. Numerous studies have established that circulating markers of inflammation are predictive of atherosclerosis and resulting cardiovascular disease events.\(^1\)\(^,\)\(^4\) Thus, inhibition of vascular inflammation and monocyte-endothelial interactions is emerging as a novel strategy to prevent and treat atherosclerosis.

Several lines of evidence suggest that LA may exert antiinflammatory and hence antiatherosclerotic effects.\(^1\)\(^,\)\(^2\)\(^,\)\(^5\)\(^,\)\(^6\) In the present study, we found that LA strongly inhibited atherosclerotic lesion formation in the aortic arch and thoracic aorta of 2 murine models of human atherosclerosis and that this antiatherosclerotic effect of LA was associated with decreased expression of VCAM-1, ICAM-1, TNFα, and IL-6 and decreased macrophage accumulation in the arterial wall. Although these data cannot establish cause and effect, they strongly suggest that LA inhibits atherosclerotic lesion development, in part, by suppressing vascular inflammation and monocyte recruitment. The antiinflammatory effects of LA observed here also are consistent with our previous results showing that LA inhibits TNFα-induced expression of adhesion molecules and MCP-1 and adherence of monocytes to human aortic endothelial cells\(^1\)\(^,\)\(^2\)\(^,\)\(^16\) and suppresses lipopolysaccharide-induced inflammatory responses in C57BL/6 mice.\(^1\)\(^,\)\(^7\) These studies also revealed a common underlying mechanism for the antiinflammatory effects of LA, ie, inhibition of nuclear factor-κB activation.\(^1\)\(^,\)\(^6\)\(^,\)\(^16\)

The extent of the antiatherosclerotic effect of LA differed at different aortic sites. Thus, LA strongly inhibited atherosclerotic lesion formation in the aortic arch and thoracic aorta but had less of an effect in the aortic sinus. These observations are consistent with the stronger inhibitory effects of LA on VCAM-1 and ICAM-1 expression and macrophage accumulation in whole aorta than the aortic sinus and buttress the concept of a causal relationship between the antiinflammatory and antiatherosclerotic effects of LA. Furthermore, our findings are consistent with previous reports that probucol and other antioxidants fed to murine models of atherosclerosis are more effective at inhibiting atherosclerotic lesion formation at distal aortic sites than the aortic root.\(^4\)\(^,\)\(^7\)\(^,\)\(^8\)

On possible limitations of our study, it should be noted that we were unable to detect LA in serum of mice at the end of the 10-week treatment period, probably because of the short half-life of LA in blood (≈30 minutes).\(^9\) LA rapidly breaks down into at least 5 main metabolites in rodents; however, little is known whether these metabolites, LA, or the reduced form of LA, dihydrolipoic acid, is the active agent in vivo.\(^9\) Another limitation is the relatively large dose of LA used. Because a 20-g mouse consumes ≈3 g of chow per day, 0.2% (wt/wt) LA in the rodent diet on a kilogram-of-body-weight basis translates to ≈20 g of LA for a 70-kg person. However, such direct extrapolation based on kilograms of body weight may be inappropriate because mice have an ≈10-times-higher metabolic rate—and eat much more per kilogram of body weight—than humans. Therefore, the equivalent human dose may be ≈2000 mg of LA per 70-kg person. Human clinical studies have used up to 1800 mg/d for 6 months, which was considered safe and did not cause significant side effects.\(^2\)

**Conclusion**

The present study provides several important new findings: Dietary LA supplementation inhibits atherosclerotic lesion formation in apoE- and apoE/LDLR-deficient mice, likely through mechanisms involving the antiobesity, antihypertriglyceridemic, and antiinflammatory effects of LA. LA supplementation may be a useful adjunct in the prevention and treatment of atherosclerotic vascular diseases.

**Sources of Funding**

The work described here was supported by National Institutes of Health grants ES11542 (Dr Zhang), HL60886 (Dr Frei), and HL079382 (Dr LeBoeuf) and National Center for Complementary and Alternative Medicine Center of Excellence grant AT002034 (Drs Frei, Zhang, and Hagen).

**Disclosures**

None.

**References**


The recognition that inflammation is a key mechanism in the pathogenesis of atherosclerosis and its clinical manifestations (eg, angina pectoris, myocardial infarction, and stroke) has significant implications for cardiovascular disease prevention, treatment, and research at the basic, clinical, and population levels. It is now clear that chronic inflammatory processes and immune mechanisms contribute to the development of atherosclerosis at all stages of the disease. Obesity and hyperlipidemia, as risk factors for atherosclerosis, also are strongly linked to increased vascular inflammation. The present study provides several important new findings: Dietary \( \alpha \)-lipoic acid supplementation inhibits atherosclerotic lesion formation in apolipoprotein E− and apolipoprotein E-low-density lipoprotein receptor−deficient mice, 2 widely accepted animal models of human atherosclerosis; and the inhibition of atherosclerosis by \( \alpha \)-lipoic acid was associated with reduced weight gain, decreased plasma triglycerides, and decreased vascular inflammation. Although our results obtained in animal models cannot be directly extrapolated to humans, they strongly suggest that \( \alpha \)-lipoic acid supplementation may be useful as an inexpensive but effective intervention strategy that targets inflammation, obesity, and hyperglycemia, thereby reducing known risk factors for the development of atherosclerosis and other inflammatory vascular diseases in humans.
Dietary α-Lipoic Acid Supplementation Inhibits Atherosclerotic Lesion Development in Apolipoprotein E–Deficient and Apolipoprotein E/Low-Density Lipoprotein Receptor–Deficient Mice
Wei-Jian Zhang, Karyn E. Bird, Timothy S. McMillen, Renee C. LeBoeuf, Tory M. Hagen and Balz Frei

_Circulation._ 2008;117:421-428; originally published online December 24, 2007; doi: 10.1161/CIRCULATIONAHA.107.725275

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/3/421

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2008/01/04/CIRCULATIONAHA.107.725275.DC1
http://circ.ahajournals.org/content/suppl/2008/01/04/CIRCULATIONAHA.107.725275.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/
Supplemental Fig. 1.