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
experimental animals and humans have shown increased expression of adhesion molecules and MCP-1 in developing and established atherosclerotic lesions.\textsuperscript{4–6} Conversely, genetic deficiencies of adhesion molecules, MCP-1, or TNFα in mice are associated with decreased atherosclerosis.\textsuperscript{7–10} The early atherosclerotic events and the initiation of lesion formation appear particularly dependent on VCAM-1.\textsuperscript{1,2} Human studies also demonstrated that increased plasma levels of soluble VCAM-1 and ICAM-1 are correlated with clinical manifestations of coronary atherosclerosis.\textsuperscript{11} 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.\textsuperscript{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.\textsuperscript{18–21} LA has been used safely in patients with diabetes mellitus and has been shown to significantly improve diabetic neurovascular and metabolic complications.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{−/−}) and apoE/IDLRL–deficient (apoE/IDLRL\textsuperscript{−/−}) mice, 2 well-established animal models of human atherosclerosis.

\section*{Methods}
A full description of all Methods can be found in the online-only Data Supplement.

\section*{Animals and Experimental Procedures}
Female C57BL/6, apoE\textsuperscript{−/−}, and apoE/IDLRL\textsuperscript{−/−} 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\textsuperscript{−/−} 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/IDLRL\textsuperscript{−/−} mice were fed ad libitum Purina 5001 chow diet (Dyets No. 611000) containing 4% hydrogenated coconut oil without (control) or with 0.2% (wt/wt) R-LA (a gift from Dr Hans Tritschler, Viatris Inc, Frankfurt, Germany). Pair feeding of apoE/IDLRL\textsuperscript{−/−} 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.

\section*{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.

\section*{Results}
LA Lowers Body Weight Gain in ApoE\textsuperscript{−/−} and ApoE/IDLRL\textsuperscript{−/−} Mice
ApoE\textsuperscript{−/−} 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/IDLRL\textsuperscript{−/−} 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=21 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).

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.
mice (Figure 4A and 4B). The lesion areas in apoE/− mice were 175.8±14.1 μm² x 10³ in the control group and 138.0±14.1 μm² x 10³ 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 μm² x 10³ in the control group and 96.2±7.5 μm² x 10³ 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 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/− 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 immunohistological staining for endothelial cells (von Willebrand factor), smooth muscle cells

![Figure 3](image3.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.

![Figure 4](image4.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 present study demonstrates that dietary supplementation with LA inhibits atherosclerotic lesion formation by inhibiting expression of adhesion molecules and proinflammatory cytokines and monocyte recruitment to the arterial wall.

**Discussion**

The antiatherogenic effect of LA was associated with “antiobesity,” antihypertriglyceridemic, and antiinflammatory effects (ie, reduced body weight gain, serum triglycerides, aortic expression of cellular adhesion molecules and proinflammatory cytokines, and aortic macrophage accumulation).

The antiobesity effect of LA is consistent with previous data showing that LA effectively lowered body weight gain and adipose fat mass in C57BL/6J mice and genetically obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Interestingly, LA was effective in treating obesity in these rats. The reduction in body weight gain by LA appears to be due to suppression of hypothalamic AMP-activated protein kinase, resulting in reduced appetite and food intake as oral LA. In our study, the apoE/LDLR−/− mice fed a high-fat/high-cholesterol diet but increased total 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 Ross1 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.11 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.12–17 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 cells10 and suppresses lipopolysaccharide-induced inflammatory responses in C57BL/6 mice.17 These studies also revealed a common underlying mechanism for the antiinflammatory effects of LA, i.e., inhibition of nuclear factor-κB activation.16,17

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 propucol 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.47,48

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).19 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.19 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.22

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, antihypertri-glyceridemic, and antiinflammatory effects of LA. LA supplementation may be a useful adjunct in the prevention and treatment of atherosclerotic vascular diseases.

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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, are also strongly linked to increased vascular inflammation. The present study provides several important new findings: Dietary α-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 α-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 α-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.
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Supplemental Fig. 1.