Original Article

α-Lipoic Acid Prevents the Increase in Atherosclerosis Induced by Diabetes in Apolipoprotein E–Deficient Mice Fed High-Fat/Low-Cholesterol Diet

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Considerable evidence indicates that hyperglycemia increases oxidative stress and contributes to the increased incidence of atherosclerosis and cardiovascular complications in diabetic patients. To examine the effect of α-lipoic acid, a potent natural antioxidant, on atherosclerosis in diabetic mice, 3-month-old apolipoprotein (apo) E-deficient (apoE$^{-/-}$) mice were made diabetic by administering streptozotocin (STZ). At 4 weeks after starting the STZ administration, a high-fat diet with or without α-lipoic acid (1.65 g/kg) was given to the mice and to nondiabetic apoE$^{-/-}$ controls. At 20 weeks, markers of oxidative stress were significantly lower in both the diabetic apoE$^{-/-}$ mice and their nondiabetic apoE$^{-/-}$ controls with α-lipoic acid supplement than in those without it. Remarkably, α-lipoic acid completely prevented the increase in plasma total cholesterol, atherosclerotic lesions, and the general deterioration of health caused by diabetes. These protective effects of α-lipoic acid were accompanied by a reduction of plasma glucose and an accelerated recovery of insulin-producing cells in the pancreas, suggesting that part of its effects are attributable to protecting pancreatic β-cells from damage. Our results suggest that dietary α-lipoic acid is a promising protective agent for reducing cardiovascular complications of diabetes. *Diabetes* 55:2238–2244, 2006

Diabetes is a common metabolic disorder (1) that can lead to serious multiple organ damage, including atherosclerotic cardiovascular disease. Diabetic patients have a two- to eightfold increased risk of coronary artery disease compared with nondiabetic individuals (2). Diabetes is usually accompanied by an increased production of reactive oxygen species and free radicals, or by impaired antioxidant defenses (3), which is widely accepted as important in the development and progression of diabetes complications (4). This is particularly relevant to the risk of cardiovascular disease because there is strong evidence that oxidation of LDL and other lipids by free radicals is one of the most important factors for the initiation of atherosclerosis (5,6). Oxidative stress also facilitates endothelial cell dysfunction (7). However, evidence linking antioxidant vitamins to diabetes complications in humans is still inconclusive. For example, although treatment with a high dose of vitamin E (1,800 IU/day) appeared to be effective in normalizing retinal hemodynamic abnormalities and improving renal function in type 1 diabetes in a small randomized trial (8), vitamin E at a dose of 400 IU/day had no effect on cardiovascular outcomes or nephropathy in high-risk patients with diabetes, as reported in the HOPE (Heart Outcomes Prevention Evaluation) study and MICRO-HOPE (Microalbuminuria, Cardiovascular, and Renal Outcomes-Heart Outcomes Prevention Evaluation) substudy (9). Similarly, across a wide range of intake, vitamin C does not appear to be associated with improved cardiovascular risk factor status (10).

α-Lipoic acid functions as a cofactor in multienzyme complexes, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain α-ketoacid dehydrogenase (11). α-Lipoic acid and its reduced form, dihydrolipoate, are potent antioxidants. They are amphiphilic and widely distributed in both cell membrane and cytosol, and they readily cross the blood-brain barrier. They chelate transition metals and can be regenerated by several enzymes, including lipoamide reductase, glutathione reductase, and thioredoxin reductase. Additionally, α-lipoic acid/dihydrolipoate recycles other antioxidants, such as glutathione, vitamin C, vitamin E, and coenzyme Q10 (12). α-Lipoic acid has been used in Germany for patients with diabetic neuropathy for >30 years, and a recent meta-analysis showed that treatment with 600 mg α-lipoic acid per day for 3 weeks is safe and reduces neuropathic deficits by a clinically meaningful degree in diabetic patients with symptomatic polyneuropathy (13,14). However, the effects of α-lipoic acid treatment at doses that produce a significant reduction of oxidative stress have not been clearly demonstrated in diabetic cardiovascular diseases.

The current study examined the effect of dietary α-lipoic acid supplement on atherosclerosis, which is known to be enhanced by streptozotocin (STZ)-induced diabetes in apolipoprotein (apo) E-deficient mice (15). We show here that dietary α-lipoic acid, started after the induction of diabetes by STZ administration, leads to significant changes in biomarkers of oxidative stress and completely prevents the accelerated atherosclerosis seen in diabetic apoE$^{-/-}$ mice that did not receive the supplement.

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apo, apolipoprotein; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substance.

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RESEARCH DESIGN AND METHODS

Mouse experiments were carried out under protocols approved by the institutional animal care and use committee. Male 12-week-old apoE$^{-/-}$ mice on a C57BL/6J genetic background were made diabetic with intraperitoneal injections of STZ (Sigma, St. Louis, MO) at 40 mg/kg body wt in 0.1 mol/l citrate buffer (pH 4.5) for 5 consecutive days. A control group received buffer alone. Mice with blood glucose levels >300 mg/dl at 4 weeks after the initial STZ administration were considered diabetic and were used in this study. Both STZ-administered and control apoE$^{-/-}$ mice were maintained on regular chow until 4 weeks after starting the STZ administration, when they were switched to a semisynthetic diet high in fat (23% wt/wt) but low in cholesterol (0.05% wt/wt), with or without 1.65 g/kg α-lipoic acid (D03120501, D03120502; Research Diets, New Brunswick, NJ). The calorie source of the high-fat diet (45% fat, 35% carbohydrate, and 20% sucrose) is similar to diets consumed by humans in Western societies. Mice had ad libitum access to diet and water and were monitored closely for their health. The animals were killed for analyses at 20 weeks after starting the STZ administration.

Biochemical analysis. The mice were fasted for 4 h, and blood samples were collected from the retro-orbital sinus before STZ injections and every 4 weeks thereafter. Plasma levels of glucose, total cholesterol, and triglycerides were measured with commercially available kits (Wako, Richmond, VA). Plasma lipoprotein profiles were determined, using fast-protein liquid chromatography (Amersham Pharmacia Biotech, Piscataway, NJ). Lipoproteins were isolated from pooled plasma by ultracentrifugation (16).

Plasma lipid peroxide content was determined, using the thiobarbituric acid reactive substance (TBARS) assay (17). Erythrocyte glutathione was measured with an assay kit (Calbiochem, San Diego, CA). Serum paraoxonase activity was determined spectrophotometrically at 270 nm with phenylacetate as the substrate (18).

Mice were individually placed in metabolic cages monthly for 3 consecutive days after starting the STZ administration. Water and food consumption, urine volume, and body weight were monitored for a 24-h period. Urinary 8-isoprostane levels were determined using an enzyme immunosorbent assay kit (Cayman, Ann Arbor, MI).

Atherosclerotic lesion analysis. Atherosclerotic lesion analyses followed the standard protocol in our laboratory (16). Frozen sections of the proximal aorta were stained with Sudan IV and counterstained with hematoxylin. The areas of atherosclerotic lesions were measured, using National Institutes of Health 1.59 Imaging Software (19). The average of lesion areas in sections from four anatomically defined positions was taken as the lesion size of each animal.

Immunohistochemistry. Immunostaining was performed on the pancreas using a standard avidin/biotin complex method with guinea pig polyclonal anti-insulin (1:1,000 dilution; Dako, Carpinteria, CA) and biotinylated anti–guinea pig immunoglobulin (1:400 dilution; Vector Laboratories, Burlingame, CA) (20). For the negative control, nonimmune serum was substituted for primary antibody. At least 10 islets per mouse from five mice from each experimental group were analyzed, using National Institutes of Health ImageJ 1.34 software (available online from http://rsb.info.nih.gov/ij).

Statistics. All values are the means ± SE. Statistical analysis was carried out using JMP software (SAS, Cary, NC). The effects of diabetes and α-lipoic acid and their interactions were analyzed using two-way ANOVA, and the Tukey-Kramer honestly significant difference was used for multiple comparisons. Effects of the duration of diabetes were assessed by multiple ANOVA of repeated measures of each mouse.

RESULTS

STZ induced diabetes in apoE$^{-/-}$ mice. At 3 months after starting STZ administration, many of the diabetic mice not receiving α-lipoic acid began to show signs of sickness, including lethargy. Of the 29 mice in this group, 3 were killed prematurely before the end of the study when they began to show signs of severe dehydration and marked body weight loss. Gross and histological examinations of these mice showed no inflammation or necrosis in hepatic and renal tissues or any vascular abnormalities such as thrombi formations. In contrast, all 16 α-lipoic acid–fed diabetic animals appeared healthy throughout the study period and reached the termination of the experiment, 20 weeks post–STZ administration.

Body weight changes during the experimental period of diabetic and nondiabetic mice are illustrated in Fig. 1*A*. Although the high-fat diet caused a significant weight gain

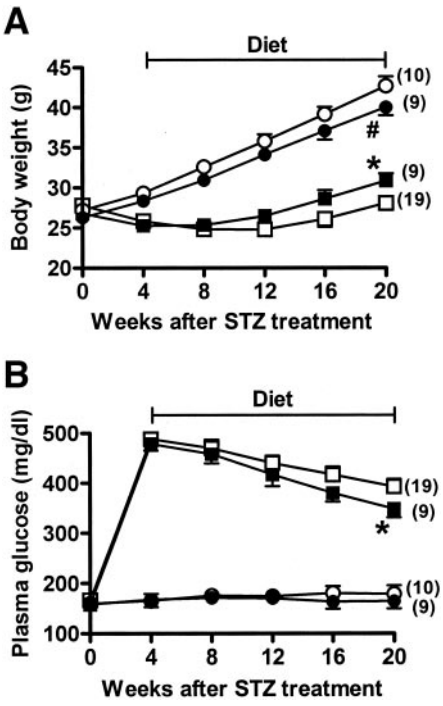

FIG. 1. Effect of dietary α-lipoic acid on body weight (*A*) and plasma glucose levels (*B*). The diet without α-lipoic acid (open symbols) or with α-lipoic acid (filled symbols) was provided at 4 weeks after administration with STZ (squares) or with the buffer (circles) at day 0. The number of animals in each group is in parentheses. Data are the means ± SE. **P* < 0.05 vs. mice without α-lipoic acid in the diabetic group. #*P* < 0.05 vs. mice without α-lipoic acid in the control group.

in the nondiabetic mice, dietary α-lipoic acid significantly slowed this gain, despite the group consuming a similar amount of diet daily (4.8 ± 0.3 g with α-lipoic acid vs. 4.9 ± 0.4 without α-lipoic acid). At the end of the experiments, the control animals without α-lipoic acid weighed 42.7 ± 1.0 g compared with 40 ± 0.5 g for those receiving α-lipoic acid ($P < 0.02$). The induction of diabetes in mice by STZ caused a significant reduction in body weight compared with nondiabetic mice. The diabetic mice regained weight after beginning the high-fat diet, and dietary α-lipoic acid accelerated weight gain among the diabetic mice (Fig. 1*A*). At the end of the 4-month high-fat diet period, diabetic mice denied α-lipoic acid weighed 28.1 ± 0.5 g, and those given α-lipoic acid weighed 30.9 ± 0.7 ($P < 0.007$).

Taken together, these results strongly suggest that dietary α-lipoic acid supplementation significantly improves the overall health of the diabetic mice.

Plasma glucose levels. Plasma glucose levels at 4 weeks after STZ administration were elevated in diabetic mice (488 ± 9 mg/dl) from preadministration levels (164 ± 8 mg/dl), but glucose levels decreased during the study period (Fig. 1*B*). Dietary α-lipoic acid significantly accelerated this recovery process. The difference in plasma glucose levels in the mice with and without α-lipoic acid was small, but at all times the levels in the mice with α-lipoic acid were less than those in mice without α-lipoic acid. At 20 weeks, the end of the study, their glucose levels differed significantly (393 ± 8 mg/dl without α-lipoic acid vs. 355 ± 12 with α-lipoic acid, $P < 0.003$). Thus, the dietary α-lipoic acid supplementation has a small glucose-lowering effect in the diabetic apoE$^{-/-}$ mice.

Plasma lipids. Mice fed the high-fat diet with low cholesterol had a higher plasma cholesterol level (343 ± 9 mg/dl,

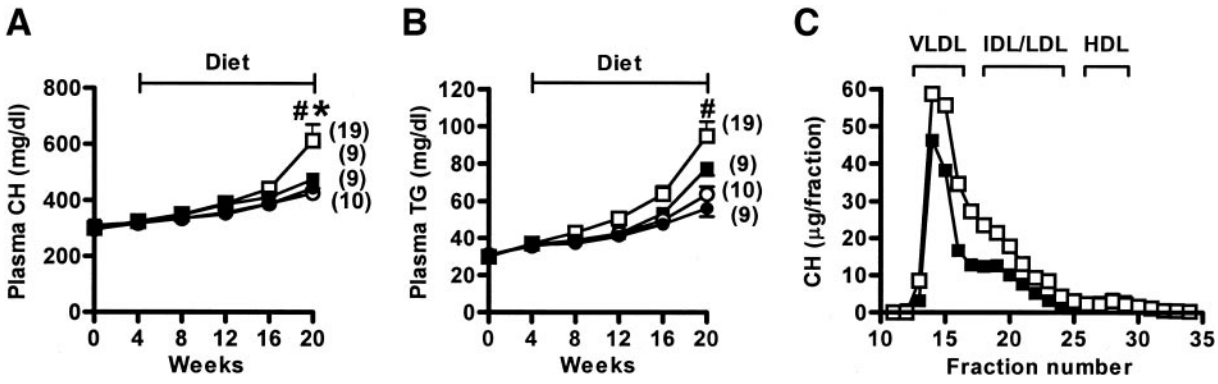

FIG. 2. Effect of dietary α-lipoic acid on plasma lipid levels. Cholesterol (*A*) and triglyceride (*B*) of diabetic (squares) and nondiabetic (circles) mice without (open symbols) or with (filled symbols) α-lipoic acid diet. **P* < 0.01 vs. diabetic mice without α-lipoic acid. #*P* < 0.01 diabetic mice vs. nondiabetic mice. Data are the means ± SE. The numbers in parentheses indicate the number of animals in each group. *C*: Plasma lipoprotein distribution. Cholesterol in each fast-protein liquid chromatography fraction (0.5 ml) from plasma (100 μl) of diabetic apoE$^{-/-}$ mice with (filled squares) or without (open squares) α-lipoic acid was measured. CH, cholesterol; IDL, intermediate-density lipoprotein; TG, triglyceride.

$n = 10$) than mice fed normal chow (317 ± 9, $n = 10$), but the difference did not reach significance ($P = 0.07$). Neither STZ administration nor hyperglycemia significantly altered the plasma cholesterol and triglyceride levels of the mice with or without α-lipoic acid during the initial 4 months after STZ administration (Fig. 2*A* and *B*). However, at 5 months post–STZ administration, plasma cholesterol levels (685 ± 57 mg/dl) of diabetic mice without α-lipoic acid were significantly higher ($P < 0.001$) than those of nondiabetic mice (452 ± 20 mg/dl), and three of the mice had >1,000 mg/dl cholesterol accompanied by increased triglycerides. The plasma cholesterol levels of mice receiving dietary α-lipoic acid (474 ± 10 mg/dl) remained the same as in nondiabetic mice. Diabetes caused a small but significant increase in plasma triglycerides ($P < 0.05$ by ANOVA), but α-lipoic acid had no significant effect ($P = 0.23$) (Fig. 2*B*). Fast-protein liquid chromatography showed that the distribution of plasma lipoproteins in the diabetic apoE$^{-/-}$ mice with α-lipoic acid was not different from that in nondiabetic apoE$^{-/-}$ mice. In contrast, the diabetic mice without α-lipoic acid had ~60% more cholesterol in VLDL, intermediate-density lipoprotein, and LDL compared with the diabetic apoE$^{-/-}$ mice with α-lipoic acid (Fig. 2*C*). α-Lipoic acid had no effect on the levels of HDL cholesterol.

These data demonstrate that prolonged untreated diabetes increases plasma cholesterol and lipoprotein remnant particles in apoE$^{-/-}$ mice and that α-lipoic acid supplementation prevents these alterations.

Oxidative stress biomarkers. The levels of erythrocyte glutathione and plasma TBARS in 2-month-old apoE$^{-/-}$ mice on regular chow were 28.3 ± 1.5 and 12.3 ± 0.6 mg/ml, respectively. Feeding the high-fat diet to these mice for ≥4 weeks did not alter levels significantly (28.3 ± 1.1 and 13.3 ± 0.6 mg/ml, respectively; $n = 26$). As expected, however, diabetes increased oxidative stress, as evidenced by the lower erythrocyte glutathione in diabetic mice than in nondiabetic controls at 5 months post–STZ administration. Dietary α-lipoic acid significantly increased erythrocyte glutathione in both the nondiabetic and diabetic animals by 33% ($P < 0.0001$ for effect of diabetes, $P < 0.0001$ for the effect of α-lipoic acid, and $P = 0.17$ for interaction of the two factors) (Fig. 3*A*). Likewise, plasma levels of TBARS were markedly higher in diabetic than in

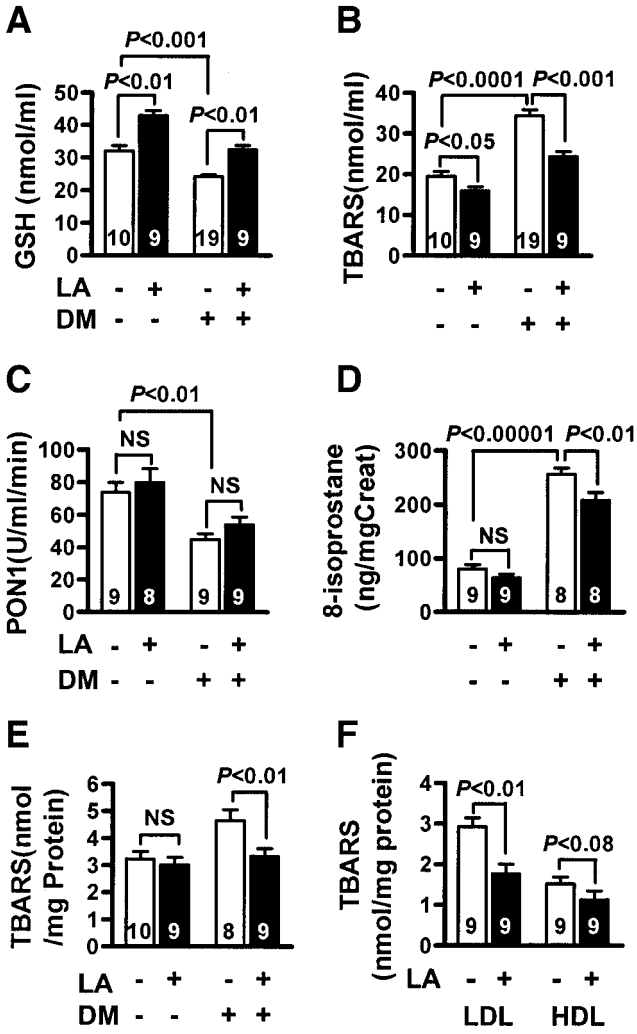

FIG. 3. Reduction of systemic oxidative stress by α-lipoic acid (LA). Panels show erythrocyte glutathione (*A*), plasma TBARS (*B*), plasma paraoxonase activity (*C*), urinary 8-isoprostane (*D*), TBARS level in liver (*E*), and oxidation of plasma LDL and HDL (*F*) in apoE$^{-/-}$ mice with (■) and without (□) α-lipoic acid at the 5-month time point. The numbers inside the bars indicate the number of animals. Results are the means ± SE. DM, diabetes; GSH, glutathione; PON1, paraoxonase.

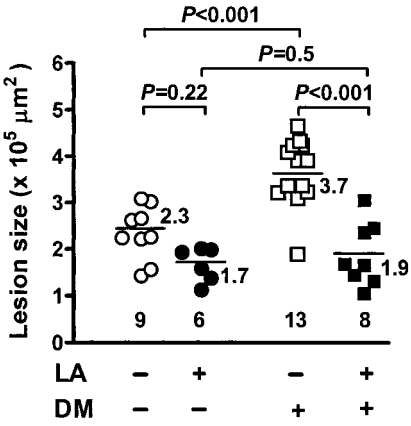

FIG. 4. Aortic atherosclerotic lesion. Each point represents the lesion size (means ± SE) of the individual mouse. Horizontal bars indicate an average of each group with lesion size (μm^2) shown on the right. □, diabetic apoE$^{-/-}$ mice without α-lipoic acid; ■, diabetic mice with α-lipoic acid; ○, nondiabetic apoE$^{-/-}$ mice without α-lipoic acid; ●, nondiabetic mice with α-lipoic acid. DM, diabetes; LA, α-lipoic acid.

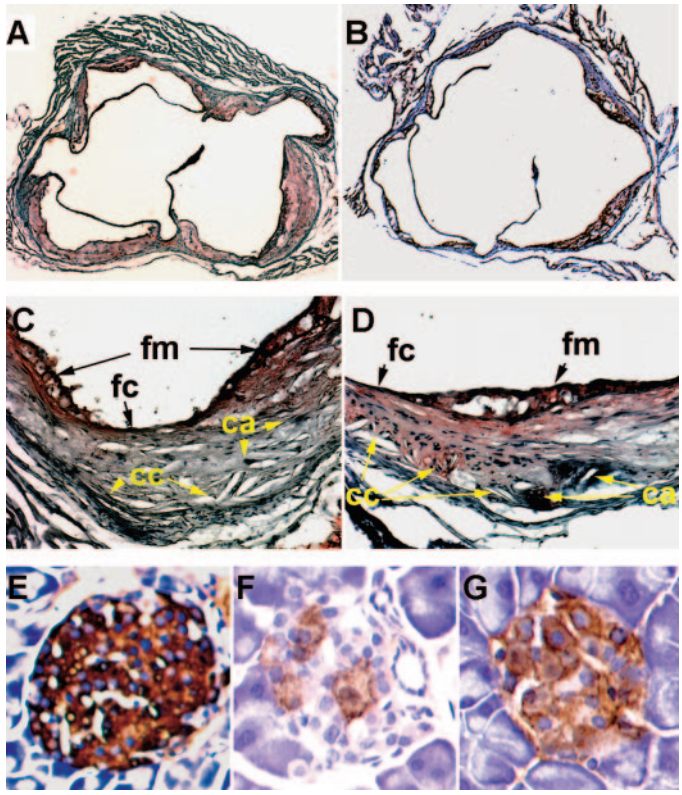

FIG. 5. Atherosclerotic plaques at the aortic sinus (*A–D*) and immunostaining of β-cells in pancreas (*E–G*). Representative photographs of proximal aortas in the diabetic apoE$^{-/-}$ mice without α-lipoic acid (*A* and *C*) and with α-lipoic acid (*B* and *D*) are shown. *C* and *D* illustrate plaques of similar sizes. Sections were stained with Sudan IV and counterstained with hematoxylin. Original magnification was ×4 for *A* and *B* and ×20 for *C* and *D*. Arrows in *C* show fibrous caps (fc), cholesterol clefts (cc), foamy macrophages (fm), and calcification (ca). Insulin-immunoreactive β-cells are shown in a nondiabetic mouse (*E*), diabetic mice without α-lipoic acid (*F*), and α-lipoic acid diet (*G*).

nondiabetic mice. Dietary α-lipoic acid significantly lowered plasma TBARS in both the nondiabetic and diabetic mice, but the decrease was more prominent in diabetic (31%) than in nondiabetic mice (18%, $P < 0.0001$ for diabetic effect, $P < 0.0001$ for the α-lipoic acid effect, and $P < 0.01$ for interaction of two factors) (Fig. 3*B*). α-Lipoic acid effects remained significant when plasma TBARS were normalized by cholesterol ($P < 0.02$) or by triglycerides ($P < 0.01$), even when the three extremely hyperlipidemic mice were omitted from analyses. Dietary α-lipoic acid significantly lowered liver TBARS in diabetic mice, but the reduction was not significant in nondiabetic mice (Fig. 3*E*).

Diabetes reduced serum paraoxonase activity in apoE$^{-/-}$ mice by ~40% compared with the nondiabetic mice ($P < 0.05$) (Fig. 3*C*). Paraoxonase catalyzes the breakdown of phospholipid and cholesteryl-ester lipid peroxides in both LDL and HDL. Paraoxonase activity of α-lipoic acid–fed mice trended to be higher, but the effect of diet was not significant. Additionally, diabetic mice excreted markedly more urinary 8-isoprostane than nondiabetic controls ($P < 0.001$ for diabetes effect) (Fig. 3*D*). The overall α-lipoic acid effect on urinary 8-isoprostane was also significant ($P < 0.007$) with diabetes and α-lipoic acid interaction ($P = 0.03$), indicating that dietary α-lipoic acid had a larger effect in diabetic than in nondiabetic mice. Finally, TBARS normalized by protein in both LDL and HDL fractions from the diabetic mice without α-lipoic acid were 2 and 1.2 times as high, respectively, as those of the respective fractions from the diabetic mice with α-lipoic acid (Fig. 3*F*).

These results demonstrate that oxidative stress is markedly increased in the diabetic apoE$^{-/-}$ mice and that dietary α-lipoic acid supplementation limits this increase.

Atherosclerotic lesion development. We next evaluated how dietary α-lipoic acid supplementation affects atherosclerotic lesion development in apoE$^{-/-}$ mice (Fig. 4). In the nondiabetic mice, dietary α-lipoic acid caused a small nonsignificant reduction of plaque size ($P = 0.22$) (Fig. 4). In mice without α-lipoic acid, diabetes caused a significant increase ($P < 0.001$ by Tukey-Kramer honestly significant difference) in mean cross-sectional area of the atherosclerotic lesions (Fig. 4). However, α-lipoic acid in the diet (Fig. 4) completely prevented this diabetes-induced increase of atherosclerosis in the apoE$^{-/-}$ mice. The effect of diabetes and of diet were both significant ($P < 0.01$ and $P < 0.0001$, respectively) with a highly significant interaction between the two factors ($P < 0.02$), in agreement with the much larger effect of α-lipoic acid in the diabetic than in the nondiabetic mice. Although the lesion area is larger in a typical diabetic mouse without α-lipoic acid (Fig. 5*A*) compared with that in a typical diabetic mouse with α-lipoic acid (Fig. 5*B*), the plaques in all animals were matured and complex, containing necrotic lipid cores, cholesterol clefts, calcification, and fibrous caps (Fig. 5*C* and *D*). There were no notable differences in the morphology of the plaques of similar size between diabetic and nondiabetic mice or between mice fed a diet with and without α-lipoic acid (Fig. 5*C* and *D*). The plaques were small and insignificant in other areas of aortas in all of the mice evaluated after dissection, and we did not analyze the en face lesion areas or plaques in brachiocephalic arteries. Nevertheless, our data clearly demonstrate that α-lipoic acid supplementation prevents the enhancement of atherosclerosis that is induced in apoE$^{-/-}$ mice by diabetes.

Immunohistochemistry of pancreatic β-cells. The ~20% decrease of plasma glucose levels over time in the STZ-induced diabetic mice described above could be attributable to the partial regeneration or proliferation of pancreatic β-cells (21). Immunostaining for insulin-producing β-cells showed that the number of insulin-positive

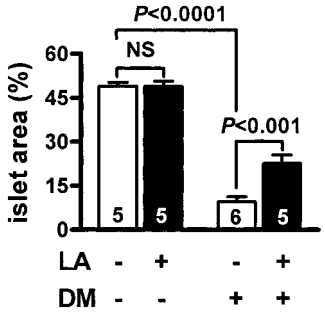

FIG. 6. Dietary α-lipoic acid facilitates β-cell recovery. Data are the percent area (means ± SE) of insulin-immunoreactive regions over entire islet areas, measured in 10 sections from five or six mice in each group. The numbers inside bars indicate the number of mice used for analysis. □, mice without α-lipoic acid treatment; ■, mice with α-lipoic acid treatment. DM, diabetes; LA, α-lipoic acid.

cells in the islets of the diabetic apoE$^{-/-}$ mice without α-lipoic acid (Fig. 4*F*) was markedly decreased at the end of the experiment relative to the nondiabetic mice (Fig. 4*E*). Relatively more immunoreactive cells were seen in diabetic mice given α-lipoic acid supplementation (Fig. 4*G*). Morphometric quantification of the ratio between insulin-immunoreactive areas and entire islet areas (Fig. 6) showed that 48.9 ± 2.3% of islets were staining positive for insulin in nondiabetic controls. Diabetes significantly decreased the number of insulin-positive cells, but mice with dietary α-lipoic acid had significantly more (21.4 ± 2.0%) than mice without α-lipoic acid (9.9 ± 2.6%; $P < 0.001$ for the effect of diabetes, $P < 0.05$ for the effect of α-lipoic acid supplement, and $P < 0.05$ for the interaction of two factors). Thus, these data show that α-lipoic acid has no effect in the absence of diabetes but partially protects β-cells from STZ-induced damage and/or accelerates their regeneration/recovery. This slight increase in insulin secretion at the 5-month end point is likely to have contributed to the protective effects of α-lipoic acid.

DISCUSSION

In this report, we have presented evidence that dietary α-lipoic acid completely prevents diabetes-induced enhancement of atherosclerosis as judged by plaque size in STZ-administered apoE$^{-/-}$ mice. These atheroprotective effects were accompanied by a substantial reduction of oxidative stress, by a small but significant decrease in plasma glucose, and by the survival or regeneration of pancreatic β-cells in the STZ-administered mice fed with α-lipoic acid. In addition, the dietary α-lipoic acid prevented a general decline in health and prevented the increases in plasma cholesterol that occurs in apoE$^{-/-}$ mice with prolonged diabetes.

Evidence linking antioxidant vitamins to cardiovascular diseases in humans is still largely controversial (22). Examination of the role of various antioxidants on atherosclerotic mice has also produced conflicting results. Some studies have shown inhibitory effects of antioxidants such as vitamin E (22,23) or a combination of vitamins C and E and β-carotene (24). In contrast, others have shown no effect of vitamin E alone (25) or in combination with β-carotene (26). In some cases, antioxidants such as probucol had a detrimental effect and increased plaque sizes (27). Our current data show that dietary α-lipoic acid supplementation significantly decreases oxidative stress, as shown by plasma TBARS, erythrocyte glutathione, and urinary 8-isoprostane, and it appears to have a small protective effect on atherogenesis in the nondiabetic apoE$^{-/-}$ mice. These observations concur with earlier reports that dietary α-lipoic acid reduces cholesterol content in the aortic tissues of cholesterol-fed rabbits (28) and that α-lipoic acid has a preventive effect on cholesterol-induced atherosclerosis in Japanese quail (29). These results from animal experiments suggest that further investigation of α-lipoic acid's effects on atherosclerosis in humans would be warranted.

In contrast to its small effects in nondiabetic apoE$^{-/-}$ mice, dietary α-lipoic acid had a marked atheroprotective effect in diabetic apoE$^{-/-}$ mice: it completely prevented the enhancement of atherosclerosis and the increase in plasma cholesterol that followed the induction of diabetes by STZ. Hyperlipidemia is a well-known risk factor for cardiovascular disease, and the lower plasma cholesterol levels of α-lipoic acid–fed diabetic mice in our experiment may have contributed to the prevention of accelerated atherosclerosis. Earlier studies showed that α-lipoic acid substantially reduced lipemia in cholesterol-fed rabbits (30,31) and prevented malondialdehyde-induced loss of lecithin/cholesterol acyltransferase activity in vitro (32). Although these results suggest that α-lipoic acid may directly affect lipoprotein metabolism, α-lipoic acid did not alter plasma lipids in the nondiabetic apoE$^{-/-}$ mice at the dose we used. In addition, the increase in the plasma lipids in diabetic mice without α-lipoic acid became evident only at the 5-month end point. In these mice, liver TBARS were also significantly elevated. Recently, Pan et al. (33) demonstrated that oxidant stress increases hepatic apolipoprotein B degradation and thus reduces plasma lipoprotein secretion. This appears to conflict with our findings in the diabetic apoE$^{-/-}$ mice, but it suggests that the diabetes-induced hyperlipidemia may be caused mainly by abnormal lipoprotein clearance. Further studies of lipoprotein metabolism in diabetic mice are clearly necessary.

At 5 months post–STZ administration, some mice developed severe hyperlipidemia. However, the atherosclerosis in these mice was not worse than in others, and eliminating these animals from analyses did not alter the significance of any parameters we examined, including atherosclerosis and biomarkers of oxidative stress. Renard et al. (20) recently generated a new transgenic mouse model of type 1 diabetes that can be induced by lymphocytic choriomeningitis virus infection. They reported that diabetes caused accelerated lesion initiation in the absence of lipid abnormalities in the LDL receptor deficiency mice on a cholesterol-free diet, whereas in diabetic mice fed a cholesterol-rich diet, the progression to advanced lesions was largely dependent on diabetes-induced dyslipidemia (20). Although our experiments differ from theirs not only in models but in diets, the above observations suggest that protection from diabetic-induced hyperlipidemia is unlikely to be the sole reason for atheroprotection by α-lipoic acid in apoE$^{-/-}$ mice.

Oxidative modification of LDL and VLDL remnants is also widely regarded as a critical event in the atherogenic process (34), and we found that α-lipoic acid effectively decreases lipid peroxidation in LDL, as judged by a substantial decrease in TBARS. Thus, the protection of lipoproteins from oxidative modification by α-lipoic acid may be an important factor for the reduced atherosclerotic lesion development in the diabetic apoE$^{-/-}$ mice. Supporting this interpretation, recent reports showed that vitamin E administration partially but significantly prevented development of atherosclerosis in diabetic BALB/c mice fed

an atherogenic diet (35). In another report, S18886, a thromboxane A2 receptor blocker, significantly attenuated the increase of diabetes-induced atherosclerosis in apoE$^{-/-}$ mice, preventing the decrease in nitric oxide production without affecting plasma glucose or lipids (36). We also note that high expression of aldose reductase accelerated diabetic atherosclerosis in mice (37) and that a combination of an inhibitor of aldose reductase and of α-lipoic acid was more efficacious for preventing diabetes-induced vascular and neural dysfunction in peripheral tissues than monotheraphy with either inhibitor (38). Thus, inhibiting aldose reductase and α-lipoic acid may affect overlapping and complementary pathways, such as glucose metabolism and glutathione biogenesis.

The therapeutic effects of α-lipoic acid in type 2 diabetic patients include enhancement of glucose uptake by muscle cells, prevention of glucose-induced protein modifications, and reduction of serum lactate and pyruvate concentrations (39). This observation raises a question of whether dietary α-lipoic acid directly stimulates the action of pyruvate dehydrogenase, for which it is a cofactor. α-Lipoic acid is synthesized in mitochondria by lipoic acid synthase, which catalyzes the insertion of two sulfur atoms into C6 and C8 positions of an octanoic acid molecule covalently attached to a specific ε-amino group of a target apoenzyme (40,41). We have previously shown that endogenous α-lipoic acid synthesis in mice is essential for embryo development and cannot be replaced by α-lipoic acid in the maternal diet (42). Although it is yet to be determined whether cells in adult tissues can utilize exogenous α-lipoic acid, this observation in mouse embryos suggests that exogenous α-lipoic acid cannot be transported into mitochondria and/or that α-lipoic acid cannot be incorporated into the core enzyme subunits. Nevertheless, even if exogenous α-lipoic acid does not directly function as a cofactor of the mitochondrial enzymes, substantial reduction of oxidative stress likely facilitates overall cellular metabolism.

STZ is the most commonly used diabetogenic agent for experimental animals (43). Although the mechanism of action is not fully understood, one of the primary effects of STZ on pancreatic β-cells is damage caused by free radicals formed when STZ decomposes inside the cell (44). We found that plasma glucose levels of the diabetic mice given α-lipoic acid had more β-cells at the end of the study period than the diabetic mice without α-lipoic acid. Previous investigations showed that in rodents, β-cells injured by STZ are able to partially regenerate, mainly as a result of ductile budding rather than mitosis of preexisting β-cells (45), and they can recover some degree of islet cell function (46). It is also possible that α-lipoic acid protects β-cells from hyperglycemia-induced dysfunction by providing a better endogenous antioxidant environment to the mice. Thus, α-lipoic acid treatment may offer a promising therapeutic alternative in an attempt to preserve pancreatic β-cell function, and this warrants studies of its effects on β-cell damage caused by a source other than STZ. The effect of dietary α-lipoic acid on plasma glucose levels in the STZ-administered animals was, however, quantitatively small, suggesting that the indirect atheroprotective effect of α-lipoic acid is smaller than its more direct effect on plaques.

In addition to preventing severe loss of body weight in diabetic mice, our experiments revealed that α-lipoic acid also prevented the weight gain that occurred in nondiabetic apoE$^{-/-}$ mice fed high-fat diet. Thus, the nondiabetic mice given diet with α-lipoic acid were significantly less obese than their littermates fed diet without α-lipoic acid, although they consumed similar amounts of diet daily. Previously, Kim et al. (47) demonstrated that α-lipoic acid significantly reduced food intake and body weight of Sprague-Dawley rats in a dose-dependent manner and that the central nervous system is the primary site of the anorexic effect of α-lipoic acid. In contrast, the nondiabetic mice in our experiments did not show any loss of appetite. This difference may be because we used a lower dose of α-lipoic acid than in the experiments by Kim et al. and/or because of different species. Because obesity is an important risk factor for the development of type 2 diabetes and atherosclerosis, an antiobesity function of α-lipoic acid may be an additional benefit.

In summary, our study demonstrates that dietary supplementation of α-lipoic acid for 20 weeks completely protects apoE$^{-/-}$ mice from enhancement of aortic atherosclerosis caused by STZ-induced diabetes. Underlying mechanisms by which α-lipoic acid exerts its atheroprotective effects include substantial decreases in oxidative stress leading to decreased LDL oxidation, protection from diabetes-induced increases in plasma cholesterol, and a small decrease in plasma glucose levels accompanied by increased protection/recovery of β-cells from damage. Although the current study cannot fully discriminate the relative contribution of these factors, together with the established clinical efficacy of α-lipoic acid on diabetic neuropathy, our results suggest that α-lipoic acid has promise as a protective agent against the cardiovascular complications of diabetes.

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