D-4F Induces Heme Oxygenase-1 and Extracellular Superoxide Dismutase, Decreases Endothelial Cell Sloughing, and Improves Vascular Reactivity in Rat Model of Diabetes

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Background—Apolipoprotein A1 mimetic peptide, synthesized from D-amino acid (D-4F), enhances the ability of HDL to protect LDL against oxidation in atherosclerotic animals.

Methods and Results—We investigated the mechanisms by which D-4F provides antioxidant effects in a diabetic model. Sprague-Dawley rats developed diabetes with administration of streptozotocin (STZ). We examined the effects of daily D-4F (100 μg/100 g of body weight, intraperitoneal injection) on superoxide (O2−), extracellular superoxide dismutase (EC-SOD), vascular heme oxygenase (HO-1 and HO-2) levels, and circulating endothelial cells in diabetic rats. In response to D-4F, both the quantity and activity of HO-1 were increased. O2− levels were elevated in diabetic rats (74.8±8×103 cpm/10 mg protein) compared with controls (38.1±8×103 cpm/10 mg protein; P<0.01). D-4F decreased O2− levels to 13.23±1×103 (P<0.05 compared with untreated diabetics). The average number of circulating endothelial cells was higher in diabetics (50±6 cells/mL) than in controls (5±1 cells/mL) and was significantly decreased in diabetics treated with D-4F (20±3 cells/mL; P<0.005). D-4F also decreased endothelial cell fragmentation in diabetic rats. The impaired relaxation typical of blood vessels in diabetic rats was prevented by administration of D-4F (85.0±2.0% relaxation). Western blot analysis showed decreased EC-SOD in the diabetic rats, whereas D-4F restored the EC-SOD level.

Conclusions—We conclude that an increase in circulating endothelial cell sloughing, superoxide anion, and vasoconstriction in diabetic rats can be prevented by administration of D-4F, which is associated with an increase in 2 antioxidant proteins, HO-1 and EC-SOD. (Circulation. 2005;111:3126-3134.)

Key Words: atherosclerosis ■ antioxidants ■ apolipoproteins ■ lipids

D-4F is a synthesized mimetic peptide of apolipoprotein A1, the major lipoprotein found in HDL. Prior studies have shown that oral administration of D-4F can reduce atherosclerotic disease independent of cholesterol levels and enhance the ability of HDL to protect against LDL oxidation.1-3 Navab et al1,2 have demonstrated that treatment with D-4F causes HDL to become antiinflammatory, stimulates HDL-mediated cholesterol efflux, and reverses cholesterol transport from macrophages. It has been shown previously that apolipoprotein A1 can restore the balance between nitric oxide (NO) and superoxide (O2−) anions in mice.4 Using a sickle cell disease model, Ou et al5 have shown that apolipoprotein A1 can improve vasoreactivity in LDL-receptor null mice. These early data indicate an antioxidant effect from the administration of apolipoprotein A1 and possibly D-4F.

The realization that heme oxygenase-1 (HO-1) is strongly induced by oxidant stress and its substrate, heme, in conjunction with the robust ability of HO-1 to guard against oxidative insult6-8 has led to examination of the antioxidant nature of HO-1 and HO-2.9 Antioxidant effects arise from the capacity of HO-1 to degrade the heme from destabilized heme proteins10 and from the elaboration of biliverdin and bilirubin, which are products of HO with potent antioxidant properties.11 Carbon monoxide (CO), an HO product, is not an antioxidant,12,13 but it has an antiapoptotic effect14 and vasodilator properties.15-16 Additionally, the induction of HO-1 prevents cell death, which is attributed to its augmentation of ion efflux and exportation of iron-binding proteins.17 The protective actions of HO-1 are not confined to overtly oxidant processes but rather extend widely to such disease processes as inflammation8,18 and atherosclerosis.19 Furthermore, upregulation of HO-1 increases the antioxidant system by decreasing O2−.20,21 However, the mechanism by which HO-1 decreases O2− is not clear.
Superoxide dismutase (SOD) is an antioxidant enzyme that converts superoxide (O$_2^-$) anions into hydrogen peroxide and molecular oxygen.$^{22}$ All mammalian tissues contain 3 forms of SOD: copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD), and extracellular SOD (EC-SOD). Under physiological conditions, the basal level of O$_2^-$ is controlled by mitochondrial and cytosolic SOD.$^{23}$ Under pathological conditions, such as heart ischemia or inflammatory blood cells, O$_2^-$ is acted on by EC-SOD instead.$^{24}$ The vascular concentration of EC-SOD is a key determinant of endothelial function; a decrease in EC-SOD is associated with a decrease in NO bioavailability and an increase in the reaction of NO with superoxide to form peroxynitrite, a potent cytotoxic oxidant.$^{22}$ In the present study, we measured EC-SOD because HO-1 has been shown to attenuate the increased level of superoxide in diabetics and to increase reduced glutathione levels.$^{21,25}$ We hypothesized that this effect might be due to an increase in another antioxidant gene such as vascular EC-SOD, which is responsible for metabolizing superoxide. Therefore, we examined the effect of HO-1 on the levels of EC-SOD.

Hyperglycemia-mediated cardiovascular complications and atherosclerosis contribute to the formation of O$_2^-$,$^{26}$ and reactive oxygen species, each of which contributes to endothelial dysfunction and apoptosis.$^{27}$ It has been shown that these abnormalities are reversible with increased expression of antioxidant enzymes or administration of antioxidant agents.$^{28}$ A reduction in antioxidant reserves has been related to endothelial cell dysfunction in diabetes, even in patients with well-controlled blood glucose levels.$^{27,29}$ Similarly, it has been shown that individuals with moderately to well-controlled type 2 diabetes mellitus demonstrate an increase in LDL oxidation.$^{30}$ Conditions associated with an elevation of oxidative stress have been shown to have an increased level of endothelial cell sloughing.$^{31-33}$ In a recent study, diabetes was also found to be associated with increased endothelial apoptosis.$^{20,21}$

The primary objective of the present study was to determine whether apolipoprotein D-4F causes an antioxidant effect as a result of increased HO-1–derived bilirubin and CO. Additional objectives were to investigate whether D-4F decreases endothelial cell sloughing and fragmentation or restores vascular reactivity in diabetic rats. The present results demonstrated that treatment with D-4F increased HO-1 protein and activity and EC-SOD. The increase in HO-1 and EC-SOD was associated with restoration in vascular reactivity and the amelioration of endothelial cell sloughing and fragmentation. These results indicate that the antioxidant effect of D-4F operates via an increase in both HO-1 and EC-SOD, both of which cause decreased oxidative stress and attenuate the vascular damage seen with diabetes.

**Methods**

**Animal Treatment**

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, Mass) weighing 170 to 190 g were maintained on standard rat diet and tap water ad libitum. After the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg body weight), diabetes was induced by a single injection, via the tail vein, of streptozotocin (STZ; Sigma; 60 mg/kg body weight) dissolved in 0.05 mol/L citrate buffer (pH 4.5). Age-matched control rats were injected with an equal volume of vehicle (sodium citrate buffer). Rats were divided into 3 groups: control, STZ, and STZ plus D-4F (daily 100 µg/100 g body weight intraperitoneal injections for 6 weeks starting the day after injection of STZ). Blood glucose level was measured 2 days after injection of STZ or vehicle. These rats were used 1 month after induction of diabetes. The Animal Care and Use Committee of New York Medical College approved all experiments.

**Measurement of Vascular O$_2^-$ Levels**

Previously described methods$^{24}$ were used in which control and diabetic vessels were placed in plastic scintillation minivans that contained 5 µm of lucigenin, for detection of O$_2^-$ and other additions, in a final volume of 1 mL of air-equilibrated Krebs solution buffered with 10 mmol/L HEPES-NaOH (pH 7.4). Lucigenin chemiluminescence was measured in a liquid scintillation counter (LS6000IC, Beckman Instruments) at ~37°C, and data are reported as counts/min per milligram of protein after background subtraction.

**Tissue Preparation for HO and EC-SOD Measurement**

Frozen thoracic aorta segments were pulverized under liquid nitrogen and placed in a homogenization buffer (10 mmol/L phosphate buffer, 250 mmol/L sucrose, 1 mmol/L EDTA, 0.1 mmol/L PMSF, and 0.1% tertigol, pH 7.5). Homogenates were centrifuged at 27,000 g for 10 minutes at 4°C. The supernatant was isolated, and protein levels were assayed (Bradford method). The supernatant was used for measurement of HO-1, HO-2, and EC-SOD–Cu/Zn-SOD protein expression and determination of HO activity.

**Western Blot Analysis**

These measurements were performed to determine HO-1, HO-2, EC-SOD, and Cu/Zn-SOD protein expression. Protein levels were visualized by immunoblotting with antibodies against rat HO-1, HO-2, EC-SOD, and Cu/Zn-SOD (Stressgen Biotechnologies Corp). Briefly, 20 µg of lysate supernatant was separated by 12% SDS/PAGE and transferred to a nitrocellulose membrane (Abersham) with a semidy transfer apparatus (Bio-Rad). The membranes were incubated with 10% milk in 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, and 0.05% Tween 20 (TBST) buffer at 4°C overnight. After they were washed with TBST, the membranes were incubated with anti-HO-1, anti-HO-2, anti-EC-SOD, and anti-Cu/Zn-SOD antibodies for 1 hour at room temperature with constant shaking. The filters were washed and subsequently probed with horseradish peroxidase–conjugated donkey anti-rabbit or anti-mouse IgG (Abersham). Chemiluminescence detection was performed with the Abersham ECL detection kit, according to the manufacturer’s instructions.

**Measurement of HO Activity**

HO activity was assayed as previously described$^{20}$ in which bilirubin, the end product of heme degradation, was extracted with chloroform and its concentration determined spectrophotometrically (Perkin-Elmer Dual UV/VIS Beam Spectrophotometer Lambda 25) using the difference in absorbance at a wavelength from 460 to 530 nm with an absorption coefficient of 40 mmol·L$^{-1}$·cm$^{-1}$.

**Detection and Quantification of Circulating Endothelial Cells**

For immunomagnetic isolation and quantification of endothelial cells, we used monodispersed magnetizable particles (Dynabeads CELLection Pan Mouse IgG kit) obtained from Dynal. The 4.5-µm diameter polystyrene beads are coated with affinity-purified pan-anti-mouse immunoglobulin G1 covalently bound to the surface. The beads were washed according to the manufacturer, with a strong magnet (MPC6, Dynal) used to remove sodium azide. Typically, 100
μL of bead suspension was coated noncovalently with 10 μg/mL RECA-1 (Novus Biologicals), a pan-rat endothelial cell–specific monoclonal antibody diluted 1:10 in PBS 0.1% BSA, by overnight incubation at 4°C with head-over-head agitation. After 3 washes with PBS-BSA to remove excess antibodies, the beads were resuspended in buffer until use. RECA-uncovered particles were used as a negative control. If the beads were stored for a long period of time, 0.1% sodium azide was added to the buffer. Beads and target cells were incubated for 1.5 hours at 4°C on a rotator. The amount of beads (4×10⁸/mL) was calculated to be in great excess of target cells (>2000 beads per target cell). Separation of beads and rosetted cells from the blood samples required a minimum of 1 minute of exposure to the magnet. Three washes were performed to completely remove nonrosetted cells. After the third wash, rosetted cells were recovered in a 150 μL solution of acridine orange (a vital fluorescent dye at final concentration of 5 μg/mL in PBS), and observations were made in a hemacytometer under both white and fluorescent blue excitation with fluorescence microscopy (Olympus IX81 F).

Assessment of Vascular Reactivity

The thoracic aorta was removed and placed in cold Krebs-bicarbonate solution, cleaned of fat and loose connective tissue, and sectioned into 3-mm-long rings. Two rings per aorta were mounted on stainless steel hooks and suspended in 5-mL tissue baths. The bath was filled with Krebs solution at 37°C, pH 7.4, with 95% O₂/5% CO₂ as a gas phase. Aortic rings were stretched to a 2 g initial tension and were equilibrated for 60 minutes. Tension development was measured by isometric force transducers (GRASS FT03) connected to an amplifier. The Krebs buffer solution in the tissue bath was replaced every 15 minutes, and the tension was readjusted each time. At the end of the equilibration period, the maximal force generated

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**Figure 1.** A and B, Western blot and densitometry analysis of HO-1 and HO-2 proteins in aorta from control and diabetic rats and rats treated with D-4F (daily 100 μg/100 g body weight IP) for 6 weeks. Immunoblots were performed with antibodies against rat HO-1 and HO-2. Data are representative of 6 separate experiments. C and D, Mean band density normalized relative to α-actin (n=6, *P<0.03 vs STZ and P<0.05 vs control; †P<0.001 vs control). E, HO activity in control, diabetic, and D-4F-treated rats. HO activity was determined 24 hours after last D-4F injection. Results are mean±SE, n=4, *P<0.0001 vs control, †P<0.0001 vs diabetic rats.
by addition of a depolarizing solution of 60 mmol/L KCl was determined. To evaluate acetylcholine-induced vasodilation, the rings were preconstricted with phenylephrine (3.5×10⁻⁷ mol/L) to obtain a stable plateau, and then a cumulative dose-response curve to acetylcholine or NONOate was obtained.

**Statistical Analyses**

Data are presented as mean±SEM for the number of experiments. Statistical significance (P<0.05) between experimental groups was determined by the Fisher method of analysis of multiple comparisons. For comparison between treatment groups, the null hypothesis was tested by a single-factor ANOVA for multiple groups or unpaired t test for 2 groups.

**Results**

**Effect of D-4F on HO Activity and HO-1 and HO-2 Protein**

To define the effect of D-4F on vascular HO-1, we measured HO activity and the protein levels in control and diabetic rats. Compared with controls, the induction of diabetes had no significant effect on either HO-1 or HO-2 protein (Figure 1). Treatment with D-4F resulted in a significant increase in the amount of HO-1 protein compared with control (290%, P<0.001) and untreated diabetic rats (277%, P<0.05). Administration of D-4F had no effect on HO-2. Because HO-1 converts heme to equimolar amounts of CO and bilirubin,20 we measured HO activity by formation of bilirubin. HO activity in control vessels was 0.81±0.03 nmol bilirubin formed/mg protein and decreased to 0.59±0.02 nmol bilirubin formed/mg protein in diabetic vessels (P<0.05). The stimulatory effect of D-4F on HO-1 protein was associated with an increase in HO activity (Figure 1E) in both diabetic (1.14±0.04 nmol bilirubin) and control (01.11±0.02 nmol bilirubin) rats (P<0.0001).

**Effect of D-4F on Superoxide Formation**

Because D-4F increases HO-1-derived bilirubin formation, we compared the effect of D-4F on superoxide formation. A comparison was made between superoxide levels in the tissues of D-4F–treated diabetic rats, untreated diabetic rats, and controls. Compared with controls, diabetic rats showed an elevation in aorta O₂⁻ formation from 38.1±8 to 74.8±8×10⁴ cpm/mg protein (P<0.01). D-4F was a very potent inhibitor of O₂⁻ formation. Administration of D-4F decreased O₂⁻ by 85% in diabetic rats (Figure 2). D-4F also decreased O₂⁻ compared with controls, although not significantly.

**Endothelial Cell Sloughing**

Endothelial cells were isolated from peripheral blood as described above and counted under microscopy. Cells were counted if they had at least 10 immunomagnetic beads attached, fluoresced under ultraviolet light after staining with acridine orange, and maintained the round to oval shape and

![Figure 2](attachment:figure2.png)  
**Figure 2.** Aortic O₂⁻ production in diabetic rats treated with D-4F as described in Methods. Results are mean±SE, n=4. *P<0.01 versus control rats, †P<0.01 versus TZ rats.

![Figure 3](attachment:figure3.png)  
**Figure 3.** Morphology of endothelial cells with Dynabeads under light microscopy (A) and fluorescent microscopy (B); cells were obtained from diabetic rats after 6 weeks.
20- to 50-μm size typical of endothelial cells. Representative photographs of endothelial cells are shown in Figure 3. As seen in Figure 4, the number of circulating endothelial cells in controls was 5±1 cells/mL, whereas in diabetic rats, the number was 50±6 cells/mL (P=0.0001). Treatment with D-4F reduced the number of circulating endothelial cells to 20±3 cells/mL in diabetic rats (P<0.005) but had no significant effect on control rats.

Endothelial Cell Fragmentation

Under microscopic examination, there was evidence of cellular fragmentation and debris, which was bound by the RECA-1–coated beads and fluoresced after staining with acridine but was too small to be considered as whole cells (Figure 5). Quantification of these fragments (Figure 6) yielded an average of 8.3±2 fragments/mL in controls and 38±4 fragments/mL in untreated diabetic rats (P=0.009). Administration of D-4F to diabetic rats prevented endothelial fragmentation by 80% in diabetic rats; only 6.8±1 fragments/mL was seen in D-4F–treated diabetic rats (P<0.0001 versus untreated diabetics). Treatment with D-4F also decreased endothelial cell fragmentation in control rats to 2.8±1 fragments/mL (P<0.05 versus untreated control). The level of decrease in cellular fragmentation was indicative of cellular protection secondary to the D-4F–mediated decrease in O$_2^-$.

Vascular Reactivity

It has been reported that acetylcholine-evoked endothelium-mediated relaxation is impaired in diabetes. The present study confirmed a significant impairment in diabetic rats (51±7% relaxation) compared with controls (77±3% relaxation). D-4F restored vascular reactivity in diabetic rats to 85±2% relaxation (Figure 7A). In the presence of the NO donor NONOate, there were no significant changes noted between controls and diabetic rats in response to acetylcholine (Figure 7B). Aorta from diabetic rats treated with D-4F showed an even higher response to NONOate than either control or diabetic vessels at a concentration of 10$^{-6}$ mol/L (P<0.05).

**Effect of HO-1 Expression on the Antioxidant System**

In an attempt to elucidate the mechanism by which D-4F attenuates endothelial sloughing and fragmentation and decreases O$_2^-$, we examined the effect of D-4F on vascular SOD. As shown in Figure 8, the level of EC-SOD was significantly reduced in diabetic rats compared with controls (P<0.003); however, treatment with D-4F significantly restored EC-SOD protein levels (P<0.001). Treatment with D-4F also increased the amount of EC-SOD in control rats (P<0.02 versus control, P<0.0001 versus diabetic). The levels of Cu/Zn-SOD and Mn-SOD proteins were not affected by D-4F in either control or diabetic rats.

**Discussion**

This study demonstrates that D-4F participates in the attenuation of diabetic vascular complications, presumably by increases in bilirubin (an antioxidant) and CO (which is cytoprotective) secondary to increased HO-1 and/or by a robust increase in EC-SOD. This conclusion is supported by 4 important key findings.

First, D-4F was shown to decrease the formation of reactive oxygen species, namely, superoxide (O$_2^-$). Prior study has indicated elevated O$_2^-$ in the hyperglycemic state associated with diabetes, which was attenuated with up-regulation of HO-1. A similar decrease in O$_2^-$ was seen with administration of D-4F. This confirms the antioxidant nature of D-4F and supports earlier studies. Treatment of diabetic rats with D-4F induced increases of HO-1 and significantly increased EC-SOD but had no effect on the other SOD isoforms (Cu/Zn or Mn). We believe that the action of D-4F is largely or solely through the induction of HO-1; however, the present results, which indicate that
D-4F dramatically induces HO-1 and EC-SOD, are novel, and together with our findings that D-4F dramatically reduced endothelial sloughing and improved vasoreactivity in diabetic rats, they constitute important new information about the actions of apolipoprotein A1 mimetic peptides. Although rats have a diminished level of EC-SOD compared with other species, there is evidence of EC-SOD in the vascular tissue. There is potentially an important role for EC-SOD in the attenuating reactive oxygen species and peroxynitrite generation from inducible NO synthase—endothelial NO synthase—derived NO. Peroxynitrite oxidizes and inactivates the NOS cofactor tetrahydrobiopterin into inactive molecules, such as dihydrobiopterin. This uncouples the enzyme, which then preferentially increases O$_2^-$ production over NO production. Therefore, the HO-1 gene expression–mediated increase in EC-SOD may protect endothelial NO synthase from uncoupling. This is of particular importance in diabetes, in which hyperglycemia causes increased endothelial NO synthase uncoupling. The mechanism by which the HO-1 products CO and/or bilirubin may directly regulate this uncoupled state remains unclear.

Second, administration of D-4F increased both the level and activity of HO-1. These results confirmed our previous study, which established that diabetes results in a down-regulation of HO-1 level and activity. Administration of D-4F to diabetic rats led to a reversal of this trend and a resultant increase in both the level and activity of HO-1, which strongly suggests a role for HO in the antioxidant effect of D-4F; however, D-4F–mediated HO-1 may be considered as a signaling mechanism in decreasing inducible NO synthase and O$_2^-$ with an increase in EC-SOD. Gene transfer of antisense HO-1, as a specific inhibitor of HO-1, has been shown to decrease EC-SOD and increase circulating endothelial cells and inducible NO synthase.

The third major finding indicated the vascular protective effect of D-4F administration by decreasing endothelial cell sloughing and debris. Many of the significant clinical problems associated with diabetes can be traced to vascular damage induced by hyperglycemia. Unfortunately, even patients with well-controlled blood glucose levels are not totally protected from these side effects. The increased vascular damage with resultant sloughing and fragmentation of endothelial cells typically found with diabetes was lessened in D-4F–treated animals. This demonstrates a possible clinical role for D-4F in protecting the vasculature from the damage associated with diabetes mellitus.

Finally, D-4F was shown to have positive effects on vascular reactivity to stimulation with acetylcholine. Reactivity of the blood vessels has been shown to be diminished in diabetes. Vascular reactivity in control, untreated diabetic, and D-4F–treated diabetic rats did not differ significantly in the presence of NONOate, which indicates a dependence on the bioavailability of NO.

Figure 6. Endothelial cell membrane fragments in blood obtained from control, diabetic, and D-4F–treated diabetic rats. Number of endothelial cell fragments was determined; n=5 rats. *P<0.001 vs control; †P<0.001 vs diabetic rats; ‡P<0.05 vs control.

Figure 7. A, Dose-response curves for acetylcholine-induced vascular relaxation after preconstriction with phenylephrine. Decreased relaxation in diabetics compared with controls is restored by treatment with D-4F (*P<0.05). B, Dose-response curves in presence of NONOate. D-4F–treated diabetic rats had some improvement in their reactivity compared with untreated diabetic rats (*P<0.05).
There is an ever-increasing body of evidence that indicates that inflammation and oxidative stress play a major role in the formation of atherosclerotic disease. The oxidative-modification hypothesis indicates that only after oxidation does LDL become atherogenic.40–42 After incorporation into foam cells, oxidized LDL acts to recruit inflammatory cells, including T lymphocytes and monocytes, via O$_2^-$.

There is also evidence that oxidative stress plays a role in the mechanisms for the documented risk factors of atherosclerosis, including hypertension, diabetes, and smoking.45,46 In individuals with diabetes, there is increased LDL oxidation even in the face of adequate glucose control.30 Prior study has shown that patients with documented atherosclerosis had LDL that was highly proinflammatory, independent of their total cholesterol or HDL levels.3,4 Superoxide, a marker for oxidative stress, has been implicated in many cardiovascular diseases, including atherosclerosis, and has been shown to rapidly inactivate endothelium-derived NO.47,48 Peroxynitrite, formed from the reaction of superoxide with NO, is known to promote lipid peroxidation, contributing to formation of atherosclerotic lesions.49 Superoxide-mediated impairment of NO may result in vascular smooth muscle cell proliferation and migration and the expression of proinflammatory molecules, which contribute to atherosclerosis.47,50 It is hypothesized that the elevated superoxide seen in the diabetic animals in the present study was vascular in origin, because EC-SOD is primarily vascular in nature. This hypothesis is supported by prior study showing that the apolipoprotein AI mimetic L-4F can attenuate O$_2^-$ production in cultured endothelial cells.4,5

In light of these recent discoveries, there is a growing potential for therapeutic agents that can attenuate oxidative stress. Mounting evidence demonstrates that D-4F can protect against oxidative injury, which makes it a potential therapeutic agent in the fight against atherosclerosis. With administration of D-4F, HDL becomes antiinflammatory, which increases HDL-mediated cholesterol efflux and reverses cholesterol transport from macrophages.1 In LDL-receptor null or apolipoprotein E null experimental mice fed Western diets, D-4F administration resulted in a 79% and 75% reduction in atherosclerotic lesions, respectively.3 This is in agreement with the potency of D-4F on induction of the HO-1–mediated increase in EC-SOD and subsequent decrease in O$_2^-$. The previously unknown mechanism for the antioxidant role of D-4F has now been shown to be due primarily to HO, a known antioxidant enzyme. In summation, the present results when added to the previous findings about D-4F indicate a possible clinical benefit in preventing the cardiovascular complications of diabetes.
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References


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