Long-Term Vitamin C Treatment Increases Vascular Tetrahydrobiopterin Levels and Nitric Oxide Synthase Activity

Livius V. d’Uscio, Sheldon Milstien, Darcy Richardson, Leslie Smith, Zvonimir S. Katusic

Abstract—In cultured endothelial cells, the antioxidant, L-ascorbic acid (vitamin C), increases nitric oxide synthase (NOS) enzyme activity via chemical stabilization of tetrahydrobiopterin. Our objective was to determine the effect of vitamin C on NOS function and tetrahydrobiopterin metabolism in vivo. Twenty-six to twenty-eight weeks of diet supplementation with vitamin C (1%/kg chow) significantly increased circulating levels of vitamin C in wild-type (C57BL/6J) and apolipoprotein E (apoE)–deficient mice. Measurements of NOS enzymatic activity in aortas of apoE-deficient mice indicated a significant increase in total NOS activity. However, this increase was mainly due to high activity of inducible NOS, whereas eNOS activity was reduced. Significantly higher tetrahydrobiopterin levels were detected in aortas of apoE-deficient mice. Long-term treatment with vitamin C restored endothelial NOS activity in aortas of apoE-deficient mice, but did not affect activity of inducible NOS. In addition, 7,8-dihydrobiopterin levels, an oxidized form of tetrahydrobiopterin, were decreased and vascular endothelial function of aortas was significantly improved in apoE-deficient mice. Interestingly, vitamin C also increased tetrahydrobiopterin and NOS activity in aortas of C57BL/6J mice. In contrast, long-term treatment with vitamin E (2000 U/kg chow) did not affect vascular NOS activity or metabolism of tetrahydrobiopterin. In vivo, beneficial effect of vitamin C on vascular endothelial function appears to be mediated in part by protection of tetrahydrobiopterin and restoration of eNOS enzymatic activity. (Circ Res. 2003;92:88-95.)

Key Words: tetrahydrobiopterin ■ nitric oxide synthase ■ nitric oxide ■ antioxidants ■ superoxide anion

Nitric oxide (NO) is a potent vasodilator and plays a key role in the control of the cardiovascular system. NO is mainly formed in endothelial cells from L-arginine by oxidation of its terminal guanidino-nitrogen, requiring the cofactors NADPH, (6R)-5,6,7,8-tetrahydrobiopterin (BH4), FAD, FMN, heme, and Zn2+. The formation of NO occurs via endothelial NO-synthase (eNOS) which is expressed constitutively. Relaxations in response to the abluminal release of endothelium-derived NO are associated with stimulation of soluble guanylyl cyclase (sGC) and in turn formation of cyclic guanosine 3’,5’-monophosphate (cGMP) in vascular smooth muscle cells. Inducible NOS (iNOS) enzyme can be expressed in vascular smooth muscle cells, endothelium, and macrophages. This enzyme activity is Ca2+-independent and produces large amounts of NO; it is induced by cytokines such as interleukin 1β and tumor necrosis factor-α and hence is activated in atherosclerosis and inflammatory processes. BH4 is an essential cofactor required for activity of all NOS isoforms. During activation of NOS, BH4 is needed for allosteric and redox activation of its enzymatic activity. Accumulating evidence suggests that alterations in the NO pathway, such as increased NO decomposition by superoxide anion (O2−) or altered NOS expressions, play a central role in endothelial dysfunction induced by hypercholesterolemia. This may be of major importance because NO can substantially inhibit several components of the atherogenic process, such as vascular smooth muscle cells contraction and proliferation, platelet aggregation, and monocyte adhesion. It has been shown in several studies that antioxidants, vitamin C or vitamin E, reduced vascular oxidative stress and increased NO-mediated endothelium-dependent relaxations. In addition, vitamin C increased vasodilation of forearm resistance arteries in humans with hypercholesterolemia, long-term smokers, essential hypertension, and coronary artery disease. The molecular mechanisms underlying the in vivo antioxidant effects of vitamin C are not fully understood. More recent findings in cultured endothelial cells indicate that vitamin C may increase NOS enzymatic activity by chemical stabilization of BH4. Therefore, we hypothesized that the in vivo effect of vitamin C is mediated in part by its ability to protect BH4 from oxidation and thereby increase enzymatic activity of eNOS. In this study, we compared the effects of vitamins...
Enzyme Activity of vitamin C and vitamin E were based on previous studies. 18,33

Measurement of Ca\(^{2+}\)-Dependent NOS

Aortic lesion areas were significantly reduced by 51% after treatment of apoE-deficient mice with vitamin C or E had no effect on the plasma lipid profile (percent of 80 mmol/L). Data are mean ± SEM of 5 to 12 mice.

TABLE 1. Characteristics of ApoE-Deficient and C57BL/6J Mice After 26 to 28 Weeks of Treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C57BL/6J</th>
<th>C57BL/6J+Vit C</th>
<th>C57BL/6J+Vit E</th>
<th>ApoE</th>
<th>ApoE+Vit C</th>
<th>ApoE+Vit E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.9±0.6</td>
<td>5.8±0.6</td>
<td>6.9±0.6</td>
<td>22.8±3.7(^*)</td>
<td>22.7±2.9(^*)</td>
<td>22.1±2.8(^*)</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>0.9±0.1</td>
<td>0.6±0.1</td>
<td>0.8±0.1</td>
<td>1.7±0.4(^*)</td>
<td>1.8±0.5(^*)</td>
<td>2.1±0.5(^*)</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>0.9±0.1</td>
<td>0.7±0.1</td>
<td>0.8±0.1</td>
<td>15.8±3.0(^*)</td>
<td>14.3±0.9(^*)</td>
<td>16.2±2.3(^*)</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>5.5±0.6</td>
<td>5.0±0.5</td>
<td>5.9±0.5</td>
<td>2.6±0.3(^*)</td>
<td>2.7±0.1(^*)</td>
<td>3.2±0.3(^*)</td>
</tr>
<tr>
<td>L-ascorbic acid, (\mu)mol/L</td>
<td>114±3</td>
<td>315±42(\dagger)</td>
<td>ND</td>
<td>88±7(\dagger)</td>
<td>230±58#</td>
<td>ND</td>
</tr>
<tr>
<td>(\alpha)-tocopherol, (\mu)mol/L</td>
<td>35±2</td>
<td>ND</td>
<td>47±3(\dagger)</td>
<td>49±4(\dagger)</td>
<td>ND</td>
<td>98±8#</td>
</tr>
<tr>
<td>Aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KCl contraction, g</td>
<td>1.5±0.1</td>
<td>1.5±0.1</td>
<td>1.5±0.1</td>
<td>1.4±0.1</td>
<td>1.3±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Phenylerythrine max, %</td>
<td>89±6</td>
<td>86±7</td>
<td>88±5</td>
<td>104±4</td>
<td>105±5</td>
<td>100±4</td>
</tr>
</tbody>
</table>

ApoE indicates apolipoprotein E–deficient mice; C57BL/6J, wild-type mice; ND, not determined; max, maximal response to agonist (percent of 80 mmol/L). Data are mean ± SEM of 5 to 12 mice.

Materials and Methods

Experimental Animals

Male C57BL/6J (wild-type) mice and homozygous apoE-deficient mice (4 to 5 weeks old) were obtained from Jackson Laboratory (Bar Harbor, Maine) and were fed a lipid rich Western-type diet (TD88137, Harlan Teklad)35,32 without or with vitamin C (1%/kg diet) or vitamin E (2000 IU/kg diet) for 26 to 28 weeks. The dosages of vitamin C and vitamin E were based on previous studies.18,33 Housing facilities and all experimental protocols were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic.

Plasma Vitamins C and E

A reverse-phase HPLC was used to determine plasma concentrations of vitamins C and E.

Lesion Assessment

Dissected aortas were opened longitudinally and fixed in 4% buffered paraformaldehyde for 2 hours and were stained in super-saturated Sudan IV solution for an additional 16 hours.34

Vasomotor Reactivity

Isolated aortic rings were connected to a force transducer for recording of isometric force and placed in organ baths filled with 25 mL Krebs solution (37°C; 94% \(O_2\)/6% \(CO_2\); pH 7.4).35 Concentration-dependent response curves to acetylcholine (Ach), and diethylammonium (\(Z\))-1-(\(N,\)N-diethylaminodiazem-1-i-um-1,2-diolate (DEA-NONOate) were cumulatively obtained during sub-maximal contractions to phenylephrine.

Quantification of Vascular \(O_2^*\) Production

Vascular \(O_2^*\) production was measured by lucigenin-enhanced chemiluminescence as described.35

Measurement of \(Ca^{2+}\)-Dependent NOS

Enzyme Activity

Aortas were homogenized on ice in lysis buffer pH 7.5, and \(L\)-[\(14\)C]-Citrulline formation was measured as described previously.35

Western Blot Analysis

Mouse monoclonal anti-eNOS (1:500), anti-iNOS (1:100; Transduction Labs), and anti-nitrotyrosine (0.5 \(\mu\)g/mL; Upstate Biotechnolog-
E-selectin in apoE-deficient mice while, conversely, vitamin C impaired NO-mediated endothelium-dependent relaxations to Ach in aortas of apoE-deficient mice (83±2%; P<0.05) whereas vitamin E did not have any effect (Figure 1A, left).

Endothelium-independent relaxations to the NO donor DEA-NONOate were reduced, and the concentration-response curve was shifted to right in apoE-deficient mice (pD2: 7.4; P<0.05 versus wild-type mice: 8.5). Vitamin C, but not vitamin E, in part improved the sensitivity to DEA-NONOate in apoE-deficient mice (pD2: 7.7; P<0.05 versus apoE mice; Figure 1B, right). In contrast, vitamin C reduced relaxations to the NO-donor in wild-type mice (pD2: 8.2; P<0.05 versus wild-type group: 8.5; Figure 1B, left) without affecting maximal relaxations.

Ca2+-Dependent NOS Activity
In order to evaluate the mechanisms underlying effects of antioxidants on endothelium-dependent relaxations, we measured Ca2+-dependent NOS activity in aortas of apoE-deficient and wild-type mice as determined by conversion of L-[14C]arginine to L-[14C]citrulline in tissue homogenates. Vitamin C selectively increased Ca2+-dependent NOS activity in aortas from both wild-type and apoE-deficient mice (P<0.05; Figure 2A). Interestingly, vitamin C normalized enzyme activity in apoE-deficient mice to values similar to those found in aortas from wild-type mice. Conversely, vitamin C did not affect eNOS protein expression (Figure 2B; n=3), whereas vitamin E had no significant effects on eNOS protein expression or NOS activity in either apoE-deficient or wild-type mice (Figure 2).

INOS Enzyme Activity and Protein Expression
In the aortas of wild-type mice, Ca2+-independent NOS activity was very low as compared with Ca2+-dependent NOS activity (P<0.05; Figures 2A and 3A). iNOS activity was increased in apoE-deficient mice as compared with wild-type (P<0.05; Figure 3A). In addition, iNOS protein expression was also enhanced in apoE-deficient mice (P<0.05; Figure 3B). Antioxidant vitamins did not affect iNOS protein expression (Figure 3B). Interestingly, vitamin C selectively increased iNOS enzyme activity in wild-type (P<0.05; Figure 3A) whereas it had no effect in apoE-deficient mice.

cGMP and cAMP Levels
Basal cGMP levels were reduced in aortas from apoE-deficient mice as compared with wild-type mice (P<0.05; Figure 4). Vitamin C treatment increased basal cGMP levels only in wild-type (P<0.05; Figure 4). Basal cAMP levels were not different between wild-type (30±6 pmol/mg) and apoE-deficient mice (25±2 pmol/mg) and after vitamin C treatment (34±4 and 26±3 pmol/mg) or after vitamin E treatment (33±4 and 27±4 pmol/mg), respectively.

Tetrahydrobiopterin Levels
Total aortic biopterin levels were increased in apoE-deficient mice as compared with wild-type mice (P<0.05). This increase was due to the elevation of BH4 levels (P<0.05; Figure 5A). 7,8-BH2/biopterin levels were not affected (NS; Figure 5B). The ratios of BH4 to 7,8-BH2/biopterin were not different between two groups of mice (Figure 5C).
Treatment of apoE-deficient mice with vitamin C did not affect aortic BH₄ levels. In contrast, vitamin C significantly decreased 7,8-BH₂/biopterin levels in apoE-deficient mice (P<0.05; Figure 5B). Conversely, vitamin C significantly increased BH₄ levels without affecting 7,8-BH₂/biopterin levels in wild-type mice (P<0.05; Figure 5), whereas vitamin E did not have any effect. Most importantly, vitamin C increased BH₄ to 7,8-BH₂/biopterin ratio in both apoE-deficient and wild-type mice (P<0.05; Figure 5C).

We also measured BH₄ and 7,8-BH₂/biopterin levels in the liver in order to determine whether vitamin C may affect BH₄ metabolism in tissues other than blood vessel. We found that in wild-type mice, 7,8-BH₂/biopterin was very low as compared with BH₄ (Table 2). On the other hand, 7,8-BH₂/biopterin levels were increased in apoE-deficient mice as compared with wild-type (P<0.05; Table 2). Consequently, BH₄ to 7,8-BH₂/biopterin ratio decreased in apoE mice (P<0.05). Vitamin C treatment did not have any effect on BH₄ and 7,8-BH₂/biopterin levels (NS; Table 2), whereas vitamin E slightly decreased BH₄ levels in apoE-deficient mice (P<0.05).

Figure 2. A, Bar graphs showing Ca²⁺-dependent eNOS enzyme activity in the aorta of wild-type (C57BL/6J) and apoE-deficient mice after 26 to 28 weeks on a Western-type diet with or without antioxidants. L-[¹⁴C]citrulline formation was measured in aortic homogenates as described in Materials and Methods. Results are mean±SEM (n=7). *P<0.05 vs C57BL/6J mice (ANOVA+Bonferroni’s); †P<0.05 vs C57BL/6J mice (unpaired t test); ‖P<0.05 vs apoE-deficient mice (ANOVA+Bonferroni’s). B, Representative Western blot analysis of eNOS protein expression in aortas of C57BL/6J and apoE-deficient mice. Bar graph indicates the results of relative densitometric analysis of eNOS expression as OD per mm² aortic surface (n=3 to 4). Actin blots are shown as loading controls.

Figure 3. A, Bar graphs showing Ca²⁺-independent NOS enzyme activity (iNOS) in the aorta of wild-type (C57BL/6J) and apoE-deficient mice after 26 to 28 weeks on a Western-type diet with or without antioxidants. L-[¹⁴C]citrulline formation was measured in aortic homogenates as described in Materials and Methods. Results are mean±SEM (n=7). *P<0.05 vs C57BL/6J mice; †P<0.05 vs C57BL/6J with or without antioxidants (ANOVA+Bonferroni’s). B, Representative Western blot analysis of iNOS protein expression in aortas of C57BL/6J and apoE-deficient mice. Bar graphs indicate the results of the relative densitometry as compared with actin (n=3). *P<0.05 vs C57BL/6J with or without antioxidants (ANOVA+Bonferroni’s).

Figure 4. Bar graphs showing basal cGMP levels in aortas of wild-type (C57BL/6J) and apoE-deficient mice. Results are mean±SEM (n=5 to 10). *P<0.05 vs C57BL/6J mice; †P<0.05 vs C57BL/6J with or without antioxidants (ANOVA+Bonferroni’s).
Vascular $\text{O}_2^-$ Production

Formation of $\text{O}_2^-$ was increased 3-fold in apoE aortas ($P<0.05$ versus wild-type mice; Figure 6A). Both antioxidant vitamins significantly decreased $\text{O}_2^-$ levels in apoE-deficient mice aortas ($P<0.05$ versus apoE group; Figure 6A), whereas they did not affect $\text{O}_2^-$ production in wild-type mice.

Detection of Nitrotyrosine

Western blot analysis showed an increased nitrotyrosine abundance in the aorta of apoE-deficient mice (n=4, Figure 6B), whereas in wild-type mice, nitrotyrosine could not be detected (data not shown). Both vitamin C and E reduced tissue nitrotyrosine abundance in apoE-deficient mice (Figure 6B). In order to confirm the specificity of the antibody, sodium dithionite was used to destroy the nitrotyrosine epitope (Figure 6B; lanes 5 to 7).

Discussion

This is the first study to examine in vivo effects of long-term vitamin C treatment on NOS enzymatic activity and BH$_4$ metabolism in aortas of wild-type and apoE-deficient mice.
We report a number of novel findings. First, vitamin C treatment increased total biopterin and BH$_4$ levels in aorta of wild-type mice. This increase was associated with increased enzymatic activity of eNOS, iNOS, and higher basal levels of cGMP, suggesting that vitamin C has a BH$_4$-dependent stimulatory effect on NO formation in normal arterial wall. Second, total biopterin, BH$_4$, and iNOS enzymatic activity were significantly higher in apoE-deficient mice as compared with wild-type mice. Third, supplementation with vitamin C improved endothelial dysfunction in apoE-deficient mice, reduced atherosclerotic lesions, and restored eNOS enzymatic activity. This is most likely due, in part, to the ability of vitamin C to protect BH$_4$ and to preserve biosynthesis of NO.

Fourth, in contrast to vitamin C, vitamin E did not affect vascular NOS enzymatic activity or BH$_4$ metabolism. Thus, our results demonstrate that vitamin C (but not vitamin E) is an important regulator of BH$_4$ metabolism and NOS function in vivo.

BH$_4$ is an essential cofactor required for activity of NOS. Previous studies in cultured vascular endothelial cells demonstrated that vitamin C increases eNOS activity by increasing availability of BH$_4$. Increased availability of BH$_4$ was not due to higher activity of GTP cyclohydrolase I, the rate-limiting enzyme in biosynthesis of BH$_4$. Rather, chemical stabilization of BH$_4$ by vitamin C may be the most likely explanation for previously reported observations. In the present study, we tested this concept in vivo by long-term dietary supplementation of vitamin C. Our findings support the idea that vitamin C may increase intracellular concentrations of BH$_4$ in the normal arterial wall. This, in turn, may activate NOS and increase formation of NO. Increased enzymatic activity of NOS and higher cGMP (but not cAMP) levels in arteries obtained from vitamin C-treated wild-type mice strongly suggest that formation of NO is selectively augmented by vitamin C treatment. It is interesting that iNOS is expressed in wild-type mouse arteries and its activity is very low as compared with Ca$^{2+}$-dependent NOS activity. The fact that vitamin C did not affect expression of eNOS or iNOS protein, together with a significant increase in eNOS and iNOS enzymatic activity, suggest that availability of BH$_4$ may be a regulatory mechanism designed to control levels of NO production. It appears that in vivo intracellular concentration of BH$_4$ is subsaturating for vascular NOS isoforms.

Endothelium-dependent relaxations to Ach and endothelium-independent relaxations to DEA-NONOate were impaired in the aortas of vitamin C-treated wild-type mice. This finding is consistent with reported impairment of NO-induced relaxation in eNOS transgenic mice and arteries transduced with recombinant iNOS. Vitamin C did not increase formation of O$_2^-$ in normal arteries, ruling out chemical antagonism between O$_2^-$ and NO as an explanation for impairment of relaxations mediated by NO. Downregulation of expression and function of soluble guanylate cyclase in vitamin C-treated aortas is the most likely reason behind reduced reactivity of vascular smooth muscle to NO.

Further studies are needed to determine the exact mechanism responsible for reduction of relaxations induced by endogenous or exogenous NO. Our results also call for further studies of BH$_4$ catabolism in normal arteries. Turnover of BH$_4$ in blood vessels appears to be very rapid. In isolated canine basilar arteries, incubation with a GTP cyclohydrolase I inhibitor for 6 hours resulted in 95% depletion of intracellular BH$_4$. The exact molecular mechanisms responsible for degradation of BH$_4$ that can be inhibited by vitamin C remain to be determined.

Proinflammatory cytokines, including tumor necrosis factor-α, interferon-γ, and interleukin-1β, stimulate BH$_4$ biosynthesis in cultured vascular endothelial cells. This effect is due to upregulation of GTP cyclohydrolase I transcription, expression, and function. Simmons and colleagues demonstrated that in cardiac microvascular endothelial cells cytokines cause coordinate induction of GTP cyclohydrolase I and iNOS. Cytokines play a key role in pathogenesis of atherosclerosis, and therefore, it is not surprising that in the present study we detected 2-fold increases of BH$_4$ in aortas of apoE-deficient mice. This increase in BH$_4$ was associated with about 7-fold increase in iNOS enzymatic activity. Thus, the present in vivo findings are consistent with previously obtained results in cultured endothelial cells and suggest the hypothesis that biosynthesis of BH$_4$ is coordinated with induction and increased activity of iNOS. They are also consistent with reported increased plasma levels of neopterin, a by-product of BH$_4$ biosynthesis, in patients with atherosclerosis and coronary syndromes.

In apoE-deficient mice, vitamin C treatment did not affect aortic BH$_4$ levels, but did significantly reduce the BH$_4$ fraction, suggesting that vitamin C may protect BH$_4$ from oxidation. Catabolism of BH$_4$ has not been studied in apoE-deficient mice, and we can only speculate about molecular mechanisms underlying protection of BH$_4$. In a previous study, we demonstrated that peroxynitrite causes oxidation of BH$_4$. This has been confirmed in two subsequent reports. Whether endogenous peroxynitrite contributes to oxidation of BH$_4$ in vivo is unknown. Vitamin C could lessen redox cycling of BH$_4$ by decreasing intracellular O$_2^-$ and peroxynitrite accumulation because BH$_4$ has been shown to undergo redox cycling with molecular oxygen, which results in the generation of O$_2^-$.

However, because both vitamins C and E reduced production of O$_2^-$ and nitrotyrosine, but only vitamin C had effects on BH$_4$ and NO activity, it appears unlikely that O$_2^-$/peroxynitrite-mediated oxidation is responsible for oxidation of BH$_4$. Furthermore, vitamin C was very effective in increasing BH$_4$ levels in wild-type animals despite the absence of nitrotyrosine and very low O$_2^-$ formation in their aortas. Studies in cultured vascular endothelial cells demonstrated that oxidation of BH$_4$ to quinonoid 6,7-[8H]-BH$_2$ rearrangement to 7,8-BH$_2$ and further oxidation to BH$_3$ radical to BH$_4$. In addition, vitamin C could also increase the affinity of BH$_4$ for NOS enzyme by preserving thiols on NOS protein.
and apoE-deficient mice. It is possible that the high level of oxidative stress that was found in atherosclerotic apoE-deficient mice may consume vitamin C. Indeed, plasma concentrations of vitamin C were significantly lower in apoE-deficient mice. This is consistent with results of epidemiological studies in humans demonstrating that plasma vitamin C concentrations are inversely related to increased risk for atherosclerosis. Thus, supplementation of vitamin C may help to replace oxidized vitamin C in apoE-deficient mice. Why long-term treatment with vitamin C increases NOS activity in wild-type animals is unclear and remains to be determined.

Our study is the first to examine the effect of vitamin C on endothelial dysfunction and progression of atherosclerosis in apoE-deficient mice. As expected, vitamin C improved endothelial function, and reduced O2·− and peroxynitrite formation. These effects could be independent of the effect of vitamin C on BH4 metabolism. Endothelial cells can take up reduced or oxidized forms of ascorbic acid and accumulate concentrations up to 3 to 8 mmol/L. This concentration of vitamin C can effectively scavenge O2·− and protect NO from chemical inactivation. With regard to the antiatherogenic effect of vitamin E, our results are in agreement with the previously reported ability of vitamin E to prevent development of atherosclerosis in apoE-deficient mice.

The present study demonstrates that long-term treatment of C57BL/6J mice with vitamin C increases BH4 levels in the vascular wall. This increase is coupled with increased eNOS enzymatic activity and high basal levels of cGMP. We also provide evidence that BH4 metabolism may be an important component in pathogenesis of atherosclerosis. Coordinated upregulation of BH4 availability and iNOS expression is probably designed to increase biosynthesis of NO in vascular wall exposed to proinflammatory cytokines. However, prolonged high activity of iNOS may be detrimental to vascular function due to “uncoupling” of the enzyme and subsequent increased formation of O2·−. Protection of BH4 appears to be an important mechanism that may contribute to antiatherogenic effect of vitamin C.

Acknowledgments

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References