During the past twenty years, the essential role of endothelial cells in preservation of vascular homeostasis has been well established. Protection of the vascular endothelium against harmful influences of circulating substances including excessive levels of lipids has been a major therapeutic approach in prevention of atherosclerosis. While endothelial dysfunction, defined as a loss of biologically active nitric oxide (NO) produced in the endothelium, has been recognized as a prime target for prevention and reversal of atherosclerotic process, identification of exact molecular mechanisms responsible for the loss of endothelial NO have been more difficult to determine. In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Alp et al report a series of elegant studies on genetically modified mice supporting the concept that NO synthase cofactor, tetrahydrobiopterin (BH4) deficiency mice appears to be mediated by preservation of endothelial nitric oxide synthase (eNOS) activity and production of nitric oxide in endothelial cells.

The beneficial effect of BH4 supplementation is based on the assumption that an insufficient amount of BH4 is present in diseased arteries. However, measurements of BH4 levels in arteries of atherosclerotic animals reported controversial results. Studies by Ozaki et al and Vasquez-Vivar et al reported reduced BH4 levels in aortas of hypercholesterolemic mice and rabbits, respectively. In contrast, d’Uscio and colleagues reported increased BH4 levels in aortas of ApoE-deficient mice. In the present study by Alp et al, BH4 levels of hypercholesterolemic mice were not measured in control wild-type animals; however, presented findings are consistent with the reported increase of BH4 in ApoE-deficient mice. The most likely explanation for the controversial findings is that studies by Ozaki et al and Vasquez-Vivar et al used atherosclerotic animals that had about 30 times higher circulating levels of cholesterol as compared with control animals. On the other hand, in studies by d’Uscio et al and Alp et al, total cholesterol levels were increased only about 3 times. Thus, it appears that hypercholesterolemia tends to increase BH4 levels, whereas extremely high cholesterol levels can reduce availability of BH4. This finding is consistent with existing literature demonstrating stimulatory effect of proinflammatory cytokines on biosynthesis of BH4. These findings are also in line with the reported increased plasma levels of neopterin, a byproduct of BH4 biosynthesis, in patients with atherosclerosis and coronary syndrome. Interestingly, in the present study by Alp et al, additional increase in BH4 levels in hypercholesterolemic mice due to over-expression of GTP cyclohydrolase I in endothelium of ApoE-deficient mice provided protection against atherosclerosis. This observation suggests that despite an apparent adaptive increase of BH4 levels in aorta of ApoE-deficient mice, there appears to be a relative shortage of the NOS cofactor. Genetic
supplementation of tetrahydrobiopterin preserved production of NO, reduced endothelial formation of superoxide anions, and protected vascular wall against atherosclerosis. Thus, tetrahydrobiopterin could be added to the list of endogenously produced antiatherosclerotic molecules, whereas endothelial GTP cyclohydrolase I is emerging as a potential new therapeutic target.

Results of the study by Alp and colleagues\textsuperscript{3} are interpreted as additional evidence to support "eNOS uncoupling hypothesis" as an important mechanism of endothelial dysfunction. Studies by several different groups suggest that "eNOS uncoupling" could be prevented by tetrahydrobiopterin supplementation.\textsuperscript{5,11,25} However, as correctly pointed out by Alp et al,\textsuperscript{3} direct in vivo evidence of eNOS uncoupling is missing because of limitations of methodologies available to detect vascular superoxide anions.\textsuperscript{26} Furthermore, it remains puzzling how hypercholesterolemia-induced increase in tetrahydrobiopterin concentration in vascular wall may lead to uncoupling of eNOS in ApoE-deficient mice. Further studies of mechanisms involved in control of vascular GTP cyclohydrolase I expression and activity, as well as metabolism of tetrahydrobiopterin in endothelial cells in vivo, may ultimately provide explanation for the role of tetrahydrobiopterin in eNOS uncoupling. Irrespective of the exact mechanism underlying vascular protective effect of tetrahydrobiopterin, the study by Alp and colleagues\textsuperscript{3} provides strong evidence that tetrahydrobiopterin is an important endogenous antiatherosclerotic molecule.

Acknowledgments
This work was supported in part by National Heart, Lung, and Blood Institute grants HL-53524, HL-58080, and HL-066958. The secretarial assistance of Janet Beckman is gratefully acknowledged.

References