

# Tetrahydrobiopterin Improves Endothelium-Dependent Vasodilation in Chronic Smokers

## Evidence for a Dysfunctional Nitric Oxide Synthase

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**Abstract**—Conditions associated with impaired nitric oxide (NO) activity and accelerated atherosclerosis have been shown to be associated with a reduced bioavailability of tetrahydrobiopterin (BH4). We therefore hypothesized that BH4 supplementation may improve endothelial dysfunction of chronic smokers. Forearm blood flow (FBF) responses to the endothelium-dependent vasodilators acetylcholine (ACh; 0.75, 1.5, and 3.0  $\mu\text{g}/100\text{ mL}$  tissue/min) or serotonin (5-HT; 0.7, 2.1, and 6.3  $\text{ng}/100\text{ mL}$  tissue/min), to the inhibitor of endothelial nitric oxide synthase (NOS)  $N^G$ -monomethyl-L-arginine (L-NMMA; 2, 4, and 8  $\mu\text{mol}/\text{min}$ ), and to the endothelium-independent vasodilator sodium nitroprusside (SNP; 0.1, 0.3, and 1.0  $\mu\text{g}/100\text{ mL}$  tissue/min) were measured by venous occlusion plethysmography in controls and chronic smokers. Drugs were infused into the brachial artery, and FBF was measured before and during concomitant intra-arterial infusion of BH4, tetrahydroneopterin (NH4; another reduced pteridine), or the antioxidant vitamin C (6 and 18  $\text{mg}/\text{min}$ ). In control subjects, BH4 had no effect on FBF in response to ACh, 5-HT, and SNP. In contrast, in chronic smokers, the attenuated FBF responses to ACh and 5-HT were markedly improved by concomitant administration of BH4, whereas the vasodilator responses to SNP were not affected. L-NMMA-induced vasoconstriction was significantly reduced in smokers compared with controls, suggesting impaired basal NO bioactivity. BH4 improved L-NMMA responses in smokers while having no effect on L-NMMA responses in controls. Pretreatment with vitamin C abolished BH4 effects on ACh-dependent vasodilation. In vitro, NH4 scavenged superoxide created by the xanthine/xanthine oxidase reaction equipotent like BH4 but failed to modify ACh-induced changes in FBF in chronic smokers in vivo. These data support the concept that in addition to the free radical burden of cigarette smoke, a dysfunctional NOS III due to BH4 depletion may contribute at least in part to endothelial dysfunction in chronic smokers. The full text of this article is available at <http://www.circresaha.org>. (*Circ Res.* 2000;86:e36-e41.)

**Key Words:** smoking ■ endothelium ■ nitric oxide ■ tetrahydrobiopterin ■ tetrahydroneopterin

Endothelial-derived nitric oxide (NO) plays a pivotal role in the regulation of vascular tone and in the maintenance of vascular integrity (for review, see Reference 1). Several invasive and noninvasive studies have established endothelial dysfunction in chronic smokers in coronary and peripheral conductance<sup>2,3</sup> and resistance vessels<sup>4</sup> as well as in isolated veins.<sup>5</sup> The precise mechanisms underlying endothelial dysfunction in chronic smokers remain to be established but likely involve oxygen-derived free radicals. Chronic smoking increases the excretion of free radical-catalyzed products of arachidonic acid,<sup>6</sup> and in chronic smokers, there is a strong correlation of endothelial dysfunction with autoantibody titers against oxidized LDL.<sup>4</sup> Cessation of smoking as well as therapy with antioxidants reduces isoprostane excretion<sup>6</sup> and improves endothelial dysfunction,<sup>7,8</sup> suggesting that in chronic smokers reduced NO bioactivity is at least in part mediated by increased levels of oxygen-derived free radicals.

Cigarette smoke is known to contain a large number of free radicals and pro-oxidants.<sup>9</sup> It remains to be established whether these constituents may cause endothelial dysfunction by damaging the endothelium directly or whether cigarette smoke-induced oxidative stress in turn may activate vascular superoxide-producing enzymes that may further increase the free radical burden to the vasculature in a positive feedback fashion.

Recent experimental and clinical studies demonstrated that administration of tetrahydrobiopterin (BH4) improves endothelial dysfunction in diseases with increased vascular superoxide production such as hypercholesterolemia<sup>10</sup> and diabetes mellitus.<sup>11</sup> Interestingly, in vitro studies with saphenous veins from chronic smokers showed improved endothelium-dependent relaxation after preincubation with BH4.<sup>12</sup> BH4 is a critical cofactor for NO formation and appears to modulate

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NOS activity by serving as electron donor for the hydroxylation of L-arginine.<sup>13</sup> Reduced bioavailability of BH4 has been demonstrated to cause an uncoupling of the endothelial NO synthase (NOS III) thereby generating superoxide instead of NO.<sup>14,15</sup> Preliminary experiments indicate that peroxytrite (ONOO<sup>-</sup>), which is a major constituent of the gas phase of cigarette smoke, avidly oxidizes BH4 to dihydrobiopterin, leading to impaired endothelial function.<sup>16</sup>

The primary aim of the present study was to assess whether BH4 could improve basal and stimulated NO activity in chronic smokers. Because BH4 is active only in its reduced form, one has to assume that BH4 deficiency may be due to enhanced oxidative modification of BH4. Because ascorbic acid has been shown to enhance endothelial NO production in a BH4-dependent manner,<sup>17</sup> the second objective was to test whether pretreatment with the antioxidant vitamin C is able to modulate the effect of BH4 on endothelial function. Third, we compared the antioxidant effects of the pteridine tetrahydro-neopterin (NH4) with BH4 effects in vitro and in vivo to determine whether BH4-induced improvements in forearm blood flow (FBF) in chronic smokers are secondary to its effects as a cofactor on a dysfunctional NOS III or due to its nonspecific antioxidant effects.

## Materials and Methods

### Study Population

The total study population included 45 chronic smokers and 13 control subjects. Smokers were included in the study if they had a history of 20 or more pack-years (1 pack-year defined as smoking 20 cigarettes per day for 1 year or the equivalent). Subjects with hypercholesterolemia (LDL cholesterol >75th percentile for age and sex), diabetes mellitus, arterial hypertension, or any other systemic disease predisposing them to endothelial dysfunction were excluded from the study. Further exclusion criteria were current use of antioxidants or vasoactive medication. On the day of the study, smoking was not allowed. The study protocol was approved by the local ethics committee, and informed consent was obtained from each subject.

### FBF Measurements

FBF measurements were done by venous plethysmography, as described recently.<sup>4,7</sup>

### Study Design

#### *Protocol 1: Effects of BH4 on Endothelium-Dependent and Endothelium-Independent Vasodilation in Control Subjects and Chronic Smokers*

Endothelium-dependent vasodilatation was assessed in 8 chronic smokers and 8 controls by infusing acetylcholine (ACh) in increasing concentrations of 0.75, 1.5, and 3.0  $\mu\text{g}/100\text{ mL}$  forearm tissue/min. In another group of 5 smokers and 5 control subjects, serotonin (5-HT) was used as an endothelium-dependent vasodilator in increasing doses of 0.7, 2.1, and 6.3  $\text{ng}/100\text{ mL}$  forearm tissue/min. These particular 5-HT concentrations have been recently shown to cause increases in FBF that are in contrast to ACh completely inhibited by NOS III inhibitor *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA), reflecting solely NO-mediated vasodilation.<sup>18</sup> Sodium nitroprusside (SNP) was infused to assess endothelium-independent vasodilation (0.1, 0.3, and 1.0  $\mu\text{g}/100\text{ mL}$  forearm tissue/min). During concurrent administration of BH4 (500  $\mu\text{g}/\text{min}$ ), responses to ACh, 5-HT, and SNP were established again. BH4 infusion was started 5 minutes before infusing ACh, 5-HT, or SNP. This dose of BH4 was chosen to reach plasma concentrations that have been shown to achieve maximal NO production by NOS III in vitro<sup>19</sup> and

### Clinical Characteristics of Study Subjects

	Healthy Subjects (n=13)	Chronic Smokers (n=45)
Age, y	52 $\pm$ 2	53 $\pm$ 1
Sex (male)	13	45
Pack-years	0	42 $\pm$ 2*
Mean blood pressure, mm Hg	90 $\pm$ 2	88 $\pm$ 1
Body mass index, $\text{kg}/\text{m}^2$	23 $\pm$ 1	22 $\pm$ 1
Total cholesterol, $\text{mg}/\text{dL}$	190 $\pm$ 9	193 $\pm$ 4
LDL cholesterol, $\text{mg}/\text{dL}$	128 $\pm$ 8	127 $\pm$ 5
HDL cholesterol, $\text{mg}/\text{dL}$	42 $\pm$ 4	41 $\pm$ 3
Triglycerides, $\text{mg}/\text{dL}$	130 $\pm$ 12	135 $\pm$ 8
FBF, $\text{mL}/100\text{ mL tissue}/\text{min}$	3.1 $\pm$ 0.3	3.0 $\pm$ 0.2

Data are presented as mean $\pm$ SEM. \*Apart from pack-years, no comparisons were significantly different.

to improve endothelium-dependent vasodilation in subjects with hypercholesterolemia.<sup>10</sup> Basal NO activity was estimated in all subjects by assessment of vasoconstrictor response to cumulative dose infusion of the NOS III inhibitor L-NMMA at 2, 4, and 8  $\mu\text{mol}/\text{min}$  each dose for 7 minutes. After a resting period of at least 60 minutes (to reestablish baseline FBF), BH4 was infused intra-arterially for a 10-minute period. Subsequently, the cumulative dose response to L-NMMA was repeated during coinfusion of BH4.

#### *Protocol 2: Effects of BH4 in the Presence of Vitamin C on Endothelial Dysfunction in Chronic Smokers*

Vitamin C is a strong antioxidant capable of scavenging free radicals. In a previous study, we have demonstrated that short-term administration of vitamin C could improve endothelium-dependent vasodilation in chronic smokers.<sup>7</sup> In addition, more recent studies demonstrate that ascorbic acid improves endothelial dysfunction in a BH4-dependent manner.<sup>17</sup> We therefore tested whether pretreatment with vitamin C is able to modulate BH4 effects on endothelial function. In 17 chronic smokers, ACh was tested during coinfusion with saline or BH4 (500  $\mu\text{g}/\text{min}$ ), respectively. After cessation of BH4 infusion (for 30 minutes), the dose-response curve to ACh was repeated during coinfusion with vitamin C alone (6  $\text{mg}/\text{min}$ , n=10 or 18  $\text{mg}/\text{min}$ , n=7) followed by a combination of BH4 and vitamin C.

#### *Protocol 3: Effects of NH4 and BH4 on Endothelial Dysfunction in Chronic Smokers*

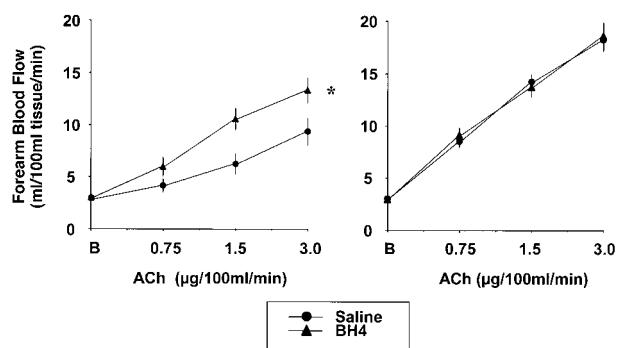
Reduced pteridines such as BH4 have been shown to be potent antioxidants and to scavenge in vitro oxygen-derived free radicals.<sup>20</sup> To test whether BH4-induced improvements on FBF in chronic smokers are due to its specific effects on the uncoupled NOS III or secondary to its antioxidant properties, we tested in an additional group of 15 smokers equimolar concentrations (50  $\mu\text{mol}/\text{L}$ ) of BH4 and NH4 on the ACh dose-response relationship on 2 separate days.

### In Vitro Experiments

The antioxidative effects of BH4 and NH4 (1 to 100  $\mu\text{mol}/\text{L}$ , respectively) were tested in vitro using lucigenin-enhanced chemiluminescence as recently described.<sup>21</sup> Superoxide was generated using the xanthine/xanthine oxidase reaction.<sup>22</sup>

### Drugs

The following substances were infused: ACh (Farmigea S.p.A), 5-HT (Sigma Chemical Co), SNP (Schwarz Pharma), L-NMMA (Calbiochem), and vitamin C (Sanorell). All drugs were freshly diluted to the desired concentration by addition of normal saline. BH4 and NH4 (Schircks Laboratories) were prepared just before administration using oxygen-free saline.



**Figure 1.** Effect of BH4 on ACh-induced vasodilation in chronic smokers and healthy control subjects. BH4 significantly improved ACh-mediated vasodilation in chronic smokers (left) but failed to change the ACh dose-response relationship in control subjects (right). Data are presented as mean±SEM. \*Significant differences in the overall dose response versus saline treatment.

### Statistical Analysis

All values are reported as mean±SEM. Group comparisons with respect to baseline characteristics were performed by unpaired *t* test. To test for differences in the overall dose-response relationship in response to ACh, 5-HT, SNP, and L-NMMA with and without vitamin C, BH4, and NH4, a two-way ANOVA for repeated measures was applied. A value of  $P < 0.05$  was considered statistically significant.

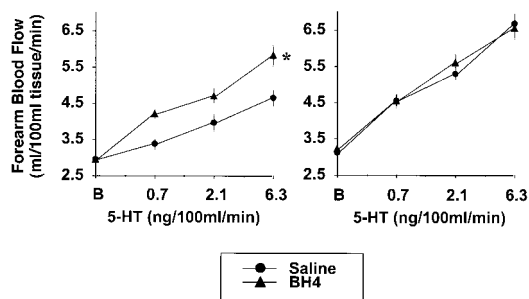
## Results

### Subjects

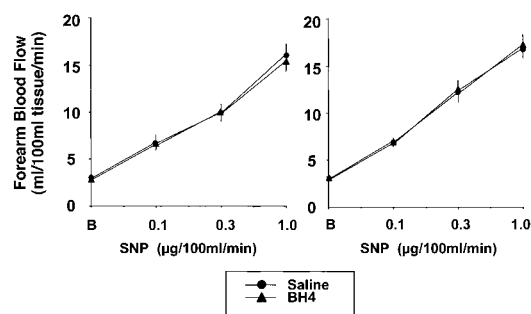
The Table summarizes the characteristics of the study population. Control subjects and smokers were matched for age.

### Effect of BH4 on Endothelium-Dependent and Endothelium-Independent Vasodilation

ACh responses were blunted in chronic smokers ( $n=8$ ) compared with control subjects ( $n=8$ ,  $P < 0.05$ ). BH4 alone did not change basal FBF in either smokers ( $2.9 \pm 0.2$  versus  $3.0 \pm 0.3$  mL/100 mL/min) or control subjects ( $3.1 \pm 0.3$  versus  $3.0 \pm 0.3$  mL/100 mL/min). There was no effect of BH4 on mean arterial pressure in either group. In smokers, concomitant infusion of BH4 increased ACh responses whereas in control subjects the FBF response to ACh was not



**Figure 2.** Effect of BH4 on 5-HT-induced vasodilation in chronic smokers and healthy control subjects. BH4 significantly improved 5-HT-induced vasodilation in chronic smokers (left) but failed to change the ACh dose-response relationship in control subjects (right). Data are presented as mean±SEM. \*Significant differences in the overall dose response versus saline treatment.



**Figure 3.** Effects of BH4 on SNP-induced vasodilation in chronic smokers and healthy control subjects. BH4 did not modify the SNP dose-response relationship in either chronic smokers (left) or control subjects (right). Data are presented as mean±SEM.

modified (Figure 1). Similar results were obtained with 5-HT as the endothelium-dependent vasodilator (Figure 2).

The FBF response to SNP was not different between control subjects and chronic smokers and was not modified by BH4 (Figure 3).

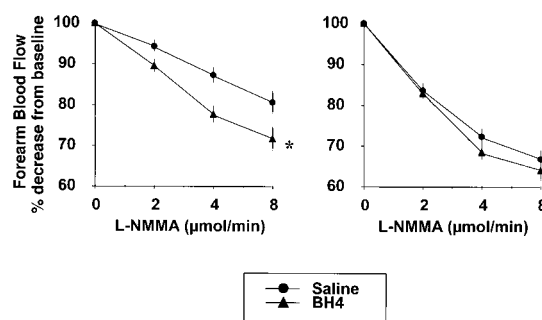
### Effect of BH4 on L-NMMA-Induced Vasoconstriction

Inhibition of NO synthesis by L-NMMA (2, 4, and 8 µmol/min) induced a dose-dependent reduction in FBF in both groups ( $P < 0.001$ ). In smokers ( $n=13$ ), however, the L-NMMA-induced reductions in FBF were significantly less compared with control subjects ( $n=13$ ,  $P < 0.05$ ) (Figure 4). Concomitant infusion of BH4 significantly increased the vasoconstrictor response to L-NMMA in chronic smokers but did not modify L-NMMA responses in control subjects.

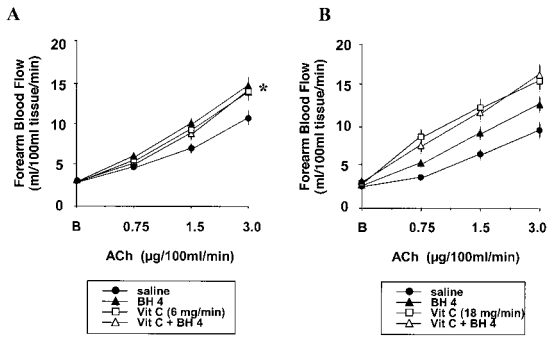
### Effect of BH4 on Endothelium-Dependent Vasodilation in the Presence of Vitamin C

In smokers ( $n=17$ ), BH4 coinfusion significantly augmented ACh-induced vasodilation (Figures 5A and 5B). Similarly, coinfusion of low and high concentrations of vitamin C (6 and 18 mg/min,  $n=10$  and  $n=7$ , respectively) augmented the ACh blood flow responses dose dependently.

BH4 effects on ACh responses were abolished by pretreatment with vitamin C.



**Figure 4.** Effects of BH4 on basal NO activity in chronic smokers and healthy control subjects. Pretreatment of chronic smokers (left) with BH4 significantly enhanced the L-NMMA-induced vasoconstriction while having no significant effect on the dose-response relationship in healthy control subjects (right). Data are presented as mean±SEM. \*Significant differences in the overall dose response versus saline treatment.



**Figure 5.** Effect of low- and high-dose vitamin C pretreatment (6 and 18 mg/min) on BH4-induced effects on the ACh dose-response relationship in chronic smokers. BH4 significantly improved ACh dose-response relationship as did low-dose vitamin C (A). After pretreatment with vitamin C, BH4 failed to augment further ACh-induced changes in FBF. Similar effects were observed with high concentrations of vitamin C (B). Data are presented as mean ± SEM. \*Significant differences in the overall dose-response relationship versus saline treatment. †Significant differences in the overall dose-response relationship versus BH4 treatment.

**Antioxidative Capacity of BH4 and NH4 In Vitro**

BH4 as well as NH4 dose dependently inhibited in vitro lucigenin-enhanced chemiluminescence generated by the xanthine/xanthine oxidase reaction. The inhibitory effect on superoxide production was not significantly different between BH4 and NH4 (Figure 6).

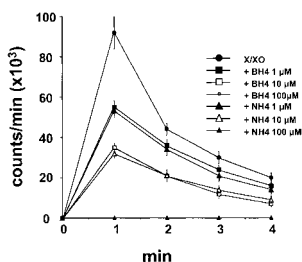
**Effects of BH4 and NH4 on Endothelium-Dependent Vasodilation**

In smokers (n=15, protocol 3), BH4 significantly augmented ACh-induced vasodilation whereas NH4 was ineffective (Figure 7).

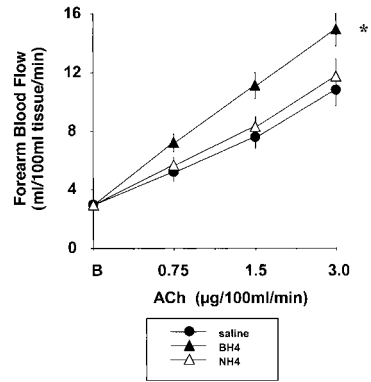
**Discussion**

The present studies demonstrate that in chronic smokers administration of the endothelial NOS III cofactor BH4 improves both basal and stimulated NO-mediated vasodilation. These data suggest that endothelial dysfunction in chronic smokers is at least in part secondary to an absolute or relative deficit in BH4 bioavailability.

With the present studies, we can show an impairment of basal and stimulated NO production in chronic smokers leading to endothelial dysfunction. Using the inhibitor of the NOS III L-NMMA, we found, in agreement with previous observations, a significantly attenuated vasoconstriction in



**Figure 6.** Effects of BH4 and NH4 on the lucigenin-enhanced chemiluminescence signal generated by the xanthine/xanthine oxidase reaction (counts/min). Data are presented as mean ± SEM from 6 to 8 experiments each.



**Figure 7.** Effect of BH4 and NH4 on the ACh dose-response relationship in chronic smokers (n=15). BH4 significantly improved ACh dose-response relationship whereas NH4 was ineffective. Data are presented as mean ± SEM. \*Significant differences in the overall dose-response relationship versus saline treatment.

smokers compared with control subjects.<sup>23</sup> We also studied the vasodilation during stimulation with the endothelium-dependent vasodilators ACh and 5-HT. 5-HT was chosen in addition to ACh because its action is in contrast to ACh completely inhibited by L-NMMA, therefore reflecting pure NO bioactivity.<sup>18</sup> ACh- and 5-HT-induced endothelium-dependent vasodilation were markedly reduced in smokers compared with age-matched control subjects while changes in FBF in response to the endothelium-independent vasodilator SNP were preserved.

The mechanisms underlying endothelial dysfunction in chronic smokers are multifactorial but likely may be a consequence of increased oxidative stress. Recent studies revealed increased excretion of the free radical-catalyzed products of arachidonic acid such as 8-epi PGF2α<sup>6</sup> in chronic smokers. Cessation of cigarette smoking as well as the use of antioxidants such as vitamin C, but not the use of cyclo-oxygenase (COX) inhibitors such as aspirin, reduced isoprostane excretion, suggesting a free radical-mediated and not a COX-mediated formation of 8-epi PGF2α.<sup>6</sup> Recently, we were able to show a close relation of autoantibody titers to oxidized LDL- and ACh-induced endothelium-dependent changes in FBF in chronic smokers with and without hypercholesterolemia, suggesting an involvement of oxidized LDL in mediating endothelial dysfunction in vivo.<sup>4</sup> Impaired endothelial-dependent vasorelaxations were markedly improved by infusing the antioxidant vitamin C, providing evidence that vitamin C improves endothelial dysfunction mainly by directly scavenging oxygen-derived free radicals. Cigarette smoke contains free radicals and pro-oxidants that may cause damage to the vascular wall via depleting antioxidants,<sup>24</sup> causing protein peroxidation<sup>25</sup> and activation of phagocyte-platelet-endothelial cell interactions.<sup>26,27</sup> It remains to be established, however, whether constituents of the cigarette smoke have direct toxic effects to the vasculature and/or whether these components may activate vascular superoxide-producing enzymes that may further increase the free radical burden to the vasculature in a positive feedback fashion.



Recently, it was demonstrated that BH4 improves endothelium-dependent vasodilation in vivo in patients with hypercholesterolemia.<sup>10</sup> BH4 is an important cofactor of the NOS III and appears to contribute to the ability of the enzyme to bind L-arginine. In the absence of BH4, there is an uncoupling of the enzyme, leading to inhibition of NO production and to NOS-mediated superoxide production.<sup>14,15</sup> Interestingly, in vitro studies revealed that BH4 improves endothelium-dependent vasorelaxation of vena saphena from chronic smokers in vitro.<sup>12</sup> We therefore postulated that BH4 may also improve endothelial dysfunction in chronic smokers in vivo.

Infusing BH4 in a concentration that has been previously shown to increase basal NO bioactivity in hypercholesterolemic patients<sup>10</sup> markedly improved the inhibitory effect of the NOS III inhibitor L-NMMA on basal FBF in chronic smokers while having no effect on L-NMMA responses on FBF in healthy control subjects. Similarly, BH4 improved ACh- and 5-HT-induced vasorelaxations in chronic smokers but did not modify endothelium-dependent vasodilation in age-matched control subjects. These data suggest that decreased basal and stimulated NO bioactivity in chronic smokers is at least in part secondary to reduced BH4 bioavailability.

The mechanisms by which BH4 is improving endothelial dysfunction remain unclear. As pointed out earlier, a recent study by Higman et al<sup>12</sup> demonstrated that smoking-induced impairment of NOS III activity in human veins could be reversed by exogenous application of BH4. The authors speculated that aromatic amines absorbed into the circulation from the combustion of tobacco are potent inhibitors of the enzymes involved in the biosynthesis of this important NOS III cofactor.<sup>12</sup> Cigarette smoke also contains free radicals such as  $\cdot\text{NO}$  and  $\text{O}_2^{\cdot-}$ , which may react with each other to form the strong pro-oxidant  $\text{ONOO}^-$ . Moreover, auto-oxidation of polyhydroxyaromatic compounds such as catechol and 1,4-hydroquinone present in cigarette tar (particulate phase) has been demonstrated to induce superoxide production in lung tissue that in turn could react with  $\cdot\text{NO}$  from the gas phase of cigarette smoke to form  $\text{ONOO}^-$ .<sup>28</sup>  $\text{ONOO}^-$  has been associated with increased oxidative reactions<sup>29</sup> and DNA damage,<sup>30</sup> and it may cause a reduction of plasma antioxidants as well. Nitration of tyrosine residues of proteins leads to the production of 3-nitrotyrosine that may be considered as a marker of  $\text{ONOO}^-$ -dependent oxidative damage.<sup>31</sup> Recent studies revealed increased plasma nitrotyrosine concentrations in chronic smokers compared with nonsmokers.<sup>32</sup> Interestingly, preliminary studies using a spectrophotometric assay indicate that  $\text{ONOO}^-$ , and not  $\text{O}_2^{\cdot-}$  or hydrogen peroxide, avidly oxidizes BH4 to dihydrobiopterin. This observation would imply that BH4 oxidation rather than intracellular BH4 depletion may induce NOS III dysfunction, which in turn may be a source of altered endothelium-dependent vasorelaxation in chronic smokers.<sup>16</sup>

To obtain mechanistic insight into the mechanisms by which BH4 improves endothelial function (depletion of BH4 levels versus functional depletion due to oxidation), we tested the effects of BH4 in chronic smokers in the presence and absence of vitamin C. Recent preliminary results have indicated that ascorbic acid is able to enhance endothelial NO

production in a BH4-dependent manner.<sup>17</sup> With the present studies, we found that pretreatment with vitamin C in low and high concentrations abolished the BH4 effects in chronic smokers, suggesting that functional depletion of BH4 due to enhanced BH4 oxidation rather than intracellular BH4 depletion accounts at least in part for endothelial dysfunction in chronic smokers. A critical question that must be addressed is whether BH4-induced improvements in endothelial dysfunction in chronic smokers are secondary to its effects on the uncoupled NOS III or whether they simply reflect antioxidant actions described for reduced pteridines.<sup>20</sup> Indeed, by using lucigenin-enhanced chemiluminescence, we found that BH4 as well as NH4 potentially scavenged superoxide generated in vitro by the xanthine/xanthine oxidase reaction in a dose-dependent fashion (see Figure 6).

To address this issue, we infused in a subgroup of 15 chronic smokers equimolar concentrations of BH4 and NH4 and tested the effects on the ACh-induced relationship. In contrast to the significant effects of BH4 on endothelial dysfunction, NH4 failed to modify ACh-induced changes in FBF in chronic smokers. These data suggest that BH4-induced improvements in endothelial dysfunction in chronic smokers reflect a specific effect on NOS III rather than being the consequence of a nonspecific antioxidant action.

The improvement of FBF by BH4 in smokers is smaller compared with the effects of the antioxidant vitamin C. One has to take into account, however, that the concentration of vitamin C given is on a molar basis  $\approx 100$ -fold higher compared with the BH4 concentration. In addition, the special purpose of our study was to determine to what extent endothelial dysfunction in chronic smokers is due to NOS cofactor deficiency and not to test the effects of BH4 when given in high concentrations where antioxidant properties may come into play.

Taken together, the present studies demonstrate that endothelial dysfunction in chronic smokers can be improved using the NOS III cofactor BH4. These data support the concept that in addition to the free radical burden of cigarette smoke, a dysfunctional NOS III due to BH4 depletion possibly as a result of BH4 oxidation may contribute at least in part to endothelial dysfunction in chronic smokers. It remains to be established whether oral treatment with the BH4 precursor sepiapterin is able to ameliorate endothelial dysfunction in chronic smokers.

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