Effects of L-Arginine on Atherogenesis and Endothelial Dysfunction due to Secondhand Smoke

Stuart J. Hutchison, Krishnankutty Sudhir, Richard E. Sievers, Bo-Qing Zhu, Yi-Ping Sun, Tony M. Chou, Kanu Chatterjee, Prakash C. Deedwania, John P. Cooke, Stanton A. Glantz, William W. Parmley

Abstract—Secondhand smoke (SHS) and hypercholesterolemia increase cardiovascular risk. We hypothesized that L-arginine, the precursor of nitric oxide (NO), might protect against atherogenesis and endothelial dysfunction caused by SHS. The effects of L-arginine supplementation (2.25% solution ad libitum) and SHS (smoking chambers for 10 weeks) were examined in 32 hypercholesterolemic rabbits. Eight normal rabbits served as controls. Acetylcholine- and nitroglycerin-induced vasorelaxation was assessed in aortic rings precontracted with norepinephrine. Hypercholesterolemia increased intimal lesion area (P=0.012), reduced endothelium-dependent relaxation (P=0.009), and reduced basal (P=0.005) and stimulated (P<0.0005) production of NOs. SHS increased intimal lesion area (P=0.01) norepinephrine-induced contraction (P=0.001) and reduced endothelium-dependent relaxation (P=0.02). SHS-induced increase in norepinephrine contraction was abolished by the inhibition of NO synthase and removal of endothelium. L-Arginine improved endothelium-dependent relaxation (P=0.001) and attenuated SHS-induced endothelial dysfunction (P=0.007) and atherogenesis (P=0.001). Basal production of nitrogen oxides correlated inversely with intimal lesion area (r=−0.66; P<0.0005) and stimulated production of NO correlated with endothelium-dependent relaxation (r=−0.66; P<0.001). SHS causes endothelial dysfunction and increased adrenergic responsiveness and atherogenesis in hypercholesterolemic rabbits. Chronic dietary supplementation with the NO precursor L-arginine mitigates these effects. The adverse vascular consequences of SHS appear to be mediated via deleterious effects on endothelial function.

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Key Words: nitric oxide ■ arginine ■ secondhand smoke ■ cholesterol ■ endothelium

Secondhand smoke (SHS), or passive smoking, constitutes a major and preventable public health risk. Epidemiological studies demonstrate an increase in coronary artery disease (CAD) events and mortality with exposure to SHS.1–7 Pooled analysis of the best-controlled studies of SHS suggests that SHS results in a relative risk of dying from heart disease as high as 1.7 (95% CI, 1.3 to 2.3).2,4 The estimated cardiac mortality in the United States from SHS exposure is 53 000 deaths per year,1–4 primarily from cardiovascular effects.

The arterial endothelial cell monolayer possesses important functions that preserve arterial function and structure. Many of these functions are mediated through the nitric oxide (NO) pathway. Endothelial NO is an important factor that contributes to resting artery tone8–10 and possesses antiplatelet11,12 and antiatherogenic properties that preserve normal arterial structure.13,14 Although the relationship of endothelial dysfunction to clinical vascular disease is not completely understood, endothelial dysfunction may exist as a pathological state preceding the development of overt atherosclerosis15 and may contribute to the pathogenesis of acute ischemic syndromes in the development of advanced atherosclerosis.16

Experimental animal studies have addressed the relationship between endothelial NO production and atherosclerosis and have examined the effects of interventions on atherogenesis. Diet-induced hypercholesterolemia in rabbits is associated with impaired endothelial NO production.17–19 Chronic dietary supplementation with L-arginine, the substrate of NO synthase and the precursor of NO, restores endothelium-mediated relaxation to normal in hyperlipidemic rabbits19 and reduces some indices of atherogenesis in the same model.14,18 Production of NO may impart an antiatherogenic effect; therefore, dietary interventions that enhance NO production may preserve arterial structure and function in the presence of 1 or more risk factors. We have previously shown in normcholesterolemia and nonatherogenic rabbits that SHS causes endothelial dysfunction that is prevented by chronic L-arginine ingestion.20 Whether L-arginine is protective against endothelial dysfunction once there is already atherogenesis is unknown.

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Several studies have sought to further define the interactions and characteristics of major risk factors for CAD. The cardiovascular risks of active tobacco smoking and elevated cholesterol appear additive.\textsuperscript{21} Similarly, active tobacco smoking and elevated cholesterol are additive risk factors for endothelial dysfunction.\textsuperscript{22} Active tobacco smokers exhibit abnormal vascular reactivity that may contribute to the pathogenesis of smoking-induced cardiovascular disease. Active smoking reduces basal NO release\textsuperscript{23} and impairs NO-mediated endothelium-dependent relaxation of the forearm\textsuperscript{24} and coronary\textsuperscript{25} vasculature. Active smoking also causes abnormal constrictor responses in the coronary vasculature.\textsuperscript{25,26} Few studies exist of the vascular effects of exposure to SHS. Noninvasive peripheral vascular studies demonstrate impaired endothelium-dependent relaxation in patients exposed to SHS.\textsuperscript{27} SHS induces atherogenesis in hypercholesterolemic rabbits\textsuperscript{28} and in cockerels.\textsuperscript{29} The adverse effects of tobacco smoke appear to be dose dependent.\textsuperscript{24} To date, no intervention has been demonstrated to prevent endothelial dysfunction or atherogenesis caused by SHS.\textsuperscript{30} No study has investigated the interactions of SHS and hypercholesterolemia on vascular reactivity.

This study sought to determine the effects of SHS exposure on endothelial function and atherogenesis in a rabbit model of experimental atherogenesis and to establish whether chronic dietary L-arginine supplementation protects against endothelial dysfunction and atherogenesis caused by SHS.

### Methods

#### Protocol

The study protocol was approved by the Committee for Animal Research of the University of California at San Francisco and was performed in accordance with the recommendations of the American Association for Accreditation of Laboratory Animal Care.

Before starting the high cholesterol diet, hematocrit, hemoglobin, and serum levels of total cholesterol, triglycerides, and HDL cholesterol were measured from blood drawn by ear venipuncture. To induce hypercholesterolemia, 40 male New Zealand White rabbits (Niatabelle Rabbits, Hayward, Calif) were fed a high-cholesterol diet (Ziegler Bros, Inc), 3% soybean oil and 0.3% cholesterol by weight, induce hypercholesterolemia, 40 male New Zealand White rabbits (Niatabelle Rabbits, Hayward, Calif) were fed a high-cholesterol diet (Ziegler Bros, Inc), 3% soybean oil and 0.3% cholesterol by weight, for 2 weeks. Serum cholesterol levels were measured after 2 weeks of cholesterol supplementation. The 8 rabbits with the lowest cholesterol levels were excluded from the study, and the 32 hypercholesterolemic (HC) rabbits with similar elevations in cholesterol were carefully freed of adventitia, opened longitudinally, and placed in SHS exposure chambers (BioClean, Duo Flo, model H 5500, Laboratory Products Inc) that measured 1.92×1.92×0.97 m (3.58 m\textsuperscript{3}) and accommodated 8 rabbits at a time.

#### Vascular Reactivity Studies

The rabbits were killed, and aortic ring segments were harvested and prepared for organ bath studies, as previously described.\textsuperscript{32} To measure the responsiveness to norepinephrine (NE) and to calculate the dose needed for precontraction, NE in increasing doses (10\textsuperscript{-9} to 10\textsuperscript{-4} mol/L) was added to each ring/bath. For each ring, the dose needed to achieve half-maximal contraction (ED\textsubscript{50}) was calculated.

After the NE contraction series, the baths were washed 3 times with fresh Krebs solution and the rings were allowed to stabilize for 1 hour. Vasodilatory responses to the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator nitroglycerine were determined, as previously described.\textsuperscript{20} To determine the responsiveness of aortic rings to NE in the absence of NO production, the rings were exposed a second time to NE (10\textsuperscript{-10} to 10\textsuperscript{-4} mol/L) in the presence of the specific and potent inhibitor of NO synthase N\textsuperscript{3}-nitro-L-arginine methyl ester 10\textsuperscript{-4} mol/L (L-NAME), which was added to the baths 15 minutes before NE. This dose of L-NAME abolishes NO-induced vasorelaxation.\textsuperscript{31} To determine the responsiveness of the aortic rings to NE in the absence of endothelium, in which most vascular NO synthase resides,\textsuperscript{2} a second aortic ring segment from each animal was mechanically deendothelialized by gentle abrasion with a wooden curette. The deendothelialized ring and the ring with endothelium were obtained from adjacent aortic segments and studied simultaneously. Functional deendothelialization was confirmed by demonstrating an absence of acetylcholine-induced relaxation. Deendothelialization was histologically confirmed in 2 rings per group at the end of the study. At the end of the organ bath experiment, the ring segments were preserved in 10\% formalin solution for intimal lesion area staining. Vascular reactivity experiments were performed by an investigator who was blinded to the rabbit treatment group.

#### Studies of NO Production

A chemiluminescence technique\textsuperscript{32} was used to obtain direct measurements of production of nitrogen oxides in 4 rings from each treatment group and from the normal group. Rings of aorta 1 cm long were carefully freed of adventitia, opened longitudinally, and placed with the endothelium facing up in 2-mL tissue culture wells to expose the media to the endothelium. Wells contained 2 mL of media composed of HBSS (Irvine Scientific Inc) supplemented with 2 mmol/L Ca\textsuperscript{2+}, 2 mmol/L Mg\textsuperscript{2+}, HEPES (20 mmol/L; Sigma), and L-arginine (100 \mu mol/L; Sigma). Culture dishes were placed on a rocking platform to ensure gentle mixing and incubated at 37°C. NO production was studied in a basal state with maximal stimulation by the calcium ionophore A23187 with the use of 2 adjacent aortic ring segments, which stimulates NO production by a non-receptor-dependent mechanism.\textsuperscript{33} This concentration was chosen because prior use of this agent had demonstrated maximal NO-mediated rabbit aortic relaxation at this dose. NO production in a basal state was determined by the removal of a 100-μL sample of the medium for subsequent measurement of nitrogen oxides and the replacement of the media with 2 mL of fresh HBSS solution at 30, 60, and 120 minutes.\textsuperscript{32} NO production in a stimulated state was determined by the use of another segment of aorta (an adjacent segment) that was incubated in HBSS media as above and contained A23187 (10 to 6 mol/L). Samples of media were also removed at 30, 60, and 120 minutes.

Nitrogen oxides (NO and 1 electron oxidation product of NO, NO\textsubscript{x}) were measured with a commercially available chemiluminescence apparatus (model 2108, Dasibi Environmental Corp) after reduction with boiling acidic vanadium (III) at 37°C.\textsuperscript{34,35} Boiling acidic vanadium quantitatively reduces NO\textsubscript{x} to NO, which is quantified by the chemiluminescence detector after reaction with ozone. Signals from the detector were analyzed by computer. Standard curves for NO\textsubscript{2} and NO\textsubscript{3} were linear over the range of 100 picomole to 5 mole. NO\textsubscript{x} production was expressed as the area under the curve of production of total nitrogen oxides from 0 to 120 minutes and was calculated with Kaleidagraph, version 3.05 (Abelbeck Software). The surface area of the segments of aorta studied for production of nitrogen oxides was...
measured so that production of nitrogen oxides could be standardized for surface area [NOx (pmol/L · 100 μL−1 · cm−2)].

**Drugs**

NE, acetylcholine, L-NAME, and the calcium ionophore A23187 were purchased from Sigma Chemical Co. Nitroglycerin was purchased from Solopak Laboratories Inc. Distilled water was used as the solvent for all agents other than A23187, which was dissolved in DMSO to create a stock solution of A23187 that was then sequentially diluted with water.

**Histological Studies**

Aortas were dissected free to 2 cm distal to the aortoiliac bifurcation. The vasculature was opened longitudinally, pinned to a corkboard with the endothelial side facing up, and placed in a normal saline bath. The aorta was fixed in 10% formalin, stained with the lipophilic stain Sudan IV, and photographed. The Sudan-stained lesions were planimetered by individuals blinded to the experimental treatment groups; we used the mean of 2 determinations.

**Monitoring SHS Exposure Inside the Chambers**

Carbon monoxide, total particulates, and air nicotine levels were measured as previously described.28

**Hematologic and Biochemical Analysis**

Total serum cholesterol, triglycerides, and HDLs were measured at the beginning and end of the 10-week SHS exposure (that is, after the 3rd and 13th weeks) (HDLS were measured at the beginning of the study before the rabbits started the high-cholesterol diet). Total serum cholesterol and triglyceride levels were determined by automated enzymatic methods (Coulter DART cholesterol reagent with the DACOS and DACOS XL analyzers, Coulter Corp), and HDL-C concentrations were measured after precipitation of other lipoprotein classes with dextran and magnesium ions (HDL-C precipitant, Cat No 236141, Ciba Corning Diagnostics Corp). The blood samples were drawn in the morning (Tuesday to Friday) after 12 hours of fasting and before SHS exposure.

**Statistical Analysis**

All results are expressed as mean±SEM unless otherwise indicated. Relaxation of aortic rings is expressed as percentage change of net developed tension ([Measured Tension−Baseline Tension]/Precontracted Tension−Baseline Tension), EC50, and slope (calculated by the Hill equation). A curve of best fit was calculated for each ring with the use of the equation for the Hill coefficient with Synergy Software version 3.0 that calculated the EC50 and slope. Response to NE was expressed as change in tension (from baseline, in grams) and was recorded and analyzed as above. All vasoreactivity data from 2 rings (1 in the SHS/HC group and 1 in the HC group) were discarded because they exhibited a response typical of deendothelialization; the relaxation to acetylcholine was absent and >2 SD different from the other rings in the group. One measurement of aortic intimal lesion area was deleted because it was >3 SD from the mean for that group (no SHS/HC/L-arginine group) and may have been caused by improper staining.

The effects of hypercholesterolemia, SHS, and L-arginine on vascular reactivity and intimal lesion area were evaluated with a general linear model ANOVA (Minitab version 10.2, State College, GLM procedure), which included cholesterol (present or absent), SHS (present or absent), and L-arginine (present or absent) as main effects and the SHS×arginine interaction. Testing the significance of the interaction term specifically permitted us to test whether the effect of SHS exposure was modified by the presence of L-arginine beyond purely additive effects. (We were unable to test for interactions between hypercholesterolemia and SHS or L-arginine because we did not expose rabbits on a normal diet to SHS or L-arginine.) For animal weights, food ingestion, and cholesterol measurements, we did not collect data on normal controls, therefore the analysis was by 2-way ANOVA with SHS and L-arginine as the factors. For air particulate matter, carbon monoxide exposure and L-arginine ingestion measurements, data were collected on only the 2 SHS-exposed or 2 L-arginine supplemented groups; thus, a t test was used to compare the 2 groups. A P value of <0.05 was considered significant. Data in profile plots are cell (raw) means and SEM. The relationship of production of nitrogen oxides to intimal lesion area and to maximal acetylcholine-induced relaxation was analyzed with a Spearman rank correlation test with the use of the Primer of Biostatistics, version 3.01 (McGraw-Hill).

**Results**

**Animal Data**

The hypercholesterolemic diet increased animal body weight (P<0.0001). SHS and L-arginine did not influence body weight. The carbon monoxide and particulate matter exposure of the 2 SHS-exposed groups (SHS/HC and SHS/HC/Arg respectively) were similar. We have previously shown that serum nicotine and cotinine levels of different groups exposed to SHS are similar.28,30 The L-arginine–supplemented groups (HC/Arg and SHS/HC/Arg) consumed similar amounts of L-arginine. Neither SHS exposure nor L-arginine significantly affected food consumption. SHS did not affect total cholesterol, triglyceride, or HDL-C levels at the end of the study. Similarly, L-arginine did not affect total cholesterol, triglyceride, or HDL-C levels. No significant SHS×arginine interactions that affected cholesterol, HDL-C, and triglyceride measurements existed (Table 1). (We have previously published cholesterol, HDL-C, and triglyceride levels of normal male New Zealand White rabbits.30)
Surface Lipid Lesions

Hypercholesterolemia increased intimal lesions (P<0.001) and SHS further increased intimal lesions (P=0.01). L-Arginine attenuated the effect of SHS on atherogenesis (P=0.001) and tended to reduce atherogenesis (P=0.09).

Vascular Reactivity Studies Relaxation

Acetylcholine dose-response curves are plotted in Figure 1A. Hypercholesterolemia impaired maximal acetylcholine-induced relaxation (see Figure 1B). SHS further impaired maximal acetylcholine-induced relaxation (Figure 1B).

**TABLE 2. Aortic Ring Response to Nitroglycerin**

<table>
<thead>
<tr>
<th>Group</th>
<th>Maximal Change in Tension, %</th>
<th>EC_{50}, mol/L</th>
<th>Slope (Hill Coefficient)</th>
<th>Group Size, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>-109±17</td>
<td>1.4×10^{-7}±3.3×10^{-8}</td>
<td>1.05±0.14</td>
<td>7</td>
</tr>
<tr>
<td>HC/Arg</td>
<td>-129±21</td>
<td>1.18×10^{-7}±2.80×10^{-8}</td>
<td>0.97±0.13</td>
<td>7</td>
</tr>
<tr>
<td>SHS/HC</td>
<td>-100±8</td>
<td>1.16×10^{-7}±2.5×10^{-8}</td>
<td>1.00±0.16</td>
<td>7</td>
</tr>
<tr>
<td>SHS/HC/Arg</td>
<td>-105±7</td>
<td>1.57×10^{-7}±3.5×10^{-8}</td>
<td>0.99±0.13</td>
<td>7</td>
</tr>
<tr>
<td>Normals</td>
<td>-141±11</td>
<td>5.3×10^{-8}±2.8×10^{-9}</td>
<td>1.22±0.08</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are mean±SE.
production of nitrogen oxides in SHS-exposed animals or significantly increase A23187 stimulated nitrogen oxide production (Figure 2B).

Relation of NO Production to Intimal Lesion Area and Endothelium-Dependent Relaxation
Atherogenesis correlated inversely with basal production of nitrogen oxides (Spearman rank correlation, \(-0.66; P<0.0005\)). Maximal acetylcholine-induced relaxation correlated with stimulated production of nitrogen oxides (Spearman rank correlation, \(-0.66; P<0.0005\)).

Contractile Responses to NE
Mean dose-response curves to NE are plotted in Figure 3A. SHS increased NE-induced tension development. When rings were mechanically denuded of endothelium by abrasion, there was no longer an effect of SHS to increase maximal contraction (Figure 3B). Similarly, inhibiting NO synthesis with L-NAME blocked this effect (Figure 3B).

Discussion
The major findings of this study were as follows: (1) Hypercholesterolemia increased intimal lesion area, reduced endothelium-dependent relaxation, and reduced basal and stimulated production of nitrogen oxides. (2) SHS exposure further increased intimal lesion area and further reduced endothelium-dependent relaxation. (3) SHS exposure increased NE-induced contraction. The SHS effect of increasing NE-induced contraction was abolished by an inhibitor of NO synthesis and by removal of endothelium, which suggested a NO synthase- and endothelium-dependent mechanism. (4) L-Arginine improved endothelium-dependent relaxation and reduced the SHS-associated impairment of relaxation, reduced the effect of SHS to accelerate intimal lesion formation, and tended to reduce atherogenesis overall. (5) Intimal lesion formation correlated inversely with basal production of nitrogen oxide levels. These observations may offer insight into the mechanisms of SHS-induced vascular disease.

Hypercholesterolemia and L-Arginine
This study confirms previously published observations that hypercholesterolemia induces endothelial dysfunction and intimal lesion formation.\(^{28,30,37}\) This study demonstrates that hypercholesterolemia impairs both NO generation with the nonreceptor dependent agent calcium ionophore and
also acetylcholine-induced receptor-dependent endothelial NO-dependent relaxation. Thus, it is likely that hypercholesterolemia impairs NO-mediated relaxation by impairing NO synthase activity or by increasing free radical formation that degrades NO and not via effects on the endothelial cell muscarinic receptor or on muscarinic receptor-NO coupling. In our study, hypercholesterolemia reduced NO levels. The level of NO was inversely related to intimal lesion formation as has been previously described.

Our study did not demonstrate that dietary L-arginine reduces atherogenesis or restores endothelial function. Other studies have also failed to show that L-arginine improves endothelial function. In our study, hypercholesterolemia did not increase NE-induced contraction despite the expected attenuation of G\textsubscript{i} protein–mediated endothelium-dependent relaxation seen with hypercholesterolemia. A similar finding of an improvement in acetylcholine-induced vasorelaxation with L-arginine supplementation, without an effect on NE-induced contraction, has been previously reported. As the literature has consistently demonstrated that hypercholesterolemia adversely influences endothelium-dependent relaxation through G\textsubscript{i} protein–sensitive mechanisms in porcine vessels, it is possible that species differences underlie the different observed responses in rabbits (our study and that of Singer et al) and in pigs.

**SHS and L-Arginine**

The important observations of this study are that L-arginine blocked SHS-induced endothelial dysfunction and atherogenesis. Several aspects of SHS-induced endothelial dysfunction were identified: impaired NO-mediated relaxation, a tendency to impair NO generation, and increased adrenoreceptor-mediated constriction. SHS did not affect endothelium-independent relaxation. SHS appears to induce endothelial dysfunction (reflected by reduced NO synthesis) without affecting smooth muscle responsiveness. We previously demonstrated that L-arginine protected against endothelial dysfunction from SHS; however, this was seen in a nonatherosclerotic preparation. There have been concerns that the benefits of interventions to preserve endothelial function may be salutary and short-lived. In this study of the rabbit hypercholesterolemia-atherogenesis model, L-arginine conferred partial protection against endothelial dysfunction and atherogenesis over at least 8 weeks.

Vasomotor tone appears to be substantially compromised by SHS. The combination of reduced vasorelaxation and increased vasoconstriction may predispose tissue to ischemia. Such perturbation of vascular reactivity by SHS may contribute to the clinically observed excess of cardiovascular mortality seen with SHS exposure.

Abnormalities of the endothelial NO pathway and/or possibly abnormalities of smooth muscle adrenergic responsiveness appear to mediate the increased responsiveness to the adrenergic agonist NE that was seen in aortic rings of SHS-exposed hypercholesterolemic rabbits. Chemical inhibition of endothelial NO synthase with L-NAME and mechanical removal of endothelial NO synthase by denudation abolished SHS-increased contraction. These findings are consistent with the phenomenon originally described by Cocks and Angus in isolated ovine and canine coronary arteries that vasoconstrictor amines such as NE are more powerful in the absence of endothelium because they release an endothelium-derived vasodilator substance that partially offsets the vasoconstrictor effect. In the present study, the greatest impairment of acetylcholine-induced NO-mediated relaxation was seen in the SHS/hypercholesterolemic aorta, and basal and stimulated NO production was subnormal in this group.

**Clinical Correlates of This Study**

Until now, there have been little available experimental data concerning the effect of passive smoking on atherogenesis and vascular reactivity in humans and on the nature of the interaction of the risks of passive smoke exposure and of hypercholesterolemia. Our results confirm human studies that showed SHS-associated endothelial dysfunction and offer a potential mechanism for the observed increased risk of CAD in passive smokers.

**Limitations of This Study**

Only male rabbits were studied. Endothelium-dependent relaxation in this study was assessed only with acetylcholine. Thus, the observations in this study do not allow us to state whether the SHS-associated impairment of endothelium-dependent relaxation was dependent on or independent of muscarinic receptors.

A treatment group supplemented with the stereoisomer d-arginine would have demonstrated that L-arginine supplementation was responsible for improved endothelial function through increased production of NO. Therefore, we cannot say that the improvement in endothelial function is necessarily due to NO generation because it may be a nonspecific, non-NO effect.

Another possibility that may account for the discrepancy of SHS impairment in mediated endothelium-dependent relaxation in the presence of a nonsignificant SHS effect on nitrogen oxide production is that oxidant stress is increased by SHS and leads to lesser availability of NO, although total nitrogen oxide production is not impaired. With the use of the same SHS rabbit model, we have demonstrated that SHS increases superoxide anion production. Therefore, SHS may not reduce NO production but may reduce availability. The differences in maximal contraction to NE were seen at concentrations above physiological, and therefore their pathophysiological relevance is unclear.

In conclusion, SHS causes endothelial dysfunction and atherogenesis. SHS reduces NO-mediated relaxation and increases adrenoreceptor-mediated constriction, thus unfavorably influencing vasomotor tone. Such disturbances may explain SHS-associated adverse cardiovascular clinical events. Chronic dietary L-arginine supplementation mitigates the deleterious effect of SHS on endothelium-dependent relaxation and atherogenesis. Thus, it appears that at least some of the adverse effects of SHS on the cardiovascular system are mediated by the NO effects on arteries.

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