Chronic Inhibition of Nitric Oxide Production Accelerates Neointima Formation and Impairs Endothelial Function in Hypercholesterolemic Rabbits

Antonio J. Cayatte, James J. Palacino, Kathleen Horten, Richard A. Cohen

Abstract To determine if endogenous local levels of nitric oxide (NO) modulate atherogenesis, we studied the effect of inhibiting NO with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) on early neointima formation in cholesterol-fed rabbits. Male rabbits were fed for 5 weeks with a 0.5% cholesterol diet alone or treated in addition during the last 4 weeks with L-NAME (12 mg/kg per day SC) via osmotic minipump. Endothelial cell function was assessed in isolated aortic rings by vascular reactivity and levels of cyclic GMP. In L-NAME-treated rabbits there was inhibition of endothelium-dependent relaxation to acetylcholine and the calcium ionophore A23187 as well as impaired cyclic GMP accumulation in response to acetylcholine. Neointima formation in the ascending thoracic aorta was assessed by determining media and intima cross-sectional areas with computerized image analysis. Compared with rabbits that consumed the cholesterol diet alone, L-NAME-treated rabbits had significant increases in lesion area (0.29±0.04 versus 0.15±0.03 mm\textsuperscript{2}) and in lesion/media ratio (0.06±0.01 versus 0.03±0.01). Plasma levels of cholesterol and fluorescent lipid peroxide products were unchanged, suggesting no difference in cholesterol metabolism or oxidation. Because arterial blood pressure was not altered by L-NAME treatment, the increased atherogenesis could not be attributed to an increase in blood pressure. These results indicated that local inhibition of NO accelerates early neointima formation possibly because of modulating monocyte recruitment or foam cell lipid accumulation. (Arterioscler Thromb. 1994;14:753-759.)

Key Words • rabbits • nitric oxide • cholesterol • endothelium • atherogenesis

Nitric oxide (NO), a potent physiological vasodilator that accounts for the biological activities of endothelium-derived relaxing factor, is synthesized in endothelial cells from the terminal guanidino nitrogen of L-arginine by the constitutive calcium-calmodulin-NADPH-dependent enzyme NO synthase (NOS). In humans and animals with hypercholesterolemia-induced atherosclerosis, endothelium-dependent vasodilation is impaired, suggesting reduced NO synthesis or action. NO has antiproliferative actions on vascular cells, supporting the hypothesis that the endothelial cell dysfunction observed in hypercholesterolemia could contribute to the initiation and progression of the atherosclerotic neointima. When administered in the diet of cholesterol-fed rabbits, the NO precursor L-arginine limits development of aortic atherosclerosis and improves endothelial cell function.

We recently reported a technique for the chronic administration to rabbits of the NOS inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) and demonstrated its persistent systemic effects on endothelial cells and microcirculation. We used this L-NAME rabbit model in this study to determine the effect of chronic inhibition of NO synthesis on atherosclerosis development during the initial weeks of cholesterol feeding. The observed acceleration of neointimal formation suggests that local NO production regulates neointimal growth in hypercholesterolemia.

Methods

L-NAME Rabbit Model and Diets

Sixteen male New Zealand White rabbits (2.6±0.0 kg) were fed a diet of chow (Agway Prolab, Agway, Country Foods Division) supplemented with cholesterol (0.3% wt/wt) and peanut oil (4% vol/wt). Cholesterol was suspended in peanut oil and then mixed with normal rabbit chow. After 1 week of feeding, the rabbits were randomly assigned to control and L-NAME-treated groups. To study the effect of L-NAME treatment alone, 4 additional rabbits fed a standard diet were treated with L-NAME. Osmotic pumps (Alzet model 2ML4) were loaded with a sterile filtered solution of L-NAME (Sigma Chemical Co) that had been dissolved in deionized water and made up to a concentration based on the initial weight of each rabbit and the factory-determined mean pumping rate of 2.6±0.1 \( \mu \)L/h to deliver 12 mg/kg per day. The pumps were surgically implanted under sterile conditions in the scapular region. After the rabbits were anesthetized with xylazine (5 mg/kg IM) and ketamine (35 mg/kg IM), the area was shaved, and an incision (approximately 2 cm) was made laterally, exposing a subcutaneous pouch into which the pump was implanted with the flow moderator pointing away from the incision. The incised area was closed with a suture. After 4

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weeks the rabbits were anesthetized with pentobarbital sodium 50 mg/kg IV; after the administration of heparin 1000 IU IV, blood was drawn via the heart, and vessels were removed for study.

**Tissue Preparation**

Immediately after exsanguination the aorta was dissected from the arch to the iliac bifurcation and rinsed with cold phosphate-buffered saline, pH 7.4, to remove traces of adherent blood. The descending thoracic aorta, free of periaortic tissue, was used for the vascular reactivity studies. The ascending thoracic portion from the heart to the left subclavian blood was drawn via the heart, and vessels were removed for study. The descending thoracic portion from the heart to the left subclavian blood was drawn via the heart, and vessels were removed for study. The descending thoracic portion from the heart to the left subclavian blood was drawn via the heart, and vessels were removed for study.

**Morphometric Analysis**

After immersion fixation 5-μm sections were cut from three random sites at the proximal, mid, and distal ascending aortic arch. For morphometric analysis of neointimal size, measurements of aortic lesion area and media area were performed at random on two elastin-stained sections of each aorta by an examiner blinded to the study. An Olympus microscope equipped with an IBM personal computer, a digitizing pad, and OPTIMAS 3.1 image-analysis software (Bioscan, Inc) was used. Media area was determined with a x10 objective. For accuracy, lesion area was determined with a x40 objective, and areas in each field were added. After correcting for magnification, the lesion/media ratio was calculated, and a mean value for the two sections was calculated for each rabbit. For gross comparison of lesion areas in the two groups, x10 magnified images of each entire elastin-stained aortic section were reflected on a digitizing pad using a microprojector, and contours of the lumen and the internal elastic lamina were traced to provide an image of the intima only. These images are presented in photographs of superimposed traces of the intima of all sections analyzed. This image-analysis technique was sensitive enough to quantify normal intimal area, because tissue segments were dehydrated in a graded series of ethanol and paraffin embedded for histological examination of neointima formation, which was done on sections prepared with van Gieson’s elastic stain. One randomly chosen rabbit from each group was anesthetized, and the aorta was perfusion fixed at 80 mm Hg pressure for en face examination. The aorta was mounted on a black background to enable visualization of early plaque localization.

**Physiological Responses**

Two rings of proximal descending thoracic aorta from each rabbit were suspended in organ chambers on stainless steel stirrups and attached to a strain-gauge transducer (model FT03, Grass Instruments, Inc) coupled to a polygraph recorder (Grass model 7D) to measure circumferential isometric force. Rings were then bathed in 37°C physiological salt solution containing (in mmol/L) 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25.0 NaHCO₃, 5.5 dextrose, and 0.026 disodium EDTA. The solution was bubbled with a 95% O₂/5% CO₂ mixture, and aortic rings were stretched stepwise to an optimal basal tension of 6 g and given 90 minutes to equilibrate; the bathing solution was changed every 20 minutes during this time. Rings were contracted to 40% to 50% of maximal tone by using phenylephrine. One ring was exposed to acetylcholine in half-log increasing concentrations (10⁻⁴ to 10⁻⁸ mol/L) to achieve maximal relaxation, washed repeatedly with buffer, and allowed 40 minutes to reequilibrate. Rings that were previously exposed to acetylcholine were exposed to L-arginine (10⁻³ mol/L) followed by a second dose of phenylephrine to establish a contraction comparable to that of the first response; this was followed by a repeated exposure to acetylcholine in half-log increasing concentrations. The second ring was contracted again with phenylephrine and then exposed to calcium ionophore A23187 in half-log increasing concentrations (10⁻⁴ to 10⁻⁸ mol/L).

**Cyclic GMP Assay**

Levels of cyclic GMP (cGMP) were assayed by using a modified protocol. Briefly, four rings of aorta were incubated in test tubes containing physiological buffer warmed to 37°C and bubbled with 95% O₂/5% CO₂ for 90 minutes with buffer changes every 30 minutes. After the first 50 minutes of incubation, one pair of rings was treated with L-arginine (10⁻³ mol/L). After an additional 20 minutes, all rings were treated with phenylephrine (10⁻⁷ mol/L). Twenty minutes later one ring from each pair was frozen to assay for basal levels of cGMP. The second ring from each pair was treated with acetylcholine (10⁻⁶ mol/L) for 1 minute to determine the agonist-stimulated rise in cGMP mediated by endothelium-derived NO. The rings were then frozen in liquid nitrogen and stored at −80°C. At the time of assay, rings were homogenized in 1 mL 6% trichloroacetic acid at 4°C and centrifuged at 12,000g for 5 minutes. The supernatant was removed from each sample, placed in a test tube, and washed four times with 4 mL water-saturated ethyl ether. The liquid samples were then frozen at −80°C and lyophilized overnight. The lyophylate was resolubilized in 1 mL 0.05 mol/L sodium acetate buffer, and 50-μL aliquots of the sample were placed in a 12×75-mm test tube. Samples were acetylated and assayed by using a cGMP radioimmunoassay kit (BT-340, Biomedical Technologies Inc). Solids left from the initial homogenization step were digested in 1 mL 0.1N NaOH overnight at 60°C to extract protein, which was assayed by using a Pierce BCA kit.

**Lipid Peroxide Assay**

Fluorescent lipid peroxidation products were assayed in the plasma of all rabbits. Briefly, rabbit blood was collected at the time of death and centrifuged to divide the plasma. Plasma was preserved with a final concentration of 1 mg/mL EDTA and frozen at −80°C until assay, when 1 mL plasma was treated with 6 mL methanol and 1 mL chloroform and vortexed. The mixture was allowed to incubate for 15 minutes at 4°C. After incubation, 4 mL 0.9% saline was added, and the mixture was centrifuged at 200g for 20 minutes at 4°C. One milliliter of the organic layer was added to 0.1 mL methanol, and fluorescent products were measured on a Perkin-Elmer model 650-10S spectrophotometer at excitation 355 nm and emission 435 nm.

**Cholesterol Assay**

Plasma cholesterol levels were assayed spectrophotometrically by using a commercially available kit (Sigma Diagnostics 352-20, Sigma Chemical Co). Briefly, 5 μL plasma and 5 μL 0.9% saline were added to 1 mL cholesterol reagent and gently mixed, and the mixture was then incubated for 15 minutes at 37°C. Samples were analyzed 30 minutes after addition of the assay solution by reading in a Perkin-Elmer Coleman 44 spectrophotometer at 500 nm. Absolute cholesterol levels were calculated by comparison with lipid control-N (Sigma Diagnostics L-2008) using deionized water added to the reagent as a blank.

**Blood Pressure Measurement**

Systolic blood pressure was measured before and after 4 weeks of L-NAME administration by using the indirect tail cuff technique. Such measurements agree within 5 to 8 mm Hg of...
TABLE 1. Effects of 4-Week L-NAME Treatment on Cholesterol-Fed Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (n=8)</th>
<th>Cholesterol/L-NAME (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>3.3±0.1</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>100±2.4</td>
<td>107±3.1</td>
</tr>
<tr>
<td>Final</td>
<td>106±3.7</td>
<td>101±3.4</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>81±5.8</td>
<td>86±5.3</td>
</tr>
<tr>
<td>Final</td>
<td>76±4.5</td>
<td>75±4.6</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1292±197</td>
<td>1510±208</td>
</tr>
<tr>
<td>Lipid peroxides, arbitrary units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>18.1±3.7</td>
<td>15.5±1.8</td>
</tr>
</tbody>
</table>

L-NAME indicates N\textsuperscript{G}-nitro-L-arginine methyl ester. All values are mean±SEM. No significant differences between groups occurred.

recordings taken from the femoral artery. An IITC rabbit tail cuff was placed over the shaved tail; the systolic pressure was measured on a NARCO physiograph (NARCO Bio-Systems, Inc) equipped with an IITC-modified 59 preamplifier. Mean blood pressure was estimated as the cuff pressure where maximal tail arterial pulsations occurred. Measurements were made after the rabbit had rested for 30 minutes in a warmed protective box.

Statistical Analysis

All data are presented as mean±SEM; n represents the number of different rabbits in each group. Relaxations are expressed as a percentage of contractions to phenylephrine and plotted against the negative log molar concentrations of acetylcholine, A23187, or sodium nitroprusside. Data for cGMP are expressed as femtomoles of cGMP per microgram protein, body weight is expressed in kilograms, blood pressure in millimeters of mercury, plasma cholesterol in milligrams per deciliter, and lipid peroxides as arbitrary units of fluorescence.

The statistical significance of the results comparing intimal area measurements between the two groups was analyzed by unpaired Student's t test.

Results

Characteristics of Cholesterol-Fed and L-NAME-Treated Rabbits

From an initial weight averaging 2.6 kg, both groups of rabbits gained weight equally over the 5-week feeding period and had similar body weights at the time of death (Table 1). There was no significant change in systolic or mean blood pressure over the 5-week cholesterol-feeding period, nor was there significant change caused by L-NAME treatment. Plasma cholesterol levels were greatly elevated in both groups of rabbits; there was no significant difference with L-NAME treatment. Plasma lipid peroxide levels showed no significant difference with L-NAME treatment (Table 1).

TABLE 2. Physiological Responses to Endothelium-Dependent and Endothelium-Independent Vasodilators in Cholesterol-Fed and Cholesterol-Fed, L-NAME-Treated Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Cholesterol/L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Contraction, g</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>7</td>
<td>8.7±0.3</td>
</tr>
<tr>
<td>Acetylcholine After L-arginine</td>
<td>5</td>
<td>9.2±0.5</td>
</tr>
<tr>
<td>A23187</td>
<td>7</td>
<td>8.7±0.2</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>7</td>
<td>8.3±0.4</td>
</tr>
</tbody>
</table>

L-NAME indicates N\textsuperscript{G}-nitro-L-arginine methyl ester; NS, not significant. All relaxations of the proximal descending thoracic aortic rings were to a 1-μmol/L concentration of the agonists. The concentration of L-arginine was 1 mmol/L. Values are expressed as mean±SEM of the contractions caused by phenylephrine; relaxation is expressed as a percent of maximal relaxation achieved by addition of sodium nitroprusside (1 μmol/L). Probability values refer to comparisons between cholesterol-fed and cholesterol-fed, L-NAME-treated rabbits.
Endothelium-Dependent Relaxations and cGMP Levels

Without altering the contractions of aortic rings caused by phenylephrine, L-NAME treatment significantly inhibited relaxations caused by acetylcholine (10^{-8} to 3 \times 10^{-6} \text{ mol/L}; Fig 1 and Table 2). Relaxations caused by A23187 (10^{-8} to 3 \times 10^{-6} \text{ mol/L}) were also significantly inhibited in aortic rings from L-NAME-treated rabbits compared with untreated cholesterol-fed rabbits (Fig 1 and Table 2). Relaxations caused by sodium nitroprusside (10^{-9} to 3 \times 10^{-6} \text{ mol/L}) were not significantly different in the two groups (Fig 1). In vitro addition of arginine (10^{-3} \text{ mol/L}) significantly increased acetylcholine-induced relaxations only in L-NAME-treated rabbits (Table 2).

Basal aortic levels of cGMP were decreased 50% in L-NAME–treated cholesterol-fed rabbits, but this did not reach statistical significance (Fig 2A). However, after treatment with L-arginine, basal levels of cGMP were significantly less in the L-NAME–treated group (Fig 2B). Acetylcholine-stimulated cGMP levels were significantly reduced by the NOS inhibitor (Fig 2A). In vitro addition of L-arginine (10^{-3} \text{ mol/L}; Fig 2B) significantly increased acetylcholine-stimulated levels of cGMP in L-NAME-treated rabbits such that they were not significantly different from those found in untreated cholesterol-fed rabbit aorta in the presence or absence of L-arginine.

Morphological Studies

The aorta of one rabbit from each group was perfusion fixed (Fig 3). Macroscopically, surfaces of isolated aortas from L-NAME–treated rabbit aortas were smooth and had a normal appearance. The aorta of the rabbit from the cholesterol-fed group had small white patches (black arrows) corresponding to neointimal lesions that were particularly visible at the intercostal branches and the larger patches in the aortic arch and the descending thoracic aorta in the L-NAME–treated rabbit.

Fig 3. Photographs of perfusion-fixed aortas from (a) a cholesterol-fed rabbit or (b) a cholesterol-fed and N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME)–treated rabbit. The aortic arch is at the lower right. The descending aorta begins at the upper left, and distal portions are arranged from left to right down to the abdominal aortic bifurcation. Note the small white patches (black arrows) corresponding to neointimal lesions that are particularly visible at the intercostal branches and the larger patches in the aortic arch and the descending thoracic aorta in the L-NAME–treated rabbit.

Fig 4. Representative photomicrographs of elastin-stained cross sections of ascending thoracic aortas from (a) rabbits fed cholesterol for 5 weeks and (b) rabbits fed cholesterol and treated with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME). A disrupted internal elastic lamina is seen underlying early neointimal lesions, which are increased in L-NAME–treated rabbit aortas. Lesions of both groups have similar cellular composition and are mostly composed of a continuous subendothelial layer of foam cells (original magnification ×125; bars=80 \mu m).
thoracic aortas were free of large plaques in the two
groups. Small patches of white neointimal lesions could be
seen only in the aorta of the L-NAME-treated cholesterol-fed rabbits.

Microscopic intimal lesions appeared as slightly
raised focal areas typical of fatty streaks that separated
the endothelium from the internal elastic lamina. The
lesions were composed primarily of foam cells and were
of similar cellular composition in both groups (Fig 4).

Initial comparison of the superimposed contours of
the neonintima of one section from each rabbit in the two
groups suggested that L-NAME-treated rabbit aortas
had increased lesion cross-sectional area (Fig 5). Mor-
phometric analysis substantiated this initial comparison.
Calculated lesion areas (Fig 6) were significantly in-
creased in cross sections of L-NAME-treated rabbit aortas
(0.29±0.04 versus 0.15±0.03 mm², P<.02, n=6
and n=7, respectively). Similarly, analysis of lesion/
media area ratio revealed a significant increase in
L-NAME-treated rabbit aortas (0.06±0.01 versus
0.03±0.01 mm², P<.05). The cross-sectional area of the
aortic media in both groups was similar (5.6±0.5 versus
L-NAME, 4.7±0.4 mm²). No visible aortic intimal thick-
ening or cell infiltration occurred in normocholesterol-
emic, L-NAME-treated rabbits. The intima that ap-
peared to be normal measured 0.04±0.01 mm (n=4).

Discussion

In the present study chronic administration of
L-NAME significantly increased neointimal size and
impaired endothelial function. As the rabbits’ weight
and plasma cholesterol levels were unchanged by ad-
ministration of L-NAME, it is unlikely that the effect
of the NOS inhibitor on atherogenesis resulted from any
nutritional effect.15 Moreover, the L-NAME dose was
chosen because it does not increase systolic blood
pressure in rabbits, whereas higher doses of NOS
inhibitors given to rats acutely16 or chronically17 can
increase systolic blood pressure. In this study mean
blood pressure was also estimated and was not altered
by the dose of L-NAME used. Thus, the known ability of
30- to 40-mm Hg increases in blood pressure to accele-
rate atherosclerosis11 does not explain the greater
neointimal formation observed in L-NAME-treated rab-
bits. In addition, because administration of L-NAME had
no independent inflammatory or atherogenic effect in
rabbits fed a standard diet, it is likely that L-NAME
accelerates mechanisms associated with hypercholesterol-
emia-induced atherosclerosis.

Active local concentrations of L-NAME delivered systemically by the technique used are demonstrable in
normal rabbits10 and in hypercholesterolemic rabbits in the
present study by showing that the endothelium-
dependent relaxations of isolated aorta caused by ace-
tylcholine and A23187 are inhibited. Normalcy of relax-
ations to sodium nitroprusside, a source of exogenous
NO, suggests that aortic tissue responsiveness to NO is
unaffected by L-NAME treatment and supports the fact
that L-NAME inhibits the synthesis of endogenous NO.
NO mediates endothelium-dependent relaxations in
blood vessels by increasing intracellular levels of cGMP
in smooth muscle cells upon stimulation of soluble
guanylate cyclase.2 cGMP levels in aortas from
L-NAME–treated rabbits were decreased, indicating
the effective inhibition of NO synthesis by endothelial
cells in response to acetylcholine. Notably, acetylcho-
line relaxations persisted in the aorta of L-NAME-
treated rabbits in the absence of a rise in cGMP. This
has been explained by a cGMP-independent response to
acetylcholine mediated by an endothelium-derived hy-
perpolarizing factor.18 The local effect of systemically
administered L-NAME on acetylcholine-induced relax-
ations and increases in cGMP was reversible by exoge-
nous L-arginine, further suggesting that the impaired
endothelial cell function caused by L-NAME was di-
rected specifically toward the L-arginine–NO pathway.
In vitro administration of L-arginine failed to affect
aortic relaxations in animals fed the cholesterol diet
alone. Five weeks of the 0.5% cholesterol diet used in
these studies caused no significant effect on endothel-
ium-dependent relaxation compared with normal rab-
bits.19 In contrast, the effect of chronic9 or acute4
administration of L-arginine to reverse aortic endothe-
lium dysfunction9 can possibly be explained because the

![Figure 5](http://atvb.ahajournals.org/)

**Figure 5.** Superimposed traces of the intima from cholesterol-fed (A; n=6) and cholesterol-fed and N\(^\text{5}\)-nitro-L-arginine methyl ester–treated (B; n=6) rabbits. Intimal lesion areas are depicted in black and were obtained from reflected microscopic images of aortic cross sections. Each group of superimposed contours represents individual traces of one elastin-stained section from each rabbit.
diet was higher in cholesterol and was maintained for a period of 10 weeks. Such diet significantly impairs endothelium-dependent relaxations, and aortas exhibit more extensive lesion involvement.9,10,18

Because L-NAME was shown to act locally on the L-arginine–NO pathway, the increase in atherogenesis caused by the drug may have accelerated any one of a number of cellular events thought to be responsible for the initiation and progression of atherosclerosis. Such cellular events affected by L-NAME could include the recruitment and adherence of blood monocytes, the oxidation of low-density lipoprotein (LDL), or the formation of macrophage foam cells.

Focal adherence of circulating blood monocytes is one of the earliest pathological events observed during atherogenesis in hypercholesterolemia.20 After adherence, monocytes migrate into the intima, and they are believed to undergo transformation to lipid-laden macrophages. Accumulating evidence suggests that NO influences endothelial cell–leukocyte interactions.22–23 Nitrovasodilators inhibit neutrophil adhesion,21 and L-NAME enhances leukocyte adhesion in the microcirculation in vivo.22 Exogenous NO inhibits monocyte chemotaxis and adhesion to cultured endothelium,23 suggesting that the decrease of NO in L-NAME–treated rabbits may have enhanced this initial step in atherogenesis.

The macrophage accumulates oxidized LDL via the scavenger receptor to become a lipid-laden foam cell, which is the hallmark of atherosclerosis.24 Oxygenderived free radicals are believed responsible for the oxidation of LDL because antioxidants such as probucol inhibit atherogenesis25 and improve endothelium-dependent relaxations.19 The plasma levels of lipid peroxides, which are elevated in hypercholesterolemic rabbits compared with their normcholesterolemic littermates,19 were unchanged by L-NAME, suggesting that L-NAME has no effect on lipid peroxidation. Nevertheless, blood levels of lipid peroxides may not accurately reflect tissue lipids, and aortic LDL peroxidation could be increased with L-NAME treatment. Moreover, because superoxide anion radicals, which can be implicated in local LDL oxidation, can be quenched by NO in the rabbit aorta,26 it is possible that free radical–mediated oxidation of LDL could be enhanced in the aorta of L-NAME–treated rabbits. The inhibitory effect of L-NAME on a possible protective role of NO in LDL oxidation could indirectly influence monocyte recruitment and adherence to the endothelium.27

Oxidized LDL and cytokines such as interleukin-1 and tumor necrosis factor, which are produced by activated monocytes, are important chemotactic factors for monocytes and induce the expression of vascular cell adhesion molecule-1 (VCAM-1), which selectively mediates adhesion of monocytes to endothelium.28 The expression of VCAM-1 on aortic endothelium is a very early event in atherogenesis, occurring after only 1 week of cholesterol feeding.29 The intracellular signals that activate endothelial cell adhesion molecule expression are not yet fully understood, but the transcriptional regulatory mechanisms for VCAM-1 gene expression may be redox sensitive and inhibited by antioxidants.30 During atherogenesis, localized vascular inflammatory reactions are believed to occur (see Reference 31 for review), and cytokines such as interleukin-1 and tumor necrosis factor are potential sources of NO and superoxide generation. Because scavengers of superoxide have been shown to increase NO half-life in vitro, NO perhaps represents an important regulatory molecule in the redox-sensitive mechanisms that activate the expression of endothelial cell adhesion proteins in response to cytokine stimulation. Thus, by affecting the production of NO, L-NAME could indirectly affect the action of these important mediators in the early stages of atherosclerosis. Indeed, another NOS inhibitor, Nω-monomethyl-L-arginine, increases adhesion of monocytes to cytokine-activated, cultured endothelium,33 further suggesting that NO may regulate monocyte adhesion when the endothelium is activated, such as in atherosclerosis. The effect of L-NAME may be due to inhibition of the activity of a Ca2+-independent NOS, which can be induced in atherosclerotic rabbit aorta as a result of cytokine activation.34,35

This study focused on the very early stages of atherosclerosis development to demonstrate an enhancement by L-NAME treatment. The fact that endothelium-dependent relaxations were normal in both the cholesterol-fed rabbits and the L-NAME–treated animals after arginine indicates that the impaired endothelial function was due to impaired NO synthesis rather than to the increased thickness of the aortic intima. This suggests that the abnormal endothelial function caused by L-NAME was key to the acceleration of atherosclerosis. This study thus supports the hypothesis that endothelial NO function is an important determinant of the progression of atherosclerosis.

Acknowledgments

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References


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