L-Arginine Augments Endothelium-Dependent Vasodilation in Cholesterol-Fed Rabbits

Xavier J. Girerd, Alan T. Hirsch, John P. Cooke, Victor J. Dzau, and Mark A. Creager

Evidence exists that an endothelium-derived relaxing factor is nitric oxide and that L-arginine is the precursor for the synthesis of nitric oxide in vitro. Whether exogenous L-arginine contributes to the modulation of vascular smooth muscle tone in vivo is still controversial. In hypercholesterolemia, resistance vessels do not relax normally in response to pharmacological stimuli that release endothelium-derived relaxing factor; bioassay experiments have suggested that impaired synthesis or release of endothelium-derived relaxing factor accounts, in part, for this blunted relaxation. We hypothesized that hypercholesterolemia reduces arginine metabolism and thereby impairs endothelium-derived relaxing factor synthesis. Accordingly, we designed a study to determine whether exogenous L-arginine could augment endothelium-dependent vasodilation of hind limb resistance vessels in anesthetized cholesterol-fed rabbits. Femoral blood flow was recorded with an electromagnetic flow probe in 16 cholesterol-fed and 12 control rabbits. The hind limb vasodilator responses to incremental intra-arterial infusions of acetylcholine (0.3–9.0 μg/kg/min) and nitroprusside (0.3–9.0 μg/kg/min) were studied before and during intravenous administration of L-arginine (10 mg/kg/min), D-arginine (10 mg/kg/min), or saline. The vasodilator response to acetylcholine was impaired in cholesterol-fed rabbits as compared with control rabbits. L-Arginine augmented vasodilation to acetylcholine in cholesterol-fed but not in control rabbits. L-Arginine did not alter the effect of nitroprusside in either group. Neither saline nor D-arginine changed the response to either acetylcholine or nitroprusside. Our data demonstrate that exogenous L-arginine normalizes the endothelium-dependent vasodilation of hind limb resistance vessels in cholesterol-fed rabbits. (Circulation Research 1990;67:1301–1308)

Considerable evidence is available that characterizes endothelium-derived relaxing factor(s) (EDRF) as nitric oxide or a labile nitroso compound that spontaneously liberates nitric oxide.1–4 Nitric oxide, the active moiety generated from several nitrovasodilators, is derived from the terminal guanidino nitrogen of L-arginine.2 Moreover, L-arginine is the precursor for the synthesis of nitric oxide and EDRF from cultured endothelial cells in vitro.5–7 Whether exogenous L-arginine can contribute to the modulation of vascular smooth muscle tone in vivo is controversial. In normal vessels, there may be sufficient amounts of endogenous L-arginine to saturate the nitric oxide–forming enzyme; the addition of L-arginine does not enhance endothelium-dependent relaxation.6–12 Some pathological disorders, such as hypercholesterolemia, however, are characterized by abnormal endothelium-dependent vasodilation.4 In hypercholesterolemia, resistance vessels do not relax normally in response to pharmacological stimuli that release EDRF.13–15 Potential mechanisms to explain this phenomenon include impaired synthesis or release of EDRF, the presence of functional or mechanical barriers that limit transport of EDRF from the endothelium to the vascular smooth muscle, inability of the vascular smooth muscle to relax in response to EDRF, and competing vasoconstrictive stimuli. It is unlikely that a mechanical barrier explains this phenomenon, because morphological lesions do not develop in the endothelium of the microvasculature.15 Bioassay experiments, however, have suggested that impaired synthesis or release of EDRF might contribute to this blunted relaxation.16,17 We hypothesized that hypercholesterolemia reduces arginine metabolism in the endothelium. Ac-
correspondingly, we designed a study to determine whether exogenous L-arginine augments endothelium-dependent vasodilatation of hind limb resistance vessels in cholesterol-fed rabbits. Our data suggest that decreased synthesis of EDRF by the vascular endothelium in hypercholesterolemic animals can be reversed by providing L-arginine, its physiological precursor.

Materials and Methods

Animals

Twenty-eight New Zealand White rabbits, weighing 2.1–2.7 kg, were studied. Animals were housed individually, and water was provided ad libitum. Rabbits were then separated into two treatment groups. Twelve were fed normal rabbit chow, and 16 were fed chow containing 2% cholesterol for 8 weeks (Farmers Exchange, Framingham, Mass.). These protocols were approved by the Harvard Standing Committee on Animals and were performed in accordance with the recommendation of the American Association for Accreditation of Laboratory Animal Care.

Experimental Protocol

On the day of the experiment, each rabbit was anesthetized with chlorpromazine hydrochloride (15 mg/kg i.m.) and sodium pentobarbital (8–12 mg/kg via the marginal ear vein). Supplemental anesthetic doses were not required, and spontaneous ventilation was maintained throughout the study. Heparin (300 IU/kg, intravenous bolus) was administered before insertion of catheters and was repeated hourly throughout the experiment. The rectal temperature was maintained at 37°C.

The right femoral vasculature was exposed for arterial and venous catheterization. A polyethylene catheter (PE90) was inserted into the femoral vein for both blood sampling and drug administration (heparin, arginine, or saline). A second catheter (PE90) was inserted into the femoral artery and advanced retrograde to a position proximal to the aortic bifurcation; this catheter was used for blood pressure determinations and vasoactive drug infusions. The aortic position of this catheter was confirmed at the end of each experiment by retroperitoneal dissection. After catheter placement, the left inguinal vasculature was exposed for hind limb blood flow determinations. A 2.0-mm electromagnetic flow probe was placed on the left femoral artery proximal to its bifurcation and coupled to a calibrated flowmeter (model SWF-4, Zepeda Instruments, Seattle). Thus, in this experimental model vasodilator drugs were administered to the hind limb vasculature with minimal surgical manipulation of the vessels under investigation.

The arterial catheter was attached to a pressure transducer (Statham P23D, Gould Electronics, Cleveland) and connected to a multichannel recorder (model 7, Grass Instruments Co., Quincy, Mass.). Phasic blood pressure (millimeters of mercury) was determined over 10 cardiac cycles. Mean blood pressure (millimeters of mercury) was calculated as (pulse pressure/3)+diastolic pressure. Heart rate (beats/minute) was calculated by the time-averaged pulse interval from the arterial pressure tracing. In this preparation, use of a single arterial catheter precluded graphic display of the blood pressure during drug infusion. Thus, blood pressure and heart rate were determined before and immediately after drug administration.

Phasic and mean femoral artery blood flow were recorded simultaneously on the multichannel recorder. The mean hind limb blood flow (milliliters/minute) was determined by an electronic filter. These recordings were analyzed by planimetry to determine average hind limb blood flow over the 2 minutes of drug infusion. End-diastolic flow was used as the zero reference value. Visual examination of the flow recordings ensured that all measurements were made in steady-state conditions. Hind limb vascular resistance was calculated as the ratio of mean blood pressure to mean hind limb blood flow and is expressed in arbitrary units.

Vascular Reactivity Studies

To assess endothelium-dependent vasodilation, acetylcholine chloride, a drug that stimulates the release of EDRF from the vascular endothelial cells, was administered intrarterially via a constant infusion pump (0.2 ml/min) at doses of 0.3, 0.9, 3.0, and 9.0 µg/kg/min, each for 2 minutes. Two minutes elapsed between each dose to reestablish basal hind limb blood flow values. To study the role of endothelium-independent vasodilation, sodium nitroprusside, a nitrovasodilator that acts directly on vascular smooth muscle cells, was administered intrarterially at doses of 0.3, 0.9, 3.0, and 9.0 µg/kg/min, each for 2 minutes. The order of administration of acetylcholine and nitroprusside was randomized according to a Latin-square design.

After acetylcholine and nitroprusside infusion, L-arginine (10 mg/kg/min; infusion rate, 0.2 ml/min) was administered intravenously to 16 animals (eight cholesterol-fed and eight control rabbits). This dose of L-arginine was determined to increase serum arginine levels from 0.1 to 3 mM when measured at 20 and 70 minutes of the L-arginine infusion. The effects of L-arginine alone on hind limb blood flow were evaluated after 20 minutes of the L-arginine infusion. L-Arginine infusion was then continued, and hind limb blood flow was again measured during its coadministration with acetylcholine and sodium nitroprusside.

Additional rabbits were studied in two additional protocols. First, a time-control experimental protocol was performed substituting normal saline for L-arginine in nine animals (five cholesterol-fed and four control rabbits). Second, because the D-enantiomer of arginine is not a substrate for nitric oxide synthesis in vitro, the protocol was repeated in three cholesterol-fed rabbits, substituting D-arginine (10 mg/kg/min) for L-arginine.
Assays

Administration of arginine is known to release insulin. Moreover, insulin may cause vasodilation. To exclude this confounding factor, venous blood samples were collected to measure insulin and arginine concentrations before and after 20 minutes of both L-arginine (n=4) and D-arginine (n=3) infusions. Serum insulin levels were determined by radioimmunoassay (Diagnostic Products Corp., Los Angeles). Serum arginine levels were determined with an amino acid analyzer (model 7300, Beckman Instruments, Inc., Palo Alto, Calif.).

Drugs

Acetylcholine chloride, sodium nitroprusside, L-arginine hydrochloride, and D-arginine hydrochloride were obtained from Sigma Chemical Co., St. Louis. Fresh stock solutions of all drugs were prepared immediately before each experiment. All drugs were diluted in normal saline.

Statistical Analysis

Data are presented as the mean±SEM. Statistical analysis included analysis of variance with multifactor repeated measures or analysis of variance of independent groups for repeated measures, followed by the Duncan post hoc multiple range test for statistical significance. In addition, regression lines developed from each dose–response relation were compared by analysis of covariance. The Wilcoxon rank sum test was used for nonparametric data. For nonserial measurements, Student’s t test was used. Statistical significance was accepted at the 95% confidence level (p<0.05).

Results

Characteristics of Control and Cholesterol-Fed Rabbits

Baseline mean arterial pressure, heart rate, hind limb blood flow, and hind limb vascular resistance did not differ between control and cholesterol-fed rabbits (Table 1). Body weights were also comparable in the two groups. The total serum cholesterol was increased 20-fold in the cholesterol-fed group compared with control animals.

Vasodilator Responses of Control and Cholesterol-Fed Rabbits

Acetylcholine infusion caused a dose-dependent and significant increase in hind limb blood flow and decrease in hind limb vascular resistance in control and cholesterol-fed rabbits. However, in cholesterol-fed rabbits, cholinergic vasodilation was attenuated compared with control rabbits (p=0.04 by analysis of covariance). The differences between the groups were significant with doses exceeding 3.0 μg/kg/min (Figure 1). In both groups of animals, mean arterial pressure was unchanged except at the highest dose of acetylcholine. Heart rate increased modestly but significantly during the highest dose of acetylcholine infusion.

Table 1. Baseline Characteristics of Control and Cholesterol-Fed Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Control diet (n=12)</th>
<th>2% Cholesterol diet (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>3.3±0.1</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>43±9</td>
<td>936±101*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>253±7</td>
<td>237±10</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>72.5±1.7</td>
<td>75.5±2.5</td>
</tr>
<tr>
<td>Hind limb blood flow</td>
<td>13.4±0.9</td>
<td>14.2±1.0</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind limb resistance</td>
<td>5.7±0.4</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td>(units)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SEM. *Significant difference between groups (p<0.001).

Effect of L-Arginine on Endothelium-Dependent Vasodilation

L-Arginine, when infused alone, had no effect on basal mean blood pressure, heart rate, hind limb vascular resistance, and hind limb blood flow in the control group (Figure 1). However, when the drug was given with a bolus of acetylcholine, a significant 20% decrease in hind limb vascular resistance was noted at each dose. This vasodilation was dependent on dose and was observed in both the control and cholesterol-fed groups. In contrast, when L-arginine was administered with acetylcholine and sodium nitroprusside, the nitroprusside caused a significant decrease in hind limb vascular resistance, but the arginine had no additive effect.
blood flow, or hind limb vascular resistance in either group. L-Arginine did not potentiate the vasodilator response to acetylcholine in the control rabbits (Figure 3). However, during the L-arginine infusion, the vasodilator response to acetylcholine was augmented in cholesterol-fed rabbits ($p=0.02$ by analysis of covariance). The decrease in hind limb vascular resistance was greater at the 0.3, 3.0, and 9.0 $\mu$g/kg/min doses (Figure 4). Thus, the vasodilator response to acetylcholine in cholesterol-fed rabbits during concomitant infusion of L-arginine was comparable to the vasodilator response to acetylcholine in control rabbits before L-arginine infusion (Table 4).

L-Arginine did not alter the vascular response to sodium nitroprusside in either control or cholesterol-fed rabbits (Figures 5 and 6).

**Effect of Normal Saline and D-Arginine Infusions**

Basal values for mean pressure, heart rate, hind limb blood flow, and vascular resistance were not significantly different in the groups that received normal saline, L-arginine, or D-arginine. Intravenous infusion of normal saline did not affect mean pressure, hind limb blood flow, vascular resistance, or heart rate responses to either acetylcholine or sodium nitroprusside in either control or cholesterol-fed rabbits.

Intravenous infusion of D-arginine alone had no effect on basal mean blood pressure, heart rate, hind limb blood flow, or hind limb vascular resistance. The hind limb blood flow and vascular resistance changes caused by acetylcholine and sodium nitroprusside were not significantly altered by D-arginine.

**Serum Insulin Response to Arginine Infusions**

During the intravenous infusion of L-arginine, serum insulin levels increased from 10±1 to 38±12 $\mu$U/ml ($p<0.05$). When D-arginine was administered, serum insulin levels increased from 14±2 to 30±6 $\mu$U/ml ($p<0.05$). The magnitude of increase in serum insulin concentrations was comparable during both L-arginine and D-arginine infusions.

**Discussion**

Results of this study can be summarized as follows: 1) in hypercholesterolemia, endothelium-dependent

### Table 2. Hemodynamic Changes Caused by Intra-arterial Administration of Acetylcholine and Nitroprusside in Control Rabbits (n=12)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>0.3</th>
<th>0.9</th>
<th>3.0</th>
<th>9.0</th>
</tr>
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<tbody>
<tr>
<td>Acetylcholine ($\mu$g/kg/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind limb blood flow (ml/min)</td>
<td>14±1.0</td>
<td>15.8±1.2</td>
<td>19.4±2.0</td>
<td>23.8±2.5*</td>
<td>28.4±3.0*</td>
</tr>
<tr>
<td>Hind limb resistance (units)</td>
<td>5.4±0.4</td>
<td>4.7±0.4</td>
<td>4.0±0.4*</td>
<td>3.4±0.4*</td>
<td>2.8±0.4*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>71±2</td>
<td>70±2</td>
<td>70±2</td>
<td>69±2</td>
<td>65±2*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>265±8</td>
<td>267±8</td>
<td>273±7*</td>
<td>278±7*</td>
<td>289±7*</td>
</tr>
<tr>
<td>Nitroprusside ($\mu$g/kg/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind limb blood flow (ml/min)</td>
<td>14.3±0.9</td>
<td>15.7±1.2</td>
<td>16.1±1.4*</td>
<td>16.5±1.6*</td>
<td>17.9±2.1*</td>
</tr>
<tr>
<td>Hind limb resistance (units)</td>
<td>5.2±0.3</td>
<td>4.9±0.3</td>
<td>4.6±0.3</td>
<td>4.3±0.4*</td>
<td>3.8±0.4*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>72±2</td>
<td>71±2</td>
<td>69±2*</td>
<td>64±2*</td>
<td>59±2*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>254±7</td>
<td>256±5</td>
<td>264±6</td>
<td>274±6*</td>
<td>285±8*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *p<0.01, baseline vs. drug. **p<0.05, baseline vs. drug.

### Table 3. Hemodynamic Changes Caused by Intra-arterial Administration of Acetylcholine and Nitroprusside in Cholesterol-Fed Rabbits (n=16)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>0.3</th>
<th>0.9</th>
<th>3.0</th>
<th>9.0</th>
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</thead>
<tbody>
<tr>
<td>Acetylcholine ($\mu$g/kg/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind limb blood flow (ml/min)</td>
<td>14.8±1.1</td>
<td>16.0±1.1</td>
<td>17.4±1.2</td>
<td>19.0±1.5*</td>
<td>22.7±1.7*</td>
</tr>
<tr>
<td>Hind limb resistance (units)</td>
<td>5.3±0.3</td>
<td>4.8±0.3</td>
<td>4.3±0.2*</td>
<td>4.0±0.3*</td>
<td>3.3±0.2*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>74±3</td>
<td>73±3</td>
<td>72±2</td>
<td>72±2</td>
<td>70±2*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>249±10</td>
<td>249±10</td>
<td>252±10</td>
<td>260±10†</td>
<td>265±9*</td>
</tr>
<tr>
<td>Nitroprusside ($\mu$g/kg/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind limb blood flow (ml/min)</td>
<td>14.9±1.0</td>
<td>16.0±1.0</td>
<td>16.1±1.1</td>
<td>16.9±1.4†</td>
<td>16.4±1.7†</td>
</tr>
<tr>
<td>Hind limb resistance (units)</td>
<td>5.3±0.3</td>
<td>5.0±0.3</td>
<td>4.8±0.3</td>
<td>4.2±0.3</td>
<td>3.9±0.2</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>74±2</td>
<td>74±2</td>
<td>72±2†</td>
<td>66±2*</td>
<td>60±2*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>245±9</td>
<td>247±9</td>
<td>257±9†</td>
<td>277±9*</td>
<td>287±9*</td>
</tr>
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</table>

Data are mean±SEM. Baseline conditions were reestablished before each dose of drug and did not differ significantly from each other.

*p<0.01, baseline vs. drug. **p<0.05, baseline vs. drug.
vasodilation may be normalized acutely by administration of L-arginine, the precursor of EDRF; 2) L-arginine does not potentiate endothelium-dependent vasodilation in normal rabbits; 3) L-arginine does not potentiate endothelium-independent vasodilation; and 4) D-arginine has no effect on endothelium-dependent vasodilation.

Effect of L-Arginine on Endothelium-Dependent Vasodilation

There is considerable evidence in the literature that EDRF is nitric oxide or a labile nitroso compound that liberates nitric oxide. The likelihood that L-arginine is a substrate for the formation of nitric oxide is supported by several lines of evidence. Porcine vascular endothelial cells, in culture, synthesize nitric oxide from the terminal guanidino nitrogen atom of L-arginine. Likewise, in the coronary circulation of the isolated rabbit heart, L-arginine enhances the release of nitric oxide induced by a submaximal dose of acetylcholine. This reaction is specific, because a number of analogues of L-arginine, including its D-enantiomer, are not substrates.

The exact mechanism whereby the terminal guanidino nitrogen atom of L-arginine is metabolized to nitric oxide is not known, but in the endothelium, a soluble enzyme requiring NADPH and a divalent cation is involved. In addition, one analogue, N\textsuperscript{G}-monomethyl L-arginine is a specific inhibitor of nitric oxide formation from L-arginine.

Whether L-arginine contributes to the modulation of vascular smooth muscle tone in vivo has been controversial. Reported studies are not in agreement. In studies involving either anesthetized rabbits or guinea pigs, intravenous administration of L-arginine caused no change in blood pressure, implying that there was no further vasodilation under basal conditions. Our results confirm these observations. On the other hand, in a perfused rat kidney model, L-arginine caused dose-dependent vasodilation; these responses occurred in the presence of endothelium and were abolished after endothelium denudation. Furthermore, administration of N\textsuperscript{G}-monomethyl L-arginine to anesthetized rabbits caused a dose-dependent increase in blood pressure, which was reversed by L-arginine. When infused in the brachial artery of normal volunteers, N\textsuperscript{G}-monomethyl L-arginine reduces forearm blood flow and attenuates the vasodilator effect of acetylcholine but not nitroglycerin.

L-Arginine and Hypercholesterolemia

In our study, L-arginine augmented the endothelium-dependent vasodilation caused by acetylcholine in cholesterol-fed rabbits. The finding that D-arginine had no effect on endothelium-dependent relaxation is consistent with our understanding that L-arginine serves as a specific substrate for the synthesis of nitric oxide in vivo. An effect of L-arginine on endothelium-independent vasodilation can be excluded because the response to sodium nitroprusside, a nitrovasodilator that relaxes vascular smooth muscle directly, was not potentiated. Likewise, these vasodilator responses to L-arginine are likely to represent a pharmacological effect, because stable baseline values were maintained throughout each experiment, and
the vasodilator effects of acetylcholine and sodium nitroprusside were comparable during the saline infusion protocol.

Impaired vasodilator responses of resistance vessels to drugs that stimulate release of EDRF have been observed in animals and humans with hypercholesterolemia.13–15 The present study confirms this observation. Potential mechanisms to explain this phenomenon include impaired synthesis or release of EDRF, the presence of functional or mechanical barriers that reduce the diffusion of EDRF, inability of the vascular smooth muscle to relax in response to EDRF, and competition of vasoconstrictive stimuli. It is unlikely that thickened intima acts as a mechanical barrier to limit transport of EDRF from endothelium to underlying smooth muscle. Histological studies have demonstrated a structurally intact endothelium, and intimal thickening in small vessels of cholesterol-fed rabbits has not been observed.15 Altered vascular smooth muscle responsiveness to EDRF can also be excluded because responses to vasodilators that act directly on vascular smooth muscle are not decreased.24–27 An impaired synthesis or release of EDRF is, however, a likely mechanism. Indeed, bioassay experiments designed for examining atherosclerotic porcine coronary arteries and aorta from cholesterol-fed rabbits have demonstrated reduced release of EDRF.16,17 Furthermore, lipoproteins inhibit endothelium-dependent vasorelaxation in vitro, perhaps by interacting with receptor-mediated release of EDRF.28,29

In this study, endothelium-dependent vasodilation was normalized by providing a large amount of l-arginine. Thus, our findings suggest that hypercholesterolemia may have interfered with the l-arginine to nitric oxide enzymatic pathway and caused an
alteration of the functional properties of the endothelial cells. This observation concurs with reports that lipoproteins may interfere with other synthetic functions of endothelial cells. In the presence of acetylated low density lipoproteins, the release of a hematopoietic growth factor from endothelial cells in culture is decreased. Thus, potentiation of endothelium-dependent relaxation by L-arginine may occur in two circumstances: first, if there is a cellular depletion of L-arginine; second, if the enzyme involved in the L-arginine–nitric oxide process is suppressed. Whether cholesterol causes an intracellular depletion of L-arginine or alters the L-arginine/nitric oxide enzyme(s) cannot be addressed by our study. However, our results demonstrate that in hypercholesterolemia, L-arginine can restore endothelial function acutely.

In this study, L-arginine did not potentiate endothelium-dependent vasodilation in the control group of rabbits. The finding that L-arginine is not effective in normal animals is in accordance with results previously reported regarding resistance vessels of normal rats and rabbits. It is also consistent with in vitro studies conducted on large vessels, which suggest that under normal conditions there is sufficient endogenous L-arginine to saturate the nitric oxide–forming enzyme. Thus, in normal conditions, the first step in the conversion of L-arginine to nitric oxide may not be rate limiting, and exogenous L-arginine would not be expected to further enhance relaxation.

**Limitations of This Study**

It is possible that L-arginine increased blood flow by a mechanism not related to nitric oxide synthesis. However, some potential mechanisms of action of L-arginine can be ruled out. L-Arginine administration is known to induce the release of insulin caused by a physicochemical effect on the pancreatic islet cells. This effect is not dependent on the metabolism of arginine and has been demonstrated to occur with nonmetabolized analogues of L-arginine. Increased insulin levels subsequent to the arginine infusion might potentially increase blood flow, because limb vasodilation has been shown to occur during intra-arterial insulin infusion in humans. In our study, however, serum insulin levels increased similarly during the infusions of d-arginine or L-arginine, yet only L-arginine potentiated the blood flow response. Thus, increased insulin release is unlikely to account for the potentiated vasodilator response to L-arginine in the cholesterol-fed rabbits.

A contribution of the vasodilator prostaglandins to the observed responses cannot be excluded. However, in resistance and conduit vessels, the cyclooxygenase inhibitor indomethacin does not change acetylcholine-induced vasodilation. Furthermore, in an experimental model similar to the one used in this study, indomethacin administration did not affect basal blood flow or blood pressure and did not alter hind limb vascular responses to acetylcholine. Thus, it is unlikely that release of prostaglandins from the vascular endothelium accounts for our findings.

In this study, drugs were infused intra-arterially to obviate systemic effects. Nonetheless, spillover into the systemic circulation must have occurred, because blood pressure decreased slightly at the higher doses of both the acetylcholine and sodium infusions. The concurrent increase in heart rate was likely a baroreflex response. Sodium nitroprusside caused less of a decrease in hind limb vascular resistance, yet a greater fall in blood pressure than acetylcholine. The greater effect of nitroprusside to increase venous capacitance may be responsible for this observation. None of these effects could account for the differences in endothelium-dependent vasodilation in hypercholesterolemic and control rabbits nor the improvement in endothelium-dependent vasodilation during administration of L-arginine.

**Conclusion**

In this study, we have demonstrated that L-arginine potentiates endothelium-dependent relaxation in the hind limb of cholesterol-fed rabbits. Thus, these data suggest that synthesis of EDRF can be increased by L-arginine, the physiological precursor of nitric oxide synthesis, in hypercholesterolemia.

**Acknowledgment**

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**References**


KEY WORDS • l-arginine • endothelium • cholesterol • endothelium-derived relaxing factor • resistance vessels
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