Chronic N⁶-Nitro-L-Arginine Methyl Ester Treatment Does Not Prevent Flow-Induced Remodeling in Mesenteric Feed Arteries and Arcading Arterioles

Debbie L. Ceiler, Jo G.R. De Mey

Abstract—Although endothelium-derived NO is an important mediator in acute flow-induced changes in arterial tone, the role of NO in chronic flow-induced changes in the resistance artery and arteriolar structure remains largely unresolved. We investigated the effects of chronic inhibition of NO synthase on arterial and arteriolar remodeling in a rat mesenteric model in which flow changes were induced. Alternating first-order mesenteric arteries were ligated to shunt blood flow through the intermittent patent arteries. Animals received no treatment (NT) or a continuous infusion of N⁶-nitro-L-arginine methyl ester (L-NAME, 25 mg/kg SC per day). After 2 weeks, local in vivo blood flow and in vitro arterial pressure-diameter relationships were assessed, as were the in situ diameters of arcading arterioles. Medial cross-sectional areas (CSAs) were measured histologically. In both groups of animals, blood flow was significantly increased in patent arteries and decreased in ligated arteries compared with control vessels. Nonetheless, in L-NAME–treated rats, patent artery flow was increased to a lesser extent, although control flow was not significantly reduced (0.18 ± 0.05 versus 0.26 ± 0.05 mL/min). In NT rats, the diameter of patent arteries was significantly larger and the diameter of ligated arteries was significantly smaller than that of control arteries. CSAs displayed the same pattern of change (11.9 ± 0.6 × 10³, 6.1 ± 0.7 × 10³, and 8.2 ± 1.0 × 10³ μm² for patent, ligated, and control arteries, respectively). Arterioles in the NT collateral pathway (218 ± 15 μm) had diameters similar to control arteriole diameters (201 ± 15 μm) but had a significantly larger CSA (6.2 ± 0.6 × 10³ versus 4.2 ± 0.4 × 10³ μm²). In L-NAME–treated rats, the flow-induced changes of the diameter and CSA in patent arteries, ligated arteries, and arcading arterioles mimicked those in NT rats. Nonetheless, control feed arteries (430 ± 21 versus 497 ± 16 μm) and arcading arterioles (156 ± 21 μm) were significantly narrower after L-NAME treatment. Thus, chronic blockade of NO oxide synthase (1) tended to reduce arterial blood flow and resulted in inward remodeling of mesenteric arteries and arterioles and (2) did not prevent arterial and arteriolar remodeling in response to imposed changes in blood flow. Endothelium-derived mediators other than NO can play a major role in flow-induced arterial remodeling. (Arterioscler Thromb Vasc Biol. 2000;20:2057-2063.)

Key Words: arterial remodeling • nitric oxide synthase • resistance arteries • arcading arterioles • collateral arteries

Acute alterations in blood flow, and thus shear stress, have been repeatedly demonstrated to result in changes in vasomotor tone, which modify arterial diameter to normalize shear stress.1,2 These vasomotor changes on acute alterations in shear stress have been shown to be endothelium dependent, with NO being one of the prominent mediators.3,4 When blood flow is chronically altered, vascular remodeling ensues; ie, the structural diameter and wall mass of a vessel change.4–8 The role of NO in flow-induced vascular remodeling has been addressed in large arteries9–13 but has received little attention in small arteries and arterioles.10 Because numerous studies have demonstrated that endothelium-derived prostaglandins and hyperpolarizing factors participate in acute flow-induced dilatation14–16 and that non-NO factors may play a more significant role in small than in large arteries,17,18 the contribution of NO to the chronic adaptations of small arteries and arterioles to blood flow changes is not predictable. Additionally, the actions of non-NO endothelium-derived mediators may become more prominent when NO levels are reduced.17

Reductions in NO, whether via decreased enzyme expression or activity, decreased availability of substrate or cofactors, increased endogenous inhibitors, or increased free radicals, have been described in several pathologies, such as hyperlipidemia, hypertension, heart failure, and diabetes.19–23 If NO does play a critical role in chronic remodeling processes in small arteries and arterioles, decreased bioavailability of NO under pathological conditions may impair adaptive changes in preexisting collateral pathways.

The present study was undertaken to assess the effects of chronic inhibition of NO synthase on arterial and arteriolar remodeling in a rat mesenteric model in which a collateral

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The abdomen was sutured in 2 layers with 3-0 silk. The animals were placed in a warm place and allowed to recover in a warm place and afterward were given free access to food (Hope Farms) and water. This surgical procedure is a slight adaptation of the one we previously published.24

Half of the rats were randomly selected to receive treatment with L-NAME (25 mg/kg SC per day) via osmotic minipumps (model 2002, Alzet, Alza Corp), which were implanted in the neck. The minipumps were implanted immediately after the arterial ligations.

A random selection of the nontreated (NT) rats and of the L-NAME-treated (L-NAME) rats were equipped with a catheter (PE-10, heat-sealed to PE-50) for arterial blood pressure measurements on day 12 after the operation. The heparinized saline-filled catheter was advanced from the femoral artery into the abdominal aorta. The catheter was exteriorized at the nape of the neck and sealed with a metal plug.

Mean Arterial Blood Pressure Measurements

On the morning of day 14 after the operation, blood pressure was measured in conscious quietly resting rats for 1 hour. The catheter was connected to a pressure transducer (CP-01, Century Technology) in conjunction with a data acquisition system on a personal computer (Hemodynamic Data Acquisition Systems, Instrumental Services, Universiteit Maastricht).

In Situ Blood Flow Measurements

Blood flow was measured subsequent to surgery and in NT and L-NAME animals at 2 weeks after surgery. Flow was measured in control, patent, and ligated arteries. A section of the intestine containing the vessel of interest was spread on a gauze compress moistened with warm saline. A segment of a first-order mesenteric artery was gently freed from the vein, fat, and connective tissue under a dissecting microscope. With the use of a micromanipulator, a transit-time ultrasonic flow probe (0.5 mm, V series, Transonic Systems) was placed around the artery. Flow was measured with a T106 flowmeter (Transonic Systems) linked to the aforementioned data acquisition system. Flow was sampled at 1000 Hz, averaged every second, and recorded for 5 minutes after the values had stabilized.

As noted before,24 a small but significant blood flow persisted in ligated mesenteric feed arteries (0.04±0.01 in ligated versus 0.26±0.01 mL/min in patent arteries). This is likely due to arterioles that branch off the feed artery and perfuse the perivascular fat and mesentery.

In Situ Arteriolar Diameter Measurements

At 2 weeks after the ligation surgery, the diameters of arcading arterioles running along the intestinal wall between 2 control arteries (CONARCs) or arcading arterioles running between a patent and a ligated artery (LIGARCs) were measured in NT and L-NAME animals (see Figure 1). Each animal was anesthetized with sodium pentobarbital (60 mg/kg IP) and placed on its stomach on a warmed mat. A left lateral incision was made, and a small section of the intestine was excised. The intestine was superfused with warm HEPES buffer (pH 7.4). The arcading arterioles were gently dissected free from surrounding fat under a dissecting microscope, and the diameter was determined with the use of a shearing monitor (Living Systems Instrumentation). Diameters were measured under basal conditions and after topical application of warmed sodium nitroprusside (SNP, 100 μL of a 100 μmol/L solution in HEPES buffer) onto the arteriole. Pilot experiments showed that this induced maximal vasodilatation of the arteriole but did not affect mean blood pressure (data not shown). On average, 2 CONARCs and 2 LIGARCs were measured per animal and subsequently averaged.

After diameter measurement, the arteriole was marked by placing a small ligature in the intestinal wall. The animal was subjected to perfusion fixation at its mean blood pressure (120 mm Hg for NT rats and 160 mm Hg for L-NAME rats; see Results section). Briefly, the aorta was clamped proximal to the superior mesenteric artery, and the abdominal aorta was retrogradely cannulated. The diaphragm was severed. The intestines were rinsed with PBS including 1 g/L SNP for 10 minutes and then perfused with 4% phosphate-buffered formaldehyde containing 1 g/L SNP for 10 minutes. The intestines were isolated and immersion-fixed overnight in 4% phosphate-
buffered formaldehyde before the arterioles were isolated for histological processing.

**Pressure Myograph Experiments**

Two weeks after the ligation experiments, the animals were euthanized with an overdose of pentobarbital. Control, patent, and ligated arteries were isolated and mounted in a pressure myograph (Living Systems). The 7-mL bath of the system was filled with warmed (37°C) oxygenated (5% CO₂ in O₂) calcium-free bicarbonate buffer containing 0.3 mmol/L EGTA, which was constantly circulating. The arteries were cannulated at their proximal end on a glass micropipette (200 μm) and affixed to the micropipette with 11-0 surgical suture. After ensuring that the arteries were filled with buffer, the distal end was ligated, creating a blind sack. The arteries were checked for leaks and then pressurized at 60 mm Hg for 1 hour.

The organ bath was situated on the stage of an inverted microscope (Nikon TMS) equipped with a black and white video camera (Stemmer). An electronic system (Living Systems) monitored the external diameter of the vessels.

After the equilibration period, the pressure was reduced to 20 mm Hg. A diameter-pressure curve was created by increasing the pressure in steps of 10 mm Hg up to 130 mm Hg and monitoring the external diameter. The length of the arterial segment relative to a fixed point was also recorded after each pressure step.

After the diameter-pressure curve was completed, the artery and bath were filled with warmed (37°C) phosphate-buffered formaldehyde (4%), and the artery was fixed at 100 mm Hg for 30 minutes.

**Data Processing**

From the pressure myograph measurements, circumferential strain was calculated according to the equation \( \frac{D_o - D_i}{D_o} \), with \( D_o \) representing the external diameter at a transmural pressure of 20 mm Hg. Maximal diameters were calculated by fitting the individual pressure-diameter curves (Graphpad Prism 1.00).

**Histological Measurements**

After fixation, the vessels were stored in ethanol until being embedded in paraffin. Cross sections (4 μm) were stained with Lawton’s solution (Boom). Medial CSA, defined as the area between the internal and external elastic laminae, was determined by semi-automated morphometry (JAVA 1.21, Jandel Scientific).

**Solutions and Drugs**

The composition of the HEPES buffer was as follows (mmol/L): NaCl 146.5, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, HEPES 15, and glucose 5.5. The composition of the calcium-free bicarbonate buffer was as follows (mmol/L): NaCl 118.5, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, and glucose 5.5. All salts and formaldehyde were from Merck; HEPES and EGTA, from Sigma Chemical Co; pentobarbital sodium, from Sanofi; and SNP, from Janssen Pharmaceuticals. All solutions were prepared in ultrapure water.

**Statistics**

Body weight and mean arterial pressure were compared by the nonparametric Mann-Whitney U test. CSAs and maximal diameters from pressure myograph experiments were compared by the non-parametric Kruskal-Wallis test. Paired testing was not possible because it was not always feasible to perform all experiments in the same animal. Differences between the pressure-diameter and circumferential strain curves of experimental groups were assessed by a 2-way ANOVA with a Dunnett post hoc test as necessary. Differentials between pressure-diameter and circumferential strain curves of experimental groups were assessed by a 2-way ANOVA with a Dunnett post hoc test as necessary. Differences were considered statistically significant at \( P<0.05 \). Data are expressed as mean±SEM. *P<0.05 vs CON of same group. † P<0.05 vs corresponding NT artery.

**Results**

**General Characteristics and Hemodynamics**

Treatment of rats with 25 mg/kg L-NAME per day between 8 and 10 weeks of age did not modify body weight (250±2 g at 8 weeks versus 252±4 g at 10 weeks) but increased the mean arterial pressure from 119±5 mm Hg to 148±4 mm Hg.

In anesthetized NT animals, average blood flow in first-order mesenteric feed arteries did not differ between 8 and 10 weeks of age (Table 1). Distal ligation of feed arteries acutely reduced blood flow (Table 1). Two weeks after ligation, blood flow in ligated arteries remained low (Table 1). In the intermittent patent arteries, blood flow was significantly increased within several minutes after the ligation were placed. The flow increase was comparable at 2 weeks after ligation (Table 1).

In 10-week-old animals that had been treated for 2 weeks with L-NAME, blood flow in control arteries tended to be reduced, but this did not reach statistical significance (Table 1). Blood flow in ligated arteries was reduced to the same extent as in NT animals. Although blood flow was significantly increased in patent arteries compared with control vessels of L-NAME rats, it remained significantly less than the blood flow in patent arteries of NT rats (Table 1).

**Mechanical Characteristics of First-Order Feed Arteries**

As shown in Figure 2A, for NT animals, ligated arteries displayed smaller external diameters along the entire length of the arterial segment relative to control arteries. The flow increase was comparable at 2 weeks after ligation (Table 1).

**TABLE 1. First-Order Mesenteric Feed Arterial Flow**

<table>
<thead>
<tr>
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<th>n</th>
<th>CON</th>
<th>LIG</th>
<th>PAT</th>
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<tbody>
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<td><strong>Arterial Flow, mL/min</strong></td>
<td></td>
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<tr>
<td>Acute</td>
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<td>0.30±0.05</td>
<td>0.03±0.01*</td>
<td>0.45±0.05*</td>
</tr>
<tr>
<td>NT</td>
<td>11</td>
<td>0.26±0.05</td>
<td>0.04±0.01*</td>
<td>0.55±0.07*</td>
</tr>
<tr>
<td>L-NAME</td>
<td>9</td>
<td>0.18±0.05</td>
<td>0.04±0.01*</td>
<td>0.29±0.05*†</td>
</tr>
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</table>

Values are mean±SEM. Acute indicates flow measured subsequent to ligation surgery; NT, nontreated, measured 2 weeks after surgery; L-NAME, 25 mg/kg SC L-NAME per day for 2 weeks, measured 2 weeks after surgery; CON, control artery; LIG, ligated artery; and PAT, intermittent patent artery. There are no significant differences between acute and NT measurements. *P<0.05 vs CON of same group. † P<0.05 vs corresponding NT artery.

**Figure 2.** Pressure-diameter curves generated in a pressure myograph under passive conditions in first-order mesenteric feed arteries. Chronic (2-week) ligation of feed arteries (ligated arteries [LGAs]) results in smaller arteries compared with control vessels (CONs) in NT rats (A) and L-NAME rats (B, 25 mg/kg SC L-NAME per day for 2 weeks). Intermittent patent arteries (PATs) are significantly larger than CONs in both groups. L-NAME CON and PAT are significantly smaller than their NT counterparts. Data are mean±SEM (n=9; except for NT CON, n=7). *P<0.05 vs CON of same treatment group. †P<0.05 vs corresponding NT artery.
pressure-diameter curve, and the maximal diameter was likewise smaller (Table 2). Patent arteries were significantly larger than control arteries (Figure 2A), although the calculated maximal diameter was not altered (Table 2). Feed artery ligation produced a similar pattern of arterial diameter changes in L-NAME animals (Figure 2B); ligated arteries were significantly smaller than control arteries, and patent arteries were significantly larger than control arteries along the entire pressure-diameter curve. Nonetheless, control arteries of L-NAME rats were significantly smaller than control arteries of NT rats along the pressure-diameter curve (Figure 2A and 2B) and in calculated maximal diameter (Table 2). Patent arteries of L-NAME rats were likewise smaller than patent arteries from their NT counterparts, when the diameter curves (Figure 2A and 2B) and the maximal diameters (Table 2) were compared. Ligated artery diameters were not different between NT and L-NAME rats.

Ligated arteries were less distensible than control arteries in NT rats, as shown by the reduced circumferential strain curves in Figure 3A. Despite their increased diameter, the distensibility of patent arteries did not significantly differ from that of control arteries in NT and L-NAME rats (Figure 3A and 3B). The smaller control and patent arteries of L-NAME animals were less distensible than control and patent arteries, respectively, from NT animals. Furthermore, the apparent stiffening of ligated arteries was less pronounced in L-NAME than in NT rats. This might partly be due to the reduction in arterial distensibility resulting from the hemodynamic consequences of NO synthase blockade, such as increased blood pressure and reduced blood flow.

**Figure 3.** Circumferential strain, \( (\Delta D/D_0) \), in first-order mesenteric feed arteries 2 weeks after ligation. LIGs are significantly less distensible than CONs in NT rats (A) and L-NAME rats (B, 25 mg/kg SC L-NAME per day for 2 weeks), CONs and PATs of L-NAME rats are less distensible than are corresponding arteries of NT rats. Data are mean±SEM (n=9; except for NT CON, n=7). \( \ast P<0.05 \) vs corresponding NT artery.

**Figure 4.** Effect of chronic (2-week) ligation of first-order mesenteric arteries on the medial CSA of first-order arteries (A) and arcading arterioles (B). In NT animals (open bars), first-order LIGs have significantly smaller CSAs than do CONs. PATs have significantly larger CSAs. LIGs and PATs from L-NAME rats (25 mg/kg SC L-NAME per day for 2 weeks, filled bars) follow the same pattern as corresponding NT arteries, but the changes are not statistically significant. CSAs of LIGARCs are significantly larger than those of CONARCs in NT and L-NAME animals. Data are mean±SEM (n=7 to 9). \( \ast P<0.05 \) vs CON of same treatment group.

**Figure 5.** Effect of chronic (2-week) ligation of first-order mesenteric arteries on the in situ measured diameter of distal arcading arterioles under basal conditions (basal) and during maximal dilatation with SNP (topical application of 100 \( \mu \)L of a 100 \( \mu \)mol/L warmed solution). CONARCs are significantly smaller than L-NAME rats (25 mg/kg SC L-NAME per day for 2 weeks) than in NT rats under basal conditions. LIGARCs are comparable in size to CONARCs in NT and L-NAME groups. All vessels dilate to SNP. Data are mean±SEM (n=13 or 14). \( \ast P<0.05 \) vs corresponding NT artery. \( \# P<0.05 \) vs basal diameter in corresponding arteriole.

| TABLE 2. Calculated Maximal Diameter of First-Order Mesenteric Feed Arteries |
|--------------------------|----------|----------|----------|
|                       | \( \text{Diameter, \( \mu \text{m} \)} \) |
|                       | \( \text{CON} \) | \( \text{LIG} \) | \( \text{PAT} \) |
| NT                     | \( 497\pm16 \) | \( 387\pm14^\ast \) | \( 527\pm17 \) |
| L-NAME                 | \( 430\pm21^\# \) | \( 398\pm17 \) | \( 489\pm19^\dagger \) |

Values are mean±SEM. \( ^\ast P<0.05 \) vs CON of same group, \( ^\dagger P<0.05 \) vs corresponding NT artery.

**Structural Characteristics of First-Order Feed Arteries**

In NT animals, the medial CSA of patent arteries (11.9±0.6\( \times 10^3 \) \( \mu \)m\(^2 \)) was significantly larger than that of control arteries (8.2±1.0\( \times 10^3 \) \( \mu \)m\(^2 \)), whereas ligated arteries (6.1±0.7\( \times 10^3 \) \( \mu \)m\(^2 \)) showed significantly smaller CSAs (Figure 4). L-NAME treatment did not alter control artery CSA (8.7±1.0\( \times 10^3 \) \( \mu \)m\(^2 \)). As seen in Figure 4A, patent and ligated arteries from L-NAME rats followed the same pattern of CSA change as their NT counterparts (10.6±1.1\( \times 10^3 \) and 7.2±0.4\( \times 10^3 \) \( \mu \)m\(^2 \), respectively). In no case, however, did these changes reach statistical significance with respect to each other or with respect to the corresponding NT artery.

**In Situ Diameter of Arcading Arterioles**

As shown in Figure 5, in NT rats, in situ external diameters of LIGARCs (218±15 \( \mu \)m) were not different from the diameters of CONARCs (201±15 \( \mu \)m). Although CONARC diameters of L-NAME rats (156±21 \( \mu \)m) were significantly smaller than corresponding CONARC diameters from NT
rats, LIGARC diameters (189±17 μm) were similar between the 2 groups (Figure 5). All arterioles dilated in response to topical application of SNP (Figure 5), and there was a trend for maximally dilated CONARCs of L-NAME rats to be smaller than respective CONARCs from NT rats (198±19 versus 242±17 μm, respectively; P=0.06; Figure 5).

**Structural Characteristics of Arcading Arterioles**

LIGARC medial CSA was increased compared with CONARC CSA in NT and L-NAME rats (Figure 4; NT rats, 6.2±0.6×10^3 μm^2 for LIGARC versus 4.2±0.4×10^3 μm^2 for CONARC; L-NAME rats, 7.1±0.6×10^3 μm^2 for LIGARC versus 3.4±0.4×10^3 μm^2 for CONARC). As in the first-order arteries, L-NAME had no effect on arteriolar CSA.

**Discussion**

The data presented indicate that chronic blockade of NO synthesis with L-NAME does not prevent arterial or arteriolar remodeling induced by ligation of first-order mesenteric feed arteries. Although NO has been shown to be an important vasodilator in acute arterial responses, reduction of NO synthesis does not perturb the chronic flow-induced remodeling process in resistance arteries.

**Mechanical and Structural Alterations 2 Weeks After Arterial Ligation**

Mesenteric arterial ligation is a model for studying flow-induced remodeling that has been used by us and others. In the present study, we adapted the model by reducing the number of ligations to allow sampling of control arteries from the other side of the mesentry. During the development of this technique, we noted no differences in blood flow or arterial structure and function between control arteries of operated animals or randomly chosen arteries of unoperated animals (data not shown). The alterations of flow in the present model induced arterial remodeling analogous to that previously reported, i.e., increased flow leads to larger arteries (diameter and CSA), whereas reduced flow does the opposite. In earlier experiments, we modified blood flow for 4 weeks compared with 2 weeks in the present study. Two weeks of altered blood flow induced milder changes in external diameters and medial area compared with the previous study. Nonetheless, inasmuch as Unthank and colleagues have shown that flow alterations lead to an initial abrupt change in vascular structure (within 1 week) followed by a slower adaptation over the subsequent months, the 2-week alteration is sufficiently long for examining mechanisms responsible for earlier structural remodeling in this model.

Ligated arteries displayed significantly reduced circumferential distensibility compared with control arteries, despite the reduction in medial mass. On the other hand, the intermittent patent arteries with increased medial CSAs displayed no change in distensibility. Thus, arterial distensibility seems not to be a direct consequence of the amount of medial mass. This is in line with our earlier findings showing that the dynamic mechanical properties of the rat thoracic aorta are not altered by a hypertrophic regimen of angiotensin II or by the remodeling induced by angiotensin receptor antagonism. Mechanical properties of arteries, including their structural luminal diameter, seem to be governed by the material properties of the vascular wall, which clearly changes during remodeling, as demonstrated in the present study and our earlier study.

Little is known about the ultrastructural basis of resistance arterial and arteriolar remodeling in general and the roles of changes in arterial smooth muscle cell size and number and of extracellular matrix components in particular. We reported signs of smooth muscle cell hyperplasia and of a reduction of smooth muscle cell volume in flow-loaded and ligated rat mesenteric feed arteries, respectively. However, the densities of collagen and elastin in the media of these vessels is particularly low, and the distinct influences of medial and adventitial collagen on luminal diameter are, if any, only poorly understood. In view of these uncertainties and of practical limitations, the structural basis of flow-related and L-NAME–induced remodeling was not addressed in the present study.

Arcading arterioles that interconnect individual arterial trees in the rat mesentery are the anatomic basis for the preservation of intestinal perfusion and integrity after feed artery ligation. The resistance in these collaterals determines the extent to which flow can be shunted away by the ligated vessels. The establishment of a pressure gradient results in an acute increase in collateral flow, which triggers an endothelium-dependent dilatation that further reduces the resistance offered by the collaterals. Nonetheless, no differences were observed between the diameters of normal arcading arterioles and those in the collateral pathway, either for arterial tone in situ or for maximal vasodilatation. Thus, because neither arteriolar tone nor structural diameter was altered in these arcading collaterals, the necessary decrease in resistance to accommodate the increase in flow must have been primarily achieved by diameter changes in vessels located more proximally on the collateral circuit. These results corroborate the earlier findings of Fath et al, who have demonstrated that arteriolar diameters at the center of the collateral-dependent region are not altered. Interestingly, we demonstrate that the medial CSA of the arcading arterioles in the collateral pathway is significantly increased. Because the external diameters were not altered, this suggests that the wall-to-lumen ratio in these vessels may have been increased. We can only speculate that the increase in medial mass results from the increase in transmural pressure that the arcading arterioles experience.

**Structural and Mechanical Alterations in Control Arteries After Chronic L-NAME Treatment**

Chronic L-NAME treatment has repeatedly been observed to result in hypertension in rats. Because cardiac output is reduced, L-NAME–induced hypertension primarily involves an increase in vascular resistance resulting from the withdrawal of the tonic dilator influence of endothelium-derived NO, which may in turn affect other vasoactive systems. Despite blood pressure elevation and activation of potentially mitogenic neurohumoral mechanisms, L-NAME–induced hypertension is not consistently accompanied by arterial hypertrophy (References 9 and 36 and the present study are in disagreement with the preceding statement, and References 11, 25, and 37 are in agreement). Furthermore, unaltered arterial diameters have been reported, with the present study reporting...
reduced diameters. These discrepancies may result from methodological differences. From the results of the present study, we suggest that L-NAME hypertension leads to pressure-induced eutrophic inward remodeling as has been observed in spontaneously hypertensive rats and human essential hypertension. This hypothesis is consistent with results from Lüscher’s group (Moreau et al) involving the basilar artery of the rat. The eutrophic-inward remodeling as opposed to hypertrophic nature of the remodeling may be due to reduced cardiac output, accompanied by reduced blood flow to the entire mesentery. Our measurements in single arteries showed a nonsignificant 30% reduction in blood flow. That blood flow reduction might contribute to arterial structural changes during chronic L-NAME treatment is further strengthened by our observation that the treatment, like arterial ligation, resulted in reduced arterial distensibility.

**Chronic L-NAME Treatment and Remodeling 2 Weeks After Arterial Ligation**

Unthank and colleagues have clearly demonstrated that acute flow-induced collateral dilatation is NO dependent and, furthermore, that NO-mediated vasodilatation is maintained throughout the period of collateral development. However, the role of NO in structural remodeling processes remains ambiguous.

In ligated arteries, which showed similar flow reduction in L-NAME and NT animals, the structural changes were remarkably similar, except that the decrease in medial CSA in L-NAME rats was not yet significant. Thus, arterial structural diameter responses to a reduction in blood flow were not prevented by chronic L-NAME treatment. Because reduced wall shear stress already decreases the activity of endothelial NO synthase, pharmacological blockade of the enzyme might not be anticipated to be effective in this setting. However, the present results are in contrast to recent results of Rudic et al involving an atriovenricular shunt model of the rabbit carotid artery. These authors observed a partial blockade of flow-induced arterial remodeling during L-NAME treatment and concluded a partial dependence of the process on endothelium-derived NO. However, the blood flow elevation that was studied was considerably larger (≈100%). Furthermore, in rabbits, the hemodynamic and arterial structural effects of chronic NO synthase blockade differ remarkably from those in rodents. In addition, structural composition of the wall and the nature of endothelium-derived mediators vary between large elastic and small muscular arteries.

On one hand, the results of the present study suggest that although NO regulates the response to flow or shear stress by modulating vascular tone, other (endothelium-derived) factors modulate the structural aspects of flow-induced alterations. On the other hand, the observed lack of involvement of NO in chronic flow-induced structural responses in arteries may be the consequence of the upregulation of alternative compensatory pathways during NO deficiency. Such a response would be analogous to acute endothelial vasodilator responses in which increased production of prostaglandins and of endothelium-derived hyperpolarizing factor has been shown to partially compensate for reductions in NO activity.

**Conclusion**

Although chronic L-NAME treatment alters normal arterial and arteriolar structure, pharmacological blockade of NO synthase does not prevent the flow-induced remodeling of resistance arteries and arterioles along a collateral pathway in the rat mesentery. Clinically speaking, these results suggest that in pathologies characterized by reduced bioavailability of NO (hyperlipidemia, hypertension, heart failure, and diabetes), adaptive flow-induced structural responses of pre-existing collateral vascular channels will not be impeded as a result of reduced NO levels. Other (endothelium-derived) mediators may play a major role in flow-induced remodeling of resistance arteries and arterioles or may substitute for NO during these chronic structural responses.

**Acknowledgments**

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