

# Endothelial Nitric Oxide Synthase Limits Left Ventricular Remodeling After Myocardial Infarction in Mice

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**Background**—To investigate the role of endothelial nitric oxide synthase (NOS3) in left ventricular (LV) remodeling after myocardial infarction (MI), the impact of left anterior descending coronary artery ligation on LV size and function was compared in 2- to 4-month-old wild-type (WT) and NOS3-deficient mice (NOS3<sup>-/-</sup>).

**Methods and Results**—Two days after MI, both strains of mice had a similar LV size, fractional shortening, and ejection fraction by echocardiography. Twenty-eight days after MI, both strains had dilated LVs with decreased fractional shortening and lower ejection fractions. Although the infarcted fraction of the LV was similar in both strains, LV end-diastolic internal diameter, end-diastolic volume, and mass were greater, but fractional shortening, ejection fraction, and the maximum rate of developed LV pressure (dP/dt<sub>max</sub>) were lower in NOS3<sup>-/-</sup> than in WT mice. Impairment of diastolic function, as measured by the time constant of isovolumic relaxation ( $\tau$ ) and the maximum rate of LV pressure decay (dP/dt<sub>min</sub>), was more marked in NOS3<sup>-/-</sup> than in WT mice. Mortality after MI was greater in NOS3<sup>-/-</sup> than in WT mice. Long-term administration of hydralazine normalized blood pressure in NOS3<sup>-/-</sup> mice, but it did not prevent the LV dilatation, impaired systolic and diastolic function, and increased LV mass that followed MI. In WT mice, capillary density and myocyte width in the nonischemic portion of the LV did not differ before and 28 days after MI, whereas in NOS3<sup>-/-</sup> mice, capillary density decreased and myocyte width increased after MI, whether or not hydralazine was administered.

**Conclusions**—These results suggest that the presence of NOS3 limits LV dysfunction and remodeling in a murine model of MI by an afterload-independent mechanism, in part by decreasing myocyte hypertrophy in the remote myocardium. (*Circulation*. 2001;104:1286-1291.)

**Key Words:** echocardiography ■ ventricles ■ hypertrophy

After a myocardial infarction (MI), ventricular remodeling occurs in both the myocardium surrounding the infarcted tissue (border zone) and the myocardium remote from the infarct to preserve cardiac output and limit wall stress. This adaptation involves left ventricular (LV) dilatation, changes in LV shape, myocyte hypertrophy, and interstitial myocardial fibrosis.<sup>1</sup> However, in some cases, progressive LV remodeling after MI leads to a deterioration in contractile function that is associated with increased morbidity and mortality.<sup>2</sup> Therapeutic strategies designed to limit ventricular remodeling after MI in patients can decrease the incidence of congestive heart failure and improve survival.<sup>3</sup>

Nitric oxide (NO) can modulate many of the processes leading to ventricular remodeling. In clinical studies, long-term administration of nitrates (NO-donor compounds) lim-

ited LV remodeling after MI.<sup>4</sup> Endothelium-derived NO causes systemic vascular relaxation,<sup>5</sup> thereby reducing cardiac preload and afterload. Recent evidence suggests that NO can increase angiogenesis,<sup>6</sup> decrease cardiac fibrosis,<sup>7</sup> and decrease angiotensin II-induced cardiac myocyte hypertrophy,<sup>8</sup> all of which could limit ventricular remodeling after MI.

NO is synthesized by a family of enzymes termed NO synthases (NOS), which include 3 known isoforms (NOS1, NOS2, and NOS3). Inhibition of various NOS isoforms has led to differing effects on myocardial structure and function.<sup>9-11</sup> However, most of the available NOS inhibitors are incompletely isoform-specific, potentially leading to uncertainty regarding the role of individual NOS isoforms in cardiac dysfunction and ventricular remodeling after MI.

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To identify the specific role of NOS3 (endothelial NOS) in ventricular remodeling of the noninfarcted myocardium after MI, we compared the changes in LV structure and function in wild-type (WT) and congenitally NOS3-deficient (NOS3<sup>-/-</sup>) mice after permanent occlusion of the left anterior descending coronary artery (LAD). Furthermore, we investigated the mechanisms by which NOS3 influences ventricular remodeling.

## Methods

### Experimental Animals

After approval by the Massachusetts General Hospital Subcommittee on Research Animal Care, we studied 2- to 4-month-old SV129/B6F1 WT mice and NOS3<sup>-/-</sup> mice with a SV129 and C57BL/6 background.<sup>12</sup> In supplemental studies, NOS3<sup>-/-</sup> mice, backcrossed 10 generations onto a C57BL/6 background, and WT C57BL/6 mice were studied.

### Experimental Protocol

MI was produced by ligation of the LAD, as previously described.<sup>13,14</sup> In additional experiments, NOS3<sup>-/-</sup> mice were treated with hydralazine (250 mg per liter of drinking water). Hydralazine was started 2 weeks before MI and continued until death. Mice were weighed before and 28 days after MI.

### Serial Echocardiographic Measurements

Echocardiograms were obtained before and 2, 15, and 28 days after MI, as described previously.<sup>13,14</sup> Measurements were made by an observer who was blinded to the experimental group (M.S.-C.). In additional studies, echocardiograms were obtained in 5 NOS3<sup>-/-</sup> mice 28 days after MI, before and 10 minutes after the administration of hydralazine (0.1 mg/kg through the carotid artery). LV end-diastolic volume, end-systolic volume, and ejection fraction (LVEF) were calculated using 3 parasternal short-axis views and the parasternal long-axis view.<sup>13,15</sup>

### Invasive Hemodynamics

Hemodynamic measurements were obtained 28 days after MI using a 1.4-French high-fidelity Millar pressure catheter, as described previously.<sup>16</sup>

### Noninvasive Blood Pressure Measurements

After a training period of 1 week, blood pressure and heart rate were measured noninvasively in awake mice (5 WT, 5 NOS3<sup>-/-</sup>, and 5 NOS3<sup>-/-</sup> mice treated with hydralazine) using a tail-cuff recording device (Visitech BP-2000, Visitech Systems).

### Histological Analysis

The left ventricle was weighed, frozen, and cut along the short axis into 10- $\mu$ m-thick slices obtained at 1 mm intervals.

### Infarction Size

After staining with hematoxylin-eosin, the infarcted circumference was traced at each level. Total infarct size was measured by a modification of the method of Pfeffer et al.<sup>17</sup>

### Capillary Density

Endothelial cells were detected with an antibody directed against platelet/endothelial cell adhesion molecule-1 (Santa Cruz Biotechnology), as described previously.<sup>6</sup> The number of capillaries per square millimeter was counted in regions with transversely-sectioned myocytes in the border zone of the MI and in remote myocardium. Three fields of 100  $\mu$ m $\times$ 100  $\mu$ m for the border zone and 200  $\mu$ m $\times$ 200  $\mu$ m for the remote myocardium were analyzed for each mouse at a magnification of 200 $\times$ . Additional sections were counterstained with hematoxylin, and the number of myocyte nuclei per field was counted.

**TABLE 1. Echocardiogram Analysis**

	Before MI	After MI		
		Day 2	Day 15	Day 28
LVID <sub>ED</sub> , mm				
WT	3.3 $\pm$ 0.1	3.6 $\pm$ 0.1*	3.7 $\pm$ 0.1*	3.7 $\pm$ 0.1*
NOS3 <sup>-/-</sup>	3.4 $\pm$ 0.1	3.6 $\pm$ 0.1	4.2 $\pm$ 0.1*†	4.4 $\pm$ 0.1*††
NOS3 <sup>-/-</sup> H	3.5 $\pm$ 0.1	3.7 $\pm$ 0.1	4.2 $\pm$ 0.2*	4.5 $\pm$ 0.2*††
FS, %				
WT	58 $\pm$ 2	38 $\pm$ 2*	47 $\pm$ 2*†	44 $\pm$ 2*
NOS3 <sup>-/-</sup>	57 $\pm$ 3	38 $\pm$ 3*	39 $\pm$ 2*	32 $\pm$ 3*††
NOS3 <sup>-/-</sup> H	58 $\pm$ 1	43 $\pm$ 3*	42 $\pm$ 3*	39 $\pm$ 3*
LVEDV, $\mu$ L				
WT	31 $\pm$ 2	34 $\pm$ 2	38 $\pm$ 2*	42 $\pm$ 3*†
NOS3 <sup>-/-</sup>	29 $\pm$ 2	34 $\pm$ 2*	55 $\pm$ 5*†	69 $\pm$ 7*††
NOS3 <sup>-/-</sup> H	33 $\pm$ 1	39 $\pm$ 2*	60 $\pm$ 6*†	73 $\pm$ 7*††
LVEF, %				
WT	62 $\pm$ 2	40 $\pm$ 3*	44 $\pm$ 2*	46 $\pm$ 2*
NOS3 <sup>-/-</sup>	60 $\pm$ 1	40 $\pm$ 3*	37 $\pm$ 3*	32 $\pm$ 2*††
NOS3 <sup>-/-</sup> H	59 $\pm$ 3	38 $\pm$ 3*	40 $\pm$ 3*	36 $\pm$ 3*
HR, bpm				
WT	416 $\pm$ 14	335 $\pm$ 38	434 $\pm$ 24	443 $\pm$ 28
NOS3 <sup>-/-</sup>	450 $\pm$ 26	355 $\pm$ 46	472 $\pm$ 23	481 $\pm$ 15
NOS3 <sup>-/-</sup> H	432 $\pm$ 35	413 $\pm$ 46	519 $\pm$ 25	476 $\pm$ 41

Values are mean $\pm$ SEM. n=16 for WT mice, n=13 for NOS3<sup>-/-</sup> mice, and n=11 for NOS3<sup>-/-</sup> mice treated with hydralazine. LVID<sub>ED</sub> indicates left ventricular end-diastolic internal diameter; FS, fractional shortening; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; HR, heart rate; and NOS3<sup>-/-</sup> H, NOS3<sup>-/-</sup> mice treated with hydralazine.

\* $P$ <0.05 vs day 0; † $P$ <0.05 vs day 2; †† $P$ <0.05 vs WT.

### Myocyte Size

Myocyte width was measured in regions of myocardium remote from the MI on slides stained with platelet/endothelial cell adhesion molecule-1 and counterstained with hematoxylin. Twenty measures were obtained at the level of the nucleus in longitudinally-sectioned myocytes.

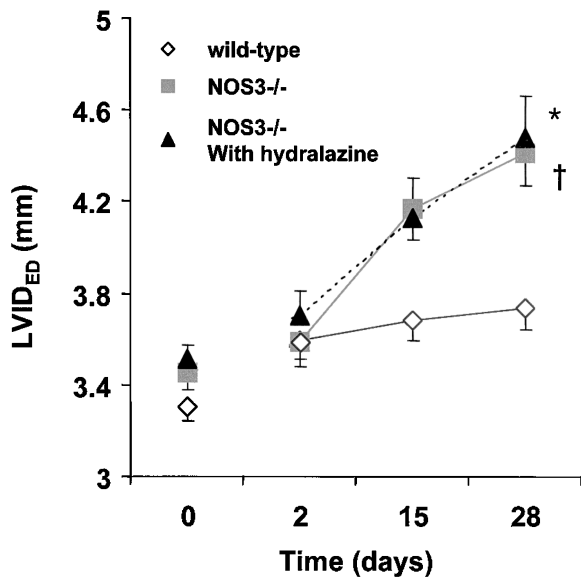
### Statistical Analysis

All data are expressed as mean $\pm$ SEM. Differences between groups were determined using 2 way ANOVA for repeated measurements. When significant differences were detected by ANOVA, a post hoc Fisher's test was employed. Analysis of survival after MI was performed with the log-rank test.  $P$ <0.05 was considered significant.

## Results

### Serial Echocardiographic Measurements

LV dimensions and function, examined using echocardiography, were similar in WT and NOS3<sup>-/-</sup> mice before MI (Table 1). Two days after MI, fractional shortening (FS) and LVEF declined similarly in both strains. Thereafter, both strains of mice demonstrated progressive LV dilatation ( $P$ <0.05 for LV end-diastolic volume). However, LV enlargement was greater in NOS3<sup>-/-</sup> than in WT mice (Table 1 and Figure 1). In WT mice, FS and LVEF did not differ between days 2 and 28 after MI, whereas in NOS3<sup>-/-</sup> mice, FS and LVEF decreased ( $P$ <0.05; Table 1). Parasternal long-axis views of a WT



**Figure 1.** Changes in end-diastolic LV internal diameter (LVID<sub>ED</sub>) in WT mice (◇), NOS3<sup>-/-</sup> mice (■), and NOS3<sup>-/-</sup> mice treated with hydralazine (▲) before and after MI induced by ligation of the LAD. NOS3<sup>-/-</sup> mice had an increase in end-diastolic LV internal diameter between day 2 and day 28 after MI, whereas WT mice did not. Pretreatment of the NOS3<sup>-/-</sup> mice with hydralazine did not prevent this enlargement. Data are shown as mean±SEM. \**P*<0.05 vs NOS3<sup>-/-</sup> mice with hydralazine at day 2 and vs WT mice. †*P*<0.05 vs NOS3<sup>-/-</sup> mice at day 2 and vs WT mice.

mouse and a NOS3<sup>-/-</sup> mouse at 2 and 28 days after MI are illustrated in Figure 2.

### Hemodynamic Measurements

Twenty-eight days after MI, mean blood pressure was greater in NOS3<sup>-/-</sup> than in WT mice (Table 2). Compared with WT mice, both the maximum rate of developed LV pressure and the maximum rate of pressure decay (dP/dt<sub>max</sub> and dP/dt<sub>min</sub>) were less in NOS3<sup>-/-</sup> mice, but the time constant of isovolumic relaxation ( $\tau$ ) was greater.

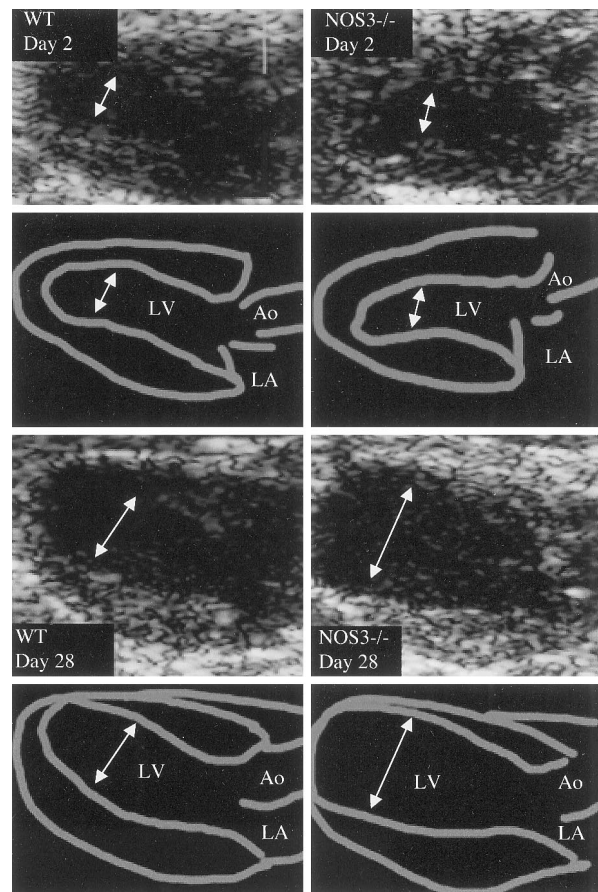
Invasive hemodynamic measurements and echocardiographic parameters after MI did not differ in SV129/B6F1 and C57BL/6 WT strains and did not differ in NOS3<sup>-/-</sup> mice with a SV129/B6F1-hybrid background and NOS3<sup>-/-</sup> mice backcrossed for 10 generations on a C57BL/6 background (data not shown).

### LV Weight and Infarction Size

Seven days after MI, the infarcted zone represented the same fraction of the LV in WT and NOS3<sup>-/-</sup> mice (Table 3). This remained true at 28 days after MI. LV weight/body weight ratios did not differ between strains before MI and 7 days after MI. At day 28, however, the LV weight/body weight ratio was greater in NOS3<sup>-/-</sup> than in WT mice (Table 3; *P*<0.05). This was not due to a loss of body weight in NOS3<sup>-/-</sup> mice, because body weight remained unchanged during the study duration in both strains (Table 3).

### Impact of Reduced Afterload on LV Remodeling in NOS3<sup>-/-</sup> Mice

Blood pressure was measured noninvasively in noninfarcted, awake mice; it was greater in NOS3<sup>-/-</sup> than in WT mice



**Figure 2.** Parasternal long-axis echocardiographic views 2 and 28 days after MI in a WT mouse and in a NOS3<sup>-/-</sup> mouse. The LV cavity size is similar in the 2 strains at 2 days after MI. By 28 days after MI, the LV of the NOS3<sup>-/-</sup> mouse is larger than that of the WT mouse (arrows). LV indicates left ventricle; LA, left atrium; and Ao, aorta.

(121±16 versus 103±19 mm Hg; *P*<0.05). Vasodilator treatment for 1 week significantly decreased blood pressure in NOS3<sup>-/-</sup> mice (96±10 mm Hg) to the level observed in WT mice.

Hydralazine treatment did not prevent LV dilatation in NOS3<sup>-/-</sup> mice subjected to LAD occlusion (Table 1 and Figure 1). Hydralazine treatment attenuated the decrease in FS and LVEF (Table 1). This effect of hydralazine on LVEF was reproduced when the drug was administered in the short-term: in 5 NOS3<sup>-/-</sup> mice at 28 days after MI, the FS increased from 29±3% to 38±2% by 10 minutes after intra-arterial administration of hydralazine (*P*<0.05), without changes in end-diastolic LV internal diameter and LV end-diastolic volume. Long-term treatment with hydralazine did not improve LV contractile function in NOS3<sup>-/-</sup> mice at 28 days after MI (Table 2). Compared with WT mice, diastolic function remained impaired in hydralazine-treated NOS3<sup>-/-</sup> mice, as reflected by a greater value of  $\tau$  and a lower dP/dt<sub>min</sub> (*P*<0.05 for both). The infarcted zone represented the same fraction of the LV in hydralazine-treated and untreated NOS3<sup>-/-</sup> mice, as well as WT mice, but the LV weight/body weight ratio was increased in both groups of NOS3<sup>-/-</sup> mice (*P*<0.05). There was no change of body weight in hydralazine-treated NOS3<sup>-/-</sup> mice after MI (Table 3).

**TABLE 2. Hemodynamic Parameters at 28 Days After MI**

	HR, bpm	MAP, mm Hg	LVESP, mm Hg	LVEDP, mm Hg	dP/dt <sub>max</sub> , mm Hg/s	dP/dt <sub>min</sub> , mm Hg/s	τ, ms
WT (n=16)	512±30	106±5	123±6	8±1	12 416±587	10 361±734	6.6±1.4
NOS3 <sup>-/-</sup> (n=13)	455±21	121±5*	141±4*	11±3	10 452±520*	8434±609*	11.3±2.0*
NOS3 <sup>-/-</sup> H (n=11)	419±43	99±8	118±10	9±3	9961±762*	7124±659*	10.7±2.5*

Values are mean ± SEM. HR indicates heart rate; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; dP/dt<sub>max</sub>, maximum rate of developed left ventricular pressure; dP/dt<sub>min</sub>, minimum rate of developed left ventricular pressure; τ, time constant of isovolumic relaxation; and NOS3<sup>-/-</sup> H, NOS3<sup>-/-</sup> mice treated with hydralazine.

\**P*<0.05 vs WT mice.

### LV Capillary Density

Before MI, LV capillary density did not differ in NOS3<sup>-/-</sup> and WT mice (Table 3). Twenty-eight days after MI, the density of capillaries in the remote myocardium decreased in NOS3<sup>-/-</sup> but not in WT mice (Table 3). Decreased capillary density was also present in the remote myocardium of hydralazine-treated NOS3<sup>-/-</sup> mice after MI (Table 3). The density of capillaries was decreased in the infarct border zone 28 days after MI in both strains, but to a lesser extent in WT than in NOS3<sup>-/-</sup> mice (2.2±0.1 versus 1.8±0.1×10<sup>3</sup> capillaries/mm<sup>2</sup>; *P*<0.05), even after hydralazine treatment (1.9±0.1×10<sup>3</sup> capillaries/mm<sup>2</sup>; *P*<0.05 versus WT).

### Myocyte Size

Before MI, myocyte width in the remote myocardium did not differ in WT and NOS3<sup>-/-</sup> mice (Table 3). Twenty-eight days after LAD occlusion, myocyte width was increased in NOS3<sup>-/-</sup> mice (Figure 3), whether or not they were treated with hydralazine (Table 3). Myocyte width did not increase in WT mice after MI (Figure 3 and Table 3). The density of myocyte nuclei did not differ in WT and NOS3<sup>-/-</sup> mice at baseline (1080±59 and 950±33 nuclei/mm<sup>2</sup> in WT and NOS3<sup>-/-</sup> mice, respectively), and it decreased in both strains 28 days after MI. The decrease in the density of myocyte nuclei was more marked in NOS3<sup>-/-</sup> mice and NOS3<sup>-/-</sup> mice treated with hydralazine (458±39 and 492±36 nuclei/mm<sup>2</sup>, respectively) than in WT mice (735±126 nuclei/mm<sup>2</sup>; *P*<0.05 versus both groups of NOS3<sup>-/-</sup> mice).

### Survival After MI

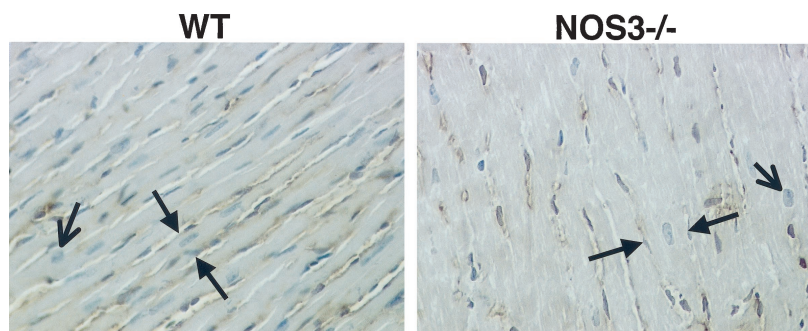
The survival rate after MI was higher in WT than NOS3<sup>-/-</sup> mice (*P*<0.01; Figure 4). Five mice (25%) died in the WT group, and 18 mice (58%) died in the NOS3<sup>-/-</sup> group. The mortality rate at 28 days for NOS3<sup>-/-</sup> mice treated with hydralazine was 42% (*P*=0.08 versus WT).

### Discussion

Ventricular remodeling is a critical determinant of the prognosis of patients after MI. Understanding the mechanisms regulating ventricular remodeling is likely to lead to new therapeutic approaches to decrease morbidity and mortality after MI. We examined a mouse MI model to investigate the role of a single NOS isoform, NOS3, in modulating ventricular function and remodeling after MI. NOS3 is known to be present in murine myocardium,<sup>18</sup> and we observed that after MI, NOS3 expression was preserved in the noninfarcted myocardium for up to 28 days (data not shown). In NOS3<sup>-/-</sup> mice, occlusion of the LAD caused greater LV remodeling and more marked LV dysfunction than in WT mice.

Before MI, LV dimensions and systolic function did not differ between WT and NOS3<sup>-/-</sup> mice, confirming previous reports.<sup>18</sup> Two days after LAD occlusion, LV dimensions increased and systolic function decreased to a similar extent in both strains of mice. However, 28 days after MI, echocardiographic measurements revealed that LV dilatation and systolic dysfunction were more marked in NOS3<sup>-/-</sup> than in WT mice. Invasive hemodynamics similarly demonstrated that LV systolic and diastolic functions were impaired to a greater extent in NOS3<sup>-/-</sup> mice than in WT mice. LV hypertrophy, as reflected by LV mass and myocyte width, was greater in NOS3<sup>-/-</sup> than in WT mice 28 days after MI. Importantly, NOS3 deficiency was associated with increased murine mortality after MI.

Because NOS3<sup>-/-</sup> mice are known to have an increased systemic blood pressure,<sup>12</sup> we considered the possibility that the increased LV remodeling observed in NOS3<sup>-/-</sup> mice after MI was attributable to increased afterload. To test this hypothesis, the LV response to LAD occlusion was measured in NOS3<sup>-/-</sup> mice that were treated long-term with hydralazine. Although systemic blood pressure in hydralazine-treated NOS3<sup>-/-</sup> mice did not differ from that in WT mice, LV



**Figure 3.** Myocyte width in the noninfarcted myocardium in WT and NOS3<sup>-/-</sup> mice 28 days after MI. Longitudinally sectioned myocytes are shown at a magnification of 600×. Myocyte nuclei (open arrow) are stained blue. Myocyte width at the level of the nucleus (shown between solid arrows) was increased in NOS3<sup>-/-</sup> compared with WT mice.

TABLE 3. Pathological Findings

	Body Weight, g		MI Size, % Heart		LV/BW, mg/g			Capillary Density, $\times 10^3/\text{mm}^2$		Myocyte Width, $\mu\text{m}$	
	Before MI	Day 28	Day 7	Day 28	Before MI	Day 7	Day 28	Before MI	Day 28	Before MI	Day 28
WT	26 $\pm$ 1	27 $\pm$ 1	29 $\pm$ 9	23 $\pm$ 3	3.6 $\pm$ 0.1	3.7 $\pm$ 0.1	3.6 $\pm$ 0.1	2.9 $\pm$ 0.1	2.7 $\pm$ 0.1	17 $\pm$ 1	17 $\pm$ 1
NOS3 <sup>-/-</sup>	28 $\pm$ 1	28 $\pm$ 1	27 $\pm$ 10	27 $\pm$ 3	3.7 $\pm$ 0.1	3.9 $\pm$ 0.1	4.4 $\pm$ 0.2*†	2.9 $\pm$ 0.1	2.3 $\pm$ 0.1*†	17 $\pm$ 1	23 $\pm$ 1*†
NOS3 <sup>-/-</sup> H	24 $\pm$ 1	26 $\pm$ 1	NA	30 $\pm$ 4	NA	NA	4.2 $\pm$ 0.2*	NA	2.1 $\pm$ 0.1*	NA	22 $\pm$ 1*

Values are mean $\pm$ SEM. Body weight, LV weight/body weight (LV/BW), and MI size (when applicable) were measured in 5 mice of each strain before MI, 7 mice of each strain 7 days after MI, and 13 WT, 9 NOS3<sup>-/-</sup> and 11 hydralazine-treated NOS3<sup>-/-</sup> (NOS3<sup>-/-</sup> H) mice 28 days after MI. Capillary density and myocyte width were measured in a subgroup of 5 WT mice before and 28 days after MI, 5 NOS3<sup>-/-</sup> mice before and 28 days after MI, and 5 hydralazine-treated NOS3<sup>-/-</sup> mice 28 days after MI. NA indicates not applicable.

\* $P < 0.05$  vs WT mice; † $P < 0.05$  vs before MI.

enlargement, systolic and diastolic dysfunction, and LV hypertrophy were more marked after MI in the former. The mortality after MI tended to be greater in hydralazine-treated NOS3<sup>-/-</sup> than in WT mice, but this difference did not reach statistical significance. After MI, both FS and LVEF were greater in hydralazine-treated NOS3<sup>-/-</sup> mice than in untreated NOS3<sup>-/-</sup> mice. This selective improvement was reproduced when hydralazine was administered short-term, suggesting that the improvement was attributable to hydralazine's effect on ventricular loading conditions. In contrast,  $dP/dt_{\text{max}}$ , a relatively load-independent measure of systolic function, was unaffected by hydralazine administration.

We also considered the possibility that increased MI size after LAD occlusion caused greater LV remodeling in NOS3<sup>-/-</sup> mice. After myocardial ischemia and reperfusion, Jones et al<sup>19</sup> observed larger MIs in NOS3<sup>-/-</sup> than in WT mice, whereas Yang et al<sup>20</sup> reported similar MI sizes in the 2 mouse strains. In our study, we observed no difference in MI size or LV weight between the 2 strains at 7 days after permanent LAD occlusion. Hence, the impact of NOS3 on LV remodeling is not attributable to differences in MI size after LAD occlusion.

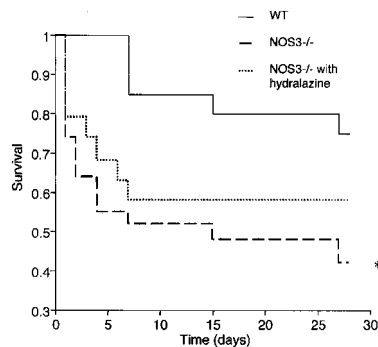
In WT mice after MI, the LV dilated and the density of myocyte nuclei in tissue sections declined, without an increase in LV mass or myocyte diameter. These findings suggest that myocytes in the remote myocardium elongate in

response to MI. Similar eccentric myocyte hypertrophy has been reported in the hearts of rats with small MIs.<sup>21</sup> In contrast, in NOS3<sup>-/-</sup> mice, even when treated with hydralazine, the LV dilated and increased in mass, which was associated with an increase in myocyte diameter. These findings suggest that in NOS3<sup>-/-</sup> mice, myocytes undergo concentric and eccentric hypertrophy, similar to that seen in rats with large MIs.<sup>21</sup> The mechanism by which NOS3 reduces LV hypertrophy after MI remains unknown. Numaguchi and colleagues<sup>22</sup> reported that long-term administration of a NOS inhibitor in rats caused cardiac hypertrophy that was not prevented by afterload reduction with hydralazine. NO reportedly decreases the hypertrophic response of cultured cardiac myocytes in response to stimuli such as angiotensin II<sup>8</sup> and decreases the expression of the angiotensin II type 1 receptor.<sup>23</sup>

It is unknown why, despite increased myocyte hypertrophy, systolic function is more impaired in NOS3<sup>-/-</sup> mice (with or without hydralazine treatment) than in WT mice. We observed that in the remote myocardium, capillary density is preserved in WT but decreased in NOS3<sup>-/-</sup> mice. It is conceivable that relative myocardial ischemia due to decreased capillary density may contribute to the greater impairment of LV function in NOS3<sup>-/-</sup> mice after MI.

Several therapeutic approaches to limiting ventricular remodeling in MI have been developed, such as the administration of angiotensin-converting enzyme inhibitors, which may act in part by increasing NO production by cardiac NOS3.<sup>24</sup> Recently, a NOS3 gene polymorphism, G894T, which alters enzyme function,<sup>25,26</sup> was associated with an increased risk of coronary artery disease.<sup>27</sup> If our observations in mice can be extrapolated to humans, it is possible that patients with congenital or acquired NOS3 deficiencies may be at increased risk for developing pathological ventricular remodeling after MI. Moreover, it may be possible to predict who is likely to respond to therapeutic agents that act via NOS3 and who may not respond and should be treated with NO-donor compounds. Finally, our studies suggest that strategies designed to increase cardiac NOS3 expression, for example by gene transfer, may benefit patients presenting with MIs by preventing LV remodeling and its associated increased morbidity and mortality.

In summary, our results demonstrate the importance of NOS3 in limiting LV dilatation, dysfunction, and hypertrophy in murine MI, possibly by limiting the hypertrophic



**Figure 4.** Survival of WT mice (solid line), NOS3<sup>-/-</sup> mice (hatched line), and NOS3<sup>-/-</sup> mice treated with hydralazine (dotted line). Life span is represented by the Kaplan-Meier method. Survival was decreased in NOS3<sup>-/-</sup> mice compared with WT mice (\* $P < 0.01$ ). There was a trend toward decreased survival in the NOS3<sup>-/-</sup> mice treated with hydralazine compared with WT mice ( $P = 0.08$ ).

response to MI, and they suggest new strategies for preventing detrimental LV remodeling in patients after MI.

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