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**Key role of the NO-pathway and MMP-9 in high blood flow-induced remodeling of rat resistance arteries.**

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Short title: flow-remodeling in resistance arteries

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Abbreviations: Angiotensin converting enzyme: ACE; Doxycycline: DOX; L-NAME (N(omega)-nitro-L-arginine methyl ester): LN; matrix metalloproteinases: MMPs; Nitric oxide: NO; high flow: HF, low flow: LF; normal flow: NF; NO synthase: NOS; Perindopril: P; physiological salt solution: PSS

## **ABSTRACT**

Objective: Blood flow is altered in metabolic and ischemic diseases with dramatic consequences. Resistance arteries structure and function remodel in response to chronic blood flow changes through a mechanism remaining mainly unknown. We hypothesized that the NO pathway and MMPs activation might play a role in flow (shear stress)-induced microvascular remodeling.

Methods and Results: Mesenteric resistance arteries were ligated in order to alter blood flow in vivo for 4 or 14 days: arteries were submitted to high (HF), low (LF) or normal flow (NF). Rats were treated with L-NAME, the angiotensin converting enzyme inhibitor perindopril or the MMPs inhibitor doxycycline. After 14 days, outward hypertrophic remodeling occurred in HF arteries in association with eNOS overexpression. MMP9 activity increased in the early phase (day 4). HF-remodeling was prevented by L-NAME, eNOS gene knockout and doxycycline. L-NAME prevented eNOS overexpression and MMPs activation whereas doxycycline only prevented MMPs activation. In LF arteries diameter reduction was associated with a decreased eNOS expression without change in MMPs expression and activation. LF-remodeling was reduced by perindopril.

Conclusions: In resistance arteries high flow induced diameter enlargement and wall hypertrophy associated with the sequential activation of eNOS and MMP9.

Key words: Microcirculation (resistance arteries), remodeling, blood flow, shear stress, endothelium, nitric oxide, matrix metalloproteases.

## **CONDENSED ABSTRACT**

Blood flow is altered in metabolic and ischemic diseases with dramatic consequences. A chronic increase in blood flow (shear stress) in mesenteric resistance arteries induced outward hypertrophic remodeling due to the sequential activation of eNOS and MMP9. Lowering blood flow induced a diameter reduction reflecting the loss of flow-dependent dilation.

## INTRODUCTION

Cardiac output generates blood flow in large compliance arteries followed by small resistance muscular arteries able to adapt their diameter to the metabolic need of the downstream located tissues. Resistance arteries are subjected to mechanical forces, pressure and flow (shear stress), inducing respectively myogenic tone and flow-dependent dilation.<sup>1</sup> Flow stimulates the endothelium to produce contractile (PGH<sub>2</sub>, TXA<sub>2</sub>, endothelin, reactive oxygen species) and relaxing factors including nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI<sub>2</sub>). Long term changes in blood flow induce arterial wall remodeling in order to normalize shear stress. In large conductance arteries, remodeling is associated with neointimal hyperplasia<sup>2</sup> and depends on NO production<sup>3</sup> and matrix metalloproteases (MMPs) activation.<sup>4</sup> In arterioles or resistance arteries, flow-dependent remodeling is involved in physiological processes, such as blood vessel growth during development,<sup>5</sup> exercise training<sup>6</sup> or pregnancy<sup>7</sup> and in pathological situations including hypertension,<sup>8</sup> diabetes,<sup>9</sup> ischemic diseases<sup>10</sup> or tumor growth.

In resistance arteries blood flow reduction induces inward remodeling and reduced contractile capacity whereas chronic increases in blood flow triggers outward hypertrophic remodeling.<sup>11,12,13</sup> The mechanisms involved in flow-induced remodeling have been mainly investigated in large elastic arteries and in cultured endothelial cells which may not be relevant for arterioles *in vivo*.<sup>3</sup> Studies in small arteries show the central role of shear stress,<sup>14</sup> circumferential wall stress,<sup>12, 15</sup> transient de-differentiation and turnover of smooth muscle cells<sup>13</sup> and growth factors.<sup>16</sup> The role of NO is essential in large arteries as shown in arteriovenous fistula in the rabbit or the rat<sup>17, 18</sup> or in ligated carotid arteries in mice lacking eNOS.<sup>19</sup> NO takes part differently in remodeling along the vascular tree.<sup>20</sup> In resistance arteries, the role of NO remains controversial. Although eNOS expression increases in resistance mesenteric arteries submitted to high flow,<sup>14, 21</sup> chronic NO-synthesis inhibition with L-NAME does not prevent remodeling.<sup>22</sup> Nevertheless, in this latter study chronic L-NAME induces a large increase in blood pressure preventing the use of a fully blocking dose of L-NAME.<sup>23</sup> In addition, high blood pressure due to L-NAME induces remodeling by its own,<sup>24</sup> thus interfering with flow-induced remodeling. Thus we aimed to further investigate the role of the NO-pathway in flow-induced remodeling of resistance arteries using higher doses of L-NAME and mice lacking the gene encoding for eNOS.

In addition, NO has a key role in the control of matrix metalloproteases (MMPs) activity involved in flow-induced remodeling,<sup>3, 25</sup> in large elastic arteries. Thus the absence of effect

of NO in microvascular remodeling would also exclude MMPs from the process in resistance arteries. Nevertheless, no study has yet been conducted in resistance arteries undergoing remodeling to test the role of MMPs. Thus, we assessed the hypothesis that MMPs stimulated by NO could play a role in flow-induced microvascular remodeling. We conducted a functional and a biochemical study in resistance arteries chronically submitted to low or high blood flow, for 4 and 14 days, in order to determine the role of NO and MMPs in remodeling. These two time points have been chosen to study early and late mechanisms of flow-remodeling. Indeed Buus et al<sup>13</sup> showed that the remodeling induced by a decrease in blood flow occurred after only 2 days whereas the remodeling induced by an increase in blood flow was a slower process requiring approximately 2 weeks to be completed. Changes in flow were performed by alternative ligations of mesenteric arteries in mice or rats.<sup>11,21</sup> NO-blockade was obtained with high dose of L-NAME (LN) in drinking water in parallel with an antihypertensive treatment, perindopril (P), preventing deadly rises in blood pressure.<sup>23</sup> Mice lacking the gene encoding for eNOS were used as well. Doxycycline (DOX) was used to prevent MMPs activity. We found that both eNOS and MMPs activation are essential for resistance arteries remodeling induced by a chronic increase in blood flow, whereas low flow-induced remodeling was prevented by perindopril.

## **MATERIALS AND METHODS**

### **In vivo Ligation of Rat Mesenteric Arteries**

Adult male rats Wistar were anesthetized (sodium pentobarbital, 50 mg/kg IP) and submitted to surgery to modify blood flow in the mesenteric arteries, as previously described.<sup>11,21</sup> Arteries were submitted to high (HF), low (LF) or normal flow (NF) (please see [www.ahajournals.org](http://www.ahajournals.org), figures IA, IB).

60 rats were divided in 4 groups and treated in drinking water as described below:

1. DOX (n=15) : doxycycline (40 mg/kg/d);
2. LN+P (n=15): L-NAME (60 mg/kg per day) plus perindopril (6 mg/kg/d);
3. P (n=15): perindopril (6 mg/kg/d);
4. CT (n=15): control without treatment.

Treatments were started 24 hours before surgery.

After 14 days, rats were euthanized and mesenteric arteries collected for functional (n=8 per group) and histomorphometric (n=4 per group) studies. In a separate series of experiments, at 4 and 14 days, biochemical (n=7 per group) and immunohistochemical (n=6 untreated rats only) studies were performed.

In addition, mice lacking the gene encoding for eNOS (-/-) (n=5) and their littermate controls (+/+) (n=4) were submitted to arterial ligation during 14 days as described above.

The procedure followed in the care and euthanasia of the study animals was in accordance with the European Community standards on the care and use of laboratory animals (authorization nb 00577).

### **Arterial Diameter Measurement in Isolated Arteries**

Segments of mesenteric arteries were cannulated at both ends in a video-monitored perfusion system as previously described.<sup>21</sup> Briefly, arteries were bathed and perfused with a Ca<sup>2+</sup>-free physiological salt solution (PSS) containing EGTA (2 mmol/L) and sodium nitroprusside (10 µmol/L). Diameter changes were measured when intraluminal pressure was increased from 10 to 150 mm Hg.

### **Western Blot Analysis of eNOS**

Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were incubated with the primary antibody (Transduction Laboratories, 1:2000). Proteins were visualized using the ECL-Plus Chemiluminescence kit (Amersham).<sup>21</sup>

### **Immunohistochemistry and in Situ Zymography**

Arterial segments were embedded vertically in Tissue-tek (Sakura). MMP-2 and MMP-9 were detected with primary goat polyclonal antibodies (Santa Cruz Biotechnology, 1:100) followed by the fluorescent secondary antibody (1:200). In negative control experiments the primary antibody was omitted.

For in situ zymography, sections (7 µm thick) were incubated over night (37°C) with a fluorogenic gelatin substrate (FluoProbes) dissolved to 25 µg/mL in zymography buffer (50 mmol/L Tris-HCl, 10 mmol/L CaCl<sub>2</sub>, and proteases inhibitors cocktail, pH 7.4). In control experiments sections were incubated with an inhibitor (EDTA) and an activator (APMA) of metalloproteinases.

Immunostaining and in situ gelatinolysis were revealed by the appearance of fluorescence visualized and quantified in the media using confocal microscopy.<sup>26</sup>

## Arteries Histomorphometric Analysis

Transversal sections of arteries fixed under a pressure of 75 mmHg were stained with orcein solution. Then internal and external medial circumferences were measured in order to determine media surface.<sup>11</sup>

## Statistical analysis

Results were expressed as means  $\pm$  standard error (s.e.mean). Significance of the differences between groups was determined by analysis of variance (ANOVA for consecutive measurements for pressure-diameter curves) or 1-way ANOVA followed by Bonferroni or paired t-test. P values less than 0.05 were considered to be significant.

## RESULTS

Mean arterial blood pressure was not significantly affected by treatments with L-NAME plus perindopril ( $113 \pm 10.5$  mmHg at Day 4 and  $102 \pm 9$  mmHg at Day 14) or doxycycline ( $103 \pm 9.4$  mmHg at Day 4 and  $107 \pm 7.4$  mmHg at Day 14), compared to control ( $101 \pm 3$  mmHg at Day 4 and  $109 \pm 2.6$  mmHg at Day 14). In rats treated with perindopril mean arterial pressure was not affected after 4 days ( $82 \pm 10$  mmHg) and was decreased after 14 days ( $74 \pm 4.8$  mmHg).

### Untreated rats

#### Structural properties of arteries and arterial passive diameter

Passive arterial diameter was measured in control rats' arteries 14 days after ligations. High, low and normal flow arteries were designated as HF, LF and NF arteries, respectively. In isolated mesenteric resistance arteries stepwise increases in pressure induced diameter (passive diameter) enlargement, when measured in a calcium-free PSS + EGTA (2 mM).

In control rats, passive arterial diameter was higher in HF arteries than in NF arteries, whereas in LF arteries it was significantly decreased (figure 1A).

#### Expression of eNOS

Four and 14 days after ligation, the expression of endothelial NO synthase (eNOS or NOS III) was increased in HF arteries (although not significantly at Day 14) and decreased in LF arteries compared to NF arteries (figure 1B).

### Regulation of MMP-2 and MMP-9 Expression

We used immunohistochemistry followed by confocal microscopy in order to locate MMP2 and MMP9 and to assess their expression level.

In NF arteries of control rats and in arteries of non-operated rats, a positive although low staining of MMP-2/MMP-9 was observed in the media of mesenteric arteries, at Day 4 and Day 14 (data not shown).

In HF arteries of control rats, the staining of MMP-2 was not significantly different from NF at Day 4 and Day 14 (figure 2C). The staining of MMP-9 was significantly increased at Day 4 but not at Day 14 (figure 2D).

In LF arteries of control rats, the staining of both MMP-2 and MMP-9 was not significantly different from NF at Day 4 and Day 14 (figures 2C, 2D).

### *Treated rats*

#### Structural properties of arteries (figure 3A)

In NF arteries of doxycycline or L-NAME and perindopril-treated rats, media surface was not significantly different from that in corresponding arteries of control rats. In NF arteries of rats treated with perindopril alone media surface was significantly decreased compared to that in NF control rats' arteries.

In HF arteries doxycycline and perindopril prevented media surface to increase.

In LF arteries no treatment affected media surface.

#### Arterial passive diameter

Passive arterial diameter was measured in rats treated with doxycycline, L-NAME and/or perindopril and in eNOS  $-/-$  and eNOS  $+/+$  mice 14 days after ligation.

In rats treated with doxycycline, no significant remodeling was observed in HF arteries (figures 3B, 3E). Remodeling was present in LF arteries doxycycline-treated rats (figures 3B, 3F), but it was significantly lower than in LF arteries isolated from control rats (figure 3F).

Arterial diameter in NF arteries was not affected by doxycycline as compared to untreated rats (figures 1A, 3B).

In rats treated with L-NAME and perindopril no significant remodeling was observed in HF arteries (figures 3C, 3E), whereas perindopril alone did not affect remodeling in HF arteries (figures 3D, 3E). In LF arteries remodeling was not affected by the treatment associating L-NAME and perindopril (figures 3C, 3F) but it was inhibited by perindopril alone (figures 3D, 3F). Arterial diameter in NF arteries was not affected by perindopril associated or not with L-NAME (figures 1A, 3C, 3D).

In eNOS<sup>+/+</sup> mice, passive arterial diameter was higher in HF arteries than in NF arteries, whereas in LF arteries it was decreased. In eNOS<sup>-/-</sup> mice, passive arterial diameter in HF arteries was equivalent to that in NF arteries, whereas in LF arteries it was decreased (figure 4A).

#### Expression of eNOS (figures 4B, 4C)

In HF and LF arteries of L-NAME and perindopril-treated rats, the expression of eNOS was not different from that in NF arteries, except in LF arteries after 14 days of ligation where it was decreased.

Four and 14 days after ligation, in HF and LF arteries of perindopril-treated rats, the expression of eNOS was respectively increased and decreased compared to that in NF arteries.

Four and 14 days after ligation, in HF arteries of doxycycline-treated rats, the expression of eNOS was significantly increased compared to that in NF arteries. In LF arteries, it was equivalent to that in NF arteries.

Four and 14 days after ligation, in NF and LF arteries, the expression of eNOS was not significantly affected by any treatment. In HF arteries of L-NAME plus perindopril-treated rats and doxycycline-treated rats, the expression of eNOS was significantly lower than in HF control arteries.

#### Regulation of MMP-2 and MMP-9 Activity (figure 5)

In situ zymography showed lytic areas corresponding to an activity of both MMP2 and MMP9. In NF arteries of control rats, a significant gelatinolytic activity was detected at Day 4 and Day 14. This MMPs activity was assigned a value of 100%.

In HF arteries of control rats and perindopril-treated rats, the gelatinolytic activity was significantly increased at Day 4 and was equivalent to that in NF at Day 14. In LF arteries, the gelatinolytic activity was not significantly different from NF at Day 4 and Day 14.

Four and 14 days after ligation, in L-NAME and perindopril-treated rats and doxycycline-treated rats, the gelatinolytic activity in HF and LF arteries was equivalent to that in NF.

Four days after ligation, in NF and LF arteries, the gelatinolytic activity was not significantly affected by any treatment, whereas in HF arteries, the gelatinolytic activity was prevented to increase by a treatment with L-NAME and perindopril or with doxycycline.

## DISCUSSION

This study shows a relationship between NO production, eNOS expression, MMPs activity and the remodeling of resistance arteries induced by a chronic increase in blood flow *in vivo*. On the other hand, inward remodeling induced by a chronic decrease in blood flow was associated with a decreased eNOS expression. This latter remodeling was prevented by angiotensin I converting enzyme (ACE) inhibition.

Microvascular structural and functional changes induced by chronic changes in blood flow have a key role in pathological processes such as hypertension, ischemic diseases, diabetes or tumor growth. Thus a better understanding of the mechanisms involved in flow-induced remodeling of resistance arteries is especially important.

We used a model previously described in rats<sup>11, 14</sup> and mice,<sup>21</sup> allowing the comparison of resistance arteries submitted to different blood flow levels in the same conditions and in the same animal. In these resistance arteries, chronic increases and decreases in blood flow induce outward and inward arterial remodeling, respectively.<sup>11, 13, 21</sup> These diameter changes allow normalization of wall shear stress and are accompanied by a compensatory change in medial mass, which restores circumferential wall stress.<sup>11</sup> In this model blood flow increases by approximately 60% and arterial remodeling fully normalizes shear stress.<sup>11, 21</sup> This is different from flow-induced remodeling in large arteries, obtained with arterio-venous fistulae inducing a 6-10 fold increase in blood flow and an incomplete shear stress normalization.<sup>3, 25</sup> Besides tissue specificity this difference in the amplitude of the stimulus may account for differences

in the mechanism(s) involved in the process, especially concerning the remodeling induced by blood flow reductions (see below).

As the NO pathway and MMPs are involved in the remodeling of large arteries in response to a chronic increase in blood flow, we measured changes in expression levels of eNOS.

First we demonstrated the key role of eNOS activation in high flow-induced remodeling of resistance arteries. High flow-induced remodeling was prevented by NO synthesis inhibition with L-NAME and by the absence of the gene encoding for eNOS. In order to fully block NO production we used a high dose of L-NAME in association with an ACE inhibitor, thus preventing the mortality due to L-NAME.<sup>23</sup> In addition, perindopril prevented the rise in blood pressure induced by L-NAME, which is important as high blood pressure, per se, induces remodeling,<sup>27</sup> thus rendering difficult a conclusion on flow-induced remodeling. Indeed, high blood pressure due to L-NAME induces inward eutrophic remodeling.<sup>15</sup> The present study is in agreement with previous findings obtained in large elastic arteries.<sup>3, 25</sup> On the other hand our result contrasts with a previous report<sup>22</sup> stating that, in resistance arteries, chronic L-NAME does not affect high flow-induced remodeling, using the same model. Nevertheless, the dose of L-NAME used was lower than in the present study and the rise in blood pressure due to L-NAME was interfering with flow-induced remodeling as stated above. In addition, treatment with L-NAME was started after ligations, allowing HF-remodeling to start before the inhibition of NO pathway had reached an effective level.

Another main finding of the present study is that eNOS activation and the consecutive activation of MMP9 are essential for HF-remodeling. We found that MMPs inhibition with doxycycline prevented high flow-induced remodeling of resistance arteries. In addition, HF-remodeling was also associated with a rise in MMP2/9 activity and MMP9 expression 4 days after ligation. This was followed by a return to baseline activity and expression at Day 14. These data suggest a more noteworthy involvement of MMP9 than MMP2 in high flow-induced remodeling of resistance arteries, although the activity measurement does not allow discerning between MMP2 and MMP9. After an increase in blood flow MMPs could be activated by NO<sup>3,25</sup> or directly by shear stress.<sup>28</sup> For the purpose of determining the sequence of events involved in flow-remodeling, we assessed the expression of eNOS and MMPs activity in L-NAME-treated and doxycycline-treated rats. In HF arteries, L-NAME prevented eNOS overexpression (figures 4B, C) and MMPs activation (figure 5B) whereas doxycycline inhibited only the rise in MMPs activity without preventing eNOS expression to rise in the

HF arteries (figure 5B). These data suggest that a sequential activation of eNOS and then of MMPs occurred in HF remodeling. These findings are supported by a recent study showing that eNOS mediated MMPs activation.<sup>29</sup> Without preventing eNOS overexpression in HF arteries, a downward shift in eNOS expression was nevertheless observed in the arteries isolated from doxycycline-treated rats. This observation is in agreement with previous studies establishing the inhibiting effects of doxycycline on NOS expression.<sup>30</sup>

Thus, the chronic increase in blood flow in resistance arteries stimulated eNOS expression and activated NO production that in turn enhanced MMP9 activity leading to cell proliferation, media hypertrophy and diameter enhancement.

In resistance arteries a chronic decrease in blood flow induces an important diameter reduction. This remodeling has been found hypotrophic with a reduced smooth muscle cell number and size associated with apoptosis.<sup>11, 13</sup> In the present study we did not find a significant hypotrophy although a tendency exists in the 4 groups of rats. Nevertheless, the present study and our previous works<sup>21, 31</sup> agree with the other findings associated with the LF-remodeling such as the hyporeactivity and the decreased protein expression (mainly eNOS). We found that this remodeling was not affected by NO synthesis or MMPs activity inhibition with L-NAME or doxycycline, respectively. These findings contrast with the remodeling induced by blood flow reduction in large arteries<sup>32</sup> where intimal hyperplasia develops in association with MMP2/9 activation.<sup>33</sup> Angiotensin I converting enzyme inhibition prevented low flow-induced remodeling. This finding supports the hypothesis that inward remodeling in resistance arteries results from an unbalanced vasoconstrictor tone due to the reduction in flow stimulating the endothelium followed by transglutaminase-dependent stabilization of the remodeling.<sup>34, 35</sup> This prevention of the remodeling induced by blood flow reduction can also be due to the blood pressure reduction observed in perindopril-treated rats (significant at day 14: 35mmHg blood pressure reduction compared to control). This latter hypothesis is compatible with the one given above as suppressing a vasoconstrictor influence and reducing blood pressure should increase arterial diameter. Indeed Perindopril alone reduces blood pressure and exerts a vasodilator influence thus inhibiting LF-remodeling. When perindopril is associated with L-NAME its vasodilator effect is counteracted by the vasoconstrictor influence of L-NAME, resulting in a normal blood pressure and the restoration of LF-remodeling. Our data showing a downregulation of the NO pathway are in agreement with the previous studies showing that shear stress is a main determinant of eNOS expression.<sup>36</sup>

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In addition, ACE inhibition, which did not affect the diameter enlargement induced by the chronic increase in blood flow, prevented the rise in media surface. This is especially important as arterial wall hypertrophy is correlated to the outcome of cardiovascular events. Nevertheless, in the present study, perindopril was used to prevent the rise in blood pressure due to L-NAME and the dose required, when used alone, induced a significant decrease in blood pressure as compare to control. Thus further investigations are required in order to better understand the role of the renin-angiotensin system in flow-dependent hypertrophic remodeling in resistance arteries.

In conclusion, these findings show that in resistance arteries chronic increases in blood flow, in vivo, induce a structural and functional remodeling requiring NO production and MMPs activation. On the other hand, low flow-induced remodeling was independent of NO and MMPs.

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Conflict of interest: none.

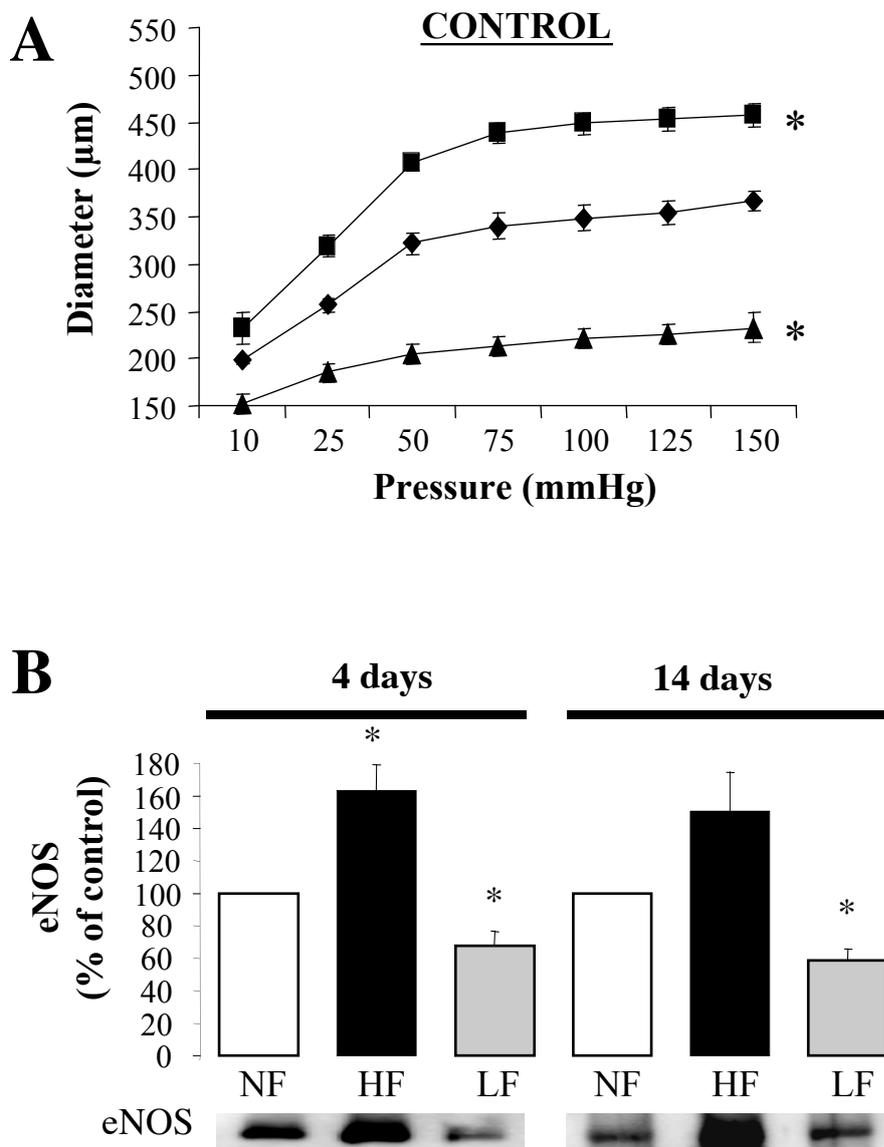
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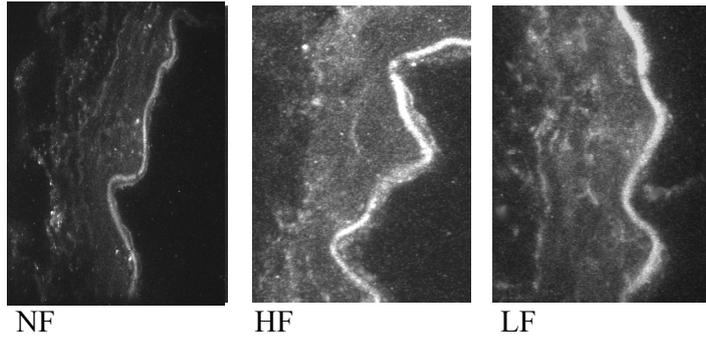
**Figure 1:**

Pressure-diameter (passive arterial diameter) relationship was determined in mesenteric arteries isolated from untreated rats submitted to arterial ligation for 14 days (A). Values are means $\pm$ SEM.

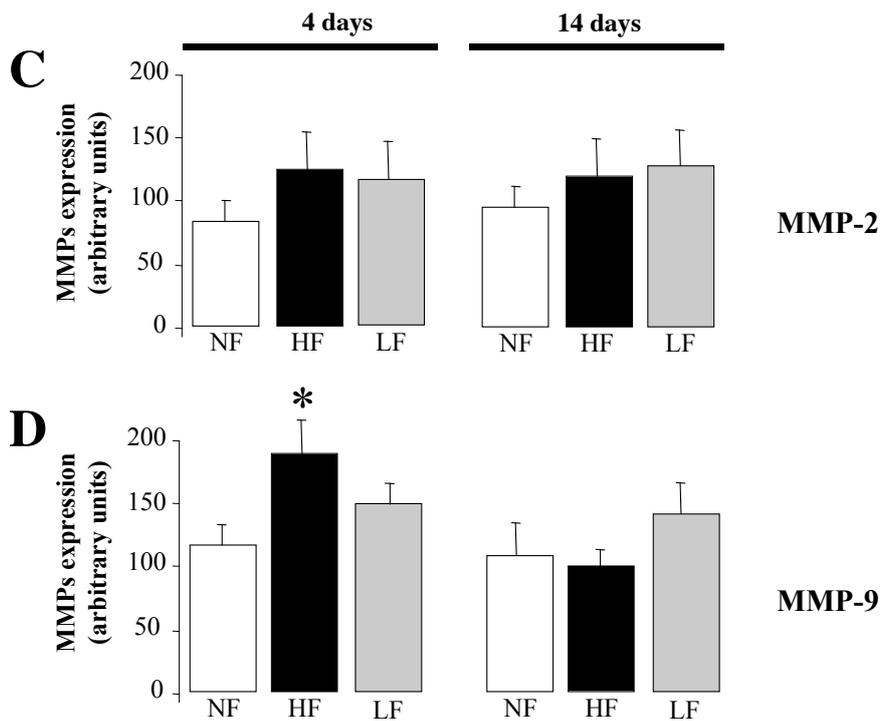
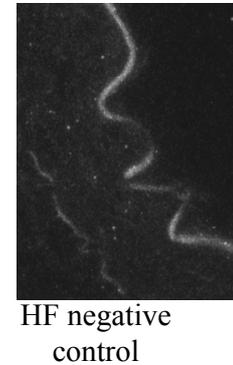
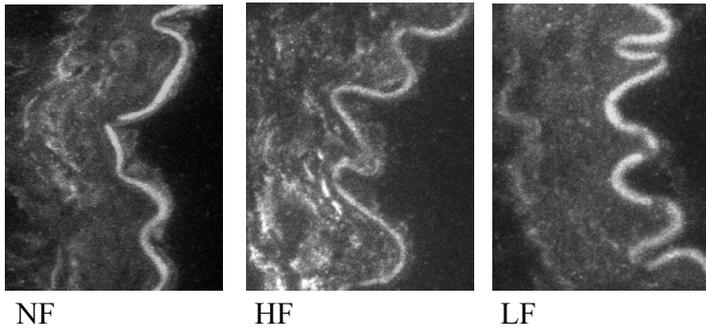
Endothelial nitric oxide synthase (eNOS) expression in mesenteric arteries isolated from untreated rats 4 and 14 days after ligations (B). Protein expression is given as percentage of NF arteries (mean  $\pm$  SEM is presented). A typical Western-blot is shown below each graph. n=7 per group.

\*P<0.05, LF or HF versus NF arteries

**A**  
MMP-2  
(4 days)



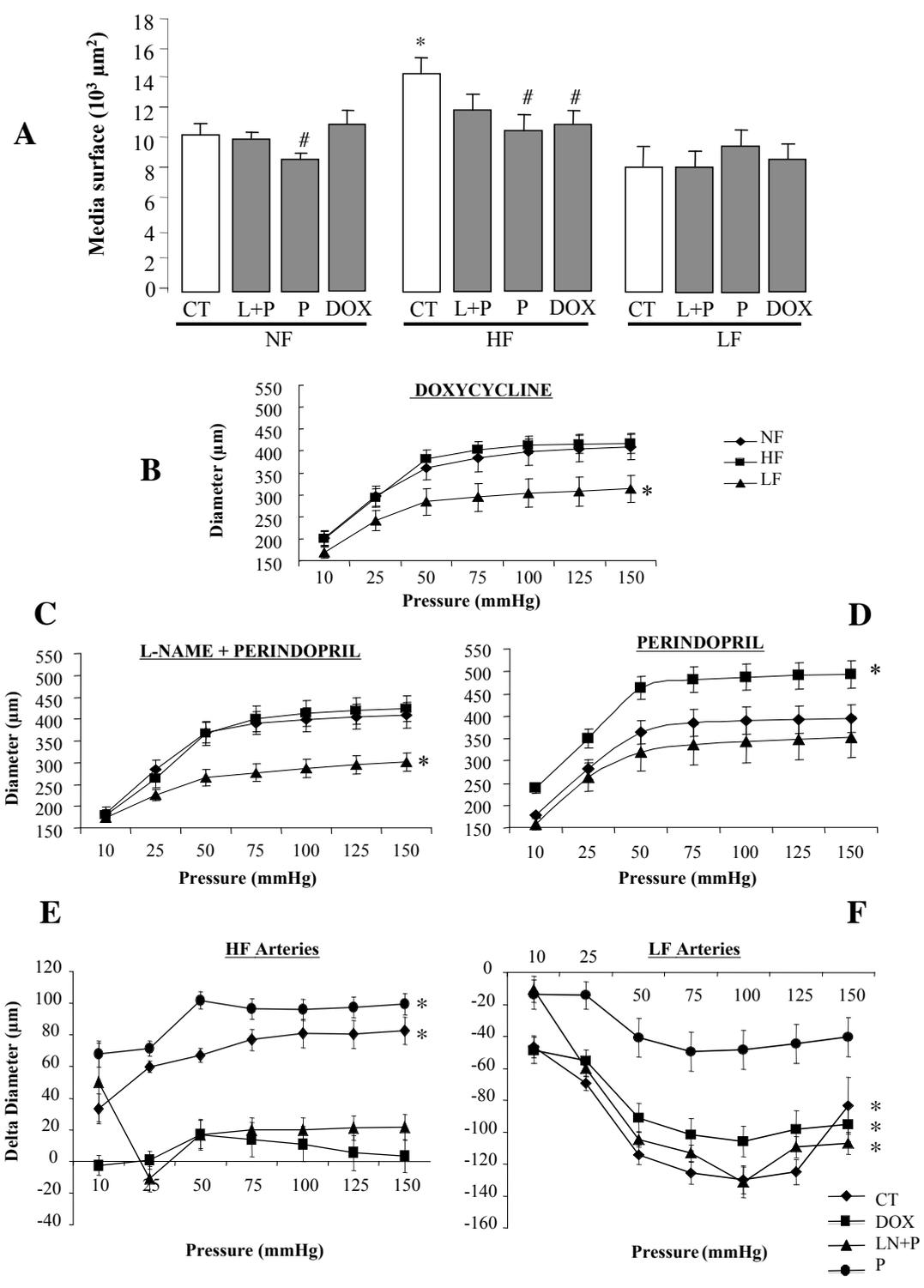
**B**  
MMP-9  
(4 days)



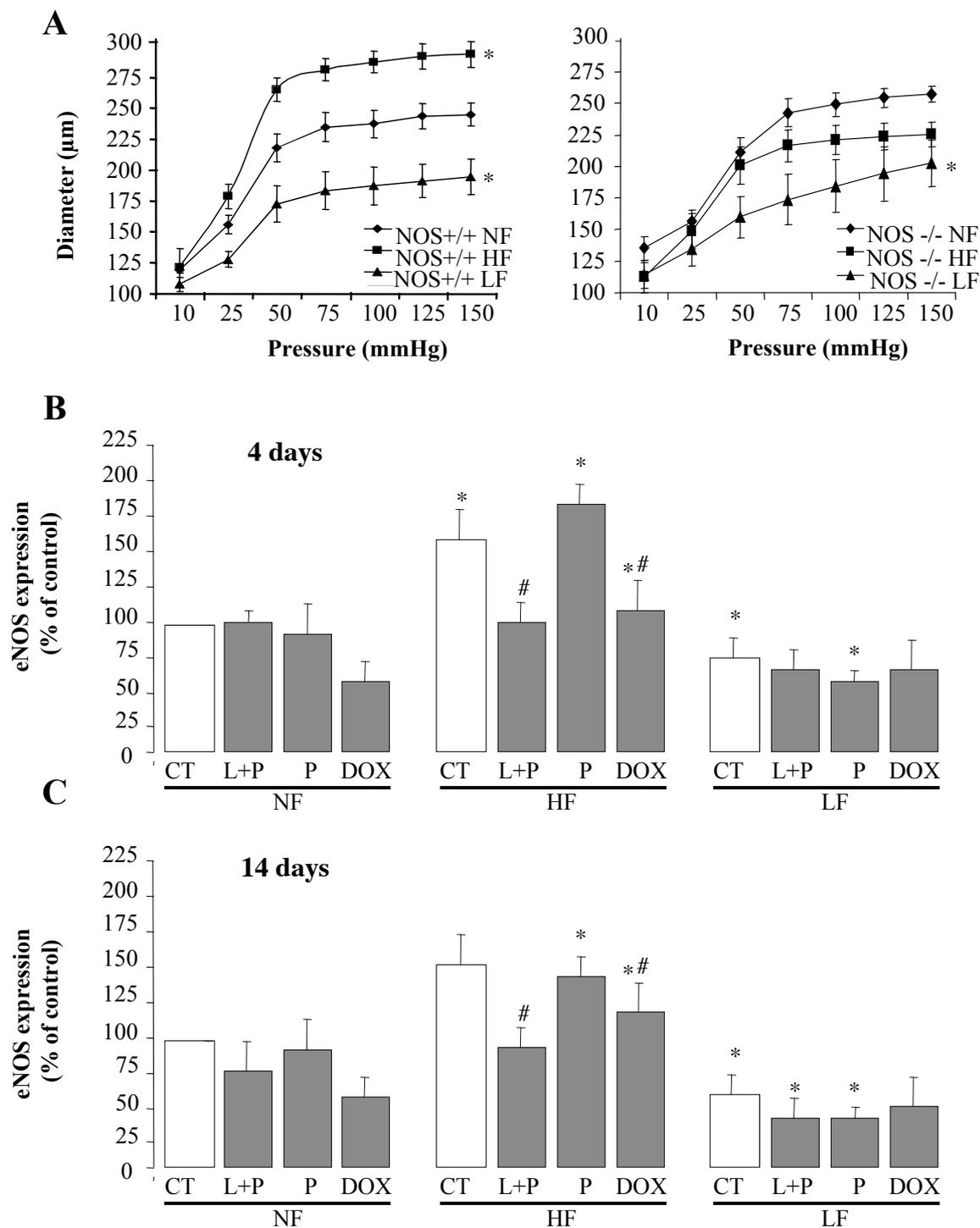
**Figure 2:**

Immunostaining of MMP-2 (A) and MMP-9 (B) was performed in the tunica media of NF, HF and LF mesenteric resistance arteries isolated from untreated rats 4 and 14 days after ligations. Immunostaining was visualized using confocal microscopy (typical images obtained after 4 days are shown in panel A for MMP2 and B for MMP9). A negative control without the primary antibody is presented. Quantification of MMP-2 and MMP-9 staining 4 days (C) and 14 days (D) after ligation was performed using 3-4 sections per artery. n=6 rats per group. Values are means±SEM.

\*P<0.05, HF or LF versus NF.



**Figure 3:** Media surface (A) of isolated mesenteric arteries submitted to arterial ligation for 14 days. Arteries were isolated from untreated rats (CT) and from rats treated with L-NAME plus perindopril (LN+P), perindopril alone (P) and doxycycline (DOX). n=6 rats per group. Pressure-diameter (passive arterial diameter) relationship was determined in mesenteric arteries isolated from rats submitted to arterial ligation for 14 days. Arteries were isolated from rats treated with doxycycline (B), L-NAME and perindopril (C) and perindopril alone (D). Diameter changes due to chronic increases or decreases in blood flow was expressed as the difference in diameter between HF and NF arteries (E) and between LF and NF arteries (F). n=8 rats per group. Values are means±SEM. \*P<0.05 for HF or LF versus NF arteries, #P<0.05 for HF, LF or NF in treated groups versus the corresponding artery in the control group.



**Figure 4:**

Pressure-diameter (passive arterial diameter) relationship determined in mesenteric arteries isolated from control +/+ (left panel) and eNOS-knockout -/- (right panel) mice submitted to arterial ligation for 14 days (A).

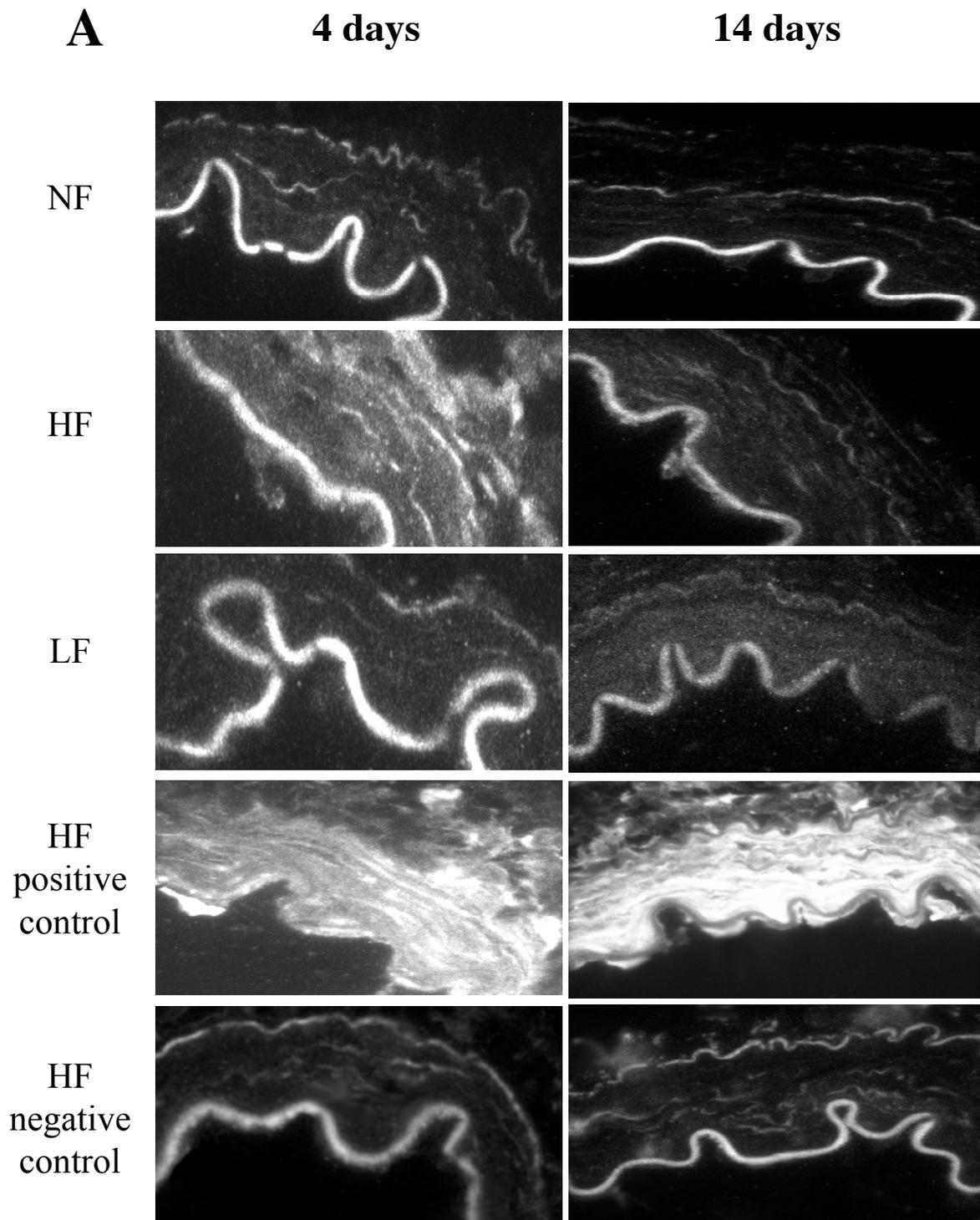
\*P<0.05, eNOS-knockout (n=5 mice) versus control mice (n=4 mice).

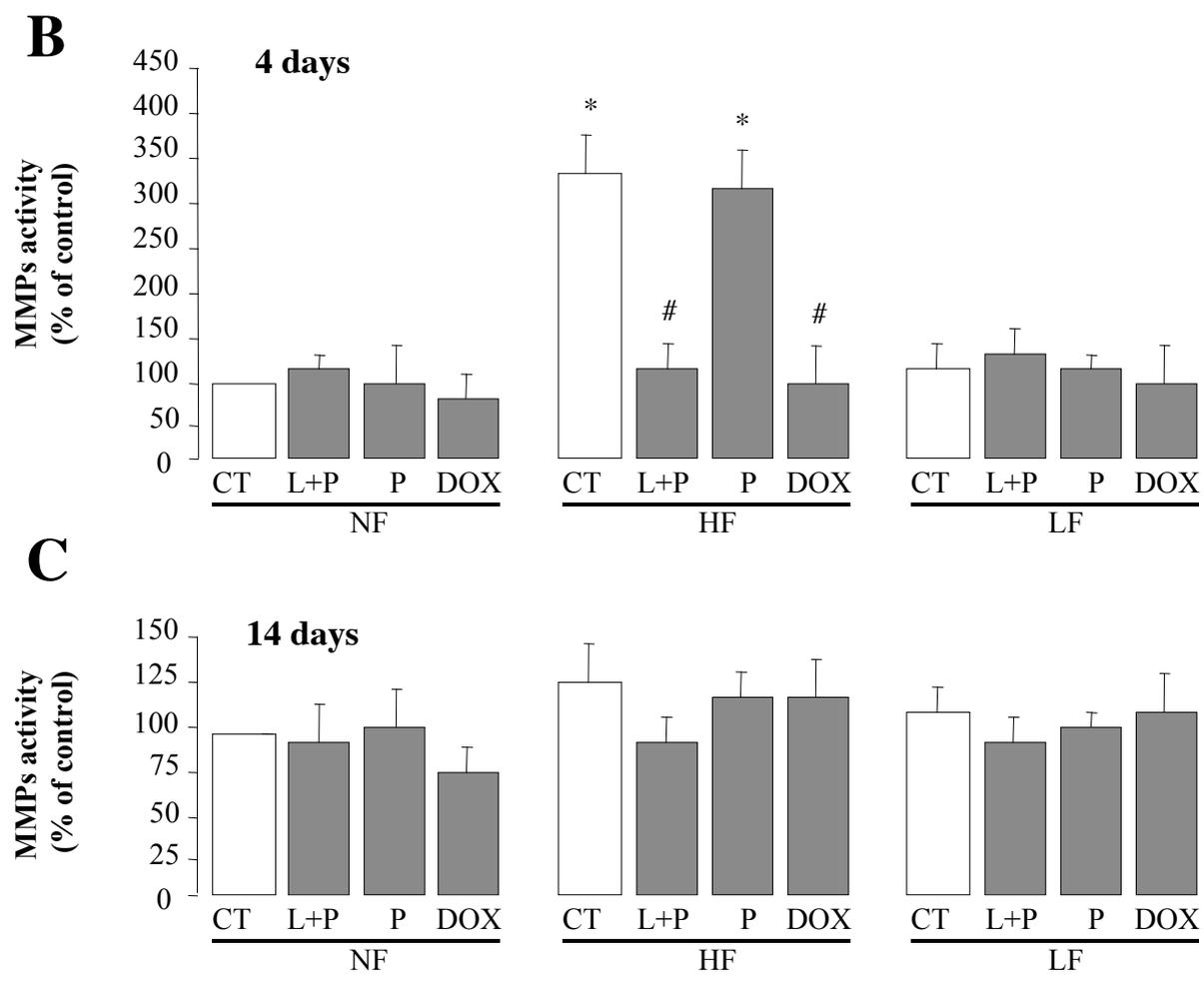
Endothelial nitric oxide synthase (eNOS) expression in NF, HF and LF arteries isolated from untreated rats and rats treated with L-NAME and perindopril, perindopril alone or doxycycline. Arteries were isolated 4 (B) and 14 days (C) after ligations. Protein expression is given as percentage of NF arteries (mean ± SEM is presented).

\*P<0.05, HF or LF versus NF (n=7 rats per group).

#P<0.05 for HF, LF or NF in treated groups versus the corresponding artery in the control group.

Figure 5





**Figure 5:**  
 Gelatinase activity in NF, HF and LF arteries 4 and 14 days after ligations, using confocal microscopy. Positive and negative controls are represented (A).  
 Quantification of gelatinolytic activity was performed using image density analysis in untreated rats and rats treated with L-NAME and perindopril, perindopril alone or doxycycline (6 rats per group). Data is presented as percentage of NF arteries 4 (B) and 14 days (C) after ligations (mean ± SEM).  
 \*P<0.05, HF or LF versus NF.  
 #P<0.05 for HF, LF or NF in treated groups versus the corresponding artery in the control group.

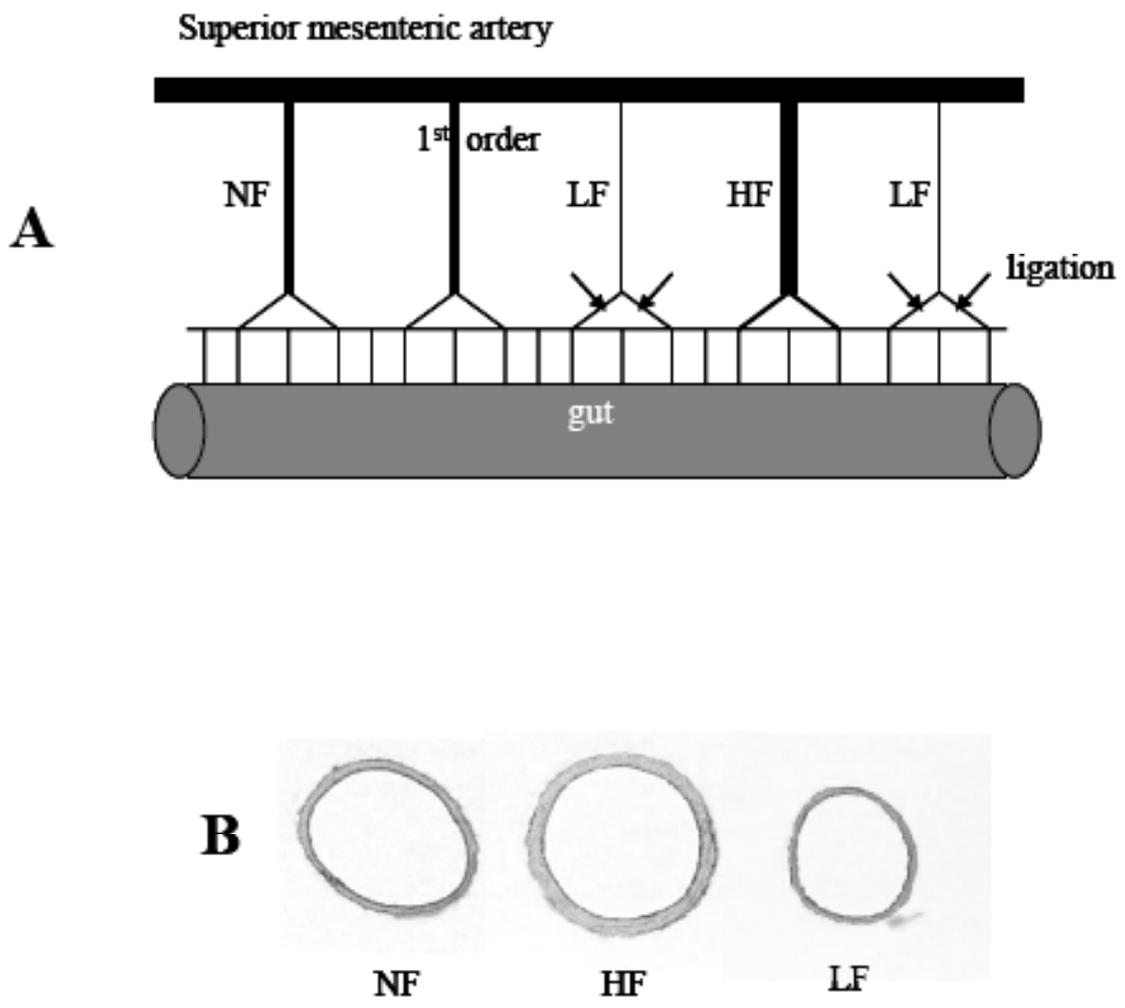


Figure I (supplement data)

Diagram of the mesenteric circulation in rats (A). The locations of the ligations of the second order

mesenteric artery branches are indicated by arrows. Ligated arteries were designated as low flow (LF) arteries. The artery located between two ligated vessels was designated as a high flow (HF) artery. Other arteries had a normal flow (NF).

Typical examples of histological cross sections of arteries exposed for 14 days to NF, HF or LF in control rats vessels (B).