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Role of estrogens and age in flow-mediated outward remodeling of rat mesenteric arteries


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Tarhouni K, Freidja ML, Guihot AL, Vessieres E, Grimaud L, Toutain B, Lenfant F, Arnal JF, Loufrani L, Henrion D. Role of estrogens and age in flow-mediated outward remodeling of rat mesenteric resistance arteries. Am J Physiol Heart Circ Physiol 307: H504–H514, 2014.—In resistance arteries, a chronic increase in blood flow induces hypertrophic outward remodeling. This flow-mediated remodeling (FMR) is absent in male rats aged 10 mo and more. As FMR depends on estrogens in 3-mo-old female rats, we hypothesized that it might be preserved in 12-mo-old female rats. Blood flow was increased in vivo in mesenteric resistance arteries after ligation of the side arteries in 3- and 12-mo-old male and female rats. After 2 wk, high-flow (HF) and normal-flow (NF) arteries were isolated for in vitro analysis. Arterial diameter and cross-sectional area increased in HF arteries compared with NF arteries in 3-mo-old male and female rats. In 12-mo-old rats, diameter increased only in female rats. Endothelial nitric oxide synthase expression and endothelium-mediated relaxation were higher in HF arteries than in NF arteries in all groups. ERK1/2 phosphorylation, NADPH oxidase subunit expression levels, and arterial contractility to KCl and to phenylephrine were greater in HF vessels than in NF vessels in 12-mo-old male rats only. Ovariectomy in 12-mo-old female rats induced a similar pattern with an increased contractility without diameter increase in HF arteries. Treatment of 12-mo-old male rats and ovariectomized female rats with hydralazine, the antihypertensive compound, restored FMR remodeling and normalized arterial contractility in HF vessels. Thus, we found that FMR of resistance arteries remains efficient in 12-mo-old female rats compared with age-matched male rats. A balance between estrogens and vascular contractility might preserve FMR in mature female rats.

RESISTANCE ARTERIES control local blood flow to organs. They are able to adapt in response to changes in hemodynamic conditions, even in the adult. A chronic increase in blood flow (shear stress) in these vessels induces outward hypertrophic remodeling associated with increased endothelium [nitric oxide (NO)]-dependent dilation, as shown in male (2, 5, 33) and female rats (37). Chronic increases in blood flow occur in physiological conditions such as growth, chronic exercise, or pregnancy as well as in ischemic disorders, where they contribute to collateral arteries growth (7, 36). Flow-mediated outward remodeling of resistance arteries involves a transient inflammatory response (1) and oxidative stress (2) that favors the formation of peroxinitrite, which then activates metalloproteinases and extracellular matrix digestion (2, 8, 14, 16, 19). The final step, leading to diameter enlargement, requires a dilator stimulus (17). Flow-mediated outward remodeling is also associated with a compensatory increase in wall mass (11, 32) due to ANG II type 1 receptor activation of ERK1/2 (11).

As shown in male rats, flow-mediated remodeling of mesenteric resistance arteries does not occur in male rats aged 12 mo (40) or 24 mo (15). Although no diameter enlargement occurred in arteries after increasing blood flow, endothelium-mediated dilation in the same arteries submitted to high flow (HF) could be increased, as shown in 2-yr-old male rats (47). Nevertheless, these experiments were performed in male rats (37). Nevertheless, we have recently shown that flow-mediated remodeling in 3-mo-old female rats requires the activation of endothelial estrogen receptor-α (37).

An epidemiological study (22) has demonstrated that women, before menopause, are better protected than men against cardiovascular diseases. Both animal and human studies (9, 28) have shown that the decline in ovarian function is associated with decreased NO production. Indeed, stimulation of the NO pathway explains, at least in part, the effect of estrogens on the vascular wall (35, 46). Thus, we hypothesized that flow-mediated remodeling would occur in 12-mo-old female rats and not in 12-mo-old male rats due to the activation of estrogen-dependent pathways increasing endothelium-dependent relaxation and/or reducing smooth muscle contractility.

We investigated flow-mediated remodeling in 3- and 12-mo-old male and female rats. To induce remodeling, mesenteric arteries were alternatively ligated so that blood flow increased in vivo in one artery only (32).
METHODS

Animal protocol. Three- and twelve-month-old male and female Wistar rats (Charles River, L’Arbresles, France) were anesthetized [isoflurane (2.5%)], and submitted to surgery to increase blood flow in one mesenteric artery, as previously described (14). Briefly, three consecutive first-order arteries were used. Ligatures were applied to second-order branches (11). The artery located between the two ligated vessels was designated as the HF artery. Arteries located at distance of the ligated arteries were used as control [normal flow (NF)]. Rats were treated with tretoprenorphine [Temgesic (0.1 mg/kg sc)] before and after surgery.

The protocol conformed with European Community standards on the care and use of laboratory animals (Authorization No. 00577) and with National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). The protocol was approved by the ethical committee (Permit No. CEEA PiD 2008.10).

Fourteen days after surgery, rats were anesthetized [isoflurane (2.5%)]. Arterial blood pressure was measured in the carotid artery (13), and blood flow was measured in NF and HF mesenteric arteries using a Transonic flow probe (18). The mesentery was then quickly removed and placed in ice-cold physiological salt solution of the following composition (in mM): 130 NaCl, 15 NaHCO3, 3.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 11 glucose, 1.6 CaCl2, and 5 HEPES (pH 7.4, PO2: 160 mmHg, PCO2: 37 mmHg). HF and NF mesenteric arteries were gently dissected and divided into two segments, proximal for the functional experiment and distal for histological and biochemical experiments.

In another series of experiments, 12-mo-old female rats were ovariec-tomized (38) and 12-mo-old male rats were orchidectomized. After 1 wk, rats were submitted to the protocol described above.

Other groups of 12-mo-old male rats and ovariec-tomized female rats were treated with hydralazine (200 mg/l in drinking water, 3 wk) (20), candesartan (2 mg/kg sc) before and after surgery. Animals were treated with hydralazine (200 mg/l in drinking water, 3 wk) (11), or tempol (10 mg·kg–1·day–1, 3 wk) (2) (11). These rats were submitted to mesenteric arteries ligature as described above. Treatments started 1 wk before surgery. The total duration of the treatments was 3 wk.

Finally, 12-mo-old male rats were orchidectomized and, 1 wk later, submitted to surgery as described above.

Blood was collected in rats for estradiol (E2) measurement using a commercially available kit (Estradiol EIA Kit no. 58225, Cayman Chemical).

Pressure-diameter relationship in mesenteric arteries in vitro. Arterial segments were cannulated at both ends and mounted in a video-monitored perfusion system (Living System, LSI, Burlington, VT) as previously described (21). Briefly, cannulated arterial segments were bathed in a 5-ml organ bath containing Ca2+-free physiological salt solution containing EGTA (2 mmol/l) and sodium nitroprusside (SNP; 10 µmol/l). Pressure steps (10–150 mmHg) were then applied to the arterial segment to determine passive arterial diameter. Pressure and diameter were measured continuously using a video dimension analyzer (LSI), and data were collected using a Biopac data-acquisition system (Biopac MP100 and Acqknowledge software, La Jolla, CA). Pressure was then set at 75 mmHg, and the arterial segment was then fixed with formaldehyde to measure media cross-sectional area (CSA) and wall thickness as previously described (43).

Pharmacological profile of isolated NF and HF arteries. Other arterial segments (2 mm long each) were dissected and mounted in a wire myograph (Danish Myo Technology) (12). After three contractions induced by KCl (80 mmol/l), cumulative concentration-response curves to phenylephrine (0.001–10 µmol/l), SNP (0.001–10 µmol/l), and ACh (0.01–10 µmol/l) were performed. Cumulative concentration-response curves to ACh were obtained before and after incubation (20 min) with the NO synthase (NOS) inhibitor N-nitro-L-arginine methyl ester (L-NAME; 100 µmol/l). ACH-dependent relaxation was performed after precontraction of the arteries with phenylephrine and serotonin to 50% of their maximal contractile response.

Western blot analysis. The remaining segments of HF and NF arteries were collected, quickly frozen, and pulverized in liquid nitrogen. The sample powders obtained were resuspended in lysis buffers. Vessel extracts were incubated on ice for 30 min and then centrifuged (14,000 rpm, 20 min at 4°C).

Proteins (30 µg total protein/sample) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were then incubated with the primary antibody (BD Biosciences, endothelial NOS (eNOS): 1:500, MnSOD: 1:1,000, CuSOD: 1:1,000, ERK1/2: 1:1,000, phospho-ERK1/2: 1:1,000, p67phox: 1:1,000, p22phox: 1:1,000, gp91phox: 1:1,000, and [β-actin: 1:5,000]) and incubated with horseradish peroxidase-conjugated secondary antibody (Amersham) at room temperature. Proteins were visualized using an ECL-Plus Chemiluminescence kit (Amersham) (5, 42).

Statistical analysis. Results are expressed as means ± SE. Data for concentration-response curves to phenylephrine, ACh, and SNP are expressed as the maximal extent of inhibition (I₅₀% max) or maximal effect of the drug (E₅₀% max) and EC₅₀ or IC₅₀. Significance of the differences between groups was determined by ANOVA (two-way ANOVA for consecutive measurements for pressure-diameter curves or one-way ANOVA followed by a Bonferroni test or paired t-test for the other groups). P values of <0.05 were considered significant.

RESULTS

Characteristics of experimental animals. Mean arterial blood pressure was not significantly affected by age or sex (Table 1). Body weight was greater in 12-mo-old male rats than in 3-mo-old male rats and in 12-mo-old male rats than in 12-mo-old female rats (Table 2). Treatment of 12-mo-old male rats and ovariec-tomized female rats with hydralazine, candesartan, or tempol did not significantly affect body weight and mean blood pressure (Table 1). E₂ blood levels were higher in female rats than in male rats and was not significantly affected by age (Table 1).

Blood flow measured in mesenteric arteries, 14 days after arterial ligation, was significantly greater in HF arteries than in NF arteries in all groups without a significant effect of age or sex (Table 1).

Shear stress calculated in these arteries was normalized in HF arteries (not different from NF vessels) in all groups except in 12-mo-old male rats (Table 1).

Table 1. Body weight, mean arterial pressure, and blood estradiol as well as blood flow and shear stress in mesenteric arteries submitted to HF and NF measured in male and female rats aged 3 or 12 mo

<table>
<thead>
<tr>
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<th>12 mo old</th>
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<tr>
<td>Female Rats</td>
<td></td>
<td></td>
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<tr>
<td>Body weight, g</td>
<td>307 ± 12</td>
<td>360 ± 17*</td>
<td>342 ± 18</td>
<td>438 ± 24†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>95 ± 4</td>
<td>95 ± 5</td>
<td>94 ± 4</td>
<td>98 ± 5</td>
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<tr>
<td>Blood estradiol, pg/ml</td>
<td>19.4 ± 3.3</td>
<td>17.2 ± 2.1</td>
<td>7.1 ± 1.7†</td>
<td>6.4 ± 1.4†</td>
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<tr>
<td>Blood flow, µl/min</td>
<td>354 ± 42</td>
<td>370 ± 53</td>
<td>389 ± 50</td>
<td>402 ± 63</td>
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<tr>
<td>Male Rats</td>
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</tr>
<tr>
<td>Blood flow, µl/min</td>
<td>684 ± 74‡</td>
<td>725 ± 66‡</td>
<td>754 ± 76‡</td>
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<td>Shear stress, dyne/cm²</td>
<td>49.1 ± 7.0</td>
<td>43.4 ± 5.1</td>
<td>53.9 ± 6.8</td>
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<td>NF artery</td>
<td>44.6 ± 6.2</td>
<td>41.5 ± 5.3</td>
<td>49.1 ± 5.4</td>
<td>62.9 ± 6.0‡</td>
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<td>Ratio of HF to NF × 100</td>
<td>90.9</td>
<td>95.6</td>
<td>91.2</td>
<td>159.3</td>
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Values are means ± SE; n = 12 rats/group. Blood flow was measured in normal flow (NF) and high-flow (HF) arteries in all groups. MAP, mean arterial pressure. *P < 0.05, 12- vs. 3-mo-old rats; †P < 0.05, male rats vs. female rats; ‡P < 0.05, HF vs. NF arteries.

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Arterial diameter and structure. Passive arterial diameter was significantly greater in HF arteries than in NF arteries in 3-mo-old female and male rats as well as in 12-mo-old female rats (Fig. 1, A–C). In 12-mo-old male rats, diameter was equivalent in HF and NF vessels (Fig. 1D).

In both 3- and 12-mo-old male and female rats, CSA was greater in HF arteries than in NF arteries. Nevertheless, neither age nor sex significantly affected CSA and wall-to-lumen ratios in NF or HF arteries (Fig. 1E). As arterial diameter and CSA both increased in HF arteries in female rats (3 and 12 mo old) and in 3-mo-old male rats, the wall-to-lumen ratio was equivalent to that measured in NF vessels (Fig. 1F). In contrast, in 12-mo-old male rats, the wall-to-lumen ratio was significantly greater in HF arteries than in NF vessels (Fig. 1F).

### Table 2. Body weight and MAP measured in 12-mo-old male rats and 12-mo-old OVX female rats with or without treatment with hydralazine, candesartan, or tempol

<table>
<thead>
<tr>
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<th>Hydralazine</th>
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<td><strong>OVX female rats</strong></td>
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<tr>
<td>Body weight, g</td>
<td>380 ± 26</td>
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<td>366 ± 25</td>
<td>391 ± 30</td>
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<tr>
<td>MAP, mmHg</td>
<td>96 ± 5</td>
<td>93 ± 4</td>
<td>92 ± 4</td>
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<tr>
<td><strong>Male rats</strong></td>
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<tr>
<td>Body weight, g</td>
<td>451 ± 27</td>
<td>465 ± 31</td>
<td>441 ± 30</td>
<td>460 ± 26</td>
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<tr>
<td>MAP, mmHg</td>
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Values are means ± SE; n = 8 rats/group. OVX, ovariectomized.

**Arterial diameter and structure.** Passive arterial diameter was significantly greater in HF arteries than in NF arteries in 3-mo-old female and male rats as well as in 12-mo-old female rats (Fig. 1, A–C). In 12-mo-old male rats, diameter was equivalent in HF and NF vessels (Fig. 1D).

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eNOS expression level and endothelium-dependent relaxation. The expression level of eNOS was significantly higher in HF arteries than in NF arteries in all groups of rats (Fig. 2A).

In both NF and HF arteries, ACh induced a concentration-dependent relaxation. The $I_{\text{max}}$ for ACh was significantly higher in HF arteries than in NF arteries in all groups without significant influence of age or sex (Fig. 2B). The IC$_{50}$ for ACh

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![Fig. 1. A–D: arterial diameter measured in response to stepwise increases in intraluminal pressure in mesenteric arteries submitted to a chronic increase in blood flow (HF) or to normal blood flow (NF). E and F: cross-sectional area (CSA; E) and wall-to-lumen ratio (F) measured in NF and HF vessels fixed under a pressure of 75 mmHg. Arteries were isolated from male and female rats aged 3 or 12 mo. Values are means ± SE; n = 12 rats/group. *P < 0.05, HF vs. NF arteries.](http://ajpheart.physiology.org/)

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**Table 2. Body weight and MAP measured in 12-mo-old male rats and 12-mo-old OVX female rats with or without treatment with hydralazine, candesartan, or tempol**

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**Fig. 1. A–D: arterial diameter measured in response to stepwise increases in intraluminal pressure in mesenteric arteries submitted to a chronic increase in blood flow [high flow (HF)] or to normal blood flow (NF). E and F: cross-sectional area (CSA; E) and wall-to-lumen ratio (F) measured in NF and HF vessels fixed under a pressure of 75 mmHg. Arteries were isolated from male and female rats aged 3 or 12 mo. Values are means ± SE; n = 12 rats/group. *P < 0.05, HF vs. NF arteries.**
was not significantly different in HF arteries than in NF arteries in all groups (Fig. 2B).

The inhibition of NO synthesis with l-NAME reduced ACh-dependent relaxation in all groups by ~50%. This effect was not significantly affected by age or sex (Fig. 2C).

In NF and HF arteries, SNP induced a concentration-dependent relaxation. The $I_{\text{max}}$ and IC$_{50}$ for SNP were not significantly different in HF arteries than in NF arteries in all groups (Fig. 3).

**Expression level analyses of SOD, ERK1/2, and NADPH oxidase subunits.** Expression levels of MnSOD (Fig. 4A) and CuZnSOD (Fig. 4B) were not significantly different in HF arteries than in NF arteries in all groups.

The ratio of phospho-ERK1/2 to ERK1/2 was not significantly different in HF arteries than in NF arteries in all groups except in 12-mo-old male rats (Fig. 4C). Indeed, in 12-mo-old male rats, the ERK1/2 expression level in HF vessels was significantly greater than in NF arteries.

Expression levels of gp91$^{\text{phox}}$, p22$^{\text{phox}}$, and p67$^{\text{phox}}$ (Fig. 5) were not significantly different in HF arteries than in NF arteries in all groups except for p22$^{\text{phox}}$ and p67$^{\text{phox}}$, which were greater in HF vessels than in NF vessels in 12-mo-old male rats.

**Phenylephrine-induced contraction.** Phenylephrine induced a concentration-dependent contraction in both NF and HF mesenteric arteries. The $E_{\text{max}}$ for phenylephrine was not sig-
significantly greater in HF arteries than in NF arteries in all groups except for 12-mo-old male rats. In this group, the $E_{\max}$ for phenylephrine was significantly greater in HF arteries than in NF arteries (Fig. 6A). Nevertheless, the $EC_{50}$ for phenylephrine was not significantly different in HF arteries than in NF arteries in all groups (Fig. 6B). Similarly, KCl (80 mmol/l)-induced contractions were not significantly greater in HF arteries than in NF arteries except for 12-mo-old rats (Fig. 6C).

Flow-mediated remodeling in ovariectomized female rats. In ovariectomized 12-mo-old female rats, no significant diameter enlargement occurred in HF arteries (Fig. 7A). As a consequence, shear stress was not normalized (30.1 ± 5.2 vs. 57.7 ± 7.4 dyn/cm²). This was accompanied by a significant increase in CSA (Fig. 7B) and in the wall-to-lumen ratio (Fig. 7C). ACh-mediated relaxation was increased in HF arteries with enhanced $I_{\max}$ without a change in $IC_{50}$ (Fig. 7D). The inhibitory effects of l-NAME (Fig. 7E) and SNP-mediated relaxation (Fig. 7F) were equivalent in HF and NF vessels. Phenylephrine-mediated contractions were greater in HF arteries than in NF vessels with an increase in $E_{\max}$ and without a change in $EC_{50}$ (Fig. 7G). The ratio of phospho-ERK1/2 to ERK1/2 (Fig. 7H) and p67phox expression level (Fig. 7I) were both significantly greater in HF arteries than in NF arteries. In orchidectomized 12-mo-old male rats, arterial diameter was equivalent in HF and NF arteries (Fig. 7J).

Flow-mediated remodeling in 12-mo-old rats treated with a vasodilator or an antioxidant. In both 12-mo-old male rats and 12-mo-old ovariectomized female rats treated with hydralazine (Fig. 8, A and B), tempol (Fig. 8, C and D), or candesartan (Fig. 8, E and F), arterial diameter was significantly higher in HF arteries than in NF arteries. After treatment of 12-mo-old male rats with hydralazine, tempol, or candesartan, phenylephrine-mediated contraction in HF arteries was equivalent to that measured in HF arteries isolated from untreated 3-mo-old rats and lower than in untreated 12-mo-old rats. Consequently, shear stress in HF arteries was equivalent to that in NF vessels (e.g., in hydralazine-treated 12-mo-old rats: 31.5 ± 5.1 vs. 35.3 ± 5.7 dyn/cm², NF vs. HF arteries). A similar observation was made in ovariec-tomized 12-mo-old female rats (Fig. 8G).

DISCUSSION

The main findings of the present study were that a diameter enlargement in response to a chronic increase in blood flow in vivo was observed in arteries of 3-mo-old male and female rats and in arteries of 12-mo-old female rats, whereas it was absent in arteries of 12-mo-old male rats. An increase in arterial contractility, rather than endothelium dysfunction, in arteries of 12-mo-old male rats is likely to explain this effect. Estrogens in 12-mo-old female rats opposed this increased contractility.

The ability of resistance arteries to remodel in response to a chronic increase in blood flow is reduced in aging (15, 40), although increasing chronically blood flow remains associated with increased endothelium-mediated vasodilatation in HF arteries, as shown in the present study and in previous works (15, 47). Indeed, we found that eNOS expression levels were not different between 3 and 12 mo of age in mesenteric arteries of both male and female rats. Nevertheless, due to the lack of diameter enlargement of HF arteries of 12-mo-old male rats, the wall-to-lumen ratio, an index of hypertrophy (24) associated with cardiovascular disorders (30, 34, 41), was greater than in 3-mo-old rats. Indeed, the increase in CSA allows normalization of wall stress in HF arteries. In 3-mo-old male rats, this compensatory increase in CSA (6) depends on ANG II type 1 receptor-dependent phosphorylation of ERK1/2 (11).

Twelve months is about half the lifespan of the rat. Our finding that diameter expansion in response to a chronic increase in flow did not occur in 12-mo-old male rats is in agreement with observations showing that the incidence of cardiovascular events increases in men aged 40 yr and more (31). On the other hand, an epidemiological study (22) has demonstrated that premenopausal women are better protected against cardiovascular diseases than men. Estrogens exert their effect on blood vessels, at least in part, through the activation of eNOS and the consecutive production of NO. Estrogens stimulate eNOS activity acutely through a membrane-association nongenomic effect (27), leading to acute dilation, as shown in the rat aorta (4). Estrogens also increase NO bioavailability chronically through a reduction in ROS production (45). In the present study, no obvious changes in NO-dependent dilation or eNOS expression were found in 12-mo-old male rats compared with 3-mo-old male and female rats. Thus, a change in the NO pathway is not likely to explain the absence of diameter expansion. Consequently, we investigated oxidative stress, which is involved in flow-mediated remodeling (2, 8, 16, 19) and arterial contractility (10). Our data showed a larger contractile response to phenylephrine in HF arteries of 12-mo-old male rats. This was associated with greater expression levels of NAD(P)H oxidase subunits (p22phox and p67phox).
and greater ERK1/2 phosphorylation. These observations are in line with previous works showing that ERK1/2 activation (23, 25, 26, 39) and oxidative stress (10) are involved in smooth muscle contractility. This increased contractility observed in HF arteries, not in NF vessels, might oppose diameter expansion in 12-mo-old male rats. This is in agreement with a previous study (48) showing a rise in NO production in HF arteries, which was opposed by O$_2^\cdot$/H$_2$O$_2$ produced by NAD(P)H oxidase in spontaneously hypertensive rats. This is also in agreement with our previous study showing in 2-yr-old male rats that hydralazine, without affecting systemic blood pressure (20), restored flow-mediated diameter enlargement in rat mesenteric arteries (15). Indeed, we confirmed this hypothesis by submitting 12-mo-old male rats and ovariectomized female rats to chronic treatment with hydralazine. This treatment restored HF remodeling and reduced the increased contractility.

As arteries in 12-mo-old female rats remained able to increase their diameter in response to a chronic increase in blood flow, we hypothesized that estrogens could oppose the increase in contractility observed in HF arteries. Indeed, estrogens activate NO production in cultured endothelial cells and induce vasodilation in isolated arteries (4). This hypothesis was supported by our finding showing that no diameter enlargement occurred in 12-mo-old ovariectomized female rats in association with hypertrophy, increased contractility, and higher p22$^{phox}$ and p67$^{phox}$ expression with higher ERK1/2 phosphorylation. This is a pattern similar to that observed in 12-mo-old male rats. Thus, estrogens most likely mediate the difference between 12-mo-old male and female rats observed in the present study. A similar effect can be obtained with other vasodilator agents, in male or in female rats, as shown by the effects of hydralazine, candesartan, or tempol in 12-mo-old male or female rats. Our observations agree with those of a previous study (29) showing that impaired flow-mediated remodeling in diseased conditions could be restored with losartan.
In summary, we found that maturity in male rats was associated with a lost capacity of resistance arteries to respond to a chronic increase in blood flow by a diameter enlargement mainly because of the increased contractility observed in the HF artery. This increase in contractility was counteracted by the action of estrogens in age-matched female rats. Consequently, in 12-mo-old female rats, mesenteric arteries remain able to increase their diameter in response to chronic increases in blood flow in vivo. This finding may certainly contribute to better understanding the effect of estrogens in premenopausal women. The effect of estrogens on the mechanism of flow-mediated outward hypertrophic remodeling of resistance arteries is summarized in Fig. 9.

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Fig. 5. A–C: expression levels of gp91phox (A), p22phox (B), and p67phox (C) using Western blot analysis in mesenteric arteries submitted to HF or NF. Arteries were isolated from male and female rats aged 3 and 12 mo. Values are means ± SE; n = 12 rats/group. *P < 0.05, HF vs. NF arteries.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Fig. 6. A–C: vascular reactivity to phenylephrine (PE; A and B) and KCl (80 mM; C) measured in mesenteric arteries submitted to HF or NF. Arteries were isolated from male and female rats aged 3 or 12 mo. A: Wall force (mN) after maximal contraction to PE (E\text{max}) is shown. Values are means ± SE; n = 12 rats/group. *P < 0.05, HF vs. NF arteries.

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Fig. 7. A–I: arterial diameter in response to stepwise increases in intraluminal pressure (A), CSA (B), wall-to-lumen ratio (C), ACh-mediated relaxation (D), inhibitory effect of L-NAME (E), SNP-mediated relaxation (F), PE-mediated contraction (G), ratio of phospho-ERK1/2 to ERK1/2 (H), and p67phox expression level (I) were measured in mesenteric arteries submitted to a chronic increase in blood flow (HF). Control arteries were submitted to NF. Arteries were isolated from ovariectomized (OVX) 12-mo-old female rats. In the last group, diameter in NF and HF arteries was measured in castrated 12-mo-old male rats (J). Values are means ± SE; n = 10 rats/group for OVX female rats and 8 rats/group in castrated male rats. *P < 0.05, HF vs. NF arteries.


Fig. 8. Arterial diameter was measured in response to stepwise increases in intraluminal pressure in mesenteric arteries submitted to a chronic increase in blood flow (HF) or NF. Arteries were isolated from 12-mo-old male and OVX female rats. Rats were treated with hydralazine (Hydra; A and B), tempol (Temp; C and D), or candesartan (Cand; E and F). PE-mediated contraction was measured in HF arteries in the six groups of treated rats compared with 3-mo-old male rats (3-m and cont) and untreated 12-mo-old male rats (cont) and compared with intact 12-mo-old female rats. G: E_{max} values. Values are means ± SE; n = 8–10 rats/group. *P < 0.05, HF vs. NF arteries.
Fig. 9. A chronic increase in blood flow in mesenteric resistance arteries induces outward hypertrophic remodeling within ~7 days. The chronic increase in blood flow first induces a moderate inflammatory response with production of monocyte chemotactic protein (MCP)-1 (1, 37) followed by macrophage accumulation in the perivascular area (1). This first step leads to oxidative stress (1, 2, 17, 37), which results in the production of peroxinitrite (ONOO) and then to the activation of matrix metalloproteinases (MMPs) (14). MMPs induce then a partial dissociation of the extracellular matrix (ECM). In the last step, vasodilator agents, such as NO produced by eNOS (14), PGI2 produced by cyclooxygenase (COX)-2 (3), and/or carbon monoxide (CO) produced by heme oxygenase (HO)-1 (17, 18), increase arterial diameter until the normalization of wall shear stress (44). In parallel, activation of the ANG II type 1 receptor (AT1R) and of MAPK ERK1/2 allow a compensatory hypertrophy aiming at normalization of wall strain induced by the diameter expansion (11). The effect of estradiol (E2) on flow-mediated outward hypertrophic remodeling is multiple with the activation of eNOS overexpression (37) and with the reduction of oxidative stress and ERK1/2 activation, thus allowing preserving outward remodeling in mature male rats (present study).