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Rémi Varin, Paul Mulder, Vincent Richard, Fabienne Tamion, Corinne Devaux, Jean-Paul Henry, Françoise Lallemand, Guy Lerebours and Christian Thuillez

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# Exercise Improves Flow-Mediated Vasodilatation of Skeletal Muscle Arteries in Rats With Chronic Heart Failure

## Role of Nitric Oxide, Prostanoids, and Oxidant Stress

Rémi Varin, BS; Paul Mulder, PhD; Vincent Richard, PhD; Fabienne Tamion, MD;  
Corinne Devaux, BS; Jean-Paul Henry, BS; Françoise Lallemand, BS;  
Guy Lerebours, MD, PhD; Christian Thuillez, MD, PhD

**Background**—Flow-mediated dilatation (FMD) of the peripheral arteries may be impaired in chronic heart failure (CHF), and this could contribute to the increased peripheral resistance and exercise intolerance that occur with this disease. Physical exercise improves the FMD of large conduit arteries in CHF, but whether a similar impairment also occurs in smaller arteries is unknown. The mechanisms of the changes in FMD after CHF or exercise are also unknown.

**Methods and Results**—FMD was assessed in isolated, perfused, and preconstricted gracilis muscle arteries from sham-operated rats or CHF rats (coronary artery ligation) who were either sedentary or exercised (30-minute swimming period twice a day for 10 weeks, starting 7 days after ligation). In animals with hemodynamic and echographic signs of CHF, FMD was abolished and converted into vasoconstriction (percent change in diameter after 370  $\mu$ L/min flow: sham,  $42 \pm 5\%$ ; CHF,  $-4 \pm 3\%$ ;  $P < 0.05$ ). Exercise partially restored FMD ( $18 \pm 3\%$ ;  $P < 0.05$  versus CHF). In sham rats, FMD was abolished by the nitric oxide-synthase inhibitor *N*<sup>ω</sup>-nitro-L-arginine (L-NA) but unaffected by the cyclooxygenase inhibitor diclofenac or the free radical scavenger N-(2-mercaptopropionyl)-glycine (MPG). In arteries from sedentary CHF rats, FMD was not modified by L-NA, but it was partially restored by diclofenac or MPG. In exercised CHF rats, FMD was abolished by L-NA and only moderately improved by diclofenac or MPG. Likewise, endothelial nitric oxide synthase mRNA expression (determined by reverse transcription polymerase chain reaction at the level of the gracilis muscle) was reduced by CHF, and this was prevented by exercise.

**Conclusions**—CHF abolishes the FMD of small arteries by impairing the nitric oxide pathway, increasing oxidant stress, and releasing a prostanoid-contracting factor. Exercise partially restores FMD by increasing expression of endothelial nitric oxide synthase and preventing the production of vasoconstrictor prostanoids and free radicals. Such restoration of FMD might contribute to the increase in exercise capacity after physical exercise in CHF. (*Circulation*. 1999;99:2951-2957.)

**Key Words:** exercise ■ dilatation ■ heart failure ■ muscle, skeletal ■ prostaglandins ■ nitric oxide synthase

Exercise capacity is reduced in patients with chronic heart failure (CHF) because of modifications in the complex interplay of cardiac, systemic, and/or local vascular mechanisms.<sup>1-3</sup> A reduced increase of nutrient blood flow in response to a given level of exercise, caused by the impairment of the dilator response of small skeletal muscular arteries, can explain this diminished exercise capacity. Furthermore, physical exercise augments exercise capacity and increases limb blood flow during exercise in patients with CHF.<sup>4</sup>

Recent *in vivo*<sup>4,5</sup> and *in vitro*<sup>6-8</sup> studies show that CHF induces a marked reduction in the production and/or release of endothelium-derived vasodilator factors, such as nitric oxide (NO), in response to acetylcholine. However, whether CHF also affects the release of NO induced by a more

physiological stimulus, such as flow (ie, flow-mediated vasodilatation) at the level of resistance arteries, is largely unknown. Moreover, although impaired endothelium-dependent vasodilatation may simply be the consequence of decreased production of NO, other factors may also contribute, such as oxygen-derived free radicals or prostanoids. However, the relative contribution of these different factors in the impaired flow-mediated vasodilatation observed in heart failure is unknown.

The impaired endothelium-dependent vasodilatation of the peripheral resistance arteries observed in CHF may be related in part to long-term adaptations secondary to the long-term decrease in blood flow.<sup>9,10</sup> Thus, it is possible that repeated increases in blood flow, as is the case with physical exercise, may improve flow-mediated vasodilatation in CHF. Indeed,

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From the Department of Pharmacology, VACOMED, Rouen University Medical School, Rouen, France.

Correspondence to C. Thuillez, MD, PhD, Service de Pharmacologie, CHU de Rouen, 76031 Rouen Cedex, France. E-mail Christian.Thuillez@chu-rouen.fr

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exercise increases flow-dependent, NO-mediated vasodilatation of peripheral conduit arteries in human CHF.<sup>11</sup> However, whether a similar improvement occurs at the level of smaller peripheral arteries is unknown. Moreover, the relative roles of NO and of other vasoactive factors in the changes induced by exercise are also largely unknown.

Thus, we investigated in a rat model of CHF whether physical exercise affects flow-mediated dilatation of peripheral muscular arteries; we also investigated the relative roles of NO, prostanoids, and oxidant stress in these changes.

## Methods

### Animals and Treatment

Myocardial infarction was produced in 10-week-old male Wistar rats (Charles River, Saint Aubin Les Elbeuf, France) by left coronary artery ligation using the method of Pfeffer et al<sup>12</sup> that has been modified in our laboratory.<sup>13,14</sup> Seven days after ligation, rats with CHF were randomized into 2 groups (either sedentary or exercised). Physical training consisted of a 30-minute swimming period in the morning (in a 150-L water tank; water temperature, 27°C to 28°C), which was followed at least 6 hours later by a second 30-minute swimming period. Swimming sessions were supervised to avoid floating and/or clinging of individual animals. Sedentary rats were not placed in the water tank. This protocol was applied 5 days a week for 10 weeks, starting 7 days after ligation.

### Hemodynamic Parameters Assessed in Anesthetized Rats

After 10 weeks, rats were anesthetized with pentobarbital (50 mg · kg<sup>-1</sup> IP). The right carotid artery and the right external jugular vein were cannulated with a micromanometer-tipped catheter (SPR 407, Millar Instruments) and advanced into the aorta and thoracic vena cava, respectively, to record arterial and central venous pressures. The aortic catheter was then advanced into the left ventricle (LV) to record LV pressure and its maximal rate of rise (dP/dt<sub>max</sub>). All tracings were recorded on a physiological recorder (Windowgraph, Gould, France). At the end of the hemodynamic studies, rats were euthanized, and the heart was taken out, weighed, and placed in Bouin fixative. Cardiac collagen density was then assessed, as described previously,<sup>3,15</sup> in the viable tissue (ie, septum) of the LV. Perivascular collagen was excluded from the measurement.

Transthoracic Doppler echocardiographic studies were performed as previously described<sup>14,16</sup> using an echocardiographic system equipped with a 7-MHz transducer (Acuson 128 XP/10C). Measurements were performed by a single observer (P.M.) who was blinded to prior results and treatment groups.

### In Vitro Vascular Studies

After assessment of the hemodynamic parameters, an artery of the gracilis muscle was carefully isolated under a dissection microscope and transferred to an arteriograph filled with oxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>), PSS composed of (in mmol/L): NaCl 119, NaHCO<sub>3</sub> 24, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.18, MgSO<sub>4</sub>·7H<sub>2</sub>O 1.17, CaCl<sub>2</sub> 1.6, and glucose 5.5. The artery was mounted on 2 glass micropipettes (Living Systems Instrumentation). Both proximal (inflow) and distal (outflow) micropipettes were connected with silicone tubing to a pressure-servo syringe system. The vessel segments were stretched to their in vivo length, and intraluminal pressure was set to 30 mm Hg, as described previously.<sup>3,17</sup>

### Experimental Procedure

After the equilibration period, the pressurized arteries were precontracted by adding phenylephrine, after which, cumulative concentrations of acetylcholine (10<sup>-9</sup> to 10<sup>-4</sup> mol/L) were added at 0 flow. The vessels were then washed and again precontracted with phenylephrine to assess basal flow-mediated dilatation (FMD). For this purpose, perfusate flow rate was increased from 0 to 370 μL · min<sup>-1</sup>

**TABLE 1. Primer Sequences and Sizes of Expected PCR Products**

Genes	Primer Sequences	Size, bp
eNOS		
Upper (5')	TTCCGGCTGCCACCTGATCCTAA	325
Lower (3')	AACATGTGCTCTGCTCGAGGCA	
GAPDH		
Upper (5')	CAGAGCAAGAGAGGCATCCT	535
Lower (3')	GGCAGCTCATAGCTCCTTCTC	

in a stepwise manner by changing the inflow and outflow pressures. Each flow rate was maintained for ≈2 minutes to allow the vessel to reach steady-state diameter. Three separate series of experiments were performed in arteries obtained from different rats; in these experiments, the roles of prostaglandins, NO, and free radicals were assessed using the cyclooxygenase inhibitor diclofenac (10<sup>-5</sup> mol/L), the NO synthase inhibitor *N*<sup>ω</sup>-nitro-L-arginine (L-NA; 10<sup>-5</sup> mol/L) or the free radical scavenger *N*-(2-mercaptopropionyl)-glycine (MPG; 10<sup>-5</sup> mol/L), respectively. In each vessel, FMD was assessed twice: first in the absence of inhibitor (basal values) and then 20 minutes after the administration of the corresponding inhibitor. Preliminary experiments showed that repetitions of the flow-diameter curves in the absence of treatment led to similar responses, thus ruling out a time-related effect.

At the end of each experiment, maximal vasodilatation was assessed by the response to sodium nitroprusside (10<sup>-4</sup> mol/L) under zero-flow conditions.

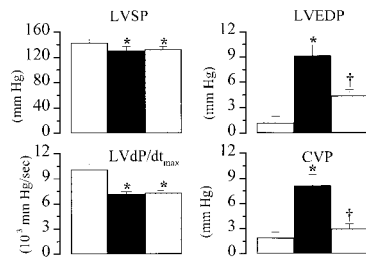
### Semiquantitative PCR

Total RNA was extracted from rat muscle using acid guanidinium thiocyanate–phenol–chloroform extraction.<sup>18</sup> Then, 4 μg of total RNA were reverse transcribed in 30 μL (final volume) of a reaction buffer made of the following (in mmol/L): Tris-HCl 30 (pH 8.3), DTT 10, KCl 85, MgCl<sub>2</sub> 3, and each dNTP 10 (each), as well as 80 U of RNasin (Promega), 800 U of reverse transcriptase (GIBCO BRL), and 8.3 pmol of random hexamer primer (Pharmacia). The mixture was incubated for 60 minutes at 37°C and then heated at 95°C for 5 minutes. Ten aliquots of the reverse transcription (RT) product was used for the polymerase chain reaction (PCR) amplification. This was added to 50 μL of PCR mix containing 5 μL of 10× PCR buffer, 1 μL of dNTPs (2 mmol/L each), 1 μCi of <sup>33</sup>P-dATP, 5 μL of MgCl<sub>2</sub> (2.25 mmol/L), 1.25 U of DNA *Taq* polymerase (Promega), and 2 μL of 3' and 5' DNA-specific primers (100 mmol/L for endothelial NO synthase [eNOS] and 50 mmol/L for GAPDH [Bioprobe Systems]). Primers were chosen to have a GC content of 40% to 60%. The primer sequences and the sizes of the expected PCR products are shown in Table 1.

The number of PCR cycles was 30 for eNOS and 20 for GAPDH. PCR products were electrophoresed through 7.5% polyacrylamide gel. Molecular weight markers were from Pharmacia. Gels were stained in deionized water containing 0.5 mg/mL ethidium bromide, illuminated with UV light, and photographed using Polaroid films. They were measured by quantitative scanning densitometry of autoradiographs (Biocom).

### Statistical Analysis

All reported values are given as mean ± SEM. The responses to acetylcholine and sodium nitroprusside and the responses to flow are expressed as percentages of the reversal of the phenylephrine-induced constriction. Differences between the sham, sedentary, and exercised CHF rats, as well as the effect of the pretreatment with L-NA, diclofenac, and MPG were determined using ANOVA for repeated measurements (SYSTAT software, SPSS Inc). Moreover, the hemodynamic, echocardiographic, and morphometric parameters in the sham, sedentary, and exercised CHF rats were compared by *t*



**Figure 1.** LVSP, LVEDP, CVP, and LVdP/dt<sub>max</sub> in sham-operated rats (open bars) and in infarcted rats, either sedentary (solid bars) or exercised (hatched bars). Results are expressed as mean±SEM. \**P*<0.05 vs sham; †*P*<0.05 vs sedentary CHF rats (*n*=10 in each group).

test or by ANOVA followed by a Tukey test for multiple comparisons. Differences were considered significant at the level *P*<0.05.

## Results

### Mortality and Exclusions

A total of 94 rats (27 sham, 35 CHF[sedentary], and 32 CHF plus exercise [Ex]) were included in the study. During the treatment period, 8 sedentary and 6 exercised rats died. Furthermore, 9 animals were excluded from the study because of technical difficulties during dissection.

### Hemodynamic Measurements in Anesthetized Rats

Figure 1 illustrates the cardiac hemodynamics and central venous pressure (CVP) measured in anesthetized animals 10 weeks after ligation. As compared with sham-operated animals, CHF decreased LV systolic pressure (LVSP) and LV dP/dt<sub>max</sub> and increased both LV end-diastolic pressure (LVEDP) and CVP. Compared with sedentary CHF rats, physical exercise did not modify LVSP and LV dP/dt<sub>max</sub>; however, exercise did significantly reduce both LVEDP and CVP.

### Echocardiographic Studies

Ten weeks after surgery, exercise reduced the CHF-induced increases in LV end-diastolic and systolic diameters, as well as LV wall thickness (Table 2). Exercise also tended to

**TABLE 2. Echocardiographic Parameters**

	Rats		
	Sham-Operated	Sedentary CHF	Exercised CHF
PWD, mm	2.0±0.1	1.5±0.1*	1.8±0.1†
PWT, %	73±5	40±3*	48±5*
LVDD, mm	6.7±0.1	10.3±0.1*	9.6±0.2*†
LVSD, mm	3.7±0.2	8.8±0.2*	8.1±0.3*†
FS, %	45±2	15±1*	19±3*
SV, mL/beat	0.61±0.02	0.45±0.02*	0.53±0.02*†
CO, mL/min	246±6	182±6*	199±8*
CI, mL·min <sup>-1</sup> ·kg <sup>-1</sup>	472±16	324±13*	416±18†

PWD indicates posterior wall diastolic thickness; PWT, posterior wall thickening; LVDD, left ventricular diastolic diameter; LVSD, left ventricular systolic diameter; FS, fractional shortening; SV, stroke volume; CO, cardiac output; and CI, cardiac index.

\**P*<0.05 vs sham rats; †*P*<0.05 vs sedentary CHF rats.

**TABLE 3. Internal Gracilis Artery Diameter at Baseline, After Precontraction by Phenylephrine, and After Endothelium-Independent Dilatation by Sodium Nitroprusside**

Rat Group	Internal Diameter, μm		
	Baseline	Phenylephrine	Sodium Nitroprusside (10 <sup>-4</sup> mol/L)
Sham-operated	243±11	104±5	223±8
Sedentary CHF	241±7	105±6	234±7
Exercised CHF	238±7	108±4	228±9

improve LV fractional shortening and posterior wall thickening. Compared with sedentary rats with CHF, exercise also increased cardiac output, cardiac index, and stroke volume.

### In Vitro Vascular Studies

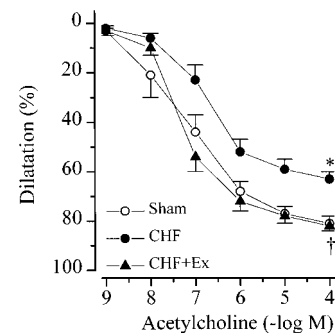
The basal values of arterial diameter before or after phenylephrine (precontracted) were similar in the 3 groups (Table 3).

#### Acetylcholine-Mediated Dilatation

Figure 2 illustrates the responses to acetylcholine in the 3 groups. Compared with sham-operated animals, CHF induced a moderate but significant impairment in the vasodilator response to acetylcholine (maximal responses: sham, 81±3%; CHF, 63±3%; *P*<0.05). Exercise normalized the dilator response to acetylcholine (82±2%). Neither CHF nor exercise affected the response to the NO donor sodium nitroprusside (Table 3).

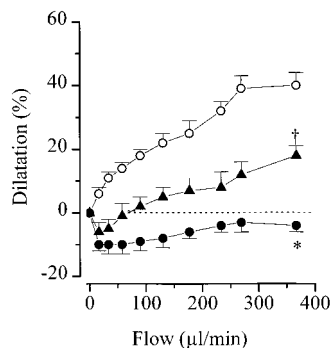
#### Flow-Mediated Dilatation

Figure 3 illustrates the changes in arterial diameter in response to stepwise increases in intraluminal flow at the baseline. In arteries isolated from sham animals, increases in flow induced a progressive dilatation with a maximum dilatation of 42±5% at 370 μL·min<sup>-1</sup>. CHF abolished FMD and converted it into a vasoconstriction. This constrictor response was more marked at low levels (at 17 μL·min<sup>-1</sup>, it was -8±3%) than at high levels of flow (at 370 mL·min<sup>-1</sup>, it was -4±3%). Exercise restored a significant degree of flow-mediated vasodilatation, and this effect was observed at all levels of flow except the lowest. At the highest level of



**Figure 2.** Concentration-response curves to acetylcholine in isolated, pressurized gracilis arteries taken from sham-operated rats (○) or CHF rats, either sedentary (●) or exercised (▲). Results are expressed as mean±SEM. \**P*<0.05 vs sham; †*P*<0.05 vs sedentary CHF rats (repeated measures ANOVA; *n*=15 in each group).





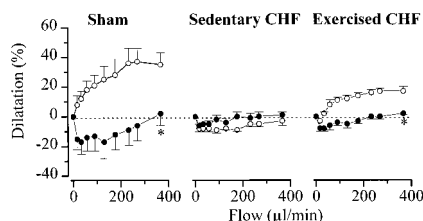
**Figure 3.** Flow-response curves in isolated, pressurized, and precontracted gracilis arteries taken from sham-operated rats (○) or CHF rats, either sedentary (●) or exercised (▲). Results are expressed as mean±SEM. \* $P<0.05$  vs sham; † $P<0.05$  vs sedentary CHF rats (repeated measures ANOVA;  $n=15$  in each group).

flow ( $370 \mu\text{L} \cdot \text{min}^{-1}$ ), the increase in diameter was  $18 \pm 3\%$ . However, this vasodilator response remained significantly lower than that obtained in the arteries of sham-operated rats. Finally, in contrast to rats with CHF, pilot experiments showed that exercise did not affect FMD in sham rats ( $370 \text{ mL} \cdot \text{min}^{-1}$ : sedentary sham,  $42 \pm 5\%$ ; exercised sham,  $35 \pm 9\%$ ;  $n=7$ ;  $P=\text{NS}$ ).

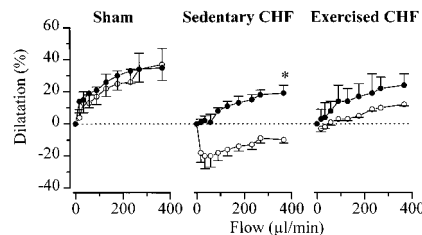
#### Mechanisms of Flow-Mediated Vasodilatation

Figure 4 illustrates the effects of the NO synthase inhibitor L-NA on FMD in the 3 groups. In arteries isolated from sham-operated animals, FMD was abolished by L-NA. Indeed, at the highest value of flow ( $370 \mu\text{L} \cdot \text{min}^{-1}$ ), L-NA reduced FMD from the basal value of  $42 \pm 5\%$  to  $2 \pm 8\%$  ( $P<0.05$ ). In sedentary CHF rats, FMD was not affected by L-NA (FMD at  $370 \mu\text{L} \cdot \text{min}^{-1}$  before and after L-NA:  $-4 \pm 3\%$  and  $1 \pm 2\%$ , respectively). Similar to sham animals, the FMD of arteries from exercised CHF rats was abolished by L-NA. Indeed, at the highest value of flow tested, L-NA reduced FMD from  $18 \pm 3\%$  to  $2 \pm 1\%$  ( $P<0.05$ ).

Figure 5 shows the effects of the cyclooxygenase inhibitor diclofenac on FMD in the 3 groups. In sham-operated animals, FMD was unaffected by diclofenac (FMD at  $370 \mu\text{L} \cdot \text{min}^{-1}$  before and after diclofenac:  $37 \pm 10\%$  and  $35 \pm 8\%$ , respectively). In contrast, in sedentary CHF rats, diclofenac partially restored flow-induced dilatation (FMD at  $370 \mu\text{L} \cdot \text{min}^{-1}$  before and after diclofenac:  $-10 \pm 2\%$  and  $19 \pm 5\%$ , respectively;  $P<0.05$ ). Diclofenac also slightly and nonsig-



**Figure 4.** Flow-response curves in isolated, pressurized, and precontracted gracilis arteries taken from sham-operated rats or CHF rats (either sedentary or exercised) in basal conditions (○) or in the presence of the nitric oxide inhibitor L-NA (●). Results are expressed as mean±SEM. \* $P<0.05$  vs basal value (repeated measures ANOVA;  $n=6$  in each group).



**Figure 5.** Flow-response curves in isolated, pressurized, and precontracted gracilis arteries taken from sham-operated rats or CHF rats (either sedentary or exercised) in basal conditions (○) or in the presence of the cyclooxygenase inhibitor diclofenac (●). Results are expressed as mean±SEM. \* $P<0.05$  vs basal value (repeated measures ANOVA;  $n=6$  in each group).

nificantly improved FMD in arteries from exercised CHF rats. This effect seemed limited compared with that observed in sedentary CHF rats (FMD at  $370 \mu\text{L} \cdot \text{min}^{-1}$  before and after diclofenac:  $12 \pm 1\%$  and  $19 \pm 3\%$ , respectively).

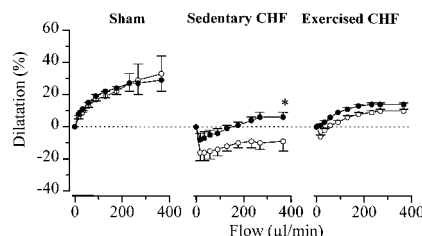
The effects of MPG on FMD are shown in Figure 6. MPG did not affect the responses obtained in sham rats. In CHF rats, MPG abolished the flow-induced vasoconstriction and reestablished a moderate degree of active vasodilatation (FMD at  $370 \mu\text{L} \cdot \text{min}^{-1}$  before and after MPG:  $-9 \pm 6\%$  and  $6 \pm 3\%$ , respectively;  $P<0.05$ ). MPG also slightly and non-significantly improved FMD in arteries taken from exercised CHF rats (FMD at  $370 \mu\text{L} \cdot \text{min}^{-1}$  before and after MPG:  $10 \pm 1\%$  and  $14 \pm 1\%$ , respectively). Again, this effect was less marked than in arteries from sedentary CHF rats.

#### Expression of eNOS

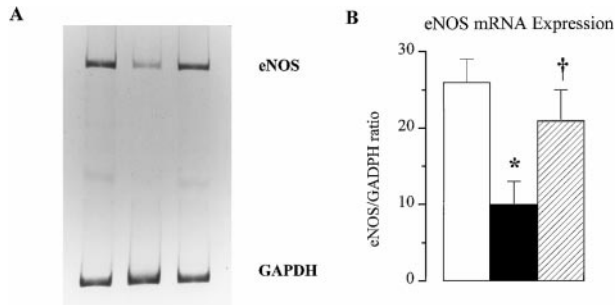
The effect of CHF or exercise on eNOS mRNA expression in the gracilis muscle is shown in Figure 7. In pilot experiments, we verified (by immunohistochemistry) that eNOS was absent from the skeletal muscle and was confined to endothelial cells (data not shown). eNOS mRNA expression was significantly reduced in the CHF group, and it was normalized by exercise (eNOS/GAPDH ratio: sham,  $26 \pm 3$ ; CHF,  $10 \pm 3$  [ $P<0.05$  versus sham]; exercise,  $21 \pm 4$  [ $P<0.05$  versus sedentary CHF and NS versus sham]).

#### Cardiac Histomorphometry

Table 4 shows the values for infarct size, scar length, scar surface, heart weight, body weight, heart weight/body weight ratio, and collagen density in the noninfarcted LV. Infarct size, scar length, and the scar surface of animals euthanized after



**Figure 6.** Flow-response curves in isolated, pressurized, and precontracted gracilis arteries taken from sham-operated rats or CHF rats (either sedentary or exercised) in basal conditions (○) or in the presence of the free radical scavenger MPG (●). Results are expressed as mean±SEM. \* $P<0.05$  vs basal value (repeated measures ANOVA;  $n=6$  in each group).



**Figure 7.** RT-PCR analysis demonstrating the effects of CHF and exercise on eNOS mRNA expression in gracilis muscles. A, Representative RT-PCR analysis of eNOS mRNA expression. B, Bar graph of eNOS/GAPDH ratio of mRNA expression in sham-operated rats (open bars) and in CHF rats, either sedentary (solid bars) or exercised (hatched bars). Results are expressed as mean ± SEM. \* $P < 0.05$  vs sham; † $P < 0.05$  vs sedentary CHF rats ( $n = 6$  in each group).

10 weeks were identical in sedentary and exercised CHF rats. Compared with sham-operated animals, CHF induced significant increases in heart weight, heart weight/body weight ratio, and LV collagen density. Exercise did not modify heart weight, but it did decrease body weight, and thus it increased the heart weight/body weight ratio. Exercise also decreased LV collagen density.

### Discussion

The present study performed using the rat model of CHF induced by coronary artery ligation shows (1) that CHF abolishes FMD at the level of small peripheral arteries and unmasks a flow-mediated vasoconstriction. This alteration is mainly because of an altered release of bioactive NO, together with the release of a vasoconstrictor prostanoid. The altered release of NO is associated with a reduced expression of eNOS and with the production of reactive oxygen species, which are known to inactivate NO. The study also shows (2) that physical exercise markedly increases FMD in CHF, both through an increased release of NO (associated with an increased eNOS expression and a decreased production of reactive oxygen species), and a decreased release of vasoconstrictor prostanoids.

In our experiments, 10 weeks after induction of myocardial infarction, the increase in LVEDP and CVP and the marked decrease in LVSP and LV  $dp/dt_{max}$  illustrated the presence of

CHF. Moreover, echographic results showed marked LV dilatation and a decrease in fractional shortening, cardiac output, and stroke volume. In this context of severe CHF, we were also able to demonstrate significant beneficial cardiac effects of long-term exercise, both in terms of hemodynamics (reduced LVEDP and CVP, increased cardiac index) and in terms of remodeling (decreased LV dilatation assessed by echocardiography and decreased LV collagen density). Moreover, the fact that infarct size, which is the major determinant of the severity of CHF in rats,<sup>12</sup> was similar in the 2 groups of rats suggests that the hemodynamic and vascular changes observed in our study are not caused by initial differences in the severity of CHF before the start of exercise but represent true effects of exercise.

In CHF, most of the evaluation of endothelial dysfunction has been performed using pharmacological stimuli such as acetylcholine.<sup>3-6,8</sup> Indeed, in our experiments, we confirmed that CHF moderately reduced the vasodilator response to acetylcholine. However, apart from 1 study in human large peripheral arteries,<sup>11</sup> no experiments have assessed the effect of CHF on the endothelial responses to changes in intraluminal flow, which represent a major physiological stimulus for endothelium-dependent vasodilatation. In this context, our experiments demonstrate that CHF abolished the dilator response to changes in intraluminal flow and even unmasked a flow-induced vasoconstriction. In human peripheral conduit arteries, CHF reduces but does not abolish flow-mediated vasodilatation.<sup>11</sup> Although comparisons between the 2 studies must be performed with caution, they do suggest that CHF affects the endothelial function of small arteries more markedly than that of large conductance vessels, as previously demonstrated with acetylcholine.<sup>8</sup>

One of the major mechanisms by which CHF affects FMD is through a decrease of flow-induced, NO-mediated vasodilatation. Indeed, in arteries isolated from sham rats, the NO synthase inhibitor L-NA abolished FMD, but L-NA did not affect FMD in arteries isolated from CHF rats, suggesting that NO-mediated vasodilatation was absent in this situation.

The impaired NO-mediated vasodilatation could be caused either by a decreased production of NO or an increased degradation of NO, for example, secondary to an increased production of reactive oxygen species that are potent inactivators of NO.<sup>19</sup> In our experiments, evaluation by RT-PCR of eNOS mRNA expression at the level of the gracilis muscle (in which eNOS expression is limited to endothelial cells) shows that CHF markedly reduces this expression; this is in agreement with previous results obtained at the level of the dog aorta.<sup>20</sup> Likewise, the fact that a free radical scavenger improves FMD in arteries from rats with CHF but not in those isolated from sham rats suggests that CHF is indeed associated with an increased production of reactive oxygen species. This is in agreement with recent human data showing that intra-arterial infusion of vitamin C improves brachial artery dilatation in response to hyperemia in humans with CHF, suggesting that oxidant stress is involved in impaired vasodilatation in this context.<sup>21</sup> Taken together, our data suggest that the impaired NO-mediated response in CHF is due at least in part both to a decreased expression of eNOS and to an increased degradation of NO by reactive oxygen species.

**TABLE 4. Histomorphometric Parameters**

	Rats		
	Sham-Operated	Sedentary CHF	Exercised CHF
Infarct size, % of LV	...	36 ± 3	35 ± 2
Scar length, mm	...	8.6 ± 0.6	8.1 ± 0.6
Scar surface, mm <sup>2</sup>	...	6.5 ± 0.5	7.1 ± 0.6
Body weight, g	528 ± 32	527 ± 14	453 ± 8†
Heart weight, g	1.25 ± 0.09	1.54 ± 0.08*	1.61 ± 0.08*
Heart weight/body weight, mg/g	2.16 ± 0.09	2.62 ± 0.15*	3.19 ± 0.15†
LV collagen density, %	2.32 ± 0.11	3.22 ± 0.19*	2.72 ± 0.19†

\* $P < 0.05$  vs sham rats; † $P < 0.05$  vs sedentary CHF rats.

However, it is not known whether other factors contribute to this impairment, for example, changes in the transduction pathway linking shear stress to activation of NO synthase.

In the context of marked decrease of NO, long-term physical exercise partially restored FMD. In these arteries, FMD could be abolished by the NO-synthase inhibitor L-NA, suggesting that it is mediated by NO. Because the impaired eNOS mRNA expression that we observed in CHF was prevented by exercise, we suggest that the increased NO response after exercise is due, at least in part, to prevention of the impaired eNOS expression. In addition, the fact that the effect of MPG was attenuated in arteries from exercised CHF rats compared with those of sedentary CHF animals suggests that exercise also attenuates the production of reactive oxygen species, and this could also contribute to the increased NO-mediated responses through a reduced degradation of NO.

Several hypotheses could explain the modifications of eNOS expression after CHF or exercise. First, a long-term increase in flow (because of an arteriovenous fistula) is associated with increased endothelium-dependent relaxations to acetylcholine<sup>9,10</sup> and with increased aortic eNOS expression.<sup>22</sup> Thus, it is likely that the long-term decrease in tissue flow observed in our model of CHF (as indirectly demonstrated by the decreased cardiac output) is a major stimulus for decreased eNOS expression.<sup>23</sup> In contrast, the increased tissue flow induced by exercise (which increased cardiac output in our experiments) is a trigger for the increased eNOS expression in this context. This hypothesis is also supported by recent experiments performed in dogs without CHF, in which long-term exercise increases eNOS expression and production of NO.<sup>24</sup>

Alternatively, changes in eNOS expression might reflect modifications of eNOS mRNA stability. Indeed, tumor necrosis factor- $\alpha$ , which is elevated in CHF,<sup>25–27</sup> decreases eNOS expression by shortening the half-life of mRNA.<sup>28</sup> Furthermore, CHF may be associated with local tissue hypoxia,<sup>29</sup> which reduces eNOS expression.<sup>30</sup> Thus, in theory, both the increased production of cytokines and the development of hypoxia may contribute to altered eNOS expression in CHF.

Our results with MPG indirectly suggest that increased oxidant stress may also contribute to the impaired NO-mediated responses in CHF. Because NO inactivates oxygen-derived free radicals, especially superoxide anions, it is possible that the reduced production of NO may by itself increase oxidant stress, as demonstrated in cultured endothelial cells after NO synthase inhibition.<sup>31</sup> Alternatively, increased oxidant stress in CHF may be a consequence of the activation of the renin-angiotensin system, as angiotensin II may induce activation of reduced NADH/NAPDH oxidases, leading to severe endothelial dysfunction.<sup>32</sup>

In our experiments, the cyclooxygenase inhibitor diclofenac partially restored FMD in arteries from CHF rats, but it had no effect in arteries from sham animals. Thus, in addition to the decreased NO-mediated response, a concomitant production of a vasoconstrictor prostaglandin also contributes to impaired FMD and is probably responsible for the flow-induced vasoconstriction that was observed in this situation. A similar increased cyclooxygenase-dependent contraction

has already been observed in spontaneously hypertensive rats.<sup>33</sup> The fact that the effect of diclofenac was attenuated by exercise suggests that it also attenuates the production of vasoconstrictor prostanoids, and this could also contribute to improved FMD after exercise.

In conclusion, our study demonstrates that CHF abolishes flow-mediated vasodilatation of skeletal muscular arteries and unmasks a vasoconstrictor response. Long-term physical exercise partially restores flow-mediated vasodilatation. This impaired response seen in CHF is due both to decreased production of NO (associated with decreased eNOS expression and increased inactivation of NO by free radicals) and to the production of a vasoconstrictor prostanoid. The beneficial effect of physical exercise on FMD is related to an increase in eNOS mRNA expression, decreased oxidant stress, and a blunted release of the vasoconstrictor prostanoids. Changes in the FMD of such peripheral arteries may have important consequences, both in terms of local tissue perfusion and of exercise tolerance.

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