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

Role of Nitric Oxide, Prostanoids, and Oxidant Stress

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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 μL/min flow: sham, 42±5%; CHF, −4±3%; P<0.05). Exercise partially restored FMD (18±3%; P<0.05 versus CHF). In sham rats, FMD was abolished by the nitric oxide–synthase inhibitor N\textsubscript{\text{\textregistered}}-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.\textsuperscript{1–3} 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.\textsuperscript{4}

Recent in vivo\textsuperscript{4,5} and in vitro\textsuperscript{6–8} 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.\textsuperscript{9,10} 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,
exercise increases flow-dependent, NO-mediated vasodilatation of peripheral conduit arteries in human CHF. 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, prostanooids, 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 ligature using the method of Peiffer et al that has been modified in our laboratory. 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⁻¹ · 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/dtmax). 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 10% buffered formalin. From this tissue, the LV septum was removed for histological studies, and the LV cavity was filled with 10% buffered formalin and weighed.

In Vitro Vascular Studies

Four femoral arteries from each animal were removed. The arteries were carefully cleaned from adherent connective tissue, cut into 2–3 mm segments, and mounted in 5-mL organ baths. Each segment was placed in a 5-mL organ bath containing modified Krebs-Henseleit buffer (95% O₂/5% CO₂, 37°C), composed of (in mmol/L): NaCl 119, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.18, CaCl₂ 2.54, and 5% glucose. The tissues were allowed to equilibrate for 30 minutes before the start of the experiment. After equilibration, the pressurized arteries were preconstricted with phenylephrine, after which, cumulative concentrations of phenylephrine (10⁻¹⁰ to 10⁻⁴ mol/L) were added at 0.5-minute intervals. The responses to the corresponding concentration of phenylephrine were determined by measuring the change in diameter of the precontracted vessels using an image analysis system (Leica, Microsystems). The time required for a 50% relaxation of the phenylephrine-induced contraction was determined.

Experimental Procedure

After the equilibration period, the pressurized arteries were preconstricted by adding phenylephrine, after which, cumulative concentrations of acetylcholine (10⁻¹⁰ to 10⁻⁴ mol/L) were added at 0.5-minute intervals. The responses to acetylcholine were determined by measuring the change in diameter of the precontracted vessels using an image analysis system (Leica, Microsystems).

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 were analyzed using a 2-way ANOVA. The responses to the corresponding concentration of phenylephrine were determined by measuring the change in diameter of the precontracted arteries using an image analysis system (Leica, Microsystems).

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 role of prostaglandins, NO, and free radicals were assessed using the cyclooxygenase inhibitor diclofenac (10⁻⁴ mol/L), the NO synthase inhibitor N²-nitro-L-arginine (L-NA; 10⁻⁴ mol/L) or the free radical scavenger N-(2-mercaptobenzyl)-glycine (MPG; 10⁻⁴ 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 (10⁻⁴ mol/L) under zero-flow conditions.

Semi quantitative PCR

Total RNA was extracted from rat muscle tissue using acid guanidinium thiocyanate–phenol-chloroform extraction. 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), DT T 10, KCl 85, MgCl₂, 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 [³²P]-dATP, 5 μL of MgCl₂ (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.

TABLE 1. Primer Sequences and Sizes of Expected PCR Products

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer Sequences</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>Upper (5’) TCCGGGCTGACACCTGACCTAA</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td>Lower (3’) AACATGCTCCTTGCGAGGCGA</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Upper (5’) CAGAGGAGAGGGATCTCT</td>
<td>535</td>
</tr>
<tr>
<td></td>
<td>Lower (3’) GGGAGCTCATAGCTTCTTTC</td>
<td></td>
</tr>
</tbody>
</table>

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 role of prostaglandins, NO, and free radicals were assessed using the cyclooxygenase inhibitor diclofenac (10⁻⁴ mol/L), the NO synthase inhibitor N²-nitro-L-arginine (L-NA; 10⁻⁴ mol/L) or the free radical scavenger N-(2-mercaptobenzyl)-glycine (MPG; 10⁻⁴ 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 (10⁻⁴ mol/L) under zero-flow conditions.

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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
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 max 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 max; 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 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 (preconstricted) 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 \( \mu \text{L} \cdot \text{min}^{-1} \). CHF abolished FMD and converted it into a vasoconstriction. This constrictor response was more marked at low levels (at 17 \( \mu \text{L} \cdot \text{min}^{-1} \), it was −8 ± 3%) than at high levels of flow (at 370 \( \mu \text{L} \cdot \text{min}^{-1} \), it was −4 ± 3%). Exercise restored a significant degree of flow-mediated vasodilation, and this effect was observed at all levels of flow except the lowest. At the highest level of

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**Table 2. Echocardiographic Parameters**

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

\( \text{PWD} \) indicates posterial wall diastolic thickness; \( \text{PWT} \), posterial wall thickening; \( \text{LVDD} \), left ventricular diastolic diameter; \( \text{LVSS} \), left ventricular systolic diameter; \( \text{FS} \), fractional shortening; \( \text{SV} \), stroke volume; \( \text{CO} \), cardiac output; and \( \text{CI} \), cardiac index.

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

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

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Baseline</th>
<th>Phenylephrine</th>
<th>Sodium Nitroprusside (10^{-4} \text{ mol/L})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>243 ± 11</td>
<td>104 ± 5</td>
<td>223 ± 8</td>
</tr>
<tr>
<td>Sedentary CHF</td>
<td>241 ± 7</td>
<td>105 ± 6</td>
<td>234 ± 7</td>
</tr>
<tr>
<td>Exercised CHF</td>
<td>238 ± 7</td>
<td>108 ± 4</td>
<td>228 ± 9</td>
</tr>
</tbody>
</table>

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**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).
Exercise-Improved Flow-Mediated Dilatation

Figure 3. Flow-response curves in isolated, pressurized, and preconstricted 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 4. Flow-response curves in isolated, pressurized, and preconstricted gracilis arteries taken from sham-operated rats (○) or CHF rats, either sedentary or exercised (SP) in basal conditions (E) or in the presence of the NO synthase inhibitor L-NA (●). Results are expressed as mean±SEM. *P<0.05 vs basal value (repeated measures ANOVA; n=6 in each group).

Flow (370 μL · min⁻¹), the increase in diameter was 18±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 mL · min⁻¹: sedentary sham, 42±5%; exercised sham, 35±9%; n=7; P=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 μL · min⁻¹), L-NA reduced FMD from the basal value of 42±5% to 2±8% (P<0.05). In sedentary CHF rats, FMD was not affected by L-NA (FMD at 370 μL · min⁻¹ before and after L-NA: -4±3% and 1±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±3% to 2±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 μL · min⁻¹ before and after diclofenac: 37±10% and 35±8%, respectively). In contrast, in sedentary CHF rats, diclofenac partially restored flow-induced dilatation (FMD at 370 μL · min⁻¹ before and after diclofenac: -10±2% and 19±5%, respectively; P<0.05). Diclofenac also slightly and nonsignificantly improved FMD in arteries from exercised CHF rats. This effect seemed limited compared with that observed in sedentary CHF rats (FMD at 370 μL · min⁻¹ before and after diclofenac: 12±1% and 19±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 μL · min⁻¹ before and after MPG: -9±6% and 6±3%, respectively; P<0.05). MPG also slightly and nonsignificantly improved FMD in arteries taken from exercised CHF rats (FMD at 370 μL · min⁻¹ before and after MPG: 10±1% and 14±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±3; CHF, 10±3 [P<0.05 versus sham]; exercise, 21±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...
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 inactive 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/dtmax 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, is 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. 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, 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 vasodilation. 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. 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.

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. 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. 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. 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

<table>
<thead>
<tr>
<th></th>
<th>Sham-Operated</th>
<th>Sedentary CHF</th>
<th>Exercised CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size, % of LV</td>
<td>...</td>
<td>36±3</td>
<td>35±2</td>
</tr>
<tr>
<td>Scar length, mm</td>
<td>...</td>
<td>8.6±0.6</td>
<td>8.1±0.6</td>
</tr>
<tr>
<td>Scar surface, mm²</td>
<td>...</td>
<td>6.5±0.5</td>
<td>7.1±0.6</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>528±32</td>
<td>527±14</td>
<td>453±8†</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.25±0.09</td>
<td>1.54±0.08*</td>
<td>1.61±0.08*</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>2.16±0.09</td>
<td>2.62±0.15*</td>
<td>3.19±0.15†</td>
</tr>
<tr>
<td>LV collagen density, %</td>
<td>2.32±0.11</td>
<td>3.22±0.19*</td>
<td>2.72±0.19†</td>
</tr>
</tbody>
</table>

*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 acetylcholine6,10 and with increased aortic eNOS expression.22 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.23 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.24

Alternatively, changes in eNOS expression might reflect modifications of eNOS mRNA stability. Indeed, tumor necrosis factor-α, which is elevated in CHF,25–27 decreases eNOS expression by shortening the half-life of mRNA.28 Furthermore, CHF may be associated with local tissue hypoxia,29 which reduces eNOS expression.30 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 synthesize inhibition.31 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.32

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.33 The fact that the effect of diclofenac was attenuated by exercise suggests that it also attenuates the production of vasoconstrictor prostanooids, and this could also contribute to improved FMD after exercise.

In conclusion, our study demonstrates that CHF abolishes flow-mediared 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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References


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