Abstract—The aim of the present study was to determine whether cardiac nitric oxide (NO) production changes during the progression of pacing-induced heart failure and whether this occurs in association with alterations in myocardial metabolism. Dogs (n=8) were instrumented and the heart paced until left ventricular end-diastolic pressure reached 25 mm Hg and clinical signs of severe failure were evident. Every week, hemodynamic measurements were recorded and blood samples were withdrawn from the aorta and the coronary sinus for measurement of NO metabolites, O₂ content, free fatty acids (FFAs), and lactate and glucose concentrations. Cardiac production of NO metabolites or consumption of O₂ or utilization of substrates was calculated as coronary sinus-arterial difference times coronary flow. In end-stage failure, occurring at 29±1.6 days, left ventricular end-diastolic pressure was 25±1 mm Hg, left ventricular systolic pressure was 92±3 mm Hg, mean arterial pressure was 75±2.5 mm Hg, and dP/dt max was 1219±73 mm Hg/s (all P<0.05). These changes in hemodynamics were associated with a fall of cardiac NO metabolite production from 0.37±0.16 to −0.28±0.13 nmol/beat (P<0.05). O₂ consumption and lactate uptake did not change significantly from control, while FFA uptake decreased from 0.16±0.03 to 0.05±0.01 μEq/beat and glucose uptake increased from −2.3±7.0 to 41±10 μg/beat (P<0.05). The cardiac respiratory quotient also increased significantly by 28%. In 14 normal dogs the same measurements were performed at control and 1 hour after we injected 30 mg/kg of nitro-L-arginine, a competitive inhibitor of NO synthase. O₂ consumption increased from 0.05±0.002 mL/beat at control to 0.071±0.003 mL/beat after nitro-L-arginine, while FFA uptake decreased from 0.1±0.01 to 0.06±0.01 μEq/beat, lactate uptake increased from 0.15±0.04 to 0.31±0.03 μmol/beat, glucose uptake increased from 8.2±5.0 to 35.4±9.5 μg/beat, and RQ increased by 23% (all P<0.05). Our results indicate that basal cardiac production of NO falls below normal levels during cardiac decompensation and that there are shifts in substrate utilization. This switch in myocardial substrate utilization also occurs after acute pharmacological blockade of NO production in normal dogs. (Circ Res. 1998;83:969-979.)

Key Words: MVO₂ ■ lactate ■ free fatty acid ■ glucose ■ respiratory quotient

One of the proposed mechanisms of cardiac dysfunction in heart failure attributes a major role to an excessive production of nitric oxide (NO) in the heart and, specifically, in myocytes.1,2 Circulating proinflammatory cytokines, found in high concentrations in plasma of patients with heart failure,3 would stimulate the expression of inducible NO synthase (iNOS) with consequent overproduction of NO. NO does not necessarily imply that cardiomyocytes are exposed to toxic concentrations of NO. Indeed, it is the amount of NO and not the enzyme isotype generating it that determines the degree to which cardiac function would be depressed. Numerous studies suggest that NO release is rather low in heart failure. In clinical and animal studies2,10 pharmacological blockade of NO synthases (NOS) did not alter baseline hemodynamics, indicating that, rather than being characterized by overproduction, systemic NO synthesis could already be minimal. Several investigations, including our own, in patients and in animal models of heart failure report an impairment of endothelial release of NO in large arteries and in coronary microvessels.13-14 The reduced vascular NO production of NO, sufficient to cause a negative inotropic action during heart failure, has not been provided. The finding that iNOS is expressed in tissue from failing hearts does not necessarily imply that cardiomyocytes are exposed to toxic concentrations of NO. Indeed, it is the amount of NO and not the enzyme isotype generating it that determines the degree to which cardiac function would be depressed. Numerous studies suggest that NO release is rather low in heart failure. In clinical and animal studies2,10 pharmacological blockade of NO synthases (NOS) did not alter baseline hemodynamics, indicating that, rather than being characterized by overproduction, systemic NO synthesis could already be minimal. Several investigations, including our own, in patients and in animal models of heart failure report an impairment of endothelial release of NO in large arteries and in coronary microvessels.13-14 The reduced vascular NO production of NO, sufficient to cause a negative inotropic action during heart failure, has not been provided. The finding that iNOS is expressed in tissue from failing hearts does not necessarily imply that cardiomyocytes are exposed to toxic concentrations of NO. Indeed, it is the amount of NO and not the enzyme isotype generating it that determines the degree to which cardiac function would be depressed. Numerous studies suggest that NO release is rather low in heart failure. In clinical and animal studies2,10 pharmacological blockade of NO synthases (NOS) did not alter baseline hemodynamics, indicating that, rather than being characterized by overproduction, systemic NO synthesis could already be minimal. Several investigations, including our own, in patients and in animal models of heart failure report an impairment of endothelial release of NO in large arteries and in coronary microvessels.13-14 The reduced vascular NO production of NO, sufficient to cause a negative inotropic action during heart failure, has not been provided. The finding that iNOS is expressed in tissue from failing hearts does not necessarily imply that cardiomyocytes are exposed to toxic concentrations of NO. Indeed, it is the amount of NO and not the enzyme isotype generating it that determines the degree to which cardiac function would be depressed. Numerous studies suggest that NO release is rather low in heart failure. In clinical and animal studies2,10 pharmacological blockade of NO synthases (NOS) did not alter baseline hemodynamics, indicating that, rather than being characterized by overproduction, systemic NO synthesis could already be minimal. Several investigations, including our own, in patients and in animal models of heart failure report an impairment of endothelial release of NO in large arteries and in coronary microvessels.13-14 The reduced vascular NO
production is likely the result of reduced gene expression of constitutive NOS.\textsuperscript{15} Under these circumstances, cardiomyocytes, each surrounded by 3 capillaries, would lose, during heart failure, the tonic exposure to NO from the neighboring endothelium, and, despite the expression of iNOS, the overall action of this molecule could be markedly attenuated. In the present study the first aim was to determine whether cardiac NO production changes during development and progression of pacing-induced heart failure. The time course of pacing-induced dilated cardiomyopathy, as produced in our laboratory, is highly reproducible and offers the possibility of an accurate monitoring over time of the changes of hemodynamics and humoral factors leading to heart failure. Since our previous studies suggest that NO may regulate myocardial metabolism,\textsuperscript{16} to determine the relationship between NO production and changes in cardiac O$_2$ consumption (MVO$_2$) and substrate utilization during the development of cardiac decompensation was the second goal of our study. To establish whether this relationship was determined by a pure association of events or by a cause-effect sequence, cardiac substrate uptake was also measured in a group of normal dogs after competitive inhibition of NO production with the use of nitro-\textsuperscript{L}-arginine (NLA), a specific NOS inhibitor.

**Materials and Methods**

**Surgical Procedure and Instrumentation**

Male dogs (n=22) weighing 25 to 27 kg were sedated with acepromazine maleate (1 mg/kg IM), anesthetized with sodium pentobarbital (25 mg/kg IV), and ventilated with room air. A thoracotomy was performed in the left fifth intercostal space. Catheters (Tygon) were placed in the descending thoracic aorta and left atrial appendage for pressure measurements. A third catheter was inserted in the coronary sinus (CS) with the tip leading away from the right atrium. During the surgery, a blood sample was taken from the CS catheter and immediately measured on a pH/blood gas analyzer. If the PaO$_2$ was >25 mm Hg, the catheter was repositioned and tied into place. A solid-state pressure gauge (P6.5, Konigsberg Instruments, Inc) was inserted in the left ventricle through the apex. A Doppler flow transducer (Parks Electronics) was placed around the left circumflex coronary artery, and a pair of 3-MHz piezoelectric crystals was placed on opposing endocardial surfaces at the base of the left circumflex coronary artery\textsuperscript{19} to obtain cardiac production per minute of NOx.

**Cardiac Metabolites**

Blood samples from aorta and CS were collected into plastic syringes treated with either heparin or EDTA and immediately stored on ice. Special care was taken to withdraw blood slowly from the CS catheter to avoid potential contamination of the sample with right atrial blood. Blood gases were measured in a blood gas analyzer (model 170, Corning). PaO$_2$ was multiplied by 0.003 and added to O$_2$ content measured by a hemoglobin analyzer (CO-Oximeter, Instruments Laboratory) to obtain total oxygen content (vol/vol). Hematocrit levels were obtained by centrifugation. Lactate was measured from whole blood with a YSI 1500 sport lactate analyzer (Yellow Springs Instrument Co). Glucose and free fatty acid (FFA) concentrations were determined in plasma after centrifugation of the blood samples at 1000g for 15 minutes at 0°C. Glucose was measured with a Beckman glucose 2 analyzer (Beckman Instruments). FFA analysis was performed on plasma from EDTA-treated samples with the use of a calorimetric assay (NEFA C kit from WAKO Pure Chemical Industries, Ltd) and a spectrophotometer (Kontron Instruments). The CS-arterial concentration difference of O$_2$, lactate, glucose, and FFA contents was multiplied by MCBF to calculate cardiac consumption. We have described these methods previously.\textsuperscript{17}

**Cardiac Production of CO$_2$ and Respiratory Quotient**

Total cardiac CO$_2$ concentration in arterial and CS blood was calculated according to well-established methods as described by Davenport\textsuperscript{20}:\begin{equation} [\text{CO}_2]_h = [\text{CO}_2]_p \times \left(1 - \text{Htc}\right) + [\text{CO}_2]_e \times \text{Htc} + [\text{carbamino-CO}_2]_p \end{equation} where [\text{CO}_2]_p is the CO$_2$ concentration (mmol/L) in whole blood, [\text{CO}_2]_e is the plasma CO$_2$ concentration, [\text{CO}_2]_h is the concentration of CO$_2$ dissolved in red cells, [carbamino-CO$_2$] is the concentration of CO$_2$ bound to hemoglobin, and Htc is the hematocrit expressed in decimals. [\text{CO}_2]_e, either in plasma or in red cells, was calculated by adding bicarbonate concentration to the concentration of physically dissolved CO$_2$. The latter is given by $a \times \text{pCO}_2$, where $a$ is 0.0301 and 0.025 for plasma and
red cells, respectively. Bicarbonate concentration, $[HCO_3^-]$, was calculated by the equation of Henderson-Hasselbach:

$$\text{pH} = 6.1 + \log([HCO_3^-]/[aPCO_2]).$$

pH of red cell cytoplasm was calculated on the basis of the plasma pH, according to the relation between the two, mathematically described by Duhm. We calculated [carbamino-CO$_2$], in millimoles per liter, as

$$0.00588 \times \left(\frac{[Hb]}{3}(1-RHb)\right) + 0.0105 \times \left(\frac{[Hb]}{3}RHb\right),$$

where $[Hb]$ is the hemoglobin concentration in grams per liter and RHb is the fraction of reduced hemoglobin as measured by the hemoglobin analyzer. Total [CO$_2$] in arterial and CS blood was converted in volume percent, multiplying the millimoles per liter by 2.226. Consequently, cardiac production of CO$_2$ (MCO$_2$) was obtained as follows: $(CS-A)[CO_2] \times MCBF$, where CS-A indicates CS-arterial difference. Finally, the respiratory quotient (RQ) was given by $MCO_2/MVO_2$, which can be simplified as follows: $(CS-A)[CO_2]/(A-CS)[O_2]$. Values of RQ range between 0.707, corresponding to oxidation of FFA as the only substrate, and 1, corresponding to oxidation of carbohydrates as the only substrate.

For some comparisons, RQ was also calculated from the percentage of calories derived from carbohydrates (% carb) over the total calories produced by complete oxidation of substrates, according to the linear correlation determined by Zuntz, Schumburg, and Lusk and described by the equation $RQ = 0.002907 \times (% \text{carb}) + 0.7048$. The caloric equivalent is 2338 cal/mmol for FFA, 326 cal/mmol for lactate, and 686 cal/mmol for glucose. For FFA, the caloric equivalent of palmitate was used, since it represents the main fatty acid consumed by the heart. This second method of RQ determination is valid if uptaken substrates are almost completely oxidized, as occurs in normal hearts.

### Induction of Heart Failure and Protocol

Heart failure was induced in 8 dogs. On the day of the experiment, dogs were weighed and placed on the laboratory table. Hemodynamic measurement and blood samples were taken in presence of stable heart rate and blood pressure. After this experimental protocol was performed as control, the heart was paced at 210 bpm for 3 weeks, and then the pacing rate was increased to 240 bpm until overt heart failure was observed. We used external pacemakers (model EV4543, Pace Medical), carried by the dog in a vest. The protocol was repeated each week for the first 3 weeks and every other day after the third week. All experiments were performed with the pacers turned off and the heart in spontaneous rhythm. Dogs were killed when LV end-diastolic pressure (LVEDP) reached 25 mm Hg and clinical signs of severe decompensation were observed.

### Competitive Inhibition of NOS

Hemodynamic measurements and arterial and CS blood samples were taken from 14 normal dogs placed on the laboratory table. The heart was paced at 132 ± 1 bpm, which corresponded to the spontaneous heart rate observed in end-stage failure. Cardiac pacing was then stopped, and a dose of 30 mg/kg of NLA, a specific NOS inhibitor, was given to the dogs intravenously. As previously established, this dose reduces the vasodilation to acetylcholine by 60% in conscious dogs and abolished the basal release of NOx by the heart. Hemodynamic changes were monitored during the following 45 minutes. At this time cardiac pacing was started again, and blood samples were taken after allowing 15 minutes of stabilization, ie, 1 hour after NLA injection.

### Statistical Analysis

Data are presented as mean ± SEM. Statistical analysis was performed with the use of commercially available software (Sigma Stat 2.0). Changes in hemodynamics and metabolites over time were tested by one-way ANOVA followed by Dunnett's test. NO production, MVO$_2$, and substrate consumption were normalized by heart rate and expressed as per beat. The normalization was used for a better comparison of data at different times, since spontaneous heart rate changed during the evolution of heart failure. Data expressed as per minute, however, are also provided. Correlations between groups of values were evaluated calculating the best fit, based on least-

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Figure 1. Changes in mean arterial pressure (MAP) (A), LVEDP (B), LV systolic pressure (LVSP) (C), and dP/dt$\max$ (D) during the evolution of pacing-induced heart failure. There were statistically significant changes in all parameters after 7 days of pacing. Data are mean ± SEM. *P < 0.05 vs control.
TABLE 1. Changes in Hemodynamics During the Evolution of Heart Failure

<table>
<thead>
<tr>
<th>Pacing Time, d</th>
<th>0 (Control)</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>24±1</th>
<th>27±1.4</th>
<th>29±1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean LcxCBF, mL/min</td>
<td>28±3.0</td>
<td>22±2.8</td>
<td>24±2.5</td>
<td>29±2.8</td>
<td>29±3.5</td>
<td>31±4.4</td>
<td>30±3.5</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>83±7</td>
<td>88±9</td>
<td>100±8</td>
<td>105±3</td>
<td>119±7*</td>
<td>119±4*</td>
<td>126±5*</td>
</tr>
<tr>
<td>LDCR, (mm Hg/mL/min)</td>
<td>3.7±0.2</td>
<td>3.6±0.6</td>
<td>3.8±0.2</td>
<td>2.6±0.3</td>
<td>2.6±0.4</td>
<td>2.3±0.5*</td>
<td>2.1±0.2*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>41.7±2.1</td>
<td>42.9±2.4</td>
<td>42.2±2.1</td>
<td>44.9±2.5</td>
<td>43.6±2.2</td>
<td>46.4±1.8*</td>
<td>45.6±2.5*</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>35.4±2.1</td>
<td>38.4±2.3</td>
<td>38.1±2.2</td>
<td>40.9±2.7</td>
<td>40.5±2.1</td>
<td>42.8±2.1</td>
<td>43.2±2.5*</td>
</tr>
<tr>
<td>% LV shortening</td>
<td>15±1.4</td>
<td>11±1.0*</td>
<td>10±1.4*</td>
<td>9±1.4*</td>
<td>7±0.9*</td>
<td>8±1.6*</td>
<td>5±0.9*</td>
</tr>
<tr>
<td>LV work, mm Hg×mm</td>
<td>884±70</td>
<td>434±32*</td>
<td>399±53*</td>
<td>324±45*</td>
<td>233±35*</td>
<td>230±47*</td>
<td>148±28*</td>
</tr>
<tr>
<td>Triple product, (mm Hg×mm/s)×10^-6</td>
<td>34±4.1</td>
<td>19±2.6*</td>
<td>22±3.4*</td>
<td>18±1.7*</td>
<td>19±2.3*</td>
<td>18±1.1*</td>
<td>15±1.7*</td>
</tr>
</tbody>
</table>

LcxCBF indicates left circumflex coronary blood flow; HR, heart rate; LDCR, late diastolic coronary resistance; LVEDD, left ventricular end-diastolic diameter; and LVESD, left ventricular end-systolic diameter. Data are mean±SEM.

*P<0.05 vs control.

Results

Pacing-Induced Heart Failure

End-stage heart failure was characterized by a LVEDP of 25±1 mm Hg and by severe clinical signs such as dyspnea, ascites, pale mucosa, and lethargy. The mean time to euthanasia was 29±1.6 days.

Hemodynamics

Changes over time of mean arterial pressure, LV systolic and diastolic pressure, and dP/dt max are shown in Figure 1. There were significant changes in hemodynamics after the first week of pacing. For instance, mean values of dP/dt max, percent diameter shortening, and LV work decreased by 36%, 30%, and 51%, respectively. LVEDP increased exponentially over the entire period of pacing, with a faster increment after 21 days. After the first week, mean arterial pressure, LV systolic pressure, dP/dt max, and LV work showed a slow decrease, with a sharp drop after 27±1.4 days. Values of other hemodynamics are summarized in Table 1. It is important to note that mean CBF did not change significantly during the progression of heart failure. Late diastolic coronary resistance fell at 3 weeks and was statistically different from the control after 24±1 days. Changes in the triple product resembled those of dP/dt max.

Cardiac Production of NO

Figure 2 displays the net cardiac NOx production per beat during the course of heart failure. Positive at control, NOx production changed significantly after 24±1 days. The production became negative, ie, there was an apparent uptake of NO metabolites during the late stage of heart failure. Arterial and CS concentrations of NOx were, respectively, 3.36±0.8 and 4.87±1.2 μmol/L at control and 4.96±0.8 and 4.43±0.9 μmol/L at 29±1.6 days. At 29±1.6 days, the CS-arterial
difference of NOx concentration was \(-0.52\pm0.22\) \(\mu\text{mol/L}\) versus \(0.78\pm0.40\) \(\mu\text{mol/L}\) at control \((P<0.05)\). Values per minute of NOx production were \(34\pm12\) \(\text{nmol/min}\) at control and \(-35\pm14\) \(\text{nmol/min}\) in end-stage failure. An inverse, linear relationship (coefficient \(r^2=0.90\) and \(P<0.05\)) was found between mean values of NO production and LVEDP after the second week of pacing (Figure 2B), indicating that the progressive ventricular dysfunction, indicated by an increase in end-diastolic pressure up to 25 mm Hg, was associated with a fall in cardiac NO production.

**Cardiac Metabolism**

Arterial PO2 and concentration of \(\text{O}_2\), FFA, lactate, and glucose, at different times, are presented in Table 2. Consumption per beat of \(\text{O}_2\), FFA, glucose, and lactate at different times are shown in Figure 3. \(\text{Pa}_2\) and \(\text{O}_2\) content fell significantly at 29±1.6 days, and consumption per beat of \(\text{O}_2\) also tended to decrease, although there was no statistically significant change. \(\text{O}_2\) consumption per minute was \(5.46\pm0.36\) mL/min at control and \(5.05\pm0.72\) mL/min at 29±1.6 days \((P=\text{NS})\). A significant decrease of FFA uptake was observed after the third week, while glucose uptake increased significantly only at 27±1.6 days. FFA and glucose uptake per minute were, respectively, \(12.5\pm1.5\) \(\mu\text{Eq/min}\) and \(-0.16\pm0.6\) \(\text{mg/min}\) at control and \(6.3\pm0.7\) \(\mu\text{Eq/min}\) and \(5.3\pm1.4\) \(\text{mg/min}\) at 29±1.6 days \((P<0.05)\). It is important to note that after the first significant fall at 24±1 days, FFA uptake was stable, while glucose uptake continued to rise. This is also reflected by the relationships between mean values of NOx production per beat and mean values of FFA uptake and glucose uptake after the second week of pacing (Figure 4). The first relationship is exponential, with a flat portion in end-stage failure, whereas the second is linear,

**TABLE 2. Arterial Blood PO2, \(\text{O}_2\) Content, and Cardiac Substrate Concentration During the Evolution of Heart Failure**

<table>
<thead>
<tr>
<th>Pacing Time, d</th>
<th>0 (Control)</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>24±1</th>
<th>27±1.4</th>
<th>29±1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial (\text{Po}_2), mm Hg</td>
<td>93±1.7</td>
<td>91±3.0</td>
<td>89±3.2</td>
<td>98±3.2</td>
<td>84±5.3</td>
<td>85±5.9</td>
<td>74±4.9*</td>
</tr>
<tr>
<td>Arterial (\text{O}_2) content, mL/dL</td>
<td>15.3±0.6</td>
<td>15.9±1.2</td>
<td>15.8±0.7</td>
<td>14.3±1.0</td>
<td>12.8±0.6</td>
<td>12±0.56*</td>
<td>12±0.6*</td>
</tr>
<tr>
<td>Arterial FFA, mEq/L</td>
<td>0.57±0.08</td>
<td>0.54±0.09</td>
<td>0.43±0.09</td>
<td>0.45±0.06</td>
<td>0.32±0.06*</td>
<td>0.31±0.05*</td>
<td>0.29±0.04*</td>
</tr>
<tr>
<td>Arterial lactate, mmol/L</td>
<td>0.88±0.11</td>
<td>0.95±0.10</td>
<td>1.03±0.10</td>
<td>0.84±0.10</td>
<td>0.96±0.10</td>
<td>1.05±0.07</td>
<td>0.97±0.10</td>
</tr>
<tr>
<td>Arterial glucose, mg/dL</td>
<td>80±2.2</td>
<td>77±1.9</td>
<td>78±2.6</td>
<td>81±2.1</td>
<td>79±1.5</td>
<td>85±2.1</td>
<td>85±2.8</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

*\(P<0.05\) vs control.
Data are mean ± SEM, *P < 0.05 vs control.

**Compared Rates of Change in Cardiac NO\textsubscript{x} Production, FFA and Glucose Uptake, and RQ**

Since all the significant changes over time in cardiac NO\textsubscript{x} production, FFA and glucose uptake, and RQ occurred during cardiac decompensation, ie, after the third week of pacing, the best curve fit was calculated for each series of data points, from day 21 to day 29 ± 1.6 of pacing (Figure 5). A curve fit was also calculated for MVO\textsubscript{2}. From the mathematical function describing these curves, it was possible to calculate the time corresponding to a fixed change in each variable. We have arbitrarily chosen a change equal to 30% of the difference between values at 21 and at 29 ± 1.6 days. The time corresponding to that variation was defined as \( t_{30} \) and was used as an index of the threshold for change in production, consumption, and RQ. The regression lines are shown in Figure 5. The coefficients for the curve fits and \( t_{30} \) are listed in Table 4. A 30% change occurred first for the decrease in FFA uptake at 21.5 days, followed by a simultaneous decrease in NO\textsubscript{x} production and MVO\textsubscript{2} at 22.6 days, and, 1 day later, by an almost simultaneous increase in glucose uptake and RQ.

**Competitive Inhibition of NOS in Normal Animals**

**Hemodynamics**

During cardiac pacing at 132 ± 1 bpm, CBF, mean arterial pressure, LV systolic pressure, and dP/dt\textsubscript{max} were, respectively, 33 ± 1.5 mL/min, 113 ± 3 mm Hg, 132 ± 3 mm Hg, and 2715 ± 89 mm Hg/s at control and 41.5 ± 2.9 mL/min, 152 ± 3.5 mm Hg, 169 ± 4 mm Hg, and 2336 ± 152 mm Hg/s after NLA (all \( P < 0.05 \) versus control).

**Cardiac Metabolism and RQ**

Changes in MVO\textsubscript{2} and FFA, glucose, and lactate uptake at control and 1 hour after NLA are shown in Figure 6. To facilitate a comparison with the corresponding data in heart failure, values are displayed as per beat. MVO\textsubscript{2} and FFA, lactate, and glucose uptake per minute were, respectively, 7.0 ± 0.6 mL/min, 13.5 ± 1.1 \( \mu \text{Eq/min} \), 16.3 ± 2.7 \( \mu \text{mol/min} \), and 1.1 ± 0.7 mg/min at control and 9.3 ± 0.9 mL/min, 7.5 ± 1.3 \( \mu \text{Eq/min} \), 41.4 ± 4.5 \( \mu \text{mol/min} \), and 4.9 ± 1.3 mg/min after NLA. All these changes were statistically significant (\( P < 0.05 \)). The arterial concentrations of FFA, lactate, and glucose were, respectively, 0.55 ± 0.06 mEq/L, 1.01 ± 0.09 mmol/L, and 89.6 ± 2 mg/dL at control and 0.28 ± 0.05 (\( P < 0.05 \)), 1.1 ± 0.08 (\( P = \text{NS} \)), and 83.5 ± 1.4 mg/L (\( P = \text{NS} \)) after NLA.

RQ was 0.754 ± 0.05 at control and 0.93 ± 0.06 after NLA (\( P < 0.05 \)). The RQ calculated by using the theoretical per-

**TABLE 3. CS-Aorta Difference of O\textsubscript{2} and CO\textsubscript{2} Content and RQ at Control and After 3 Weeks of Pacing**

<table>
<thead>
<tr>
<th>Pacing Time, d</th>
<th>Aorta-CS difference of O\textsubscript{2}, mL/dL</th>
<th>CS-aorta difference of CO\textsubscript{2}, mL/dL</th>
<th>RQ, CO\textsubscript{2}/O\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>10.6 ± 0.38</td>
<td>7.7 ± 0.79</td>
<td>0.734 ± 0.06</td>
</tr>
<tr>
<td>21</td>
<td>10.6 ± 1.04</td>
<td>8.5 ± 0.64</td>
<td>0.8 ± 0.04</td>
</tr>
<tr>
<td>24 ± 1.2</td>
<td>10.2 ± 0.35</td>
<td>8.8 ± 0.96</td>
<td>0.87 ± 0.09</td>
</tr>
<tr>
<td>27 ± 1.4</td>
<td>9.7 ± 0.77</td>
<td>9.2 ± 0.90</td>
<td>0.96 ± 0.08*</td>
</tr>
<tr>
<td>29 ± 1.6</td>
<td>8.9 ± 0.74</td>
<td>9.1 ± 1.2</td>
<td>1.02 ± 0.05*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. *\( P < 0.05 \) vs control.

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**Figure 4. Relationships between mean values of cardiac production of NO\textsubscript{x} and cardiac uptake of FFA (A) and glucose (B) after the second week of pacing. For FFA-NOX, the best fit is described by the following generic equation: \( 1/y=b+ (a+x)^{-2} \). Data are mean ± SEM. \(*P < 0.05 \) vs control.**

**Figure 5. The coefficients for the curve fits and \( t_{30} \) are listed in Table 4. A 30% change occurred first for the decrease in FFA uptake at 21.5 days, followed by a simultaneous decrease in NO\textsubscript{x} production and MVO\textsubscript{2} at 22.6 days, and, 1 day later, by an almost simultaneous increase in glucose uptake and RQ.**

**Figure 6. To facilitate a comparison with the corresponding data in heart failure, values are displayed as per beat. MVO\textsubscript{2} and FFA, lactate, and glucose uptake per minute were, respectively, 7.0 ± 0.6 mL/min, 13.5 ± 1.1 \( \mu \text{Eq/min} \), 16.3 ± 2.7 \( \mu \text{mol/min} \), and 1.1 ± 0.7 mg/min at control and 9.3 ± 0.9 mL/min, 7.5 ± 1.3 \( \mu \text{Eq/min} \), 41.4 ± 4.5 \( \mu \text{mol/min} \), and 4.9 ± 1.3 mg/min after NLA. All these changes were statistically significant (\( P < 0.05 \)). The arterial concentrations of FFA, lactate, and glucose were, respectively, 0.55 ± 0.06 mEq/L, 1.01 ± 0.09 mmol/L, and 89.6 ± 2 mg/dL at control and 0.28 ± 0.05 (\( P < 0.05 \)), 1.1 ± 0.08 (\( P = \text{NS} \)), and 83.5 ± 1.4 mg/L (\( P = \text{NS} \)) after NLA. RQ was 0.754 ± 0.05 at control and 0.93 ± 0.06 after NLA (\( P < 0.05 \)). The RQ calculated by using the theoretical per-
The major findings of the present study are as follows: (1) the onset of cardiac decompensation, during pacing-induced heart failure, occurs only after the third week of pacing (26 ± 1.4 days); (2) the transition to decompensated heart failure is associated with a significant fall of cardiac NO production; (3) there are profound changes in myocardial metabolism at this time; and (4) after acute inhibition of NO production in healthy, conscious dogs there is an alteration in cardiac substrate utilization similar to that in heart failure. To our knowledge, this represents the first study in which the time course for transition to decompensated heart failure has been determined and a marked decrease of total cardiac NO production during the process leading to cardiac decompensation has been demonstrated. Furthermore, this is the first study to indicate that acute inhibition of NOS alters substrate utilization by the heart.

A significant fall in LV dP/dt max, percent diameter shortening, and work occurred at 1 week of pacing and was not associated with cardiac decompensation. At this time there was no change in blood gases and no change in cardiac NO production, MVO 2, or substrate utilization. It was only later, at 22 to 23 days, that dramatic changes in hemodynamics, clinical state, gas exchange, and cardiac metabolism were observed. We defined this time as the onset of cardiac decompensation. Although some clinical studies propose an increased NO production by iNOS in myocardiocytes as a possible maladaptive mechanism and cause of cardiac dysfunction in heart failure,1,2,7 no direct measure of NO release was provided. Very recently, the only clinical study in which cardiac NOx production was measured reported no changes in patients with heart failure versus normal subjects.24 Any possible increase in NO, regardless of the isotype or localization of the enzyme that generates it, must be reflected by

Discussion

The percentage of calories derived from carbohydrate oxidation was 0.76 ± 0.02 at control and 0.84 ± 0.03 after NLA (P < 0.05). This result indicated that, if all the substrates were completely oxidized after NLA, 47% of the developed calories would derive from carbohydrates, but, on the basis of the RQ calculated from real CO 2 production and MVO 2, the effective percentage was 77%.

Figure 5. Best curve fits describing the time course of NOx production (A), MVO 2 (B), FFA uptake (C), glucose uptake (D), and RQ (E) during cardiac decompensation. Data are mean ± SEM. The mathematical functions of the curve are reported in Table 4.
augmented NO metabolites in the coronary venous blood, since the primary method for clearing NOx is filtration by the kidney. A previous study from our laboratory has demonstrated a direct correlation between arterial NOx and creatinine concentration during pacing-induced heart failure, indicating that the rise in plasma NOx was due to a decreased renal function. NO concentration in cardiac tissue must reach very high values to impair cardiomyocyte function. Such high levels are clearly present, for example, in endotoxic shock in humans and in dogs. We did not detect a significant increase in NO metabolites during the progression to cardiac failure. On the contrary, the onset of cardiac decompensation was characterized by the first statistically significant reduction of cardiac NOx production. Previous studies provided strong evidence that changes in NOx production reflect changes in NO synthesis. In particular, we have shown that cardiac NOx production can increase in response to enhanced coronary flow during dynamic exercise and can be abolished by blocking NOS with NLA. Moreover, a recent study in which the isotope ¹⁸O was used demonstrated that NOS inhibition by 30 mg/kg of NLA reduces by 91% the formation of NOx in vivo. It is possible that the changes in cardiac NOx production observed in the present study were simply due to an altered distribution of coronary flow, with enhanced perfusion of regions producing less NO. This possibility can be ruled out on the basis of a prior study on the distribution of coronary flow in pacing-induced heart failure. Those authors used radioactive microspheres and found a homogeneous decrease in LV and right ventricular flow compared with control, with reduced subendocardial flow reserve but unaltered baseline endocardial-epicardial ratio. In the present study cardiac NOx production decreased significantly from day 21 to pacing time corresponding to 30% change from day 21. Linear fit described by the generic function $y = a + (b \times \text{time})$. Exponential fit described by the generic function $y^2 = a + (b \times e^{-\text{time}})$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$a$</th>
<th>$b$</th>
<th>$r^2$</th>
<th>$P$</th>
<th>$t_{30}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOx production</td>
<td>1.545</td>
<td>-0.065</td>
<td>0.91</td>
<td>&lt;0.05</td>
<td>22.6</td>
</tr>
<tr>
<td>MVO$_2$</td>
<td>1.048</td>
<td>-0.023</td>
<td>0.94</td>
<td>&lt;0.05</td>
<td>22.7</td>
</tr>
<tr>
<td>FFA uptake†</td>
<td>0.0025</td>
<td>99.11×10$^5$</td>
<td>0.99</td>
<td>&lt;0.05</td>
<td>21.5</td>
</tr>
<tr>
<td>Lactate uptake*</td>
<td>0.07</td>
<td>0.003</td>
<td>0.1</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>Glucose uptake*</td>
<td>-72.25</td>
<td>3.69</td>
<td>0.98</td>
<td>&lt;0.05</td>
<td>23.6</td>
</tr>
<tr>
<td>RQ*</td>
<td>0.16</td>
<td>0.02</td>
<td>0.95</td>
<td>&lt;0.05</td>
<td>23.3</td>
</tr>
</tbody>
</table>

$t_{30}$ is the pacing time corresponding to 30% change from day 21.

$*$Linear fit described by the generic function $y = a + (b \times \text{time})$.

†Exponential fit described by the generic function $y^2 = a + (b \times e^{-\text{time}})$.

**Figure 6.** Changes in myocardial MVO$_2$ (A) and cardiac uptake of FFA (B), lactate (C), and glucose (D) 1 hour after administration of 30 mg/kg IV of NLA in 14 normal, conscious dogs. Data are mean±SEM. *$P<0.05$ vs control.
production was positive at control, as previously reported by us,17,18; however, it became negative during heart failure, resulting in an apparent cardiac uptake of NOx. The uptake was obviously apparent, since the heart is not the organ responsible for NOx clearance. Nitrites and nitrates, end products of NO oxidation, would rapidly accumulate in the cardiac tissues, reaching toxic concentrations. A possible explanation of the apparent uptake is the redistribution of NOx between plasma and erythrocytes. It is known, for example, that NO3− can exchange with Cl− contained in red cells in <10 seconds, even at 0°C.30 Preliminary data from an ongoing study in our laboratory indicate that part of NOx accumulates in red cells and that the intraerythrocyte fraction is proportional to bicarbonate concentration. The latter is responsible for NOx clearance. Nitrites and nitrates, end products of NO oxidation, would accumulate in red cells and that the intraerythrocyte fraction is proportional to bicarbonate concentration. The latter is inversely proportional to the PO2. Therefore, the percentage is proportional to bicarbonate concentration. The latter is inversely proportional to the PO2. Therefore, the percentage of NOx accumulates in red cells and that the intraerythrocyte fraction is proportional to bicarbonate concentration. The latter is inversely proportional to the PO2. Therefore, the percentage of NOx accumulates in red cells and that the intraerythrocyte fraction is proportional to bicarbonate concentration.

Our findings support the studies demonstrating depressed production of NO in heart failure.31,32 In particular, defective NO-mediated control of coronary circulation has been well documented in animal models and in humans:12,13 NOS blockade did not cause baseline hemodynamic changes in patients with heart failure and in dogs with pacing-induced heart failure, indicating that the basal release of NO was already depressed.2–10 In dogs with pacing-induced heart failure, endothelial NOS gene expression and synthesis were reduced.15 Cardiac microvessels (≤70 μm in diameter) have the potential to produce large amounts of NO, as demonstrated in vitro, and the release of NO from coronary microvessels of failing hearts was also depressed.14

It is possible that the decreased cardiac NOx production observed in the present study mainly reflects the endothelial dysfunction in microvessels. In a previous study we have hypothesized that NO released by coronary microvessels/capillaries is a paracrine factor that might play an important role in modulating metabolism of the adjacent cardiomyocytes.16 The mean distance between capillary endothelial cells and cardiomyocytes is <8 μm,33 whereas the maximal diffusion distance of NO is 200 to 600 μm.34 Other studies showed that NO can modulate calcium sensitivity of myofilaments, most likely through an increase in cGMP.35,36 In particular, some authors have shown that reduced endogenous NO or augmented exogenous NO do decrease or increase, respectively, LV diastolic compliance in vitro and in vivo.37,38 This phenomenon was independent of end-diastolic volume.

Taken together, all these observations would be complemented by the findings of the present study in explaining at least 2 aspects of the pathophysiology of heart failure: diastolic dysfunction and altered metabolism. Our data would also support the known therapeutic efficacy of organic nitrates and angiotensin-converting enzyme inhibitors in the treatment of this syndrome.39–41 The highly significant correlation shown in this study, between progressive rise in LVEDP and decrease in cardiac NOx production, likely reveals the loss of control by NO of ventricular relaxation and venous return. In fact, the therapeutic properties of NO donors in heart failure are primarily based on the reduction of ventricular preload.

The alterations of myocardial metabolism in heart failure are complex and little understood. Our experiments in vitro have shown that basal myocardial MVO2 was 54% higher in failing tissue than in normal tissue.16 In the present study MVO2 did not change significantly over time, but in end-stage failure, LV work and triple product, indexes of cardiac MVO2, were decreased by 83% and 55% versus control, respectively. Moreover, the failing heart would consume ≥12% more oxygen if it used FFA as the primary substrate instead of glucose. Studies performed in isolated, working canine hearts and in human hearts documented an increase of cardiac MVO2, not related to total mechanical energy, in failing versus normal hearts at a given level of cardiac contractility.42,43 Such an increase was interpreted as an elevated basal metabolism and/or augmented oxygen cost of contractility of the failing heart. The first interpretation receives additional support from our data, since an inhibitory function of NO on mitochondrial respiration has been documented in vitro44,45 and, indirectly, in vivo.17,46 A failing heart, with reduced NO production, would lose this tonic modulation and basal metabolism would be consequently elevated. No data, on the other hand, document a possible involvement of NO in the control of oxygen cost of contractility. This cost is due to excitation-contraction coupling, ie, to the ATP consumed for Ca2+ cycling and reuptake by the sarcosomal membrane. However, several investigations in vitro demonstrated a modulatory action of NO, through the second messenger cGMP, on calcium channels of the sarcomplasmic reticulum.47,48 Its role and importance are not clear yet, but if NO modulates intracellular Ca2+ influx in vivo, then a lack of NO would imply more energy expenditure at each excitation-contraction cycle, in particular at higher levels of contractility, when enhanced active calcium reuptake is required. In a previous study performed in our laboratory,17 blockade of NO synthesis during exercise caused an increase in myocardial MVO2 at equal levels of cardiac work.

The present data show a profound change in cardiac substrate utilization during decompensated heart failure. During the fourth week of pacing, we observed a 70% reduction of FFA uptake associated with a steep rise in glucose utilization. The time to a 30% change in substrate uptake (t30), after 21 days of pacing, indicates that the fall in FFA consumption preceded either the decrease in NOx or the increase in glucose consumption. It is important to note, however, that the t30 for FFA uptake is heavily influenced by the type of curve fit (exponential), and thus we cannot exclude that the real 30% decrease, after 3 weeks, occurred at a later time. Glucose was very likely oxidized, as indicated by the increase in respiratory quotient versus control. Moreover, lactate was always taken up rather than produced by the heart, indicating that this model of heart failure is not characterized by anaerobic glycolysis. A healthy heart utilizes mostly FFA and, to a lesser extent, lactate during fasting.49 The RQ values that we found at control, 0.73 to 0.76, indicate in fact that oxidation of FFA generated 80% to 90% of the total energy.22 Previous studies have already shown that during the development of heart failure, cardiac muscle relies on glycolysis as the primary source of energy.50–52 The present study provides...
the first direct evidence of changes in cardiac substrate utilization during pacing-induced heart failure. The shift to glucose by the failing heart is considered a recapitulation of fetal metabolism. It is probably an adaptive although insufficient response. Myocardial glucose oxidation in heart failure might in part compensate for the elevated basal MVO₂. ATP yield from carbohydrate oxidation is greater for a given rate of MVO₂ because of the higher ATP/ΔO₂ ratio compared with that of fatty acid oxidation.⁴⁶ To obtain the same amount of ATP by oxidizing, for instance, palmitate, one of the most abundant FFA in the blood, a failing heart would consume 12% more oxygen. An increased efficiency, ie, ratio of total mechanical energy to MVO₂, has in fact been shown in hearts isolated from dogs with pacing-induced heart failure.⁴⁵ Another advantage of glucose utilization, as proposed by some authors,⁵¹ is that glycolytically derived ATP is preferentially used for Ca²⁺ reuptake into the sarcoplasmic reticulum, and this is essential for optimal diastolic relaxation. We cannot exclude, however, that the observed switch of energy source also plays a role in the progression of heart failure.

The significant and linear correlation between fall in cardiac NOx production and increase in glucose uptake represents an important new finding. It raises the question of whether such correlation is a pure association of events or if there is any cause-effect relationship. The preference for glucose, in our group of dogs, might be in part explained by the simultaneous reduction of the competitive FFA uptake, a reduction that could be related to the significant fall in arterial plasma FFA levels. In humans, for instance, cardiac FFA utilization diminishes when the concentration of these substrates in arterial plasma falls below the threshold of 0.3 mmol/L, which really corresponds to 0.3 mEq/L.⁴⁹,⁵⁴ In our study, the minimum level reached during cardiac decompensation was not <0.3 mEq/L, yet the threshold in dogs has not been established. Moreover, a low plasma level of FFA can explain the reduction in uptake but not the RQ value of 1 that was found in end-stage failure. This RQ indicated that the residual uptake of FFA did not correspond to their oxidation, since the heart oxidized only carbohydrates. In fact, another cause of reduction in FFA oxidation, in a rat model of heart failure, has been recently attributed to the downregulation of mitochondrial fatty acid β-oxidation enzymes,⁵⁸ and an accumulation of long-chain acylcarnitine in tissue from failing heart has been documented.⁵⁶

To directly address whether changes in NO production can result in switch of substrate utilization by the heart, NLA was administered to normal, conscious dogs. There was an acute and dramatic change in cardiac substrate utilization similar to that observed during cardiac decompensation. These results strongly suggest that the correlation between changes in cardiac NOx release and metabolism, during pacing-induced heart failure, is due to a cause-effect relationship. The switch in substrate utilization 1 hour after NLA administration resembles very closely that observed during decompensated failure. NLA caused a fall of FFA concentration in plasma that could explain the decrease in FFA uptake. However, the increase in RQ was higher than it would have been if substrates were completely oxidized. NOS blockade caused an inhibition of FFA oxidation. We cannot exclude that the fall in plasma concentration of FFA, occurring either in end-stage failure and after NOS inhibition, is also related to decreased production of NO. To date, no mechanisms have been described that could explain the role of NO in FFA uptake and oxidation. On the other hand, it has been demonstrated in vitro that NO can stimulate ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase,⁶⁷ the enzyme responsible for the first oxidation-reduction reaction of glycolysis. NO-related ADP-ribosylation likely inhibits enzymatic activity, although its role in vivo has not been defined. In heart failure or after NOS blockade, the lack of NO would remove such inhibition to the glycolytic pathway. These observations are curious since several investigations demonstrated that NO potentiates insulin-mediated uptake of glucose in skeletal muscle⁶⁸ and, indeed, patients with heart failure present moderate insulin resistance.⁵⁹

In conclusion, we have shown that the onset of cardiac decompensation in pacing-induced heart failure is associated with a dramatic fall in cardiac NO production. This fall is also accompanied by a switch in myocardial substrate utilization. A quantitatively similar shift in substrate utilization occurs acutely after inhibition of NO production with the use of NLA in normal dogs. These findings suggest that one of the pathophysiological mechanisms involved in cardiac decompensation might be the lack of NO, an important modulator of cardiac function, coronary vascular function, and myocardial metabolism.

Acknowledgments

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References


Reduced Nitric Oxide Production and Altered Myocardial Metabolism During the Decompensation of Pacing-Induced Heart Failure in the Conscious Dog
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