Oxidative capacity in failing hearts

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In normal myocardium, it is unclear whether peak O\textsubscript{2} utilization is ultimately limited by maximal oxidative ATP synthetic capacity or by the maximal capacities of the myosin and other ATPases to utilize ATP. In failing myocardium, abnormalities of excitation-contraction coupling as well as downregulation of \(\beta\)-adrenergic receptors and the downstream adenyl cyclase system (25) make it even more difficult to determine whether ATP synthetic capacity limits contractile performance. Hence, the hypothesis that primary "energy starvation" limits function in heart failure (14) remains to be rigorously tested in vivo despite evidence that left ventricular (LV) hypertrophy (LVH) and congestive heart failure (CHF) are associated with abnormalities of myocardial energy metabolism (2, 21, 22, 38, 39).

Consequently, the present study was performed to determine whether administration of a classical mitochondrial uncoupling agent (2,4-dinitrophenol (DNP)) could further increase myocardial oxygen consumption (MV\text{O}_2) in hearts with compensated LVH or overt cardiac failure that were already functioning at a high work state produced by catecholamine stimulation. DNP accelerates intramitochondrial metabolism proximal to ATP synthase by decreasing the proton gradient across the inner mitochondrial membrane (17, 30). In response to DNP, MV\text{O}_2 increases in concert with intermediary metabolism and electron transport activity to maintain the mitochondrial proton gradient that drives ATP synthesis (15, 17, 30). Although it is unlikely that DNP can define the maximal oxygen utilization capacity in the intact heart (8), it can be used to determine whether there is a reserve capacity of the reaction sequences that generate the mitochondrial proton gradient required to support ATP synthase activity. We hypothesized that if the capacities of the energetic reactions proximal to ATP synthase constrained mechanical performance during high work states, then DNP would not cause a further increase of MV\text{O}_2 and might cause deterioration of mechanical performance and myocardial high-energy phosphate (HEP) content. Measurements of myocardial HEP levels were made with \(^{31}\text{P}\) NMR spectroscopy. Because of concerns that abnormalities of perfusion or diffusion might limit oxygen delivery and thereby impair respiratory rates in the hypertrophied or failing hearts, myocyte oxygenation was assessed using \(^1\text{H}\) NMR spectroscopy.

METHODS

Experiments were performed in accordance with the "Position of the American Heart Association on Research Animal Use," adopted November 11, 1984, and protocols were approved by the Animal Care Committee of the University of Minnesota.

Production of LVH. Sixteen Yorkshire pigs at ~45 days of age were anesthetized with pentobarbital sodium (25–30 mg/kg iv).
and 31P measurements. A capillary containing 15 mg/kg iv), intubated, and ventilated with a respirator. A right thoracotomy was performed in the third intercostal space, and the ascending aorta, ~1.5 cm above the aortic valve, was mobilized and encircled with a polyethylene band 2.5 mm in width. While LV and distal aortic pressures were simultaneously measured, the band was tightened until a 70-mmHg peak systolic pressure gradient was achieved across the narrowing. The chest was then closed, the pneumothorax was evacuated, and the animals were allowed to recover. LVH occurred progressively as the area of aortic constriction remained fixed in the face of normal body growth. Two months after being banded, animals were returned to the laboratory for study.

Experimental preparation. Ten normal pigs and twenty-one pigs with LVH were premedicated with morphine sulfate (1 mg/kg sc) and anesthetized with pentobarbital (30 mg/kg iv, followed by an infusion of 4 mg·kg⁻¹·h⁻¹). A smaller dose of pentobarbital (~20 mg/kg iv) was used for animals with CHF to prevent loss of animals from general anesthesia. Animals were intubated and ventilated with a respirator with supplemental oxygen; arterial blood gases and pH were measured in the physiological range. The pericardial cradle (3.0 mm outer diameter) filled with heparin-saline was introduced into the right femoral artery and advanced into the ascending aorta. A left thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. A heparin-saline-filled catheter was introduced into the LV through the apical dimple and secured with a purse-string suture. A similar catheter was placed into the left atrium through the atrial appendage. A catheter was also introduced into the anterior coronary vein via the right atrial appendage. An NMR surface coil was sutured to the anterior LV wall overlying the region perfused by the left anterior descending coronary artery. The surface coil was constructed of a single turn of copper wire and incorporated a 33-pF capacitor; surface coils were 28 mm in diameter. The surface coil leads were connected to a balanced-tuned circuit external and perpendicular to the thoracotomy incision. The pericardial cradle was released, and the heart was allowed to assume its normal position. The animals were then placed in a Lucite cradle and positioned within the magnet. Arterial blood gases were measured every 15 min, and the respirator was adjusted to maintain the PO2, PCO2, and pH in the physiological range.

Myocardial blood flow. Myocardial blood flow was measured with microspheres (15 μm in diameter) labeled with 141Ce, 51Cr, 52mNb, 85Sr, or 46Sc (NEN; Boston, MA) as previously described (3). Blood flow was expressed as milliliters per minute per gram of myocardium. A capillary containing 15 μl of 3 M phosphonoacetic acid was placed at the coil center to serve as a reference. The proton signal from water was used to homogenize the magnetic field and adjust the position of the animal in the magnet so that the coil was at or near the magnet and gradient isocenters. Spectra were obtained with the image-selected in vivo spectroscopy (ISIS) method, which defined a column 1.8 × 1.8 cm² perpendicular to the LV wall. 31P signal excitation was achieved with a 90°, adiabatic, B1-insensitive, BIR-4 pulse. All chemical shifts were measured relative to the PCr peak, which was assigned a chemical shift of ~2.55 ppm relative to 85% phosphonoacetic acid at 0 ppm.

Resonance intensities were quantified using integration routines provided by SISCO software. The ATP/γ resonance was used for ATP determination. Because data were acquired with the transmitter frequency positioned between the ATP/γ and PCr resonances, off resonance effects on these peaks were virtually nonexistent. Numerical values for PCr and ATP were expressed as ratios of PCr to ATP (PCr/ATP). P levels were measured as changes from baseline values (ΔPi) using integrals obtained in the region covering the Pi resonance and are presented as ΔP/PCr. ADP levels in each animal were calculated by subtracting the total creatine and ATP levels, and 31P NMR determined PCr and intracellular pH levels as previously described (16).

1H NMR spectroscopic technique. Determination of deoxy-myoglobin (Mb-δ) using the 1H NMR method has previously been reported in detail (5, 38). In brief, radiofrequency transmission and signal detection were performed with the dually tuned 28-mm-diameter surface coil. A single-pulse collection sequence with a frequency-selective gaussian excitation pulse (1 ms) was used to selectively excite the N-δ proton signal of the proximal histidine in Mb-δ. A short repetition time (25 ms) was used due to the short T1 of Mb-δ. Each spectrum is acquired in 5 min (10,000 free induction decays). Although the short T1 of Mb-δ and fast acquisition prevent gating to the cardiac cycle, the signal loss due to motion was negligible due to the inherently broad line width of the Mb-δ peak.

Hemodynamic measurements. Aortic and LV pressures were monitored with pressure transducers positioned at the midstest level. Data were recorded on an eight-channel direct writing recorder (Coulbourne Instrument; Lehigh Valley, PA). LV pressure was recorded at normal and high gain for measurement of end-diastolic pressure.

Experimental protocol. Hemodynamic measurements and NMR spectra were first obtained under basal conditions. Midway through the 10-min NMR data-acquisition period, a microsphere injection was performed for determination of myocardial blood flow. After baseline data were obtained, dobutamine and dopamine were infused simultaneously (each 20 μg·kg⁻¹·min⁻¹ iv) to induce a high cardiac work state. After ~10 min was allowed to achieve a steady state, all measurements were repeated. After completion of data acquisition, while the dobutamine and dopamine infusion continued, four sequential 5-min infusions of DNP (each 2 mg/kg iv) were given; all measurements were repeated after a 10- to 15-min stabilization period after the first infusion (2 mg/kg) and after the final infusion (cumulative dose 8 mg/kg). Microspheres were administered for measurement of myocardial blood flow during baseline conditions, during catecholamine infusion, and during the DNP infusion.

Tissue preparation. With the use of a forceps precooled to −7°C, at the end of the experiment, an epicardial biopsy was taken from five normal, four LVH, and four CHF ventricles for subsequent analysis of ATP and creatine content using HPLC techniques (31). The heart was then fixed in 10% buffered formalin. The myocardium beneath the surface coil was sectioned into three transmural layers from epicardium...
to endocardium, weighed on an analytic balance, and placed into vials for counting of radioactivity. Similar specimens were obtained from the lateral and posterior LV wall to insure that the sample from beneath the surface coil was typical of the entire LV.

**Data analysis.** Numerical values for PCr and ATP during each experimental condition were expressed as PCr/ATP. MV˙O₂ was calculated from the measured blood flows and the difference in O₂ content between aortic and anterior coronary vein blood samples. Hemodynamic data were measured from the chart recordings. Hemodynamic, biochemical, and blood flow data were analyzed with one-way ANOVA with replications. A value of $P < 0.05$ was required for significance. When the ANOVA yielded a significant result, individual comparisons were made using the method of Scheffé. Data are reported as means $\pm$ SE.

**RESULTS**

**Animal model.** Nine of twenty-one pigs with aortic banding developed CHF as evidenced by ascites (range 100–2,000 ml). The remaining 12 pigs with aortic banding formed the compensated LVH group. The anatomic data are summarized in Table 1. The ratio of LV weight to body weight was increased by 51% in hearts with LVH ($P < 0.05$ vs. control) and increased by 101% in hearts with CHF ($P < 0.05$ vs. LVH). The ratio of right ventricular weight to body weight was increased in both LVH and CHF groups ($P < 0.05$). Figure 1 shows a typical short-axis section of a normal LV (Fig. 1A) with a wall thickness of 0.8 cm compared with a typical LVH heart with concentric hypertrophy (Fig. 1B) in which wall thickness was 2.0 cm and the cavity size was decreased.

**Hemodynamic data.** Hemodynamic data are shown in Table 2. At baseline, LV systolic pressure and the rate-pressure product (RPP) were significantly higher in hearts with aortic stenosis than in normal hearts (Table 2). LV end-diastolic pressure was significantly increased only in hearts with CHF. In all three groups, heart rate, LV systolic pressure, and RPP increased significantly during catecholamine stimulation. During high-dose DNP infusion, RPP increased significantly in the LVH and CHF animals but not in the normal group.

**Myocardial blood flow and oxygen consumption data.** Mean myocardial blood flow and the transmural distribution of perfusion were similar in normal, LVH, and CHF hearts at baseline (Table 3). Myocardial blood

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<tr>
<td>Normal</td>
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<tr>
<td>LVH</td>
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<td>CHF</td>
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</tbody>
</table>

Values are means $\pm$ SE; n, no. of pigs. LVH, left ventricular (LV) hypertrophy; CHF, congestive heart failure; RV, right ventricular. $^*P < 0.01$ vs. normal; †$P < 0.05$ vs. LVH.
flow and M\(\text{VO}_2\) increased significantly in all three groups during catecholamine infusion. M\(\text{VO}_2\) increased further in the normal and LVH groups during DNP infusion but not in the CHF group.

**HEP measurements.** HEP data are summarized in Table 4. In normal animals, \(^{31}\text{P}\) spectra recorded during the control period were characterized by high PCr/ATP. Basal PCr/ATP was significantly decreased in hearts with LVH and further decreased in CHF hearts (Table 4, \(P < 0.05\) vs. normal). \(P_i\) was too low to identify at the signal-to-noise ratio of the spectra under basal conditions in any group. Infusion of dopamine-dobutamine caused significant reductions of PCr/ATP in normal and LVH hearts without a significant change in the already low PCr/ATP in CHF hearts. Catecholamine infusion was associated with the appearance of a \(P_i\) resonance in all three groups of animals. As shown in Table 4, the addition of DNP to dobutamine-dopamine caused no further change of PCr/ATP in the normal and LVH groups. However, in the animals with CHF, DNP caused a significant further decrease of PCr/ATP, from 1.61 \(\pm\) 0.06 to 1.47 \(\pm\) 0.11 (\(P < 0.05\)). In the normal animals, the addition of DNP during catecholamine infusion caused no change in \(\Delta P/\Delta\text{Cr}\). In contrast, in both the LVH and CHF groups, the addition of DNP resulted in significant increases of \(\Delta P/\Delta\text{Cr}\) (Table 4). As shown in Table 5, both ATP and total creatine levels (chemically measured) were reduced in the CHF group, whereas calculated [ADP] was significantly increased in both the LVH and CHF groups.

**Mb-\(\delta\) measurements.** No Mb-\(\delta\) resonance was observed in any group under any of the experimental conditions.
conditions, although transient occlusion of the coronary artery supplying the area of myocardium beneath the NMR coil consistently produced a large Mb-δ resonance.

**DISCUSSION**

The major findings in the present study are as follows: 1) basal state values of PCr/ATP were reduced in both groups with aortic banding (corresponding to increased free ADP), with the most severe reductions being present in the CHF group; 2) when DNP was administered during catecholamine infusion, MV̇O₂ increased significantly in the normal and LVH groups but not in the CHF group; and 3) DNP had no additional effect on PCr/ATP in the normal and LVH groups but caused a significant further reduction of PCr/ATP in the CHF group. These findings suggest that near-maximal oxidative capacity had been approached during catecholamine stimulation in the CHF hearts but not in normal hearts or hearts with compensated hypertrophy.

**Swine pressure-overload hypertrophy model.** In the present study, a supravalvular aortic stenosis was applied in juvenile swine to produce an initial pressure gradient of ~70 mmHg across the constriction (35). The animals subsequently developed myocardial hypertrophy as the degree of aortic narrowing remained fixed in the face of normal body growth. Approximately 40% of the animals developed CHF as manifested by an increased LV end-diastolic pressure, exercise intolerance, and ascites; the increase in LV/body weight was twice as great in animals that developed CHF as in those with compensated LVH. With the use of a similar but milder degree of aortic constriction in swine, Massie et al. (19) found that a stenosis that produced a 25-mmHg pressure gradient across the aortic narrowing resulted in a 38% increase of LV mass in 6 mo with no evidence of heart failure in any of the animals. The more severe aortic narrowing makes the present model useful for study of the response of physiological and metabolic variables to severe pressure overload and the evolution to overt CHF.

**Previous evidence of bioenergetic abnormalities in hypertrophied and failing myocardium.** Myocardial PCr/ATP is decreased in patients (22) and in large and small animal models of LVH secondary to pressure and volume overload and postinfarction LV remodeling (11, 21, 23, 36, 37, 39). Furthermore, abnormalities in oxidative phosphorylation have been described in in vitro studies of failing myocardium (6, 32). Consistent with these observations, we (18, 24) recently reported that ATP synthase and adenine nucleotide translocator (ANT) protein expression is decreased in animals with CHF secondary to postinfarction remodeling or pacing-induced heart failure. In swine with moderate pressure overload produced by ascending aortic constriction, Massie et al. (19) found that basal PCr/ATP tended to be decreased in animals with LVH and that PCr/ATP decreased significantly and similarly in normal and LVH hearts during the increased cardiac work state produced by dobutamine infusion. They observed that animals with LVH had greater glucose uptake during dobutamine stimulation; however, a greater fraction of the glucose was oxidized in the LVH animals, indicated that the preference for glucose in the LVH hearts was not the result of ischemia. This finding is in agreement with the absence of Mb-δ in the present study, indicating adequate oxygen availability. Thus the animals in that study behaved similarly to the animals with compensated LVH in the present study.

**Do metabolic limitations constrain myocardial function?** During basal conditions, [ADP] was higher in the LVH and CHF hearts than in the normal group. This indicates that the kinetic relationship between [ADP] and the ATP synthesis rate (as indicated by MV̇O₂) is altered in LVH and CHF myocardium (37). Altered substrate preference might contribute to the increased [ADP] in the overloaded hearts. Hypertrophied and failing hearts demonstrate increased glucose utilization (4); an increase in [ADP] has been found in perfused hearts when glucose is the sole substrate (9). Furthermore, swine hearts with CHF secondary to postinfarction LV remodeling demonstrate decreased expression of F₁,Fₐ,ATPase and ANT1 proteins (18, 24). The increased [ADP] may reflect successful kinetic adaptation to the reduced ATP synthase and ANT1 protein expression or activity. However, the ability to increase contractile performance and oxygen consum-

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**Table 4. Myocardial high-energy phosphates**

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<thead>
<tr>
<th></th>
<th>Normal</th>
<th>LHV</th>
<th>CHF</th>
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<tbody>
<tr>
<td>ΔP/PCr</td>
<td>n.</td>
<td>d.</td>
<td>d.</td>
</tr>
<tr>
<td>Baseline</td>
<td>12</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>DpDp DNP (0.2 mg/kg iv)</td>
<td>0.25 ± 0.07†</td>
<td>0.25 ± 0.08‡</td>
<td>0.49 /- 0.12‡</td>
</tr>
<tr>
<td>DNP (8 mg/kg iv)</td>
<td>0.29 ± 0.11</td>
<td>0.27 ± 0.08</td>
<td>0.46 ± 0.15</td>
</tr>
<tr>
<td>DNP (8 mg/kg iv)</td>
<td>0.35 ± 0.17</td>
<td>0.45 ± 0.10‡</td>
<td>0.72 ± 0.09‡</td>
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<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>LHV</th>
<th>CHF</th>
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<tbody>
<tr>
<td>PCr/ATP</td>
<td>n.</td>
<td>d.</td>
<td>d.</td>
</tr>
<tr>
<td>Baseline</td>
<td>12</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>DpDp DNP (0.2 mg/kg iv)</td>
<td>2.07 ± 0.07</td>
<td>1.86 ± 0.09</td>
<td>1.68 ± 0.07†</td>
</tr>
<tr>
<td>DNP (8 mg/kg iv)</td>
<td>1.85 ± 0.10*</td>
<td>1.67 ± 0.09†</td>
<td>1.61 ± 0.06</td>
</tr>
<tr>
<td>DNP (8 mg/kg iv)</td>
<td>1.89 ± 0.11</td>
<td>1.75 ± 0.09</td>
<td>1.47 ± 0.11‡</td>
</tr>
<tr>
<td>DNP (8 mg/kg iv)</td>
<td>1.91 ± 0.16</td>
<td>1.68 ± 0.05</td>
<td>1.46 ± 0.09</td>
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</table>

Values are means ± SE; n, no. of pigs. *P < 0.01 vs. normal; †P < 0.05 vs. baseline; ‡P < 0.05 vs. DbDp.

**Table 5. Myocardial ATP, total creatine, and calculated free ADP levels**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>LVH</th>
<th>CHF</th>
</tr>
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<tbody>
<tr>
<td>[ATP] μmol/g dry wt</td>
<td>n.</td>
<td>d.</td>
<td>d.</td>
</tr>
<tr>
<td>Baseline</td>
<td>6</td>
<td>21.9 ± 3.2</td>
<td>113 ± 16.5</td>
</tr>
<tr>
<td>[Cr] μmol/g dry wt</td>
<td>n.</td>
<td>d.</td>
<td>d.</td>
</tr>
<tr>
<td>Baseline</td>
<td>5</td>
<td>18.5 ± 2.6</td>
<td>119 ± 18.3</td>
</tr>
<tr>
<td>[ADP] μmol/g dry wt</td>
<td>n.</td>
<td>d.</td>
<td>d.</td>
</tr>
<tr>
<td>Baseline</td>
<td>5</td>
<td>15.6 ± 3.0*</td>
<td>86 ± 15.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs. *P < 0.05 vs. normal; †P < 0.05 vs. LVH.
tion in response to catecholamine stimulation in the present study indicates that, under basal conditions, the hypertrophied and failing hearts did have significant cytosolic and mitochondrial metabolic reserve capacity to augment the ATP synthetic rate.

During catecholamine infusion, $\text{MV}_\text{O}_2$ approximately doubled in all groups. The animals with compensated LVH tolerated the increased need for ATP synthesis during catecholamine stimulation with no greater decrease of PCr/ATP than the normal hearts, and the already low PCr/ATP in the CHF group did not decrease further. The normal $\text{MV}_\text{O}_2$ and functional responses of the CHF hearts to catecholamine stimulation were surprising given the depressed adrenergic responsiveness generally observed in failing hearts (25). It is possible that there is less downregulation of $\beta$-adrenergic receptor pathways in the present swine pressure overload model than in CHF of other etiologies. In addition, the aortic constriction appeared to contribute to the increase of RPP, because, although aortic pressure did not increase in the CHF hearts during catecholamine infusion (unlike the normal hearts in which aortic pressure did increase), LV systolic pressure did increase significantly during catecholamine infusion. In any event, the ability of the hypertrophied and failing hearts to respond to catecholamine stimulation was not less than that of normal hearts.

Use of partial mitochondrial uncoupling to unmask a mitochondrial functional abnormality. The mitochondrial uncoupling agent DNP was used to examine whether mitochondrial oxidative capacity (proximal to ATP synthase) was limited in LVH or CHF hearts during catecholamine stimulation. DNP decreases the proton gradient across the mitochondrial inner membrane that is required to support ATP synthesis. This results in compensatory augmentation of mitochondrial carbon substrate and oxygen consumption to maintain the proton gradient sufficient to sustain mitochondrial function (15, 30). Thus DNP would be expected to increase $\text{MV}_\text{O}_2$ and the rates of the supporting metabolic reactions that cause electrons to be delivered to cytochrome oxidase if the rates of these reactions are not already maximal. That is, an increase of $\text{MV}_\text{O}_2$ in response to DNP administration would indicate the presence of a functional reserve proximal to ATP synthase. In the present study, DNP administered during continuing catecholamine infusion elicited a $\sim 22\%$ increase in $\text{MV}_\text{O}_2$ in normal hearts and in hearts with compensated LVH, indicating a reserve oxidative capacity. Furthermore, the increased $\text{MV}_\text{O}_2$ produced by DNP did not tax oxygen delivery to the myocardium, because myocardial Mb-δ remained below the level of detection. Taken together, these data indicate that during DNP infusion, there was no limitation of cytosolic oxygen availability and that the reserve oxidative capacity (i.e., beyond the level reached during catecholamine administration alone) was present in normal hearts and in hearts with compensated hypertrophy.

In contrast to the normal hearts and hearts with compensated hypertrophy, in the failing hearts administration of DNP during catecholamine infusion caused no further increase in $\text{MV}_\text{O}_2$. Furthermore, DNP caused a significant decrease of the already low PCr/ATP. DNP tended to increase RPP in all groups of animals, and this was significant in the LVH and CHF groups during the high dose. This increase in cardiac work may have contributed to the increased $\text{MV}_\text{O}_2$ in the normal and LVH groups as well as to the decrease in PCr/ATP in the failing hearts. The failure of $\text{MV}_\text{O}_2$ to increase in response to DNP in the CHF group suggests that the maximal capacity to utilize $\text{O}_2$ had been approached during catecholamine stimulation. The fall of PCr/ATP would be consistent with this view and suggests that a compensatory increase of [ADP] occurred because respiration was not able increase to compensate for the increased proton leak across the inner mitochondrial membrane caused by DNP (30). Alternatively, it is possible that $\text{MV}_\text{O}_2$ failed to increase during DNP because the failing hearts had already become partially uncoupled during the high workload produced by catecholamine administration. Although the absolute increase in $\text{MV}_\text{O}_2$ in response to dobutamine-dopamine was greater in the CHF hearts, the relative increase (compared with the baseline value) was similar in normal (+102%) and CHF hearts (+105%) but tended to be less in LVH hearts (+70%). The mechanism for the greater $\text{MV}_\text{O}_2$ per gram of myocardium during baseline conditions in the aortic banded animals is uncertain, although we (1) have previously observed that $\text{MV}_\text{O}_2$ was also significantly higher in dogs with LVH secondary to aortic banding studied during awake conditions. It is likely that the increase in $\text{MV}_\text{O}_2$ during dobutamine-dopamine in the CHF hearts brought them closer to abolishing their respiratory reserve compared with the normal hearts or the hearts with compensated LVH.

Our findings are in agreement with previous in vitro studies that have demonstrated that the maximal oxidative capacity of mitochondria (33) or skinned fibers from failing myocardium is decreased compared with normal myocardium (32). However, the current data indicate that such abnormalities are not sufficiently severe to constrain in vivo performance at the moderately high work states induced by catecholamine administration. Thus during basal conditions and with moderate increases of cardiac workload bioenergetic abnormalities in failing myocardium were not functionally limiting, and the decreased contractile performance was likely the result of primary contractile abnormalities of the myocytes (34) as well as an unfavorable chamber geometry (13). Therefore, during catecholamine administration, the rate of ATP expenditure, rather than limitation of mitochondrial function, appeared to determine $\text{MV}_\text{O}_2$ in all groups of hearts. However, the DNP data suggest that maximal $\text{MV}_\text{O}_2$ had been approached in the CHF group during catecholamine infusion. Calculations based on study of the oxidative capacity of isolated mitochondria (18) suggest that the maximal oxygen consumption capacity of...
normal swine myocardium should be ~24.3 μmol·g wet wt⁻¹·min⁻¹ or ~54 ml·100 g wet wt⁻¹·min⁻¹ (20). Because this value is nearly twice the maximum MVo₂ values achieved in the present study, a significant reduction of mitochondrial capacity in the LVH group might not have been detected with the present experimental protocol.

DNP causes increased local metabolic demands, which in turn result in metabolic vasodilation of the resistance vessels. In peripheral tissues, DNP causes a marked increase in oxygen extraction. However, in the porcine hearts in the present study, DNP caused no significant change in coronary venous oxygen tension, indicating no consistent change in myocardial oxygen extraction. If DNP had acted as a primary coronary vasodilator, then one would have expected decreased oxygen extraction by the heart with an increase in coronary venous oxygen tension. Conversely, if the increase in coronary flow was the result of an increase in oxygen demands, then one might have expected a decrease in coronary venous oxygen tension, because presumably an error signal would be required to elicit metabolic vasodilation. This did not occur. The possibility remains that DNP caused metabolic vasodilation but that oxygen was not the mediator of that response; rather, some other metabolic signal during DNP could have caused vasodilation. It is of interest that there is a difference in the response of coronary venous oxygen tension between dogs and swine during exercise. Whereas dogs show a marked decrease in coronary venous PO₂ during exercise, swine do not, apparently because of feedforward control of the coronary circulation by the adrenergic nervous system (7). It is possible that adrenergic activation during DNP infusion similarly caused some degree of coronary vasodilation that prevented an increase in myocardial oxygen extraction in the present study.

The absence of myocardial myoglobin desaturation indicates that the increased oxygen utilization during catecholamine infusion and DNP administration did not exceed the ability to deliver oxygen. The inability to detect Mb-δ with the ¹H NMR technique indicates that myocyte PO₂ values were >15 mmHg, far above the Michaelis-Menten constant value for O₂ with respect to cytochrome oxidase (38). However, the MVo₂ values measured during maximal catecholamine stimulation are substantially lower than the MVo₂ levels that have been measured in normal hearts during heavy exercise (1). Furthermore, DNP-induced increases of MVo₂ almost certainly underestimate “true” mitochondrial reserve capacity (the maximum capacity to support ATP utilization under physiological conditions). This is because decreases of the mitochondrial proton gradient also compromise important mitochondrial functions other than ATP synthesis such as maintenance of ion gradients; these functions are also required to support ATP synthesis (26). Consequently, DNP cannot be used estimate maximal MVo₂ capacity (8). In intact tissues including myocardium, DNP can decrease the proton gradient to the point that the ATP synthesis rate required to support contractile and other cellular functions cannot be maintained despite a substantial increase in MVo₂. However, it is reasonable to assume that a significant increase of MVo₂ induced by DNP does indicate the presence of a reserve mitochondrial oxidative capacity so long as contractile function is maintained and HEP levels do not markedly deteriorate.

In summary, during the high workload produced by catecholamine stimulation, DNP was able to further increase MVo₂ in compensated hypertrophied hearts, indicating a significant energy generation reserve. Furthermore, the increase of MVo₂ in response to DNP occurred with no reduction of PCR/ATP. These findings support the view that primary abnormalities of oxidative ATP production did not constrain contractile performance at moderately high workloads in hearts with compensated pressure overload hypertrophy. In contrast, during catecholamine stimulation in failing hearts, DNP failed to increase of MVo₂ and caused a significant reduction of PCR/ATP. These findings suggest that the capacity of some rate-limiting reaction(s) in the oxidative ATP synthetic pathway may have been reached during the high workload produced by catecholamine administration.

DISCLOSURES

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