Enhancement of Mitochondrial Oxidative Phosphorylation Capability by Hypoperfusion in Isolated Perfused Rat Heart

Peter C.D. Pelikan, James T. Niemann, Guangzhi Xia, Gale Jagels, and John Michael Criley

To define alterations in myocardial mitochondrial function due to hypoperfusion, oxidative phosphorylation was simultaneously studied in 17 control (stable perfusion pressure) rat hearts and 17 hypoperfused isolated rat hearts. Hypoperfusion for 30 minutes was achieved by a reduction in coronary perfusion pressure from 77.8 ± 1.2 mm Hg (mean ± SEM) to 20.2 ± 1.8 mm Hg in the experimental group (control perfusion pressure after 30 minutes 75.6 ± 1.2). Hypoperfusion caused a reduction in left ventricular developed pressure to 20.5 ± 1.5 mm Hg (versus control 74.8 ± 3.3, p<0.001), a reduction of coronary flow rate to 4.9 ± 0.3 ml/min (versus control 19.4 ± 1.2, p<0.0001), and a drop in myocardial oxygen consumption to 0.06 ± 0.005 ml O2/min (versus control 0.17 ± 0.01, p<0.001). Myocardial lactate production was increased by hypoperfusion (3.0 ± 0.6 µmol/min) compared with controls (0.7 ± 0.5, p<0.02), but myocardial creatine kinase release was similar in the hypoperfused and control groups. Hypoperfusion was associated with an augmentation of state 3 mitochondrial respiration with glutamate and malate as respiratory substrates (448.8 ± 14.0 ng atoms O/min/mg mitochondrial protein versus controls 290.7 ± 13.4, p<0.001). When rates were normalized for mitochondrial malate dehydrogenase (MDHm), state 3 respiration was still increased in hypoperfused hearts (24.1 ± 2.1 ng atoms O/min/µU MDHm) compared with controls (15.5 ± 1.6, p<0.02). The rates of dinitrophenol-uncoupled electron transport were similar to the rates of state 3 respiration in both the hypoperfused and control groups. No alterations in mitochondrial function were found with succinate as substrate. Therefore, moderate levels of hypoperfusion enhanced mitochondrial oxidative phosphorylation when glutamate and malate were the respiratory substrates, consistent with an augmentation of the activity of electron transfer complex I and/or the rates of mitochondrial glutamate/malate uptake. This enhancement may be an adaptive mechanism to maximize ATP synthesis during or following myocardial oxygen supply/demand imbalance. (Circulation Research 1987;61:880-888)

During ischemia, limitation of oxygen delivery reduces mitochondrial oxidative phosphorylation and results in a reduction of intracellular adenosine triphosphate (ATP) content. After severe ischemia, isolated mitochondria show defects in structure and in the kinetics of oxidative phosphorylation, predominantly a reduction in state 3 respiration and an uncoupling of ATP synthesis. These changes may play a role in defining the extent of ischemia-induced loss of tissue viability and may limit cell viability during reperfusion.

The function and metabolism of myocardium subjected to a reduction in, but not complete cessation of, coronary perfusion have not been studied extensively. After coronary occlusion, coronary flow is low but persistent in parts of the area at risk. In these areas, there is less necrosis, and the time of onset of irreversible tissue injury occurs later. Hypoperfused myocardium, therefore, may have a greater likelihood of survival during reperfusion and deserves further study.

The purpose of this study was to examine the effects of myocardial hypoperfusion on left ventricular function and on the kinetics of mitochondrial oxidative phosphorylation. The isolated, perfused, beating rat heart was used as the model. Systolic and diastolic left ventricular pressures, coronary flow rate, myocardial oxygen consumption, myocardial lactate production, and creatine kinase release were monitored in control and hypoperfused hearts. After perfusion, the rates of mitochondrial oxidative phosphorylation were measured. These experiments demonstrate an unexpected increase in the maximum rate of ATP synthesis (state 3 respiration) in the mitochondria isolated from hypoperfused hearts. This may represent an adaptive mechanism that maximizes ATP synthesis during myocardial oxygen supply/demand imbalance. The biochemistry of hypoperfused myocardium may be important in the rational development of therapeutic strategies designed to increase myocardial salvage during postischemic reperfusion.

Materials and Methods
Isolated, Perfused Rat Heart

Randomly paired, male Sprague-Dawley rats (300–400 g) were anticoagulated with heparin and then anesthetized with intraperitoneal pentobarbital (15–30 mg/kg).
mg/kg). Under anesthesia, hearts were removed and immediately immersed in cooled perfusate. Within 30–60 seconds, the ascending aorta was cannulated distal to the coronary arteries and perfusion established by a modified Langendorff method.15 Perfusate contained (in mM) NaCl 140, KCl 5, CaCl₂ 1.5, MgCl₂ 1.2, HEPES 4, and glucose 10 at 37°C. The pH of the perfusate was adjusted to 7.40 with NaOH; perfusate was continuously bubbled with 100% oxygen, with measured Po₂ approximately 500 mm Hg.

Aortic and, therefore, coronary perfusion was gravity regulated via a perfusate reservoir positioned 110 cm above the isolated hearts. Perfusion pressure was strictly controlled by in-line constriction and measured with a side-arm of the aortic perfusion catheter connected to a Statham P23ID transducer (Gould Statham Instruments, Inc., Hato Rey, Puerto Rico). All hearts were paced at 375 beats/min by a Grass pacing stimulator (Grass Medical Instruments, Inc., Quincy, Mass.). The left ventricle was vented with an apical stab incision. A thin-walled rubber balloon attached to a fluid-filled length of PE 190 tubing was inserted into the left ventricular cavity through the left atrium and held in place with a purse-string ligature. The PE tubing was connected to a pressure transducer by a three-way stopcock to allow recording of left ventricular systolic and diastolic pressures. After beginning perfusion, intraventricular balloon volume was increased in increments of 0.05 ml until a maximum developed pressure was achieved and was left at that volume throughout the experiment.

A second PE 190 catheter was inserted into the right ventricle through the dissected pulmonary artery remnant. In preliminary studies, the Po₂ of perfusate withdrawn from the right atrium was found to be the same as that of perfusate withdrawn from the right ventricle. Since catheter position was more stable in the right ventricle than the right atrium, perfusate effluent was withdrawn through this tubing to measure coronary sinus Po₂. Because oxygen diffusion through the plastic tubing could have altered the O₂ content, the Po₂ measured with this system was compared with that obtained with a metal catheter/glass syringe assembly. The measured Po₂ using the plastic assembly was almost identical to that obtained with the metal/glass assembly (n = 6, R = 1.0, R² = 0.999, p < 0.001). A Corning Model 165 blood gas analyzer (Medfield, Mass.) was used for measurement of perfusate and effluent Po₂. Myocardial oxygen consumption (MV̇O₂) was calculated as follows:

\[ MV̇O₂ = k \times \text{coronary flow rate} \times (Po₂ - \text{aorta} - Po₂) \text{ coronary sinus} \]

where k is O₂ solubility constant \(3.25 \times 10^{-4} \text{ ml O}_2/\text{mm Hg Po}_2\).

The coronary effluent was also utilized to assess the rates of myocardial lactate production and creatine kinase (CK) release to determine whether hypoperfusion resulted in anaerobic metabolism and/or myocardial damage. Myocardial lactate production was calculated as follows: myocardial lactate production = coronary flow rate × coronary sinus lactate concentration. Coronary effluent lactate was measured every 10 minutes during perfusion with an automated lactate analyzer (model 23L, Yellow Springs Instrument Co., Yellow Springs, Ohio).

Myocardial CK release was also monitored every 10 minutes. Coronary effluent creatine kinase was measured using an Abbott Biochromatic Analyzer (Abbott Laboratories, Diagnostics Division, Irving, Tex.), and myocardial CK release was calculated using the following formula: myocardial CK release = coronary flow rate × coronary sinus CK concentration.

Myocardial oxygen consumption, lactate production, and CK release were not normalized for cardiac weight because the left ventricular weights were similar in the hypoperfused and control hearts (1.2 ± 0.02 versus 1.1 ± 0.02, respectively; p < 0.05) and because the relative contributions of the atria and right ventricle to coronary flow, oxygen consumption, and lactate and CK production were not evaluated separately.

Experimental Protocol

Hypoperfusion. All experiments were performed on pairs of hearts. After establishing perfusion with a coronary perfusion pressure of approximately 80 mm Hg in both hearts, the intraventricular balloon volume was adjusted to produce maximum left ventricular developed pressure (systolic minus diastolic pressure). After a 20–30-minute baseline stabilization period in both hearts, baseline measurements of left ventricular systolic and diastolic pressures, coronary perfusion pressure, coronary flow rate (measured effluent volume), and coronary sinus Po₂ were recorded. One heart from each pair was then randomly selected to undergo 30 minutes hypoperfusion (coronary perfusion pressure reduced to 18–22 mm Hg) by partial clamping of the perfusion line. During hypoperfusion, the temperature was maintained at 37°C with constant temperature water jackets in the perfusion line and heart bath, and pacing was continued at 375 beats/min. The coronary perfusion pressure in the control heart was not changed after randomization, and perfusion in this heart was continued for 30 minutes, simultaneously with the experimental heart. In both hearts, left ventricular systolic and diastolic pressures and coronary perfusion pressure were recorded every 5 minutes. Coronary flow rate and coronary sinus Po₂, CK, and lactate levels were measured every 10 minutes. The aortic perfusate Po₂ was measured at the end of perfusion. At the end of this 30-minute experimental period, perfusion was discontinued, and each heart was chilled separately in mitochondrial isolation medium as described below.

Global ischemia. Five hearts were isolated and perfused as described above. After the baseline stabilization period, during which left ventricular end-diastolic and developed pressures were measured, the coronary perfusion lines were totally clamped, reducing the coronary flow rate to zero. Thereafter, hearts were maintained at 37°C, paced at 375 beats/min, for 30 minutes with no alteration in the intraventricular
Mitochondria were isolated and studied as described below.

**Mitochondrial Isolation**

Mitochondrial isolation medium contained 70 mM sucrose, 210 mM mannitol, 0.5 mM EGTA [ethylene glycol bis-(aminoethyl ether) N,N',N'-tetra-acetic acid], 5 mM HEPES, and 1 mg/ml bovine serum albumin, at pH 7.20 and temperature 4°C. Mitochondria were isolated separately and simultaneously from the experimental and control hearts using a modification of the technique previously described by Jacobus. Briefly, after removal of the atria and the right ventricle, the left ventricle was weighed and then finely minced. After trypsin digestion and homogenization with a Teflon tissue homogenizer, cellular debris was separated by differential centrifugation at 480g for 10 minutes at 2°C. The supernatant solution containing the mitochondrial fraction was decanted and filtered. Three further centrifugations at 7,700gX 10 minutes with subsequent washings were performed to isolate a final mitochondrial pellet. This was resuspended in 50 μl of isolation medium. Protein determination was by the biuret method. The final concentration was 30–50 mg mitochondrial protein/ml.

**Oxyngraph Determinations**

To measure the rates of oxidative phosphorylation, Clark oxygen electrode studies were conducted in a 1.8-ml chamber at 37°C. The oxygraph medium contained (mM) KCl 130, KH₂PO₄ 2, MgCl₂ 1, K₂-EGTA 0.5, HEPES 5, and either glutamate 10 plus malate 10 or succinate 5 (with 5 μM rotenone) as respiratory substrates. The oxygraph medium was adjusted to pH 7.20 and 37°C. The rates of resting and stimulated oxygen uptake of 0.5 mg mitochondrial protein with glutamate/malate as substrate or 0.25 mg protein with succinate as substrate were measured with standard techniques.

The rate of endogenous respiration (state 2) was measured in the presence of phosphate ion, substrate, and oxygen before the addition of ADP to the oxygraph chamber. Thereafter, the maximum ADP stimulation rate (state 3) and recovery rate (state 4) of oxygen consumption were measured after the pulsed addition of ADP with a final concentration of 195.9 μM. Oxygen consumption rates were determined as nanogram atoms oxygen per minute per milligram mitochondrial protein. To quantify the relation of mitochondrial respiration in the absence of ADP to that in the presence of ADP, the acceptor control ratio (state 3/state 2) was calculated. In addition, the respiratory control ratio (state 3/state 4) and ADP/O ratio were calculated for each heart (modified from Chance and Williams and Lehninger). All 17 pairs of hearts perfused were thus studied with glutamate and malate as substrates, and 11 of the 17 pairs were also studied with succinate as substrate.

To define the kinetics of oxidative phosphorylation, one or two separate determinations of state 3 respiration were made after the addition of each of 6 different ADP pulses with final concentrations approximately 10, 20, 40, 75, 110, and 196 μM. The rate of state 3 respiration increases with increasing ADP concentration and asymptotically approaches V̇̇O₂, (the maximum rate of ATP production). Therefore, a double-reciprocal (Lineweaver-Burk) plot was used to calculate the V̇̇O₂m as well as the apparent Kₘ (ADP concentration at ½ V̇̇O₂m) for ADP for mitochondria isolated from each heart. Six of the 17 pairs of hearts were used to measure V̇̇O₂m and the apparent Kₘ with glutamate and malate as substrates. In 6 of the 11 pairs of hearts additionally studied with succinate (see paragraph above), V̇̇O₂m and the apparent Kₘ with succinate as the respiratory substrate were determined.

To explicate further the alterations in mitochondrial function induced by the perfusion protocol, mitochondrial fractions were also exposed to 2,4-dinitrophenol (DNP, 80 nmol pulsed addition) in the oxygraph chamber to measure uncoupled rates of electron transport. Correlation of V̇̇O₂m with the DNP uncoupled rate of electron transport allowed an assessment of the relation between alterations in the kinetics of oxidative phosphorylation and the rate of electron transport.

**Mitochondrial Malate Dehydrogenase Activity**

When rates of mitochondrial respiration are expressed relative to mitochondrial protein, the inclusion of fragmented, nonfunctional mitochondria would increase the protein content but not the rate of respiration of the isolated fraction. Thus, decreased purity would falsely underestimate the actual mitochondrial activity. To exclude this possibility, the activity of mitochondrial malate dehydrogenase (MDHm), a mitochondrial enzyme that is stable during ischemia, was assayed in selected mitochondrial fractions spectrophotometrically. This procedure allowed the expression of the rates of respiration relative to this marker enzyme rather than to the nonspecific protein concentration of the mitochondrial fraction.

**Statistics**

Statistical analyses were performed on a VAX 11/750 computer using the CLINFO program. Data from control hearts were compared with those from experimental hearts using unpaired t tests and the Wilcoxon rank sum test when data were not normally distributed. Linear regression was used to derive the equation of the double-reciprocal plot relating 1/state 3 to 1/[ADP], from which V̇̇O₂m and the apparent Kₘ were calculated. Data are presented as mean ± SEM.

**Results**

**Cardiac Function and Coronary Perfusion**

In both the hypoperfused and control hearts, left ventricular function and coronary flow (Table 1) were similar before the reduction in coronary perfusion pressure in the experimental group.

At the beginning of the period of experimental hypoperfusion, the coronary perfusion line for the randomly selected experimental heart was partially
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clamped. The coronary perfusion pressure was reduced to low but stable levels and after 30 minutes was 20.2 ± 1.8 mm Hg. As expected, the coronary flow rate in these hearts was also reduced and after 30 minutes was 4.9 ± 0.3 ml/min. In comparison, the control group coronary perfusion pressure was 75.6 ± 1.2 mm Hg, and the coronary flow rate was 19.4 ± 1.2 ml/min (*p < 0.0001 versus experimental).

In Figure 1, the marked drop in left ventricular developed pressure in the experimental hearts after reduction in coronary perfusion pressure is shown in contrast to pressure in control hearts. After 30 minutes hypoperfusion, the developed pressure was only 20.5 ± 1.5 mm Hg in the experimental group, whereas in control hearts it was 74.8 ± 3.3 (*p < 0.0001). End-diastolic pressure showed a slight initial fall after the onset of hypoperfusion and then rose. At the end of the perfusion protocol, the end-diastolic pressure was 3.5 ± 2.0 mm Hg in the experimental hearts and 3.2 ± 0.9 in the controls (*p = NS).

The 5 hearts selected to undergo 30 minutes of ischemia also had similar left ventricular function prior to cessation of coronary flow. The left ventricular developed pressure was 80.4 ± 3.6 (*p versus control = NS), and the end-diastolic pressure was 2.6 ± 2.4 (p versus control = NS). After clamping the coronary perfusion line, the coronary perfusion pressure and the coronary flow rate both fell to zero. There was a concomitant fall in developed pressure, which became unmeasurable within 1–2 minutes.

Global Myocardial Metabolism

\( \text{MVO}_2 \) was similar in the experimental hypoperfused (0.19 ± 0.01 ml/min) and control (0.18 ± 0.01) groups of hearts prior to the onset of hypoperfusion (*p = NS). However, accompanying the reduction in coronary perfusion pressure and the resultant decreases in coronary flow rate and left ventricular pressure development, there was a fall in \( \text{MVO}_2 \). After 10 minutes hypoperfusion, the \( \text{MVO}_2 \) reached a low, stable level. After 30 minutes, the \( \text{MVO}_2 \) was 0.06 ± 0.01 ml O\textsubscript{2}/min in the hypoperfused group compared with 0.17 ± 0.01 in controls (*p < 0.0001). The changes in \( \text{MVO}_2 \) in the two groups of hearts are shown in Figure 2.

Both the hypoperfused and control hearts (n = 5 pairs) produced similar levels of lactate during the baseline period (0.0 ± 0.0 μmol/min versus 0.5 ± 0.5, respectively, *p = NS). As shown in Figure 3, myocardial lactate production increased to 3.0 ± 0.6 μmol/min after 30 minutes hypoperfusion and remained low in controls, 0.7 ± 0.5 (*p < 0.02).

CK release was assessed in 4 pairs of hearts and was not found to be increased by cardiac hypoperfusion. During the baseline period, myocardial CK release was 121.1 ± 23.3 mIU/min in the experimental hypoperfused hearts and 59.4 ± 19.7 in the controls (*p = NS). At the end of 30 minutes hypoperfusion, the myocardial CK release was 51.1 ± 20.6 compared with 39.1 ± 16.4 in the controls (*p = NS).

Mitochondrial Oxidative Phosphorylation

When glutamate and malate were used as substrates (17 pairs of hearts), mitochondria isolated from hypoperfused hearts showed a marked increase in the rate of state 3 respiration compared with simultaneously perfused control hearts. Table 2 summarizes the data on oxidative phosphorylation when state 3 respiration was stimulated by addition of ADP at a final concentration of 195.9 μM. Accompanying the augmentation of state 3, there was an increase in state 4 respiration. However, there was no evidence of uncoupling of oxidative phosphorylation. In fact, the acceptor control ratio actually was higher in the hypoperfused group. The ADP/O ratio in the experimental, hypoperfused hearts was similar to that in the controls.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Developed pressure in the experimental and control groups of hearts. In the 17 experimental hearts, developed pressure dropped after coronary perfusion pressure was reduced at time zero but remained stable in the 17 control hearts (mean ± SEM).
In comparison to the augmentation in state 3 respiration observed in hypoperfused heart mitochondria, 30 minutes of ischemia resulted in a reduction in state 3 respiration to 124.6 ± 25.9 ng atoms O/Min/mg mitochondrial protein (p versus control <0.0001) with glutamate and malate as the respiratory substrates. The acceptor control index was also reduced to 4.4 ± 1.2 (p versus control <0.02).

In 11 of the 17 pairs of hearts, rates of oxidative phosphorylation were also studied using succinate as the respiratory substrate. The data for these studies are shown in Table 3. These experiments were performed in the presence of 5 μM rotenone and utilized a final concentration of ADP of 195.9 μM to stimulate state 3 respiration. The marked increase in the rate of glutamate/malate-supported state 3 respiration induced by hypoperfusion, described above, was also apparent in this selected subgroup. Nevertheless, with succinate as the substrate, no differences in states 2, 3, and 4 respiration were found when these rates were compared in experimental and control hearts. In addition, there were no alterations in the acceptor control, respiratory control, and ADP/O ratios.

With succinate as substrate, 30 minutes of ischemia reduced state 3 respiration to 135.3 ± 42.9 ng atoms O/Min/mg mitochondrial protein (p versus control <0.0001) and similarly reduced the acceptor control ratio to 2.8 ± 0.5 (p versus control <0.005).

Kinetics of Oxidative Phosphorylation
A differential effect of the substrates on the Vmax and apparent Km of state 3 respiration was also present (Table 4). The mean Vmax with glutamate and malate in the experimental, hypoperfused hearts was almost twice as high as that measured in control hearts. In addition, the apparent Km for ADP, with glutamate and malate as substrate, was also twice as high in the experimental hearts as in controls. In contrast, with succinate as substrate, the Vmax and apparent Km for ADP were both similar in control and experimental hearts. In the 6 pairs of hearts in which the kinetics of oxidative phosphorylation were measured with succinate as substrate, the rates of states 3 and 4 respiration were also determined with glutamate and malate, using a final ADP concentration of 195.9 μM, and were found to be increased in the hypoperfused hearts, similar to the increase observed in all 17 pairs of hearts.

Uncoupled Mitochondrial Respiration
The rates of uncoupled respiration, measured after the pulsed addition of 80 nmol DNP, were compared with the rates of state 3 respiration for both glutamate/malate and for succinate as the respiratory substrates. As shown in Table 5, with a given substrate, the rates of state 3 respiration and uncoupled electron transport were similar in each group of hearts. Therefore, the hypoperfusion-induced alterations in the rate...
of ATP synthesis occurred in parallel with the alteration in the rates of uncoupled electron transport.

**Mitochondrial Yield and Purity**

Because differences in mitochondrial fragility could result in the isolation of different amounts of mitochondria from control and experimental hearts, mitochondrial yield was quantified directly. In 5 pairs of hearts, the total mitochondrial protein isolated, as well as the MDHm activity, was measured in addition to the rates of oxidative phosphorylation using glutamate/malate and succinate as respiratory substrates. These data are represented in Table 6, the increased rate of state 3 respiration was evident with glutamate and malate as respiratory substrates but was not observed with succinate as substrate. These data represent further corroboration that the increased rate of mitochondrial respiration in hypoperfused hearts actually represents an alteration in mitochondrial function and is not an artifact resulting from differences of purity of the control and hypoperfused mitochondrial fractions.

In ischemic hearts, in contrast, normalization for MDHm activity revealed a mild decrease in state 3 respiration that was not statistically significant with glutamate and malate as substrates (10.2 ± 2.5 ng atoms O/min/IU MDHm, p versus control = 0.11). With succinate as substrate, ischemic heart mitochondrial state 3 respiration was reduced to 12.5 ± 4.2 ng O/min/IU MDHm (p versus control <0.01).

**Discussion**

These experiments demonstrate an unexpected augmentation of the kinetics of mitochondrial oxidative phosphorylation in hypoperfused hearts when glutamate and malate are used as tricarboxylic acid cycle substrates. These alterations in mitochondrial function were not observed when succinate was utilized as the respiratory substrate. The alterations in MDHm-stimulated oxidative phosphorylation were similar to those measured after DNP uncoupling in each group of hearts with a given tricarboxylic acid cycle substrate. In contrast to these findings, mitochondria of hearts subjected to 30 minutes ischemia, with complete cessation of coronary perfusion, had reduced rates of state 3 respiration with both glutamate/malate and succinate as respiratory substrates. These data are consistent with a hypoperfusion-induced enhancement in the activity of the reduced nicotinamide adenine dinucleotide (NADH)-coenzyme Q reductase segment of the electron transport chain (electron transfer complex I) and/or in the rate of mitochondrial glutamate and malate uptake.

The results for the hypoperfused hearts in these experiments differ from those in previous studies that have shown a reduction in mitochondrial function caused by ischemia. In dogs, 1 hour of complete
circumflex coronary artery occlusion caused accumulation of intramitochondrial granules with a concomitant reduction in decarboxylation of pyruvate. When pyruvate was used as substrate, there was a severe reduction in state 3 respiration with a simultaneous decrease in the respiratory control ratio. With succinate as the tricarboxylic acid cycle substrate, there was a 70% reduction in state 3 respiratory rate. Decreased mitochondrial oxidative phosphorylation may result from a defect in electron transfer complex I since a greater reduction in mitochondrial respiratory rate occurred with the NAD-linked substrates glutamate and malate than with succinate in canine and porcine models of severe ischemia. Other investigators have found that severe ischemia caused a progressive loss of CK activity. However, previous investigations have not demonstrated increases in the rate of state 3 respiration caused by reductions in myocardial perfusion.

The experimental design of the present investigation differs from that of previous studies. Although a wide variety of experimental models has been used to study ischemia, most experiments have compared myocardium undergoing a complete or severe reduction in coronary flow with normally perfused myocardium. In the present study, coronary perfusion pressure was reduced to less than one third of the baseline level, and coronary flow was reduced to less than one quarter of control. This relative shift in the relation between myocardial supply and demand was associated with a decrease in the NADH/NAD ratio. Decreased mitochondrial function has been observed. Although not specifically addressed in each study, it is possible that acute or chronic myocardial oxygen supply/demand imbalance may have occurred in each of these models. Increases in cardiac work were studied both in vivo and in isolated perfused hearts. Starnes et al achieved prolonged exercise training in rats, possibly inducing repeated episodes of supply/demand imbalance.

Other studies may have induced supply/demand imbalance in an ejecting, and therefore working, isolated perfused heart apparatus. In the hearts of exercised animals, cardiac output, and therefore myocardial demand, was higher than in nonexercised controls, while coronary flow was similar in the two groups. This relative shift in the relation between myocardial supply and demand was associated with increased state 3 respiration with both glutamate and succinate as substrates. Acute alterations in mitochondrial function related to coronary flow were not examined. Increases in the work of isolated perfused rat hearts, achieved by increasing aortic afterload, also have been shown to increase the activity of the key mitochondrial enzyme pyruvate dehydrogenase, possibly via a decrease in the NADH/NAD ratio.

Although the relation between cardiac work and coronary flow was not addressed in this study, myocardial supply/demand imbalance may have occurred.

Other studies may have induced supply/demand imbalance via reduced myocardial oxygen delivery, with resultant increases in mitochondrial function. Perfusion of isolated rat hearts with doxorubicin has been shown to cause an increase in coronary resistance with systolic and diastolic left ventricular dysfunction and reduction in intracellular high-energy phosphate levels similar to those observed during ischemia.

Table 6. Mitochondrial Yield, MDHm Activity, and Rates of Oxidative Phosphorylation Expressed Relative to MDHm Activity

<table>
<thead>
<tr>
<th>Mitochondrial yield (mg mito/g LV)</th>
<th>No. heart pairs</th>
<th>Control</th>
<th>Hypoperfused</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate/malate as substrate</td>
<td>5</td>
<td>10.6±0.7</td>
<td>9.7±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Succinate as substrate</td>
<td>5</td>
<td>18.3±1.2</td>
<td>20.0±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>State 3, glut/malate*</td>
<td>5</td>
<td>15.5±1.6</td>
<td>24.1±2.1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>State 3, succinate*</td>
<td>5</td>
<td>31.0±3.4</td>
<td>29.8±1.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Nanograms atoms oxygen per minute per International Unit MDHm (after addition of ADP, 195.9 μM).
hanced state 3 respiration with the respiratory substrates glutamate and malate but not succinate. Increases in state 3 respiration also have been observed when oxygen delivery to the myocardium is reduced by hypoxia in the isolated perfused rat heart. In these studies, hypoxia caused an increase in coronary flow rate, a decrease in heart rate, and decreased MVo.

Cardiac work or developed pressure was not assessed. Cardiac work or developed pressure was not assessed. Rate, a decrease in heart rate, and decreased MVo.

Increases in state 3 respiration also have been observed in the isolated perfused rat heart. In these models, the increase in state 3 respiration with glutamate/aspartate may represent an adaptive mechanism to maximize ATP synthesis during or following myocardial supply/demand imbalance (e.g., during recovery from hypoxia or extremes of exercise). The enhanced utilization of glutamate and malate during ischemia may also provide a rational basis for using these agents to improve myocardial function during reperfusion. In this light, it is interesting that previous studies have shown an improvement in left ventricular function and increase in myocardial ATP content when anoxic isolated perfused rat hearts were perfused with the tricarboxylic acid cycle intermediates fumarate, malate, and glutamate. Isolated perfused rabbit heart septae also benefit from perfusion with amino acids during and after ischemia and anoxia. Only those amino acids that accumulate as glutamate and malate were effective. In dogs, reperfusion after ischemia resulted in enhanced myocardial oxygen consumption, ventricular function, and ATP content when the reperfusate was enriched with glutamate and aspartate.

In summary, these results indicate that hyperperfusion is associated with an enhancement of the kinetics of mitochondrial oxidative phosphorylation with the NAD-linked substrates glutamate and malate. Similar alterations may occur in human beings during myocardial infarction or cyanosis and may be important in the development of, or protection from, myocardial damage.

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