Effects of High Arterial Oxygen Tension on Function, Blood Flow Distribution, and Metabolism in Ischemic Myocardium

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Background. Although oxygen inhalation therapy has long been used in the treatment of acute myocardial ischemia, experimental evidence that increased arterial PO₂ has any beneficial effect in the absence of hypoxemia is equivocal. In this study, we used a swine model of subendocardial myocardial ischemia to determine the effects of arterial hyperoxia on regional myocardial contractile function (sonomicrometry), myocardial blood flow distribution (microspheres), and regional myocardial glycolytic metabolism (carbon isotope–labeled substrates).

Methods and Results. In 10 domestic swine, the left anterior descending coronary artery was cannulated and flow to this artery was strictly controlled via a roller pump in the perfusion circuit. Arterial PO₂ was controlled by manipulating inspired oxygen concentration (FIo₂). Low-flow myocardial ischemia was induced by reducing pump flow to 50% of the control value, which diminished regional endocardial systolic shortening to 30–50% of normal. After a 15-minute period of flow stability, each animal was exposed in randomized order to two additional 15-minute experimental periods: coronary normoxia (PO₂=90–110 mm Hg) and coronary hyperoxia (PO₂>400 mm Hg). At each level of oxygenation, we measured regional myocardial function, regional myocardial blood flow and metabolism, and hemodynamic indexes of myocardial oxygen demand. Myocardial ischemia during normoxia reduced systolic shortening to 10.9±5.3% in the ischemic zone. Hyperoxia increased ischemic zone systolic shortening substantially to 15.2±4.6%. During myocardial ischemia, endocardial blood flow was decreased to 0.26±0.06 ml·g⁻¹·min⁻¹ in the ischemic zone. During hyperoxia, endocardial blood flow rose to 0.34±0.10 ml·g⁻¹·min⁻¹. The endocardial:epicardial flow ratio was 0.45±0.18 in the initial ischemia period and rose to 0.61±0.23 in the hyperoxic period. Myocardial ischemia increased regional uptake of glucose, conversion of glucose to released lactate, and net myocardial lactate release. In the ischemic myocardium, coronary hyperoxia decreased both chemically measured lactate production and isotopically measured lactate release and decreased glucose extraction and the conversion of glucose to lactate.

Conclusions. These data demonstrate for the first time that increasing arterial PO₂ to high levels during acute low-flow myocardial ischemia improves both function and flow distribution in the ischemic myocardium and decreases glycolytic metabolism in the ischemic zone. The degree of improvement in contractile function (5% absolute increase in systolic shortening or 25% change normalized to preischemic values) is consistent with the observed increase in subendocardial blood flow. (Circulation 1992;85:828–838)

Oxygen inhalation has long been used in the treatment of patients with acute myocardial ischemia, particularly when ischemia is complicated by hypoxemia. Although it has generally been accepted that O₂ therapy is beneficial, the experimental evidence that increased arterial PO₂ has any beneficial effect in the absence of hypoxemia is equivocal.

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Several experimental studies have indicated potentially beneficial effects of oxygen breathing in animal models of acute myocardial ischemia. Maroko and coworkers\(^1\) found that inhalation of 40% oxygen in dogs, initiated 30 minutes after coronary occlusion, diminished both epicardial ST segment elevation and myocardial leakage of creatine phosphokinase. The proposed mechanism of this effect was a reverse coronary steal: oxygen-induced vasoconstriction in the normal adjacent myocardium that increased pressure at the origin of coronary collaterals in the ischemic zone, thus increasing collateral blood flow.\(^2\) Ishikawa et al\(^3,4\) used a canine model of acute low-flow myocardial ischemia to test the effects of 40% and 100% oxygen inhalation on myocardial lactate metabolism and contractile function and found that oxygen inhalation during myocardial ischemia produced a dose-related reduction in myocardial lactate production, converting it to net extraction. However, myocardial contractile force measured by using the epicardial mercury strain gauge technique was not improved. In none of these experiments were sensitive measures of regional myocardial function used, such as sonomicrometry.

Human studies have also suggested that, in patients with myocardial ischemia or infarction, breathing 100% oxygen has at least temporary beneficial effects, including diminished ST elevation,\(^5\) increased cardiac output in patients presenting with low output,\(^6\) and higher heart rate threshold for pacing-induced angina.\(^7\) However, evidence that oxygen breathing improves eventual outcome in these patients is lacking.\(^8\) The only prospective, double-blind study of the use of oxygen in patients with myocardial infarction found no significant difference between a control group and an oxygen-treated group in mortality, instance of arrhythmias, systolic time intervals, or use of analgesics.\(^8\) However, patients who were ultimately found not to have transmural myocardial infarction were retrospectively excluded from that study, introducing a selection bias against measuring beneficial effects of oxygen breathing in transient ischemia.

Because no published study to date has used sensitive measures of regional myocardial blood flow, function, and metabolism to study the effects of high arterial oxygen tension in acute myocardial ischemia, we designed the current study to measure these effects. We used an acute swine model of subendocardial ischemia to test the hypothesis that high arterial oxygen tension improves contractile function and decreases glycolytic metabolism during myocardial ischemia. The left anterior descending (LAD) coronary artery was cannulated, and flow to this artery was strictly controlled via a roller pump in the perfusion circuit. Arterial P\(_O_2\) was controlled by manipulating the inspired oxygen concentration (F\(_O_2\)). Regional contractile function was assessed by sonomicrometry, and myocardial blood flow distribution was measured by using the radiolabeled microsphere technique. Carbon-labeled isotopes of glucose and lactate were infused to quantify regional myocardial glycolytic metabolism.\(^9\)

**Methods**

**Anesthesia.** This experimental protocol was approved by our animal welfare committee, and follows the guidelines for animal use provided by the American Physiological Society. Studies were performed under general anesthesia in 10 open-chest domestic swine weighing 40–50 kg. Swine were premedicated with ketamine (10 mg·kg\(^{-1}\) s.c.); anesthesia was then induced by mask with oxygen and isoflurane (1–4%). A tracheostomy was performed under deep general anesthesia, ventilation was controlled, and anesthesia was maintained with isoflurane 0.8–1.5%. Ventilation was controlled to keep P\(_{ACO_2}\) at 35–40 mm Hg (pH, 7.40±0.04). After completion of the surgical preparation, isoflurane was decreased to 0.7±0.2% and was thereafter held constant. Anesthesia was supplemented with a high-dose narcotic technique by using a loading dose of fentanyl (50 \(\mu\)g·kg\(^{-1}\)) followed by a continuous fentanyl infusion (0.5 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\)). Temperature was maintained at 36.5–37.5°C by use of a heating blanket and by warming humidified inhaled gases. Inspired gas concentration was measured by mass spectrometry (Perkins-Elmer, Pomona, Calif.). Arterial blood gases were measured using a Radiometer (Copenhagen, Denmark) ABL-II blood gas laboratory. Hemoglobin and oxyhemoglobin saturation were measured using a Radiometer hemoximeter OSM3 with internal correction made for swine hemoglobin absorption characteristics.

**Surgery and hemodynamic instrumentation.** A median sternotomy was performed and 16-gauge catheters were inserted into the central aorta, left ventricle, and left atrium. A pressure transducer–tip catheter (Millar Instruments, Houston, Tex.) was inserted through the left atrium into the left ventricle for measurement of left ventricular pressure and its first derivative with respect to time (dP/dt). Atrial pacing was used to maintain constant heart rate during ischemia. Epicardial pacing electrodes were attached to the right atrium, and before inducing ischemia, pacing was begun at a rate 20% higher than the intrinsic heart rate. Pacing then continued at the same rate throughout the experiment. All transducer signals were recorded on a Grass model 7 polygraph (Quincy, Mass.). After all surgery and instrumentation was completed, the animal was heparinized systemically (10,000 units heparin i.v. bolus and 5,000 units/hour continuous infusion).

**Sonomicrometry.** In all experiments, myocardial contractile function was quantified by using midwall segment-shortening measurements. A small epicardial incision was made and (2 mm) lensed piezoelectric crystals (Dimension 3, La Jolla, Calif.) were inserted using a Teflon guide tube to a position approximately 3 mm from the subendocardium. These crystals were inserted 9–15 mm apart, facing
each other, and oriented perpendicular to the long axis of the heart. Crystal position was confirmed by direct inspection at dissection of the heart, and function data was used only if crystals were confirmed to be properly oriented.

Systolic segment shortening was averaged over at least five heartbeats and was calculated as

\[
\text{Systolic shortening (\%)} = \frac{\text{(end-diastolic length} - \text{end-systolic length})}{\text{end-diastolic length}} \times 100
\]

End diastole was defined as the onset of positive left ventricular dP/dt; end systole was defined as the time of peak negative dP/dt.\(^\text{10}\)

**LAD coronary cannulation and perfusion.** Initial measurements of segmental function and coronary pressure were made, and the LAD coronary artery was cannulated proximally using a plastic cannula manufactured in our laboratory (3 mm o.d.). Coronary pressure was measured just distal to the tip of this cannula by a 25-gauge catheter that passed through the cannula. Oxygenated blood was withdrawn from the carotid artery and pumped into the LAD coronary artery using a Masterflex digital roller pump (Cole-Parmer, Chicago, Ill.). Flow was measured both by the digital pump tachometer and by an in-line electromagnetic flowmeter (Micron Medical, Los Angeles). Flow measurements were calibrated by timed collection in a graduated cylinder. Coronary flow was initially set to provide a mean intracoronary pressure equal to mean aortic pressure. Adequacy of perfusion was assessed by the quick return of segmental function to precannulation values. If function did not return to precannulation levels within 10 minutes, the animal was excluded from study.

**Determination of normal or control coronary flow.** After return of segmental function to precannulation values and a 20-minute stabilization period, control flow was defined as that coronary flow at which mean coronary pressure was equal to mean aortic pressure.

**Induction of myocardial ischemia.** In all experiments, before ischemia, 1 mg/kg i.v. lidocaine was administered followed by a continuous lidocaine infusion of 2 mg/min. LAD coronary flow was then lowered by decreasing roller pump flow to 50% of control values, which diminished LAD systolic shortening to 30–40% of control values. Pump flow was subsequently held constant at this level for the duration of the experiment.

**Regional blood flow using radioactive microspheres.** During ischemia, regional myocardial blood flow was measured using radioactive microspheres.\(^\text{11}\) Different species of radioactive microspheres (\(^{54}\text{Mn}, \, ^{153}\text{Gd}, \, \text{or } ^{65}\text{Zn})\), all 15 microns in diameter, were prepared in dextran solution with a small amount of Tween-80 added to maintain dispersion. Approximately 5 million microspheres were suspended by vigorous agitation for 30 seconds and then injected into the left atrium over a 20-second period. Simultaneously, a 3-minute reference blood sample was withdrawn from the descending aorta at a rate of 4 ml/min. Regional myocardial blood flow was measured by injection of microspheres at three times: during ischemia at normal arterial P\(_2\) (ischemia/normoxia), during a second period of ischemia at normal arterial P\(_2\) (ischemia/normoxia\(^\text{'})\), and during ischemia at high arterial P\(_2\) (ischemia/hyperoxia).

At the end of each experiment, the area of myocardium perfused by the cannulated LAD artery was defined by a dye infusion technique: blood stained with Evans blue dye was infused into the cannulated LAD artery at normal aortic pressures, and the remainder of the coronary circulation was perfused at the same pressure with undyed blood from the aortic root. The stained myocardial area, sharply demarcated in swine, represents the area of LAD perfusion.

Each heart was cut into four or five short-axis slices from apex to base. The site of each ultrasonic crystal pair was excised and crystal placement confirmed by inspection. Multiple transmural samples from the center of the ischemic zone and the normal zone were divided into three approximately equal portions 0.5–1 g in size, representing subendocardial, midmyocardial, and subepicardial flow. Tissue samples were weighed and counted for gamma radioactivity using a Packard (Meriden, Conn.) gamma counter. After correction for background counts and isotope overlap, myocardial blood flow was calculated for each layer\(^\text{11}\) using the equation

\[
\text{Counts}_r \times \text{Flow}_{\text{ref}} = \text{Counts}_{\text{ref}}
\]

where Counts\(_r\) = counts in the tissue sample, Counts\(_{\text{ref}}\) = counts in the reference sample, and Flow\(_{\text{ref}}\) = reference sample flow. Reference flow was calculated by dividing the net weight of the reference sample by the specific gravity of blood, 1.05.

**Measurement of Myocardial Metabolism**

Isotope infusion and sampling. The animal surgery began at 8:00 AM after a 12-hour fast. A short Teflon catheter was inserted percutaneously into a peripheral vein for isotope infusion. To quantitate myocardial substrate utilization, D-[\(^{6}\text{14}\text{C}]\text{glucose and L-[}^{13}\text{C}]\text{lactate were infused using a primed continuous infusion technique. D-[}^{6}\text{-14}\text{C}]\text{glucose was obtained from New England Nuclear, (Boston) (sp act 56.1 mCi/mmol); it was sterilized by microfiltration (0.22-\mu m bacteriologic filter [Millipore Corp., Bedford, Mass.]) and diluted with 0.9% NaCl. L-[}^{13}\text{C}]\text{lactate (>99% enrichment) was obtained from Merck, Sharp and Dohme, Montreal, Canada, as L-[}^{13}\text{C}]\text{iodide lactate and sterilized as above. Priming doses of 32 \mu Ci of [}^{6}\text{-14}\text{C}]\text{glucose and 60 mg of [}^{13}\text{C}]\text{lactate were given intravenously over 1 minute followed by a continuous infusion of [}^{6}\text{-14}\text{C}]\text{glucose at 20 \mu Ci/hour and [}^{13}\text{C}]\text{lactate at 75 mg/hour.}
At least 20 minutes is required to achieve equilibration of the arterial and coronary sinus specific activity and the myocardial CO$_2$ pool when lactate is labeled with a tracer, and 25–30 minutes is required for a glucose tracer.$^9,12$ Thus, to ensure equilibration, the first control blood samples were obtained at 30.7±3.7 minutes after the priming bolus and the start of the continuous isotope infusion in this study.

Arterial and coronary vein blood samples were drawn simultaneously. Samples were obtained for chemical concentrations of glucose, lactate, and free fatty acids (FFA) and specific activities ($^{13}$C) of glucose and lactate, $^{13}$C enrichment of lactate, $^{13}$CO$_2$, and the O$_2$ content. Duplicate samples were obtained at each observation period. Blood flow and hemodynamic measurements were performed between these duplicates during the three intervention sampling periods. Duplicate measurements were also obtained during the control period with a 5-minute interval between sampling.

**Chemical analysis.** Weighed blood samples (3.5 ml) for analysis of lactate, glucose, specific activities, and $^{13}$C lactate enrichments were mixed immediately with a measured volume of cold 7% perchloric acid (1:2 vol/vol) and centrifuged. The protein-free supernatant was removed and stored at −4°C for future analysis. Another weighed blood sample (3 ml) was obtained for $^{13}$CO$_2$ analysis. The $^{13}$CO$_2$ was collected directly from blood by a diffusion method$^9$,12 a modification of the procedure of Hagenfeldt.$^13$ The coefficient of variation and the methodology for the chemical substrate analyses, determination of specific activities, $^{14}$CO$_2$ measurement, and [$U-^{13}$C]lactate analysis have been published previously.$^9,12,14$

[$U-^{13}$C]lactate content was assessed by gas-liquid chromatography/mass spectrometry using the method of Tserng et al.$^{15}$ Lactate was isolated from deproteinized blood using two ion exchange columns (H$^+$ and formate) and then converted to the $n$-propylamide heptafluorobutyrate derivative. A standard curve was prepared using labeled and unlabeled lactic acid (0.0–2.0%) and derivatized. The lactate recovered from blood was compared with the standard curve at $m/z$ 327 versus 330 using either a Hewlett Packard GC/MS (model 5890 and model 5971A, respectively; Avondale, Pa.) with a Hewlett Packard 12-m capillary column (cross-linked methyl silicone, 0.33-µm film thickness); or a gas chromatograph (model 2400; Infratronics, Dohrmann Envirotech, Mountain View, Calif.) interfaced to a mass spectrometer (ms-12; Kratos Analytical Instruments, Ramsey, N.J.), modified for selected ion recording analysis$^{16}$ using a 10’ DB-17, 1.0-µm film thickness, Megabore column (J & W Scientific, Folsom, Calif.) at 90°C isothermal with a helium flow rate of 4 ml/min.

Samples for plasma FFA concentrations were immediately placed in iced, heparinized glass tubes, centrifuged at 4°C, and separated. The FFAs were determined using gas chromatography by a modifica-

tion of the method of Ko and Royer.$^{17}$ All isotopic and chemical analyses were performed in duplicate.

**Calculations.** The chemical extraction (micromoles per milliliter) for a given substrate was calculated from the arterial and coronary vein substrate chemical concentration as [A]–[CV], where [A] is the arterial concentration and [CV] is the coronary vein concentration.

The isotopic lactate extraction ratio (percent) for [$U-^{13}$C]lactate was calculated from the concentration of [$U-^{13}$C]lactate in the artery and coronary (cor) vein as

\[
\frac{[A] \times \% \text{^{13}C}_3 \text{ in artery} - [CV] \times \% \text{^{13}C}_3 \text{ in cor vein}}{[A] \times \% \text{^{13}C}_3 \text{ in artery}} \times 100
\]

where

\[
\% \text{^{13}C}_3 = \frac{[^{13}\text{C}_3 \text{lactate}]}{\text{chemical lactate}} \times 100
\]

(pcent $^{13}$C$_3$ is obtained directly from gas chromatography/mass spectrometry).

The myocardial isotopic lactate extraction (micromoles per milliliter) was determined from the [$U-^{13}$C]lactate extraction ratio as [A]×isotopic lactate extraction ratio (%)/100.

Myocardial lactate release (micromoles per milliliter) is the difference between the isotopic lactate and the chemical lactate extraction and was calculated as isotopic lactate extraction − ([A]−[CV]).

The oxidation of glucose labeled with $^{14}$C (micromoles per milliliter) was calculated from the myocardial production of $^{14}$CO$_2$ and the arterial specific activity as

\[
\frac{(CV-A)^{14}\text{CO}_2 \text{ dpm/ml}}{\text{arterial specific activity of glucose}}
\]

Because other substrates are labeled secondarily when tracers are infused, the (CV−A)$^{14}$CO$_2$ was corrected for oxidation of the secondarily labeled substrates as previously published.$^9,12$

Using dual carbon-labeled isotopes of glucose and lactate and measuring the specific activities of glucose and lactate and the $^{13}$C enrichment of lactate in the artery and coronary sinus allows quantitation of the conversion of exogenous glucose to lactate by the myocardium as previously reported.$^9$

**Experimental Protocol**

Swine were ventilated with air that was slightly oxygen enriched, (FiO$_2$=21–25%), sufficient to achieve normal arterial Po$_2$ (90<Po$_2 <$100 mm Hg) (see Figure 1). LAD coronary artery was cannulated at its origin and perfused via roller pump with blood drawn from the carotid artery. Low-flow myocardial ischemia was induced by reducing pump flow to 50% of the control value, which diminished regional endocardial systolic shortening to 30–50% of normal. After a 15-minute period of flow stability, each animal was exposed to two additional 15-minute
experimental periods: normoxia ($P_O_2=90–100$) and hyperoxia ($P_O_2>400$ mm Hg) (Figure 2). The order of exposure during ischemia (normoxia to hyperoxia versus hyperoxia to normoxia) was determined by lot on the day of the experiment after the experimental preparation was completed and satisfactory. The number of lots were controlled so that five animals were assigned to each order group. When indicated by protocol, arterial $P_O_2$ was quickly changed by increasing the $FIO_2$ to 1.0 to raise $P_O_2$ of the coronary perfusate blood to $>400$ mm Hg. At each level of $FIO_2$, we measured regional myocardial function (sonomicrometry), regional myocardial blood flow (radioactive microspheres), regional glycolytic metabolism, and hemodynamic indexes of myocardial oxygen demand.

**Data Analysis**

Summary data are expressed as mean±SD. Most data consisting of repeated measurements in individual animals (blood flow, contractile function, metabolic data) were analyzed using analysis of variance with repeated measures.\(^1\) When indicated by ANOVA results, multiple comparisons were made by using the Newman-Keuls test or the paired $t$ test with Bonferroni correction.

Because the two randomized periods (hyperoxia and normoxia) were administered in two possible orders, and because a time-dependent or order effect might conceivably alter the hyperoxia treatment effect, we also used a two-factor repeated-measures analysis of variance to test for an effects of order of hyperoxia administration on the dependent variables. No significant order effects were found in the dependent variables; therefore, the data for each type of experimental period have been combined for clarity of presentation, regardless of order.

**Results**

**Hemodynamics**

Heart rate was controlled by atrial pacing in all animals (see Table 1). The mean heart rate was $115±9.9$ beats per minute in the control precannulation period and did not change significantly throughout the study. Mean arterial pressure was $72.7±19.2$ mm Hg in the control and did not change significantly during this study. Left ventricular end-diastolic pressure was $6.6±3.3$ mm Hg and did not change significantly. The only hemodynamic value significantly affected by high $P_O_2$ was LAD coronary artery pressure. In the control period, LAD coronary arterial
pressure was 76.6±19.7 mm Hg. When ischemia was induced by reducing flow, LAD coronary pressure fell to 33.9±7.1 mm Hg. During the final two randomized measurement periods, LAD coronary pressure was constant during the period of normal PaO₂ but rose significantly during the period of high PaO₂ to 38.2±11.0 mm Hg.

Regional Myocardial Blood Flow

During the control preschisma period, blood flow was measured by calibrated flowmeter only. During this control period, the transmural flow to LAD-perfused myocardium was 1.1 ml/min/g. During the three periods of myocardial ischemia, regional blood flow was measured by the microsphere technique (Table 2). Endocardial blood flow was 0.26±0.06 in the first (normoxia) ischemia period and rose to 0.34±0.10 ml/g/min during the hyperoxia period (p=0.0002) (Figure 2). The LAD endocardial:epicardial flow ratio was 0.45±0.17 in the initial ischemia period and rose to 0.61±0.23 (p=0.0001). The increased flow to the LAD subendocardium and the increased endocardial:epicardial flow ratio during hyperoxia are partially accounted for by a decrease in LAD subepicardial flow in this period (LAD epicardial flow, 0.54±0.17 ml/g/min during ischemia/hyperoxia versus 0.61±0.18 ml/g/min during the ischemia/normoxia period, p=0.02).

In the nonischemic circumflex-perfused zone, the endocardial:epicardial flow ratio was 0.86 in the initial measurement period and did not change significantly throughout the experiment. Both endocardial and epicardial flows tended to decrease during hyperoxia in the circumflex zone, but neither trend reached statistical significance (p=0.055, and p=0.07, respectively).

Regional Myocardial Contractile Function

Regional myocardial contractile function, expressed as an absolute percent systolic shortening, was 21.4±5.7% in the control preischemia period. Myocardial ischemia reduced systolic shortening to 10.9±5.3% in the LAD-perfused zone. In the final two randomized measurement periods, systolic shortening was constant in the normoxic period but rose substantially to 15.2±4.6% during hyperoxia (p=0.0001) (Table 3 and Figure 3).

Metabolism

The arterial-venous chemical extraction difference for lactate represents the net balance between two major metabolic pathways. Nonoxidative glycolytic metabolism can cause myocardial lactate release/production. Alternatively, after conversion to pyruvate, lactate can serve as a substrate for oxidative metabolism in the citric acid cycle. Because both oxidative consumption of lactate and nonoxidative glycolytic metabolism can occur simultaneously, changes in the nonoxidative glycolytic pathway are not always reflected in the net chemical extraction.

Although the arterial-venous chemical difference for lactate is usually positive in normal human subjects (net extraction), we have shown that the nonoxidative pathway is active and that lactate is simultaneously released by the myocardium. Lactate is also released in the anesthetized nonischemic pig heart. In these previous experiments, ¹⁴C lactate was infused as a tracer, and the extraction ratio for

Table 1. Hemodynamics

<table>
<thead>
<tr>
<th>Experimental state</th>
<th>Control normoxia</th>
<th>Ischemia normoxia</th>
<th>Ischemia normoxia'</th>
<th>Ischemia hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats \cdot min⁻¹)</td>
<td>114±10.0</td>
<td>115±10.0</td>
<td>117±10.2</td>
<td>115±9.6</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>72.7±19.2</td>
<td>69.6±16.2</td>
<td>64.4±15.1</td>
<td>67.0±18.4</td>
</tr>
<tr>
<td>Coronary arterial pressure (mm Hg)</td>
<td>76.6±19.7</td>
<td>33.9±7.1</td>
<td>32.9±6.4</td>
<td>38.2±11.0*</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>6.6±3.3</td>
<td>7.1±3.9</td>
<td>7.2±4.1</td>
<td>6.6±3.5</td>
</tr>
</tbody>
</table>

*Significantly different from both ischemia normoxia periods, p<0.05.

Table 2. Regional Blood Flow

<table>
<thead>
<tr>
<th>Experimental state</th>
<th>Ischemia normoxia</th>
<th>Ischemia normoxia'</th>
<th>Ischemia hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD epicardial flow (ml \cdot g⁻¹ \cdot min⁻¹)</td>
<td>0.57±0.21</td>
<td>0.61±0.18</td>
<td>0.54±0.17‡</td>
</tr>
<tr>
<td>LAD endocardial flow (ml \cdot g⁻¹ \cdot min⁻¹)</td>
<td>0.26±0.06</td>
<td>0.25±0.07</td>
<td>0.34±0.10*</td>
</tr>
<tr>
<td>LAD endocardial:epicardial flow ratio</td>
<td>0.46±0.17</td>
<td>0.41±0.14</td>
<td>0.61±0.23*</td>
</tr>
<tr>
<td>Circumflex epicardial flow (ml \cdot g⁻¹ \cdot min⁻¹)</td>
<td>1.11±0.38</td>
<td>1.13±0.45</td>
<td>0.90±0.30†</td>
</tr>
<tr>
<td>Circumflex endocardial flow (ml \cdot g⁻¹ \cdot min⁻¹)</td>
<td>0.96±0.35</td>
<td>1.01±0.34</td>
<td>0.80±0.29§</td>
</tr>
<tr>
<td>Circumflex endocardial:epicardial flow ratio</td>
<td>0.86±0.08</td>
<td>0.91±0.10</td>
<td>0.89±0.12</td>
</tr>
</tbody>
</table>

LAD, left anterior descending coronary artery.

*Significantly different from both normoxia periods by repeated-measures ANOVA, p=0.0002.
†Did not reach statistical significance vs. normoxia values, p=0.07.
‡Different from normoxia' period by repeated-measures ANOVA, p<0.05.
§Strong trend toward statistical significance vs. normoxia values, p=0.055.
this isotopically-labeled lactate was calculated. The isotopically measured extraction ratio was greater than the chemically measured extraction ratio, indicating that the myocardium releases lactate even in the presence of net chemical extraction.

In the current studies, the mean chemical extraction of lactate was 0.08μmol/ml in the control preischemia period. At the same time, and despite this net chemical extraction, all 10 swine demonstrated a small amount of myocardial lactate release during the control nonischemic period (Table 4). During myocardial ischemia at normal P02, myocardial lactate release increased substantially, and the measured arterial-venous chemical lactate difference became negative, indicating net lactate production. In the final two randomized measurement periods, the effect of hyperoxia was to decrease both myocardial lactate production (Figure 4) and lactate release substantially (Table 4).

**Conversion of Glucose to Released Lactate**

By using 14C-labeled glucose and 13C-labeled lactate, we were able to quantify not only myocardial lactate release but also the conversion of glucose to release lactate. In the preischemic control period, 0.06±0.04μmol/ml of glucose was converted to lactate. During myocardial ischemia at normal P02, the conversion of glucose to lactate increased substantially to a mean value of 0.66±0.26. During the final two randomized measurement periods, high P02 decreased conversion of glucose to lactate from 0.70±0.26μmol/ml of blood (normoxia') to 0.33±0.27μmol/ml (p=0.0001).

**Glucose Extraction**

During the control nonischemic period, glucose extraction was 0.4±0.05 μmol/ml (see Figure 5). Ischemia increased glucose extraction significantly to 0.55±0.20 μmol/ml. During the final two randomized treatment periods, high P02 decreased glucose extraction in the ischemic zone to a mean value of 0.32±0.19 μmol/ml.

**Oxidation of Glucose**

Oxidation of glucose to CO2 was measured by determining the amount of 14C glucose appearing in the coronary venous blood as 14CO2. During the baseline preischemic period, glucose oxidation was essentially nil, with a mean value of 0.005±0.008 μmol/ml. Glucose oxidation did not significantly change during any experimental condition.

**Myocardial Oxygen Balance**

Arterial P02 was 92.1±5.6 mm Hg in the control preischemic period and did not change significantly during ischemia at normal FiO2. Raising FiO2 increased Pao2 to 511±70 mm Hg. In the final two randomized measurement periods, this change in Pao2 increased arterial oxygen content from 12.0±1.3 ml/dl (normoxia') to 13.8±1.8 ml/dl (hyperoxia) (p=0.0001).

Regional venous blood was sampled from the anterior interventricular vein. During the control preischemic period, mean P02 was 32.7±6.8 mm Hg. Ischemia caused the mean P02 in the anterior interventricular vein to drop to 25.3±5.2 mm Hg. Raising arterial P02 increased P02 in the anterior interventricular vein to 33.7±6.2 mm Hg and increased regional venous oxygen content from 1.7±0.5 to 3.4±1.1 ml/dl (p=0.0001).

Regional myocardial oxygen consumption was calculated as the product of total regional blood flow (pump flow) and the regional arterial minus venous O2 content difference. During the control preischemic period, regional oxygen consumption was
Experimental design, reduced regional (micromoles extraction (production))

4. Bar FIGURE shows effect of hyperoxia on lactate extraction (micromoles per milliliter) in the ischemic zone. By experimental design, ischemia induced negative lactate extraction (production) in the ischemic zone. Hyperoxia substantially reduced regional lactate production (p<0.05).

During ischemia, oxygen consumption fell to 5.6±1.2 ml O₂/100 g/min. Surprisingly, increasing PO₂ did not increase regional myocardial oxygen consumption, which was 5.2±1.0 ml O₂/100 g/min during both of the randomized measurement periods (ischemia/normoxia' and ischemia/hyperoxia) (p=NS).

During the three ischemia periods, oxygen delivery to the endocardium was calculated as the product of endocardial blood flow times arterial oxygen content. Endocardial oxygen delivery was 3.4±0.67 ml O₂/100 g/min during the initial ischemia period. During the final two randomized periods, high PO₂ increased endocardial oxygen delivery by 3.0±0.76 ml/100 g/min (normoxia') to 4.6±1.5 ml/100 g/min (p=0.0001).

Free Fatty Acid Extraction

FFA extraction by the risk zone was 23.1% during the control nonischemic period. Ischemia did not change total FFA extraction significantly, and arterial hyperoxia had no measurable effect on FFA extraction (Table 4).

![Lactate Extraction Graph](image1)

![Glucose Extraction Graph](image2)

### Table 4. Metabolic Data and Oxygen Balance

<table>
<thead>
<tr>
<th>Experimental state</th>
<th>Control</th>
<th>Ischemia normoxia</th>
<th>Ischemia normoxia'</th>
<th>Ischemia hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate extraction (µmol/ml)</td>
<td>0.08±0.13‡</td>
<td>-0.82±0.48</td>
<td>-0.68±0.24</td>
<td>-0.16±0.25*</td>
</tr>
<tr>
<td>Lactate release (µmol/ml)</td>
<td>0.13±0.08‡</td>
<td>1.11±0.37</td>
<td>0.95±0.24</td>
<td>0.44±0.29*</td>
</tr>
<tr>
<td>Glucose extraction (µmol/ml)</td>
<td>0.04±0.05</td>
<td>0.55±0.20</td>
<td>0.51±0.12</td>
<td>0.32±0.19*</td>
</tr>
<tr>
<td>Glucose oxidation (µmol/ml)</td>
<td>0.01±0.01</td>
<td>0.04±0.04</td>
<td>0.03±0.03</td>
<td>0.03±0.02</td>
</tr>
<tr>
<td>Free fatty acid extraction (%)</td>
<td>23.1±10.8</td>
<td>25.7±8.9</td>
<td>28.4±11.1</td>
<td>28.6±10.3</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>9.8±0.9‡</td>
<td>9.6±1.0</td>
<td>9.0±1.1</td>
<td>9.1±1.2</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>92.1±5.6</td>
<td>94.0±6.2</td>
<td>89.0±9.0</td>
<td>511.5±70.5*</td>
</tr>
<tr>
<td>Arterial O₂ content (ml/dl)</td>
<td>13.2±1.4</td>
<td>13.0±1.4</td>
<td>12.0±1.3</td>
<td>13.8±1.8*</td>
</tr>
<tr>
<td>PCVO₂ (mm Hg)</td>
<td>32.7±6.8‡</td>
<td>25.3±5.2</td>
<td>25.6±5.9</td>
<td>33.7±6.3*</td>
</tr>
<tr>
<td>Coronary vein O₂ content (ml/dl)</td>
<td>4.3±1.2‡</td>
<td>1.8±0.5</td>
<td>1.7±0.5</td>
<td>3.4±1.1*</td>
</tr>
<tr>
<td>MVO₂ (ml O₂/100 g/min)</td>
<td>9.6±3.6‡</td>
<td>5.6±1.2</td>
<td>5.2±1.3</td>
<td>5.2±1.0</td>
</tr>
</tbody>
</table>

*Different from corresponding (randomized) ischemia normoxia' period, p<0.05.
‡Different from all ischemia periods.
‡‡Different from all ischemia/normoxia periods but not different from ischemia/hyperoxia period.
Discussion

These data demonstrate for the first time that increasing arterial Po2 to high levels during acute low-flow myocardial ischemia improves both function and flow distribution in the ischemic myocardium and decreases glycolytic metabolism. The degree of improvement in contractile function (5% absolute increase in systolic shortening, a 25% change normalized to preischemic values), is consistent with the observed increase in subendocardial blood flow. These findings are in agreement with prior studies of the effects of subendocardial blood flow changes on regional contractile function.19,20

The increase in subendocardial blood flow found in this study was due primarily to a redistribution of blood flow within the ischemic zone. Significant hemodynamic changes were not found that could have caused this blood flow redistribution. Although the microsphere technique used in this study (left atrial injection) measured both direct and collateral flow, the observed flow redistribution is not likely to have been caused by acute changes in collateral flow for two reasons. First, swine have an innately poor collateral circulation,21 such that the innate collateral circulation can ordinarily only provide the equivalent of approximately 5% of normal flow during a coronary occlusion. It is therefore unlikely that acute changes in collateral flow would cause the mean increase of 0.08 ml/g/min in endocardial flow found in this experiment. Additionally, the driving force for collateral flow, which is the gradient between collateral feeder artery pressure and the ischemic zone artery pressure,22 actually decreased in this experiment. LAD coronary artery pressure increased during oxygen administration, which diminished the driving pressure for collateral flow and should have tended to decrease collateral flow rather than increase it.

The present study does not reveal the entire mechanism of the observed flow redistribution. The observed changes are consistent with two supportable hypotheses. First, metabolic regulation of blood flow in the ischemic zone can be invoked. Because of the large transmural variation in myocardial tissue pressures, the subendocardium is much more vulnerable to acute coronary flow and pressure reductions than is the subepicardium. In fact, during low-flow ischemia, the subendocardium can be severely ischemic at a time when the subepicardial tissue is minimally ischemic or not ischemic at all.19 Under these circumstances, when high arterial Po2 increases arterial oxygen content, the subepicardial tissue can extract sufficient oxygen with less blood flow, and the excess blood flow becomes available for redistribution to the more ischemic subendocardium. Because increasing arterial Po2 from 100 to 450 can increase blood oxygen content by 10–13%*, the equivalent 10–13% of subepicardial blood flow is potentially available for diversion to the endocardium. This hypothesis is also consistent with our finding of increased regional coronary artery pressure in this study, which suggests regional metabolic regulation.

Alternatively, the observed flow redistribution could be caused by a direct epicardial coronary vasoconstrictor effect of oxygen, a "reverse steal" within the ischemic zone. Oxygen has been found in several prior studies to cause increased coronary pressure,23–25 and studies of isolated coronary artery strips have shown that increasing coronary Po2 increases vascular tone.26,27 However, interpretation of these studies is difficult because isolated crystalloid-supersured coronary arteries may be nearly ischemic even at a high Po2.

The metabolic findings in the current study are largely explained by the observed redistribution in regional blood flow and oxygen delivery. As reported in prior studies,9,19 glucose uptake and lactate production were minimal in the control period. With the induction of myocardial ischemia, glycolytic metabolism increased, including glucose uptake and lactate production. High arterial Po2 decreased both glucose uptake and lactate production substantially. The observed decrease in lactate production confirms prior studies by Ishikawa.3,4

Myocardial Oxygen Balance

Myocardial oxygen consumption did not increase during ischemia at high Po2. This finding suggests that the observed improvement in contractile function is not due only to increased oxidative metabolism, which should require more oxygen as substrate. This finding is, however, consistent with recent isolated heart studies suggesting that the use of oxygen to generate myocardial contractile force is more efficient at higher Po2.28

An additional unexpected finding was the observation that regional myocardial venous Po2 increased to preischemic levels with the application of high arterial Po2. We expected but did not find that high arterial Po2 would lead to increased myocardial oxygen consumption because oxygen was a rate-limiting substrate, and that coronary venous Po2 would therefore not increase significantly. Our findings suggest the alternative hypothesis that during ischemia, there may be shunting at a microvascular level that could allow some arterial blood to be diverted through to the venous circulation without participation in oxygen exchange. Such microvascular shunting could potentially be caused by locally in-

*Arterial hemoglobin-O2 saturation in swine (as in humans) is approximately 96% at Po2=90 mm Hg at normal temperature and pH. (Wilford DC, Hill EP: Temperature corrections for blood gas values, in Tumbleson ME (ed): Swine in Biomedical Research. Columbia, Missouri, Plenum Press, 1986, pp 1473–1478.) At arterial Po2=450, hemoglobin-O2 saturation is 100%. Solubility of O2 in plasma is 0.003 ml/dl/mm Hg. Hemoglobin-bound O2=(1.34 ml/g Hgb)×Hgb-O2 saturation. At a normal blood hemoglobin content of 12 mg/dl, the oxygen content of swine blood at Po2=90 is 15.71 ml O2/dl, and at Po2=450, the oxygen content is 17.43 ml O2/dl; thus, arterial oxygen content is increased by 1.72 ml/dl or by 10.9% when arterial Po2 is increased from 90 to 450 mm Hg.

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tense vasodilation, mediated by high levels of a potent ischemia-related vasodilator such as adenosine, but this is speculation.

**Study Limitations**

One limitation of the current study is that microsphere measurements were made only during the three ischemia periods and not during the control perfusion period. However, during the control perfusion, total regional myocardial blood flow (pump flow divided by the area of myocardium perfused) was normal, approximately 1.1 ml/g/min during control. Additionally, microsphere measurements were made during all three ischemia periods, including the final two randomized test periods. Because our goal was to test the effects of altering arterial oxygen tension during ischemia, comparison with preischemic regional myocardial blood flow was not essential. Furthermore, our metabolic data indicate that the hearts in this study were not ischemic during the control perfusion period because they were consuming glucose and not releasing abnormal amounts of lactate as measured by this sensitive isotopic method.

An additional limitation of the current study is that the sampling site for the coronary venous sample was an epicardial site, the anterior interventricular vein. Blood sampled from this vein is an admixture of blood from the ischemic subepicardium and the better-perfused subepicardium and may also be contaminated to some degree by venous drainage from adjacent nonischemic tissue. Blood samples from this site, therefore, should best be considered to provide a qualitative index of changes in regional myocardial metabolism, as they represent the sum of the effects of the experimental intervention on several myocardial regions.

One final limitation of this study is that it was done in swine, which have a minimal innate collateral circulation that is primarily subendocardial. The great advantage of this model is that because of the poor innate collateral circulation, effects of acute changes in collateral blood flow on regional ischemia are much less likely than in the dog models used by prior investigators. Additionally, the open-chest, anesthetized swine model of acute ischemia does not exactly mimic the situation in most patients with acute myocardial infarction, as most patients are conscious and have no anesthesia or surgery.

**Clinical Implications**

We have documented for the first time that high arterial oxygen tension improves contractile function, flow distribution, and regional metabolism in the acutely ischemic myocardium. These findings suggest that the usefulness of high arterial oxygen tension as a clinical myocardial preservation measure during acute myocardial ischemia should be reevaluated. Although a number of prior studies of the effects of high PO₂ on myocardial ischemia have been performed in the past, most of these studies used canine models with an excellent innate collateral supply or were performed in humans with transmural infarction. The present study shows that high arterial oxygen tension may induce beneficial changes in myocardial blood flow distribution even in an animal model known for poor innate collateral flow.

To date, no studies have addressed the currently relevant problems of transient myocardial ischemia and acute reperfusion. Further investigation will be necessary to determine whether high PO₂ during acute, transient myocardial ischemia diminishes ultimate infarct size or posts ischemic contractile dysfunction (stunning) in humans or in relevant animal models.

**References**


KEY WORDS • hyperoxemia • glycolytic metabolism • ischemia • systolic segment shortening
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