Myocardial Oxygenation in Dogs During Partial and Complete Coronary Artery Occlusion

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Regional myocardial oxygenation was assessed during partial and complete coronary artery occlusion using near infrared spectroscopy. In eight open-chest dogs, partial occlusions resulting in an ≈42% decrease in left anterior descending coronary artery (LAD) blood flow produced an ≈21% decrease in tissue O₂ stores (tissue oxyhemoglobin plus oxymyoglobin) and no change in the oxidation level of mitochondrial cytochrome aa₃. Further reduction in LAD blood flow produced nadir levels of tissue oxyhemoglobin plus oxymyoglobin, maximal levels of deoxyhemoglobin plus deoxymyoglobin, a decline in tissue blood volume, and an ≈39% decrease in cytochrome aa₃ oxidation level. These changes were associated with an ≈52% decrease from the preischemic baseline in mean transmural myocardial blood flow, measured by radiolabeled microspheres, and an ≈41% decrease in myocardial O₂ consumption. Complete occlusion resulted in further decreases in myocardial blood flow, O₂ consumption, tissue blood volume, and cytochrome aa₃ oxidation state but also produced increases in tissue O₂ stores to above the nadir levels noted during partial occlusion. These results indicate that decreases in O₂ delivery during partial coronary occlusion increase O₂ extraction to sustain mitochondrial O₂ availability, but as little as a 52% reduction in myocardial blood flow produces maximal O₂ extraction and depletion of tissue O₂ stores. Mitochondrial O₂ availability is restricted further during complete occlusion because of limited O₂ delivery and, possibly, decreases in tissue blood volume and O₂ extraction. (Circulation Research 1993;73:458-464)

KEY WORDS • cytochrome aa₃ • myocardial oxygen stores • ischemia • near infrared spectroscopy • radiolabeled microspheres

Myocardial tissue has a limited capacity to sustain cellular oxygenation when subjected to a sudden restriction in coronary blood flow.¹ ²
To the extent that compensatory mechanisms do not meet aerobic demands, cardiac O₂ utilization declines. This is associated with an abrupt decrease in left ventricular function in experimental animals and patients with coronary vasospasm.³ ⁶

In open-chest dogs, myocardial O₂ extraction is held relatively constant at 65% to 75% under basal conditions.¹ ² Of note, complete occlusion of the left anterior descending coronary artery (LAD) in dogs produces little or no increase in O₂ extraction, based on continuous measurements of venous O₂ content from the great cardiac vein.⁷ ⁸ O₂ extraction, however, increases to a maximum of 84% when coronary artery blood flow is partially reduced.¹ These differences in O₂ extraction associated with absent versus diminished coronary blood flow reflect regional differences in tissue oxygenation that have not been well characterized.

Near infrared (NIR) spectroscopy has been used recently to measure transmural changes in myocardial oxygenation in vivo.⁹ This nondestructive approach permits the continuous measurement of changes in tissue blood volume, oxygenation of tissue hemoglobin and myoglobin, and the oxidation level of the copper complex of cytochrome aa₃. The latter principally reflects O₂ availability at the site of O₂ consumption within cardiac mitochondria. The present study uses this approach in the beating canine heart to examine the mechanisms by which myocardial oxygenation is supported when coronary blood flow is restricted and the differences in tissue oxygenation during partial vs complete coronary occlusion.

Materials and Methods
Preparation of Animals and Experimental Protocol

The experimental preparation is exactly as described previously,⁹ with modifications as indicated below. Briefly, adult mongrel dogs weighing 25 to 35 kg were anesthetized with intramuscular morphine sulfate (1.5 mg/kg) and α-chloralose (160 mg/kg for 10 minutes followed by 10 mg/kg per hour intravenously), endotracheally intubated, and ventilated on room air. Arterial blood gases were acquired periodically to ensure adequate systemic oxygenation and acid-base balance. The heart was exposed through a left thoracotomy at the sixth intercostal space, and a pericardial cradle was formed. Heparin-filled catheters (outer diameter, 3
mm) made of polyvinyl chloride were inserted into the carotid artery for systemic blood pressure measurements and withdrawal of microsphere reference samples and into the left atrium for microsphere injections. A segment of the LAD approximately 1.0 cm in length was isolated proximal to the first diagonal branch, and a pneumatic cuff occluder (made in our laboratory) was positioned around the vessel to allow total occlusion. A Howell-ST electromagnetic flowmeter probe, previously calibrated using an in vitro system, was positioned just proximal to the occluder. Two separate fiberoptic bundles, one to deliver NIR light and one to receive transmitted light, were positioned 2 cm apart on the epicardial surface within the region to be made ischemic. A heparinized 24-gauge catheter (Quik-Cath) was placed in the great cardiac vein to acquire samples of the venous effluent from the region of illumination. Phasic and mean coronary blood flow, systemic blood pressure, and lead II of the electrocardiogram were recorded on a Gould model 412 oscillograph. Simultaneously acquired NIR data were recorded using an Epson model FX100 printer. The signals were synchronized using a marker wired to the two recorders.

Approximately 30 minutes was allowed for stabilization of the preparation, during and after which all parameters were recorded continuously. Under basal conditions, radiolabeled microspheres were injected into the left atrium, and arterial blood samples were collected for measurement of tissue blood flow and O2 content. The arterial sampling process required =80 seconds. Venous blood was withdrawn simultaneously from the great cardiac vein at a rate of 0.1 mL/s. After an initial =0.7 mL of venous aspirate was discarded to account for tubing length, =0.7 mL was collected in a heparinized syringe and placed on ice for immediate measurement. The hemoglobin saturation and concentration were measured on a Co-oximeter (IL model 413), and the O2 content of venous and arterial blood was computed using a canine algorithm. LAD blood flow was reduced progressively by 5% to 10% at 20-second intervals by graded inflation of the pneumatic snare as NIR optical responses were monitored continuously. When tissue O2 stores (defined below) declined to within 10% of nadir levels, the pneumatic snare was clamped to maintain inflation, and radiolabeled microspheres were again injected. Blood samples were acquired for microsphere reference levels and arteriovenous O2 content determinations as described above. The coronary occluder was released 100 to 150 seconds after the second microsphere injection for a recovery period of =20 minutes. The LAD was then abruptly and completely occluded for 2 minutes. At 30 seconds into the occlusion, microspheres were injected for a third time, and blood samples were again acquired for microsphere reference and arteriovenous O2 content measurements.

**In Vivo Optical Monitoring**

A detailed description of NIR spectroscopy applied to the in vivo canine heart has been published previously.9 The NIR method permits the measurement of changes in absorption of the iron-porphyrin moieties of oxygennated and deoxygenated tissue hemoglobin and myoglobin (tHbO2 + MbO2, and tHb + Mb, respectively). When the tHbO2 + MbO2 and tHb + Mb signals are added together, changes in myoglobin saturation are cancelled, allowing for the measurement of changes in tissue hemoglobin volume. NIR spectroscopy exploits the fact that photon migration through tissue is relatively independent of wavelength in the 700- to 900-nm range.11 The NIR signals were analyzed using four wavelengths and a set of algorithms to deconvolute complex absorption data of overlapping spectra. The algorithms include weighting coefficients for optical density changes (ΔOD) at each of the four wavelengths as follows: \( \Delta \text{cytochrome } a + = -3.08 \Delta \text{OD}_{780} + 6.52 \Delta \text{OD}_{804} - 0.66 \Delta \text{OD}_{810} - 2.45 \Delta \text{OD}_{810} \); \( \Delta \text{tHbO}_2 + \text{MbO}_2 = -1.51 \Delta \text{OD}_{775} - 0.57 \Delta \text{OD}_{780} - 0.24 \Delta \text{OD}_{804} + 1.48 \Delta \text{OD}_{804} \); and \( \Delta \text{tHb} + \text{Mb} = -2.20 \Delta \text{OD}_{775} - 0.91 \Delta \text{OD}_{780} - 0.19 \Delta \text{OD}_{804} - 0.86 \Delta \text{OD}_{804} \). Wave-length-dependent light scattering is accounted for by deriving the algorithms from the absorption characteristics of light-scattering tissue.12,13

Measured changes in absorption (optical density) are linearly related to changes in concentration of an absorber according to the Beer-Lambert law, although absolute (molar) concentration cannot be determined since optical path length is unknown.11,14 For hemoglobin and myoglobin, the molar occupancy of heme moieties by oxygen is related linearly to changes in the concentration of tissue oxyhemoglobin plus oxymyoglobin (tHbO2 + MbO2).15 The latter quantity accounts for essentially all of the O2 “stored” in tissue hemoglobin and myoglobin (tHb + Mb).9,16,17 Accordingly, this level of “tissue O2 stores” reflects the net effect of the influx of oxygenated hemoglobin into the tissue region of illumination via small arteries and arterioles, the uptake of O2 from hemoglobin and myoglobin by mitochondria, and the eflux of remaining oxyhemoglobin via venous channels. Since most of the hemoglobin is located in capacitance vessels, changes in the oxyhemoglobin component of tissue O2 stores are most sensitive to changes in capillary and venous O2 content.17,18 Accordingly, regionally specific changes in O2 stores relate to changes in the volume of oxyhemoglobin in the venous effluent. Similarly, changes in the tHb + Mb response should relate closely to changes in the volume of deoxygenated hemoglobin in the venous blood. The NIR method does not permit estimation of the fraction of O2 in tissue hemoglobin versus myoglobin since the spectra of oxyhemoglobin and oxymyoglobin are essentially identical.9,15

Pulsed light from the NIR spectrometer is delivered to the tissue via a fiberoptic bundle (optrode). Transmitted light is collected via a second optical bundle, and the photo signals are processed into metabolic signals as described previously.9,12,16,19 Optical geometry is held constant by immobilizing the optrodes with a stereotaxic device. At the beginning of an experiment, a baseline is established for each optical parameter under well-defined basal physiological conditions. Optical changes associated with ischemia are expressed as changes in optical density (ΔOD). The NIR signals are recorded at a rate of 4 acquisitions per second, and six consecutive measurements are averaged electronically to produce each data point. Accordingly, the displayed signal represents a time-averaged response that is insensitive to movement associated with the relatively rapid cardiac cycle. Also, since coronary occlusion leads to cessation of
active contraction in the illuminated region, any contraction-related artifact decreases with ischemia. Although the decline in tissue blood volume with coronary artery occlusion could potentially alter tissue properties such as light scattering, photon path length, and absorbance, the effects would be small in dog hearts, since the contribution of vascular volume to the total tissue volume is small and since the decline in blood volume with occlusion is likely limited by the influx of blood via collateral channels. The decrease in oxidation level of cytochrome aa₃ from preischemic baseline to complete tissue deoxygenation at death is defined as the total labile signal (TLS) for each experiment. The TLS for cytochrome aa₃ represents a dynamic range that is less than the full redox range in the beating canine heart. For the tHbO₂+MbO₂, tHb+Mb, and tissue hemoglobin volume optical responses, the TLS represents the maximal dynamic range during each experiment.

Quantitation of Myocardial Blood Flow and O₂ Consumption

Regional myocardial blood flow was measured as previously described using 11.4±0.01-μm radiolabeled microspheres. After death by an overdose of thiopental sodium and potassium chloride, the positions of the fiberoptic bundles on the epicardium were marked with pins, and the hearts were removed and fixed in 10% buffered formalin. At least 3 days was allowed for proper fixation. Two contiguous 1-cm² full-thickness sections of myocardium were excised from the tissue region between the sites of photon entry and photon reception. Each section was divided into four layers of approximately equal thickness from epicardium to endocardium. The remainder of the left ventricle was divided similarly, and all sections were weighed. The tissue samples were counted using a multichannel gamma spectrophotometer (series 35-Plus, Canberra Industries Inc, Meriden, Conn) and a sample changer (Packard Instrument Co, Inc, Meriden, Conn). Myocardial blood flow was calculated and expressed in milliliters per minute per gram. The product of mean transmural blood flow (at the site of illumination) and the arterial minus venous O₂ [(A-V)O₂] content difference was used to quantitate myocardial O₂ consumption according to the Fick equation.

Data Analysis

Summed data were expressed as mean±SD. Student’s paired t test was used to determine differences in paired data. Results were adjusted according to the Bonferroni inequality to account for multiple comparisons. A value of P<.05 was considered statistically significant.

Results

Complete studies were obtained in eight dogs. Baseline hemodynamics included a heart rate of 124±28 beats per minute, an arterial systolic blood pressure of 124±14 mm Hg, and a diastolic blood pressure of 92±20 mm Hg.

NIR Signals and LAD Blood Flow During Partial and Complete Coronary Occlusion

The effect of partial LAD occlusion on myocardial oxygenation is demonstrated in the representative experiment shown in Fig. 1. Reduction of mean LAD blood flow to 76±9% of preocclusion levels produced the first detectable changes in the NIR signals: a decrease in the tissue O₂ stores (tHbO₂+MbO₂) and an increase in the tHb+Mb level. The O₂ stores decreased

FIG 1. Near infrared optical responses in canine myocardium during left anterior descending coronary artery occlusion. Representative recording is from one of eight dogs. Cyt a₃a₃ indicates cytochrome aa₃; tHbO₂+MbO₂, tissue oxyhemoglobin plus oxymyoglobin (tissue O₂ stores); tHb+Mb, tissue deoxymyoglobin plus deoxyhemoglobin; tBV, tissue blood (hemoglobin) volume; C.O., onset of complete occlusion; and ΔO.D., change in optical density. A, Incremental decreases in coronary blood flow during partial occlusion produced gradual changes in the near infrared signals, which were divided into four phases as indicated by number and vertical hatched lines (see "Results"). Horizontal hatched lines indicate nadir and maximal levels of tHbO₂+MbO₂ and tHb+Mb, respectively. B, Complete coronary occlusion produced abrupt changes in the near infrared signals.
by 21±4% and the tHb+Mb level increased by 24±7% from preocclusion levels during this first phase of partial occlusion. Of note, there was only a 6±4% decrease in tissue blood volume and no decrease in the cytochrome aa3 oxidation level during this phase, which hereafter is referred to as phase 1.

Reduction in coronary flow to 58±14% of preocclusion levels was associated with the first detectable decrease in the cytochrome aa3 oxidation level, marking the onset of a second phase of deoxygenation. During this phase, which hereafter is referred to as phase 2, the O2 stores continued to decrease, the tHb+Mb level continued to increase, and there was further decline in the tissue blood volume.

Progressive reduction in coronary flow to below 19±9% of preocclusion levels produced further decreases in cytochrome aa3 oxidation level and tissue blood volume. Of note, the onset of this third phase of deoxygenation was marked by nadir levels in the O2 stores and maximal tHb+Mb levels (phase 3).

Further reduction in coronary flow did not change the level of the tissue O2 stores in three of the eight dogs. In five of the eight dogs, however, reduction in coronary blood flow to below ~15% of preocclusion levels resulted in an actual increase in the O2 stores, thereby defining a fourth phase of deoxygenation. As shown in Fig 1, this increase was associated with a decline in the tHb+Mb level and further decreases in the tissue blood volume and cytochrome aa3 oxidation level during phase 4. Accordingly, between the onset of phase 3 and complete occlusion for all eight dogs, the mean tHbO2+MbO2 level increased by 21±4% above nadir levels (P=.03), and the mean tHb+Mb level decreased to 37±57% of maximal levels (P<.02). The tissue blood volume reached nadir levels (Figs 1, B, and 2), and the cytochrome aa3 oxidation level declined to 21±20% of preischemic baseline (P=.004 vs onset of phase 3). The mean changes in the NIR optical responses and LAD blood flow for the eight dogs at the onset of phases 1 through 3 during partial occlusion and during complete occlusion are summarized in Fig 2.

At the end of each experiment, the LAD was reoccluded. Sixty seconds into the final occlusion, ventricular fibrillation was induced by bolus administration of saturated potassium chloride. The latter did not lead to further decrease in tissue O2 stores below complete occlusion levels, as shown in the representative experiment in Fig 3. In contrast, the cytochrome aa3 oxidation level decreased significantly during essentially complete tissue deoxygenation at death (P<.05 vs complete occlusion).

**Myocardial Blood Flow and O2 Consumption During Partial and Complete Coronary Occlusion**

As indicated in “Materials and Methods,” tissue blood flow and arteriovenous O2 content measurements were determined before occlusion, during partial occlusion when tissue O2 stores had decreased to within 10% of nadir levels (approximately the onset of phase 3), and during complete occlusion. These results are shown in the Table. The baseline arterial P.O2 was 75.7±15.3 mm Hg, arterial pH was 7.42±0.05, and the venous pH from the great cardiac vein was 7.40±0.05. None of these parameters changed significantly with partial or complete coronary occlusion. Partial occlusion produced significant decreases in mean transmural myocardial blood flow (P<.001) and tissue O2 delivery (the product of blood flow and arterial O2 content) (P=.002). Also, the (A-V)O2 content difference increased (P=.003), and myocardial O2 consumption (MVO2) decreased (P=.01) from preocclusion levels.

Complete occlusion produced a further decrease in mean transmural myocardial blood flow and a decrease in O2 delivery (both P<.002 vs partial occlusion). Of note, the (A-V)O2 content difference did not increase further, and in fact, there was a trend toward a decrease, such that the difference between basal and complete occlusion levels was not significant (P=.08). The decrease in tissue blood flow from partial to complete occlusion was proportionately greater than the decrease in the (A-V)O2 content difference, resulting in a decrease in MVO2 (P<.001).

**Discussion**

NIR spectroscopy revealed four distinct phases of tissue deoxygenation associated with progressive reduction in tissue blood flow and O2 delivery in the beating
FIG 3. Near infrared optical responses in canine myocardium during left anterior descending coronary artery occlusion and ventricular fibrillation. Representative recording for one of eight dogs. Complete occlusion (C.O.) produced abrupt decreases in cytochrome aa₃ (Cyt aa₃) oxidation level and tissue O₂ stores (tissue oxyhemoglobin plus oxymyoglobin [tHbO₂+MbO₂]). Subsequent induction of ventricular fibrillation (V.F.) by bolus administration of saturated potassium chloride led to a further decrease in Cyt aa₃ oxidation level without further decline in tissue O₂ stores. ΔO.D. indicates change in optical density.

canine heart (Fig 1). Although it would be predicted that decreases in coronary blood flow lead to parallel decreases in cytochrome oxidation level and oxygenation of tissue hemoglobin and myoglobin, we demonstrate that these indicators of intramitochondrial and extramitochondrial oxygenation do not decrease in parallel when coronary blood flow is modestly or severely reduced. The unexpected changes in these indicators during phases 1, 3, and 4 suggest specific mechanisms by which myocardial oxygenation is supported when coronary blood flow is restricted and indicate important differences in tissue oxygenation during partial versus complete coronary occlusion that had not been defined previously. These findings also provide a basis for the future application of NIR spectroscopy to assess the relation of tissue oxygenation to contractile function during ischemia.

Changes in the tissue O₂ stores and tHb+Mb level were noted first when coronary blood flow had been reduced by ~24%. The decrease in the tissue O₂ stores and commensurate increase in the tHb+Mb level during this first phase were associated with little change in tissue blood volume and likely reflect increased O₂ extraction from tissue hemoglobin and myoglobin. Indeed, this degree of reduction in coronary blood flow previously has been shown to produce increases in myocardial O₂ extraction. Of note, the oxidation level of cytochrome aa₃ remained at preoclusion levels during this phase. This implies that under these experimental conditions myocardial O₂ extraction increases to sustain mitochondrial O₂ availability when coronary flow is reduced by up to ~42%. Minimal emptying of the venous network would be anticipated during this phase, since the decrease in intravascular pressure in the venules and veins would be small. This may explain the decrease in tissue blood volume of only 26%, although it is also possible that some degree of capillary recruitment occurs to maintain the size of the intravascular space and promote O₂ diffusion.

Reduction in coronary flow by more than ~42% during the second phase of partial occlusion led to a decrease in the cytochrome aa₃ oxidation level. This implies that basal O₂ availability was no longer maintained, despite the apparent increase in O₂ extraction, as reflected by the decrease in tissue O₂ stores during phase 2. It remains unclear why this degree of restriction in coronary blood flow did not result in maximal O₂ extraction to completely sustain the mitochondrial oxidation state. Possibly, O₂ extraction is limited by a decline in contractile activity and the resultant decrease in O₂ demand.

<table>
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<tr>
<th>Myocardial Blood Flow, O₂ Extraction, O₂ Delivery, and O₂ Consumption During Partial and Complete Coronary Occlusion</th>
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<tr>
<td>Mean transmural blood flow (mL/min·g⁻¹)</td>
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<tr>
<td>CaO₂ (mL O₂·dL⁻¹)</td>
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<td>Cvo₂ (mL O₂·dL⁻¹)</td>
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<td>CaO₂−Cvo₂ (mL O₂·dL⁻¹)</td>
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<tr>
<td>O₂ delivery (mL O₂·min⁻¹·100 g⁻¹)</td>
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<tr>
<td>MVO₂ (mL O₂·min⁻¹·100 g⁻¹)</td>
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CaO₂, arterial O₂ content; Cvo₂, mixed venous O₂ content from great cardiac vein; MVO₂, myocardial O₂ consumption. Values are mean±SD for eight dogs.

*P<.05 vs preischemic levels (basal).
†P<.05 vs partial occlusion levels.
‡25±14% of preocclusion levels.
bin provision is matched precisely by the rate of mitochondrial O\textsubscript{2} uptake. Under these conditions, there was little or no residual O\textsubscript{2} accumulation in tissue hemoglobin or myoglobin, and oxygen extraction was maximal at the site of illumination. Accordingly, the measured (A-V)O\textsubscript{2} content difference during phase 3 was significantly increased from preocclusion levels. This apparent increase in O\textsubscript{2} extraction was probably not more pronounced because of contributions to the great cardiac vein effluent via venous interconnections from nonischemic regions.\textsuperscript{22} Were it possible to selectively cannulate venules subtending only the region of illumination, lower values of venous O\textsubscript{2} content may have been acquired. Nevertheless, our finding that myocardial O\textsubscript{2} extraction is maximal when coronary blood flow is only partially reduced is consistent with a previous study in open-chest dogs.\textsuperscript{1}

Further verification of this state of maximal extraction must await the development of other methods that, like NIR spectroscopy, are sensitive to regional heterogeneities in oxygenation and that do not disturb physiological compensatory responses such as capillary recruitment.\textsuperscript{23}

The progressive and spontaneous decline in LAD blood flow without further inflation of the pneumatic snare during phase 3 of partial occlusion may reflect the aggregation of platelets at the stenosis site (Folts effect),\textsuperscript{24} the constriction of arterioles, the opening of collateral channels, or a decrease in compliance of the ischemic ventricular wall. The phenomenon of "critical closure"\textsuperscript{25,26} may also be involved; although were this the principal mechanism, a more abrupt decrease in blood flow would be anticipated than was found in the present study. The gradual decline in coronary blood flow that we observed precluded steady-state conditions while O\textsubscript{2} consumption was measured during phase 3. Accordingly, the measurement represents a mean value during the period required to collect blood samples for tissue blood flow and (A-V)O\textsubscript{2} content difference determinations.

As LAD blood flow was reduced progressively to zero (phase 4 in Fig 1), there was a further decrease in the cytochrome aa\textsubscript{3} oxidation level, reflecting a progressive decline in O\textsubscript{2} availability. The cytochrome aa\textsubscript{3} oxidation level and O\textsubscript{2} consumption did not decrease to zero, however, probably because of a limited provision of O\textsubscript{2} via collateral blood flow.\textsuperscript{9} Changes in substrate availability or the size of the ADP pool could have also influenced the redox state of cytochrome aa\textsubscript{3}.

The observed decreases in tissue blood volume and tHb+Mb levels during phase 4 reflect the progressive reduction in the volume of hemoglobin delivered to the tissue. Of note, the tHbO\textsubscript{2}+MbO\textsubscript{2} level increased in five of the eight dogs during this phase, suggesting that there was a decrease in O\textsubscript{2} extraction in those animals. Such a decrease in extraction is supported by the trend toward a decrease in the (A-V)O\textsubscript{2} content difference between measurements acquired during phase 3 and during complete occlusion (Table). The concept that extraction is submaximal during phase 4, as indicated by our measurements, is also suggested by a prior study in dog hearts that revealed unexpectedly modest decreases in mean regional venous hemoglobin saturation after complete coronary occlusion.\textsuperscript{27}

O\textsubscript{2} extraction may be submaximal during complete occlusion because of a decrease in O\textsubscript{2} demand as O\textsubscript{2} consumption and energy expenditure decline in ischemia. It is also possible that the observed decrease in tissue blood volume to nadir levels and contraction of the intravascular space associated with complete occlusion may alter oxymyoglobin distribution and increase distances for O\textsubscript{2} diffusion from hemoglobin to mitochondria. If true, there should be a minimum tissue blood flow and possibly a minimum tissue blood volume below which maximal O\textsubscript{2} extraction does not occur. This critical level may be reached with the transition from partial to complete occlusion.

Although every effort was made to hold the optical path length constant during these experiments, optical artifacts might have occurred under these conditions. The increase in the tHbO\textsubscript{2}+MbO\textsubscript{2} response during phase 4 cannot be explained by an influx of oxymyoglobin via collateral channels, since it is the tHb+Mb level rather than the tHbO\textsubscript{2}+MbO\textsubscript{2} level that increases as a function of collateral blood flow.\textsuperscript{9} Venous interconnections may have opened when LAD perfusion pressure declined with progression to complete occlusion, and venous blood passing through the region of illumination via such interconnections would be relatively inaccessible to the ischemic tissue for O\textsubscript{2} extraction.\textsuperscript{22}

The additional venous blood, however, would be expected to produce a commensurate increase in the tHb+Mb response rather than the decrease that was observed during phase 4. The paradoxical increase in the tHbO\textsubscript{2}+MbO\textsubscript{2} level is probably not explained by secondary dilation of arterioles after flow stoppage, causing a back flow of blood from the veins into the sampled area, since the latter would likely produce an increase in the tHb+Mb level rather than the decrease that was seen during phase 4. This phenomenon is probably not explained by a decrease in blood or tissue pH with ischemia, which would tend to decrease rather than increase the saturation of hemoglobin and myoglobin. It is also unlikely that changes in wall thickness or curvature associated with ischemia lead to the detection of oxygenated blood in the ventricular cavity, since the blood volume signal decreases rather than increases during phase 4.

Since it is not possible to distinguish hemoglobin from myoglobin using the current NIR method, we cannot determine in vivo whether myoglobin oxygenation increased concordantly with hemoglobin oxygenation during phase 4 or decreased as expected with the cytochrome aa\textsubscript{3} oxidation level.\textsuperscript{28,29} If the latter occurred, then the increase in the tissue level of oxymyoglobin exceeded the decline in oxymyoglobin in five of the eight dogs, producing a net rise in the tHbO\textsubscript{2}+MbO\textsubscript{2} response during phase 4.

In conclusion, partial and complete coronary artery occlusion are associated with distinct states of myocardial oxygenation in vivo. With modest decreases in coronary blood flow and O\textsubscript{2} delivery (phase 1), O\textsubscript{2} extraction increases to sustain mitochondrial O\textsubscript{2} availability at preocclusion levels. As LAD blood flow declines, O\textsubscript{2} availability decreases despite further increases in O\textsubscript{2} extraction (phase 2). With further reduction in blood flow, tissue O\textsubscript{2} stores reach nadir levels (phase 3), reflecting a state of maximal O\textsubscript{2} extraction that partially sustains mitochondrial O\textsubscript{2} availability and consumption. Progression to complete occlusion produces further decreases in O\textsubscript{2} availability and consumption due to limited O\textsubscript{2} delivery via collateral blood.
flow and, possibly, contracted intravascular blood volume and submaximal $O_2$ extraction.

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