Regulation of respiration in myocardium in the transient and steady state

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Chung, Youngran, and Thomas Jue. Regulation of respiration in myocardium in the transient and steady state. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1410–H1417, 1999.—1H/31P NMR has followed the metabolic response to increased work in the glucose- and pyruvate-perfused rat myocardium during a heart cycle and at the steady state. With electrical pacing and dobutamine, the heart O2 consumption increases by 56%. The phosphocreatine (PCr) level initially declines, but recovers within 15 min to its control level; the oxymyoglobin (MbO2) saturation decreases by 15%. Because the MbO2 signal reflects the intracellular PO2, the capillary-to-cell O2 gradient has increased to match the increased O2 need. However, no transient metabolic fluctuation is observed in either PCr or MbO2 throughout the entire cardiac cycle in both glucose and pyruvate-glucose-perfused hearts. No systolic-diastolic variation is detectable under either high workload or hypoxic conditions. The results reveal that neither O2 nor ADP is regulating respiration under increased energy demand in the steady or transient state.

heart cycle; myoglobin; nuclear magnetic resonance; oxygen; phosphate metabolism

The myocardium tightly regulates O2 consumption to meet the constant energy demand of its contractile activity. Such a coupling of chemical and mechanical energy is essential in maintaining normal physiological function and is mediated, in part, by the creatine kinase reaction, which buffers ATP loss. Although steady-state measurements have established the presence of metabolic and vascular controls, they do not elucidate the regulatory mechanism during a contraction. The energy demand is certainly not constant but peaks during contraction and falls during the relaxation phase. Observing only the energy balance at the transient and steady state may overlook metabolic oscillations and the associated biochemical regulation on a transient time scale. Indeed, optical studies have shown an oscillatory behavior in both NADH and oxymyoglobin during a heart cycle and at the steady state. With electrical pacing and dobutamine, the heart O2 consumption increases by 56%. The phosphocreatine (PCr) level initially declines, but recovers within 15 min to its control level; the oxymyoglobin (MbO2) saturation decreases by 15%. Because the MbO2 signal reflects the intracellular PO2, the capillary-to-cell O2 gradient has increased to match the increased O2 need. However, no transient metabolic fluctuation is observed in either PCr or MbO2 throughout the entire cardiac cycle in both glucose and pyruvate-glucose-perfused hearts. No systolic-diastolic variation is detectable under either high workload or hypoxic conditions. The results reveal that neither O2 nor ADP is regulating respiration under increased energy demand in the steady or transient state.

Indeed, the question has attracted the attention of investigators. Several studies have focused on assessing whether the high-energy phosphate metabolites actually fluctuate during a heart cycle. The results, however, are conflicting. In one study, the PCr level falls with the systole and rises with the diastole in isolated, glucose-perfused hearts (11, 30), whereas in another study the metabolic levels are unperturbed in either pyruvate-perfused or in situ myocardium (15, 18, 22, 30).

Although optical studies have detected a fluctuation in MbO2 saturation within a heart cycle, this observation has so far been inconsistent. A study of perfused hearts reported an increasing MbO2 saturation during systole, whereas another study noted that cellular PO2 actually drops during systole (14, 29). Still others have observed no change (10, 25). To our knowledge, no nonoptical measurements so far have confirmed any fluctuation in the cellular O2 level. A rise in cellular PO2 during systole is certainly consistent with the observed steady-state rise in MbO2 saturation during increased myocardial oxygen consumption (MV02) (16). However, if both the MbO2 saturation and the phosphate metabolite levels remain constant then neither ADP nor O2 plays any significant role in modulating mitochondrial energy production during a heart contraction cycle, and this raises questions about their role in regulating MV02. That observation would help narrow the research focus on the regulatory molecules, such as NADH or Ca2+.

Measuring the transient alteration in either MbO2 or high-energy phosphate levels, however, poses a technical challenge. Unless the experiments include stringent precautions, these measurements confront many artificial errors. A recent study (7) presented a gated NMR technique with sufficient time resolution to observe the energetic changes during a contraction cycle in the rat gastrocnemius muscle in vivo. Indeed, the technique has demonstrated that PCr levels fall rapidly within 16 ms after stimulation and recover during the relaxation phase. The P equals rise stoichiometrically, while the ATP signal remains constant. The data suggest that PCr hydrolysis yields substantial energy for skeletal muscle contraction, much more than previously reported in freeze-clamp experiments, which have a time resolution of ~100 ms. Such a technique is then applicable in determining the transient high-energy phosphate level changes in myocardium. Moreover, because 1H NMR methods can now detect the intracellular Mb signal (23), the opportunity also exists to determine whether cellular O2 itself is dynamically changing during a heart cycle.

We have focused on the transient response of MbO2 and high-energy phosphate metabolites during a heart contraction cycle and under enhanced workstates. Even though the steady-state level of intracellular O2 declines as the MV02 increases, along with the PCr level, neither cellular O2 nor PCr levels fluctuate throughout the entire cardiac cycle at different workstates. An infusion of either pyruvate or glucose to shift the availability of NADH for oxidative phosphorylation...
produces the same results. The observations imply that the metabolic regulatory mechanism during a cardiac contraction is not related to either \( \text{O}_2 \) or ADP and that any transient energetic changes are undetectable. The results also confirm the utility of the gated NMR technique to examine the interplay between metabolic energy and force development to develop a deeper understanding of the cellular mechanisms governing the fundamental unit of myocardial contraction.

METHODS

Animal preparation and heart perfusion. Rat heart perfusion at 37°C has been previously described in detail (8). After pentobarbital anesthesia (60 mg/kg) and heparinization (1,000 U/kg body wt) of male Sprague-Dawley rats (350–400 g), the hearts were quickly isolated and perfused with a modified Langendorff system. A peristaltic pump (Rainin Rabbit) maintained a constant, nonrecirculating perfusion rate of 18 ml/min. The perfusion medium was a modified Krebs-Henseleit buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.2 KH\(_2\)PO\(_4\), 1.8 CaCl\(_2\), 20 NaHCO\(_3\), 1.2 MgSO\(_4\), and 15 glucose. The perfusate was passed through both 5- and 0.45-µm Millipore filters, saturated with 95% O\(_2\)-5% CO\(_2\) and pH maintained at 7.4 ± 0.05. The heart was bathed in perfusate flowing from the pulmonary artery. The heart was placed in a 20-mm NMR tube and isolated with a Teflon plug with holes to permit perfusate overflow.

A saline-filled latex balloon inserted in the left ventricle was used to monitor the heart rate (HR) and left ventricular pressure (LV P) via a strain gauge transducer (Statham P23 XL) connected to an oscillographic recorder (Gould RS 3200 or Windograf). The balloon volume was adjusted to give an end-diastolic pressure of 6–8 mmHg. Hearts were paced with a bipolar platinum electrode connected to the Grass S48 stimulator. Rate-pressure products (RPP) were calculated from HR times the left ventricular developed pressure (LVDP). Perfusion pressure was monitored via saline-filled polyethylene tubing connecting the aortic cannula to a second strain gauge transducer (Statham P23 XL).

Four different perfusion conditions were used for the transient measurements of cellular \( \text{O}_2 \) and phosphate metabolite levels (Table 1). For all the experiments, the perturbation followed the control period, during which the hearts were perfused with the fully \( \text{O}_2 \)-saturated perfusate and paced at 300 beats/min. In protocol I we used the control perfusion condition with 15 mM glucose throughout the entire experiment. In protocol II we used the control condition in which hearts were stimulated to a high workload by pacing at 600 beats/min and infusing 80 ng/ml dobutamine (Abbott Laboratories). In protocol III we introduced hypoxic conditions to 300 beats/min paced hearts. In protocol IV we used 10 mM pyruvate/10 mM glucose as substrates to produce a nonlimiting NADH condition (13).

Perfusate \( \text{O}_2 \), lactate, and Mb measurement. A polyethylene catheter, inserted close to the pulmonary artery, was used to withdraw ∼50% of the perfusate flowing from the heart. A Yellow Springs Instrument 5300 meter was used to monitor the perfusate \( \text{O}_2 \) concentration in a temperature-jacketed chamber. The remaining 50% of the perfusate exited the chamber above the Teflon plug as an overflow. Parallel bench experiments empirically determined the \( \text{O}_2 \) loss in the tubing and adjusted the measured \( \text{PO}_2 \) value to reflect the venous value proximal to the heart (6). MbO\(_2\) was calculated from the corrected inflow and outflow \( \text{O}_2 \) measurements and the perfusion flow rate.

A YSI 2700 Bioanalyzer determined the perfusate lactate concentration. Samples were measured in triplicate, and the measurement linearity was assessed by two-point calibration at 5 and 28 mM lactate concentrations. Sensitivity of the measurement reached 0.01 mM lactate. The membrane current settled at ∼2 nA before sample measurements were initiated (8). Mb loss from the perfused heart was determined by optically measuring the perfusate sample at 540 nm after converting Mb to MbCN.

NMR. An AMX 400-MHz Bruker spectrometer was used to record \(^1\text{H}\)/\(^{31}\text{P}\) signals with a 20-mm \(^1\text{H}\)-X probe, where \( X \) represents nuclei from \(^15\text{N} \) to \(^{31}\text{P} \). A modified 1,3,31 binomial pulse sequence suppressed the \( \text{H}_2\text{O} \) line and selectively excited the MoB \( \text{O}_2\) Val E 11 resonance at ∼2.8 parts per million (ppm) (6, 23). The \(^1\text{H}\) 90° pulse was 65 µs, calibrated against the perfusate \( \text{H}_2\text{O} \) signal. Observing the MbO\(_2\) signal required a 40-ms acquisition time and a 45° pulse angle. The spectral width was set at 8,065 Hz; the data size was 512 words. Six thousand transients were averaged for a typical \(^1\text{H}\) spectrum, requiring 5 min of signal accumulation. The free induction decays (FID) were zero-filled to 2K and multiplied by an exponential-Gaussian window function. A spline fit then smoothed the baseline. All spectral lines were referenced to 4.67 ppm at 36°C, which was in turn calibrated against sodium-3-(trimethylsilyl)propionate-2,2,3,3-d\(_4\). For the \(^{31}\text{P}\) spectra, a typical control spectrum used a 45° pulse angle, a 0.5-s repetition time, and 256 scans/block (2.2 min). The \(^{31}\text{P}\) 90° pulse was 72 µs, calibrated against a 0.1 M phosphate solution. Spectral width was set at 6,494 Hz; the data size was 4K. FIDs were zero-filled to 8K and apodized with an exponential-Gaussian function to better resolve intracellular \( \text{P}_i \) signal. The \(^{31}\text{P}\) signals were referenced to phosphocreatine (PCR) as 0 ppm.

Gated \(^{31}\text{P}\) and \(^1\text{H}\) NMR measurement during a contraction cycle. Gated \(^{31}\text{P}\) acquisition was described previously (7). On heart stimulation, a home-built gating device sampled the 5–10 Hz output pulse and inserted a defined delay with <1 ms resolution before sending a pulse to trigger the NMR signal acquisition. Synchronization of the triggering pulses with the specific phases of the heart contraction cycle was visualized with a Tektronix 2230 digital oscilloscope. Signal averaging then occurred at each defined time point distal to the stimulation pulse and with a constant relaxation delay. The timing diagram is illustrated below.

![Timing Diagram](https://example.com/timing_diagram.png)
A delay, t1, distal to the heart stimulation, A, elapsed before a signal gated the spectrometer, time point B, to acquire an NMR signal. For each subsequent data block, t1 was incremented stepwise from 5 ms up to 190 ms. The time interval between B and C corresponds to the acquisition time, which is constant throughout all the experiments. A final time interval t2 was inserted to maintain the relationship (t1 + Acq + t2) = 0.4 s. Therefore, for the hearts paced at 300 beats/min, the 31P signals were acquired every other beat. For the hearts paced at 600 beats/min, 31P signals were acquired at 1 every 4 beats. A signal-averaged 31P NMR data block was composed of 256 transients and lasted for 2.0 min. Gated 1H NMR signals of Val E11 were accumulated every 0.2 s for 300 beats/min paced hearts and every 0.1 s for 600 beats/min paced hearts. Each block of 1H signal averaging lasted for 15 min.

Intracellular PO2 and phosphate metabolite measurement. During the control period, O2-saturated buffer oxygenated the Mb >90%. Fractional Mb oxy genation was determined from the integrated area of the Val E11 γ-methyl signal, which was normalized against control MbO2 saturation as 100%. The normalization procedure was based on the full NMR visibility of the Mb signal (23) and the saturation as 100%. The normalization procedure was based on the full NMR visibility of the Mb signal (23) and the saturation as 100%. The normalization procedure was based on the full NMR visibility of the Mb signal (23) and the saturation as 100%.

\[
P_{O2} = \frac{Y}{1-Y} \times [P_{O2}]_{50}
\]

where \([P_{O2}]_{50}\) is the partial pressure of \(O_2\) required to half saturate Mb. A \([P_{O2}]_{50}\) of 3.8 mmHg was used for the calculation (2).

**RESULTS**

Steady-state response to increased workload. Figure 1 shows the nongated steady-state 1H and 31P spectra from the perfused myocardium under different pacing conditions. In control hearts paced at 300 beats/min (workstate I), the 1H NMR signal of the Mb Val E11 and 31P signal intensities are identical to the intensities observed in spontaneously beating hearts (Fig. 1, A: spectrum a and B: spectrum a'). Typical control (workstate I) RPP and MVO2 were 29,337 ± 2,076 mmHg/min and 32.4 ± 2.0 µmol·min⁻¹·g dry wt⁻¹, respectively. As the electrical stimulation increases the HR to 450 beats/min (workstate II), no changes are noted in the MbO2 and phosphate metabolite signals, even though RPP has increased by 22% and MVO2 by 16% (Fig. 1, A: spectrum b and B: spectrum b'). Paced at 600 beats/min and infused with 80 ng/ml dobutamine (workstate III), the MbO2 is 56% and RPP by 83%.

**Fig. 1.** 1H NMR (A) and 31P NMR (B) spectra from perfused myocardium at different workstates. Spectrum a: control, 300 beats/min; spectrum b, 450 beats/min; spectrum c, 600 beats/min; spectrum d (postcontrol) 300 beats/min. At 600 beats/min, 1H NMR signal intensity of MbO2 Val E11 decreases to 85% of control.
Table 2. Steady-state changes under different myocardial workloads

<table>
<thead>
<tr>
<th>Workstate</th>
<th>Workstate II</th>
<th>Workstate III</th>
</tr>
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<tbody>
<tr>
<td>RPP, %</td>
<td>100</td>
<td>121.6 ± 9.5%</td>
</tr>
<tr>
<td>MV02, %</td>
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<td>P1, %</td>
<td>100</td>
<td>104.7 ± 17.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.15 ± 0.02</td>
<td>7.15 ± 0.02</td>
</tr>
<tr>
<td>Lactate, µmol·min⁻¹·g⁻¹</td>
<td>1.25 ± 0.28</td>
<td>2.44 ± 0.35*</td>
</tr>
<tr>
<td>Intracellular PO2, mmHg</td>
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<td>142.4</td>
</tr>
<tr>
<td>Venous PO2, mmHg</td>
<td>338.5 ± 73.5</td>
<td>291.8 ± 73.6</td>
</tr>
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Values are means ± SD; n = 11 hearts. At beginning of each heart perfusion, hearts were paced at 300 beats/min for 30 min (workstate I). During this period, typical rate-pressure product (RPP) was 29,337 ± 2,076 mmHg·min and myocardial oxygen consumption MV02 was 32.4 ± 2.0 µmol·min⁻¹·g dry wt⁻¹. Hearts were subsequently paced at 450 beats/min (workstate II), and 600 beats/min with 80 ng/ml dobutamine infusion (workstate III). RPP, MV02, MbO2, phosphocreatine (PCr), ATP, and P1 values are expressed as % of levels measured during workstate I. Lactate production rate is expressed in µmol·min⁻¹·g dry wt⁻¹. [PO2] of 3.8 mmHg was used for the calculation of intracellular PO2. Significantly different from workstate I (Student’s t test) *P < 0.05, †P < 0.001. ‡Calculated value of intracellular PO2 at 100% MbO2 saturation is indeterminate.

Fig. 2. Graph of MbO2 (●) and PCr (□) as a function of O2 consumption. As O2 consumption increases with electrical and inotropic stimulation, MbO2 and PCr levels decline. As workstate steps from I to III, MV02 increases by 56%. MbO2 level decreases to 85% and PCr to 91% of control.

Fig. 3. PCr, P1, and MbO2 levels during a heart contraction cycle (protocol I, 300 beats/min). PCr (●) and MbO2 (△) signals are normalized to intensities observed at peak systole (115 ms time point). P1 (■) signal intensity is normalized to PCr signal intensity. No significant changes in levels of these metabolites are observed throughout entire heart cycle. X-axis of graph showing 5-, 75-, 115-, 135-, 150-, and 190-ms time points corresponds respectively to mid-diastolic, end-diastolic, peak-systolic, mid-systolic, end-systolic, and second diastolic phase of heart cycle.

The MbO2 signal intensity now decreases by 15%, while PCr declines by 9% (Fig. 1A: spectrum c and B: spectrum c'). As soon as the HR resumes 300 beats/min, the 1H MbO2 signal recovers fully (Fig. 1A: spectrum d). The PCr signal, however, overshoots transiently to 123%, before it recovers to the control level (Fig. 1B: spectrum d'). In contrast, P1 undershoots initially to 65% of control level. In all workstates ATP level and pH remain constant, even though the lactate concentration has increased at high workload. The graphical analysis of MbO2 saturation and PCr level versus O2 consumption is shown in Fig. 2. A linear regression of the graph shows that every 10% increase in MV02 is accompanied by a 2.9% decrease in Mb oxygenation.

Transient PO2 and high-energy phosphate metabolite changes during a heart cycle. The experimental protocol to examine transient metabolite fluctuations within a heart cycle involves four different conditions as described in the Methods section and in Table 1. Protocol I uses glucose perfusion and a HR of 300 beats/min. The PCr, P1, and MbO2 response is shown in Fig. 3. Intracellular O2 level and high-energy phosphate levels remain unchanged throughout the entire heart cycle. ATP level and intracellular pH are also unchanged (data not shown).

In protocol II the increased heart workload enhances the energy demand between systole and diastole. At 600 beats/min and with 80 ng/ml dobutamine, the heart increases its RPP to 196% and its MV02 to 156% above the corresponding control levels. Even though at this workload, the steady-state MbO2 saturation drops by 15%, while PCr decreases by 6% and P1 increases by 83%, no transient changes in either intracellular oxygenation or phosphate metabolite levels are observed (Fig. 4).

When the perfused hearts are challenged with hypoxic conditions (protocol III), MbO2 saturation drops to 36% of the fully oxygenated state, and PCr level decreases to 62% of the normoxic level. Intracellular acidification is significant (pH = 7.03). RPP and MV02 drop to 52.0 ± 2.4 and 47.5 ± 4.0% of the normoxic control values, respectively. The hypoxic manipulation, however, still does not produce any fluctuations in intracellular O2 or phosphate metabolite levels throughout the entire heart contraction cycle (Fig. 5).

Protocol IV is similar to protocol I, except that 10 mM pyruvate is infused in addition to 10 mM glucose. Pyruvate causes the steady-state PCr-to-P1 ratio to increase significantly, an order of magnitude higher...
above the glucose control level, which is consistent with previous reports (13). However, the addition of 10 mM pyruvate still does not produce any transient changes in either the MbO2 or the high-energy phosphate signals (Fig. 6).

**DISCUSSION**

Regulation of MV˙O2. How MV˙O2 accommodates the changing energy demand of cardiac work is still a question under intense debate. Early studies of isolated mitochondria indicate that ADP is a key regulatory molecule, linking myosin ATP hydrolysis and mitochondrial ATP production thereby maintaining a cellular energy balance (5). Indeed, some perfused-heart experiments have lent support to the paradigm. As the myocardial workstate increases, the 31P NMR signal of PCr declines, reflecting an increase in ADP (4). Others, however, have only observed a transient drop in PCr at the onset of high workload, which gradually returns to the control level (26).

These conflicting observations from perfused-heart studies raise questions about the role of ADP as the regulator of myocardial respiration in vivo. Certainly the available substrate can modulate the ADP dependence. With pyruvate as the substrate, the NADH level is not limiting, and ADP maintains a simple Michaelis-Menten relationship with MV˙O2 (12, 13). Fatty acid substrates in addition to 10 mM glucose can also induce an NADH excess but an ADP-limited metabolic profile (13). With glucose as the substrate, however, NADH does become limiting, and ADP no longer shows any relationship with MV˙O2. Therefore, in the glucose-perfused heart, carbon substrate delivery to the mitochondria, not ADP, is limiting O2 consumption (12, 13, 19). In vivo myocardium experiments have raised more challenges. As work output and O2 consumption increase, the 31P NMR signal of PCr declines, reflecting an increase in ADP (4). Others, however, have only observed a transient drop in PCr at the onset of high workload, which gradually returns to the control level (26).

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The ADP independent regulation of MV˙O2 in the in vivo and the glucose-perfused heart has led researchers to postulate that these models are similar. Our study shows that as the myocardial work is raised from the basal state to workstate II, the 16% increase in MV˙O2 does not elicit any steady-state changes in the phosphate metabolite levels. Even at the workstate III, the...
marked increase in $MV_{O2}$ produces only a modest, transient alteration in the PCR and $P_i$ signals. Both signals recover their control intensities within 15 min. Given this observation, ADP does not appear to be the regulator of $O_2$ consumption in the glucose-perfused myocardium, which is in agreement with previous reports (12, 13, 19).

Steady-state phosphate responses to high workload. Nevertheless, at workstates II and III, the PCR signal intensity decreases initially and then recovers fully to the control levels within 15–20 min. Other perfused heart studies have also reported a similar PCR time course with inotropic stimulations and have ascribed the observation to a transient mismatch between the $O_2$ supply and demand at the onset of the high workload (26). However, our data indicate that hypoxia is not the cause for the temporary PCR drop, because the cellular $O_2$ level remains constant throughout the entire high-workload period. Although $MbO_2$ saturation level drops promptly to 85% of control at workstate III, it remains at that level as the PCR level declines and recovers, showing that the PCR response is not associated with the intracellular $O_2$ level nor $MV_{O2}$.

The drop in $MbO_2$ signal induced by workstate III reflects a mild functional hypoxia as the myocardium responds to a high-workload challenge. As expected for hypoxia, the glycolytic activity should increase to supplement the deficiency in oxidative ATP formation. Indeed, the lactate formation rate is elevated at high workstates and matches the observed lactate production rate in spontaneously beating myocardium subjected to hypoxic perfusate (6). Despite the increased glycolytic flux, myocardial acidification is not observed, consistent with other findings (9, 20), and stands in sharp contrast with the skeletal muscle response during increased exercise intensity, which shows significant changes in phosphate levels and pH (24). The changes in cellular $O_2$, glycolytic flux, and pH cannot account for the temporary fall and rise of the PCR level at the onset of high workload.

Steady-state $O_2$ response to high workload. At high workloads the heart consumes more $O_2$ and, convective and/or diffusive flow must enhance the $O_2$ delivery. Unlike the in situ myocardium, the constant flow perfused myocardium does not have a significant capacity to autoregulate the coronary flow. Instead, it must rely on a drop in cellular $P_O2$ to increase the $O_2$ gradient between the capillary and cytosol. That gradient alteration should enhance the diffusion driving force for $O_2$ delivery. Indeed, $MbO_2$ desaturation reflects such a shift in the $O_2$ gradient, which increases with $MV_{O2}$. Within 5 min after the onset of high myocardial workload, the intracellular $P_O2$ reaches a steady level and adjusts the $O_2$ flux to meet the enhanced demand. As the workload of the heart increases from workstate II to workstate III, intracellular $P_O2$ drops from 142 mmHg to 22 mmHg. If the perfusate venous $P_O2$ approximates the mean end-capillary $P_O2$, then the capillary $P_O2$ has changed from 292 to 203 mmHg with the workload jump. The change enhances the $O_2$ gradient from 150 to 181 mmHg, a 21% increase between workstate II and III. The corresponding $MV_{O2}$ enhancement is 34%. If $O_2$ conductance from the capillary to the cell is constant, then the $O_2$ gradient can sufficiently increase the $O_2$ flux to match the increased $MV_{O2}$ demand. The results also imply that the intracellular $O_2$ concentration is not limiting or regulating $MV_{O2}$ in the normoxic perfused heart, because there is an association between a drop in cellular $O_2$ and an increase in the $O_2$ consumption rate.

Because a constant flow perfusion eliminates vascular autoregulation, it can reduce the metabolic response to electrical pacing/inotropic stimulations. However, the experimental goal to assess the role of $O_2$ gradient in enhancing $O_2$ consumption requires a model that factors out the vascular contribution, and the state of heart oxygenation is determined simply by the $O_2$ consumption. The results demonstrate definitively that an enhanced vascular supply of $O_2$ is not mandatory to increase cardiac $O_2$ metabolism. Rather, the modulation of the $O_2$ gradient enhances the $O_2$ flux and accounts for the increased $MV_{O2}$, at least in the constant flow system.

Transient phosphate metabolite response during a heart cycle. During a myocardial contraction cycle, the energy demand should presumably rise and fall. Indeed, optical studies have detected oscillatory behavior in both NADH and $MbO_2$, and these studies imply that the high-energy phosphate metabolite level may also fluctuate during a cardiac cycle (14, 29). Several researchers have investigated whether the phosphate metabolite levels fluctuate during a cardiac cycle but have produced conflicting results. In glucose-perfused but not in pyruvate- or acetate-perfused myocardium, the PCR level dips at systole and rises at diastole (11, 30). In an isolated working heart the fluctuation is even more pronounced than in the Langendorff-perfused heart, arising presumably from the 28% increase in $MV_{O2}$ and the higher $P_i$-to-ATP ratio ($P_i$/ATP) (17, 30). In contrast, in situ myocardium experiments have failed to detect any fluctuation in the PCR signal, and the results have led to the hypothesis that the distinct substrate and $O_2$ availability of the perfused and in situ heart may account for the difference (15, 18, 22).

In a recent study of rat skeletal muscle, a gated NMR technique has achieved millisecond resolution and has monitored a significant transient drop in the PCR during a peak force development (7). The technique, however, reveals no transient fluctuation in any high-energy phosphate metabolite signals or $MbO_2$ saturation in the perfused normoxic or hypoxic myocardium with either glucose or pyruvate as the substrate. Both substrate and $O_2$ levels remain constant throughout the entire contraction cycle, consistent with the in situ myocardium observations. In contrast to skeletal muscle, the heart either maintains a balance between energy supply and demand or utilizes an undetectable amount of PCR during a contraction cycle. A normal rat cardiac cycle is completed in $<200$ ms and may therefore require a substantial glycolytic ATP contribution, because glycolytic reactions are activated much more rapidly than oxidative phosphorylation under energy-demanding conditions (1). However, computer simula-
tions have demonstrated that with glycolytic compensation alone, PCr should drop in response to a sudden work jump (1). Our results are consistent with the observed restoration time course of \( <15 \text{ s} \) between cytosolic ATP utilization and mitochondrial ATP production (15). A rapid replenishment of energy within a heart cycle requires then a fast increase in oxidative phosphorylation, in response to myosin ATPase activity. Indeed, the heart under a high workload can turn over its total ATP content in 1 s (27).

The constant PCR/ATP within a heart cycle also indicates that the ADP concentration is constant throughout the heart cycle and therefore may not play a significant role in regulating any transient flux in oxidative metabolism. In vivo myocardium studies have already cast doubts on a simple kinetic control of mitochondrial oxidative phosphorylation by ADP alone and even a model involving both ADP and Pi as bireactants (15, 28).

Transient O\(_2\) response during the heart cycle. Despite the peak energy demand and contraction ischemia during systole, some investigators have proposed that the heart might be more oxygenated during the systolic phase (21, 29), whereas other optical measurements have shown deoxygenation during systole (14). In fact, some optical studies have reported no systole-diastole differences in tissue O\(_2\) levels (10, 25). In the present study, no transient change is observed in the intracellular oxygenation within a cardiac cycle with either glucose or pyruvate as the substrate. As the workload increases to workstate III, the MV\(_2\) has also increased by 56%. That increase is much larger than the 28% enhancement observed in an isolated working heart by 56%. That increase is much larger than the 28% enhancement observed in an isolated working heart relative to a Langendorff-perfused heart (30). Although the steady-state intracellular O\(_2\) level declines at this workstate, as indicated by 15% drop in the MbO\(_2\) signal intensity, no cyclic alteration in the MbO\(_2\) saturation is observed.

The relationship between the MbO\(_2\) saturation and MV\(_2\) provides insight into the expected change in O\(_2\) consumption between diastole and systole. In the steady state, the increase in MV\(_2\) is accompanied by a drop in MbO\(_2\) signal intensity. The linear relationship, shown in Fig. 2, shows that a 10% change in the MbO\(_2\) signal corresponds to a 34% change in MV\(_2\). Given the signal sensitivity, a 10% change in the MbO\(_2\) signal intensity would certainly be detectable in the reported spectra; yet no MbO\(_2\) change is observed. The results then imply that the %MV\(_2\) increase is \(<34\%\) between the systolic and the diastolic phase of the heart cycle, consistent with the theoretical predictions that ATP consumption per each heartbeat will deplete only 2% of the total (ATP + PCr) pool (31). Additionally, the extent or the duration of any contraction-induced ischemia appears less significant than previously conceived (18, 21).

The increased workload, however, does deplete the cellular O\(_2\) and induces a functional hypoxia. Alternatively, decreasing the perfused O\(_2\) also produces a supply-side hypoxia and presents another physiological condition to observe any transient change in cellular oxygenation and metabolism during a heart cycle. The hypoxic manipulation in the study (protocol III, Table 1) produces a 64% decrease in the steady-state MbO\(_2\) saturation and a 38% drop in the PCr level. RPP drops to 52%, and MV\(_2\) drops to 48% of the normoxic control level. These parameters indicate a compromised myocardial function and energy state.

A previous study has shown a strong linear correlation between MbO\(_2\) saturation and MV\(_2\) (6). When MbO\(_2\) saturation decreases to 50% of its control level, MV\(_2\) also drops by 50%, which indicates a 50% decrease in cytochrome oxidase activity. Under hypoxic perturbations that produces a 36% MbO\(_2\) saturation, the cytochrome oxidase activity should then fall within the linear region of the presumed Michaelis-Menten reaction kinetics and should respond sensitively to any changes in O\(_2\). Yet intracellular O\(_2\) levels within a heart cycle during hypoxic perturbation still remain constant. Either O\(_2\) is not involved in regulating transient O\(_2\) consumption or the transient alteration in intracellular O\(_2\) level is small and immeasurable with NMR techniques.

Comparison between the in vivo and perfused heart response. In both transient and steady states, in vivo hearts fail to exhibit pronounced changes in phosphate metabolites with increased workloads (3, 15, 18, 22, 28). The response of the isolated perfused hearts depends on the available substrate. When perfused with pyruvate, the heart shows ADP-dependent O\(_2\) consumption in the steady state (12, 13). On the other hand, during a heart cycle, pyruvate-perfused hearts fail to show phosphate metabolite changes and therefore no systolic dependence on ADP regulation (30).

Glucose-perfused isolated hearts behave in an opposite manner. In the steady state, isolated hearts perfused with glucose do not change their phosphate metabolites with changing workload (12, 13, 19). Within a heart cycle of the glucose-perfused hearts, phosphate metabolite fluctuates (11, 30). Some researchers have noted that the in vivo myocardium resembles a glucose-perfused heart. But in the transient state, the in vivo myocardium appears to be similar to the pyruvate-perfused heart model. The conflicting results then suggest that ADP regulation is different in the steady versus the transient state. However, our experimental results show that both glucose and pyruvate-perfused hearts are not dependent on ADP regulation of systole-diastole variations. The transient metabolite fluctuations from perfused and in vivo myocardium within a heart cycle are now consistent.

In conclusion, the energy demand in the myocardium increases with higher workload and presumably during the systolic phase of the heart contraction cycle. At increased workloads, steady-state PCR declines initially but recovers to its control level within 15 min. Such a response does not arise from a time-dependent change in cellular oxygenation because intracellular O\(_2\) level falls with high workloads and remains low as long as O\(_2\) consumption is enhanced. An increase in capillary-to-cell O\(_2\) gradient then facilitates the increased O\(_2\) flux.
Over a heart contraction cycle, no variation in either phosphate metabolites or cellular oxygenation is measurable. In contrast to skeletal muscle, the myocardium does not show a significant change in oxidative metabolism between the systolic and diastolic phases of a heart contraction cycle.

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