Oxygen delivery does not limit cardiac performance during high work states

Jianyi Zhang, Yo Murakami, Yi Zhang, Yong K. Cho, Yun Ye, Guangrong Gong, Robert J. Bache, Kamil Ugurbil, and Arthur H. L. From. Oxygen delivery does not limit cardiac performance during high work states. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H50–H57, 1999.—This study tested the hypothesis that the loss of myocardial high-energy phosphates (HEP), which occurs during high cardiac work states (J. Zhang, D. J. Duncker, Y. Xu, Y. Zhang, G. Path, H. Merkle, K. Hendrich, A. H. L. From, R. Bache, and K. Ugurbil. Am. J. Physiol. 268: (Heart Circ. Physiol. 37): H1891–H1905, 1995), is not the result of insufficient intracellular O₂ availability. To evaluate the state of myocardial oxygenation, the proximal histidine signal of deoxymyoglobin (Mb-d) was determined with 1H nuclear magnetic resonance spectroscopy (MRS), whereas HEP were examined with 31P MRS. Normal dogs (n = 11) were studied under basal conditions and during combined infusion of dobutamine and dopamine (20 µg·kg⁻¹·min⁻¹ iv each), which increased rate-pressure products to >50,000 mmHg·beats·min⁻¹. Creatine phosphate (CP) was expressed as CP/ATP, and myocardial deoxymyoglobin desaturation was normalized to the Mb-d resonance present during total coronary artery occlusion. This Mb-d resonance appeared at 71 parts per million downfield from the water resonance. CP/ATP decreased from 2.22 ± 0.12 during the basal state to 1.83 ± 0.09 during the high work state (P < 0.01), whereas ΔP/CP increased from 0 to 0.21 ± 0.04 (P < 0.01). Despite these HEP changes, Mb-d remained undetectable. In contrast, when a coronary stenosis was applied to produce a similar decrease in CP/ATP, Mb-d reached 0.35 ± 0.10 of the value present during total coronary occlusion. These data demonstrate that Mb-d is readily detected in vivo during limitation of coronary blood flow sufficient to cause a decrease of myocardial CP/ATP. However, similar HEP changes that occur at high work states in the absence of coronary occlusion are not associated with a detectable Mb-d resonance. The findings support the hypothesis that the myocardial HEP changes observed at high work states are not due to inadequate O₂ availability to the mitochondria and emphasize the limitations of interpreting HEP alterations in the absence of knowing the level of myocyte oxygenation.

dehydmoglobin; high-energy phosphates; intense catecholamine stimulation; myocardium

IN THE NORMAL CANINE HEART, high-energy phosphate (HEP) compound levels do not change over moderate increases of work state [rate-pressure products (RPP) up to 35,000 mmHg·beats·min⁻¹] produced by pacing or catecholamine infusion (2, 20, 35). In contrast, at higher cardiac workloads, myocardial creatine phosphate (CP) and to a lesser extent ATP levels fall, resulting in elevated Pi and ADP levels (45). In the latter study, it was also shown that during the high work state, pharmacological hyperperfusion of the coronary vasculature was accompanied by a significant increase in myocardial O₂ consumption rate (MVO₂). In the latter study, it was also shown that during the high work state, pharmacological hyperperfusion of the coronary vasculature was accompanied by a significant increase in myocardial O₂ consumption rate (MVO₂). In the latter study, it was also shown that during the high work state, pharmacological hyperperfusion of the coronary vasculature was accompanied by a significant increase in myocardial O₂ consumption rate (MVO₂). In the latter study, it was also shown that during the high work state, pharmacological hyperperfusion of the coronary vasculature was accompanied by a significant increase in myocardial O₂ consumption rate (MVO₂).
intracellular $\text{PO}_2$ during basal and high work state conditions. To be certain that the system was capable of detecting Mb-$\delta$, data were also obtained during partial and total coronary artery occlusion when the $\text{O}_2$ supply was undeniably insufficient. The results demonstrate that, at the high work state levels evaluated, $\text{O}_2$ availability should not limit the rate of the ATP synthesis. These results have previously been reported in abstract form (44).

**METHODS**

All experimental procedures were performed in accordance with the animal use guidelines of the University of Minnesota, and the experimental protocol was approved by the University of Minnesota Research Animal Resources Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, Revised 1985).

Surgical preparation. Eleven adult mongrel dogs weighing 18–25 kg were anesthetized with pentobarbital sodium (30 mg/kg followed by 4 mg·kg$^{-1}$·h$^{-1}$ iv). The animals were intubated and ventilated with a respirator using room air supplemented with $\text{O}_2$. A heparin-filled polyvinyl chloride catheter (3.0 mm OD) was inserted into the left femoral artery and advanced into the ascending aorta. A left thoracotomy was performed through the fourth intercostal space. The pericardium was opened and the heart suspended in a pericardial cradle. Heparin-filled catheters were inserted into the left ventricle through the apical dimple and into the left atrium through the atrial appendage and secured with purse-string sutures. A 1.5- to 2.0-cm segment of the proximal left anterior descending coronary artery (LAD) was dissected free, and a hydraulic occluder constructed of polyvinyl chloride tubing (2.7 mm OD) was placed around the artery proximal to the first major arterial branch. A silicone elastomer catheter (0.75 mm ID) was placed into the LAD distal to the occluder (13). A 28-mm diameter NMR surface coil was sutured onto the epicardium of the myocardial region perfused by the LAD. The pericardial cradle was then released and the heart allowed to assume its normal position. The surface coil leads were connected to a balanced, tuned circuit, and the animals were placed into the magnet (36).

$^{31}$P NMR spectroscopic technique. Measurements were performed in a 40-cm bore, 4.7-T magnet interfaced with a SISCO (Spectroscopy Imaging Systems, Fremont, CA) computer console. The left ventricular pressure (LV) signal was used to gate NMR data acquisition to the cardiac cycle, whereas respiratory gating was achieved by triggering the NMR data acquisition to the cardiac cycle, using integrals obtained in the region covering the $\text{P}_i$ resonance. $\text{P}_i$ and CP resonance, off-resonance effects on these peaks were virtually nonexistent. The numerical values for $\text{CP}$ and $\text{ATP}$ in each voxel were expressed as ratios of $\text{CP}/\text{ATP}$. $\text{P}_i$ levels were measured as changes from baseline values ($\langle \text{P}_i \rangle$), using integrals obtained in the region covering the $\text{P}_i$ resonance. $\text{P}_i$ and CP are presented as $\langle \text{P}_i/\text{CP} \rangle$ ATP and CP values (normalized to the control resonance areas) are also shown. Because only whole wall myoglobin data are available, all $\text{HEP}$ data reported are also whole wall data.

$^1$H NMR spectroscopic technique. We have recently reported the $^1$H NMR methods in detail elsewhere (6). In brief, radiofrequency transmission and signal detection were performed with the dually tuned 28-mm-diameter surface coil. A single-pulse collection sequence with a frequency-selective, 1-ms Gaussian excitation pulse was used to selectively excite the Mb-$\delta$ resonance. This provided sufficient water suppression due to the large chemical shift difference between water and Mb-$\delta$ ($>14$ kHz), and other techniques such as chemical shift-selective pulse and inversion recovery pulse did not significantly improve water suppression. The NMR signal was optimized by adjusting the radiofrequency pulse power using the water signal as a reference. A short repetition time (TR = 25 ms) was used for the short spin-lattice relaxation (T1) of Mb-$\delta$. Each spectrum was acquired in 5 min (10,000 free induction decays). Although the short T1 of Mb-$\delta$ and fast acquisition prevent gating to the cardiac cycle, the signal loss due to motion is negligible due to the inherently broad line width of Mb-$\delta$ peak. Resonance intensities were quantified using integration routines provided by the SISCO software.

In vivo studies are potentially complicated by the presence of an overlapping analogous resonance from Mb-$\delta$. However, Chen et al. (6) demonstrated that using relatively long pulses for signal excitation, the much broader line width and shorter spin-spin relaxation (T2) Mb-$\delta$ resonance is fully suppressed, and Mb-$\delta$ is selectively detected; a similar strategy based on spin-echo sequences has also been used to selectively detect Mb-$\delta$ in the presence of hemoglobin in ex vivo preparations (42). In our earlier in vivo studies, we observed that under basal work state conditions myoglobin was fully oxygenated within the detection limits but that when coronary blood flow was reduced a Mb-$\delta$ resonance appeared and was linearly related to the decrease of blood flow (6). These preliminary studies indicated that $^1$H MRS could be employed to determine myoglobin saturation in an in vivo model.

To estimate the myoglobin $P_50$ value ($P_{50}$, at which myoglobin is half-saturated with $\text{O}_2$), the expression $[P_{50} = e^{0.0048T} \cdot 2.748]$, where $T$ is temperature) was employed as recently reported by Schenkmam et al. (37). $T$ was the
measured core temperature (−37°C). This calculation yielded a P50 value of 2.39 mmHg. Subsequent Po2 estimates were then calculated from the Hill equation using the aforementioned P50 value.

Myocardial blood flow measurements. Myocardial blood flow was measured using 15-µm-diameter radionuclide-labeled microspheres (45). Microspheres labeled with four different radioisotopes (51Cr, 85Sr, 95Nb, and 46Sc) were agitated in an ultrasonic mixer for 10 min before injection. Microsphere suspension containing 2 × 10^6 microspheres was injected through the left atrial catheter and flushed with 10 ml of normal saline. A reference sample of arterial blood was drawn from the aortic catheter at a rate of 15 ml/min beginning 5 s before microsphere injection and continuing for 120 s. At the end of the study the hearts were removed, weighed, and fixed in 10% buffered Formalin. The region of myocardium beneath the surface coil was removed and sectioned into three transmural layers from epicardium to endocardium and then weighed and placed into vials for counting. Similar myocardial specimens were obtained from the lateral and posterior LV wall to ensure that the measurements from the region beneath the surface coil were representative of the entire left ventricle. Radioactivity in the myocardial blood and reference specimens was determined using a gamma spectrometer (model 5912, Packard Instrument, Downers Grove, IL) at window settings chosen for the combination of radioisotopes used during the study. Activity in each energy window was corrected for background activity and overlap between isotopes. Knowing the rate of withdrawal of the reference blood specimen (O), and the radioactivity of the reference specimen (C), we used myocardial radioactivity (Cm) to compute myocardial blood flow (Qm) as Qm = Qr × (Cm/C).

Hemodynamic measurements. Aortic, LV, and mean LAD coronary pressure distal to the occluder were measured using Spectromed TNF-R pressure transducers positioned at midchest level. All data were recorded on an eight-channel Coulbourne R14-28 direct-writing recorder.

Study protocol. Ventilation rate, volume, and inspired O2 content were adjusted to maintain physiological values for arterial Po2, Pco2, and pH. Aortic, LV, and mean LAD blood pressures were monitored continuously throughout the study. During each intervention, myocardial blood flow and hemodynamic measurements were acquired simultaneously with the acquisition of 1H and 31P MRS. Baseline data were obtained, dobutamine and dopamine were simultaneously infused (each 20 µg·kg−1·min−1 iv) to produce a high work state. After waiting 10–15 min to achieve a steady state, we repeated all measurements. The catecholamine infusion was then stopped, and all measurements but blood flow were repeated. ~20–25 min later when repeated hemodynamic measurements were similar to those present at baseline. The occluder was then slowly inflated with a micrometer-driven syringe to reduce distal LAD pressure, whereas whole wall CP/ATP was monitored by 31P MRS every minute to match the whole wall CP/ATP present during the previous catecholamine stimulation period. When the desired reduction of CP/ATP had been achieved, the poststenotic perfusion pressure was maintained constant while all measurements were repeated. Finally, the LAD was completely occluded and all measurements were again repeated.

Data analysis. Hemodynamic data were measured from the strip-chart recordings. Transmural blood flow distribution was determined from the microsphere measurements. Data were analyzed with one-way analysis of variance for repeated measurements. A value of P < 0.05 was considered significant. When a significant result was found, individual comparisons were made using the method of Scheffé's.

RESULTS

Hemodynamic data. Hemodynamic measurements are shown in Table 1. In response to catecholamine stimulation, the heart rate and LV systolic pressure increased significantly and the RPP rose to >50,000 mmHg/min (Table 1). During the postcatecholamine restabilization period, systemic hemodynamic measurements were not significantly different from those present at baseline. During LAD stenosis and then complete occlusion heart rate, mean aortic pressure and LV end-diastolic pressure (LVEDP) values did not change significantly (relative to postcatecholamine restabilization period values), although the systolic and mean aortic pressures trended lower during complete LAD occlusion and the LVEDP trended higher. The coronary perfusion pressure distal to occluder was decreased to ~48 mmHg during partial LAD occlusion and fell to ~16 mmHg during total coronary occlusion.

Myocardial blood flow. In response to catecholamine infusion, myocardial blood flow increased to 225% of the basal level with no change in the transmural distribution of perfusion (Table 2). Blood flow was not measured during the postcatecholamine restabilization period. The coronary stenosis decreased mean myocardial blood flow to 60% of the basal level, whereas the subendocardial-to-subepicardial blood flow (Endo/Epi) ratio decreased to 0.54 (Table 2). During total occlusion mean blood flow in the LAD region (representing collateral flow) was ~5% of basal flow (Table 2).

Myocardial oxygenation and HEP levels. MRS measurements of myocardial HEP, ΔP/CP and oxygenation levels are summarized in Table 3. Figure 1 illustrates sequential sets of 31P MRS data (Fig. 1, A–D) examining myocardial HEP and P1 levels, and 1H MRS data

<table>
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<th>Table 1. Hemodynamic data</th>
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<td>Heart Rate, beats/min</td>
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<td>Dobutamine + dopamine</td>
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<td>Postcatecholamine restabilization</td>
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Values are means ± SE. LV, left ventricular; LAD, left anterior descending coronary artery. Dobutamine and dopamine were each administered at 20 µg·kg−1·min−1 iv. *P < 0.01 vs. respective controls; †P < 0.05 vs. stenosis.
(Fig. 1, E–H) of myocardial Mb-δ levels obtained from one experiment. The interleaved 31P and 1H MRS were obtained during each experimental condition. Data were obtained during 1) baseline conditions (A and E), 2) catecholamine infusion (B and F), 3) coronary stenosis (C and G), and finally 4) LAD occlusion (D and H). Under basal conditions no Mb-δ resonance was detected (Table 3 and Fig. 1). In response to catecholamine stimulation myocardial CP-to-ATP ratios fell and ΔP1-to-CP ratios increased, whereas normalized CP and ATP levels were significantly reduced (Fig. 1B and Table 3). However, Mb-δ remained undetectable (Table 3 and Fig. 1F). After cessation of the catecholamine infusion, although most HEP values were not significantly different from control values, ATP levels did not recover. Once again, no Mb-δ resonance was visible.

In the presence of a partial coronary artery stenosis, HEP and P1 changes similar to those noted at high workloads were observed (Table 3 and Fig. 1, B and C). However, in contrast to the high workload state, during coronary artery stenosis a Mb-δ resonance appeared (Table 3 and Fig. 1, F and G). Hence, these data identify two states with similarly abnormal whole wall HEP and P1 values but distinctly different levels of myocyte oxygenation. During total LAD occlusion, additional myocardial HEP loss was accompanied by a much larger Mb-δ signal (Fig. 1H). The apparent difference in the line widths in Fig. 1, G and H, most likely originates from temperature gradients in the ischemic zone due to partial coronary artery occlusion (Fig. 1G) due to inhomogeneous perfusion of the myocardial wall during partial blood flow reduction. It is well known that the chemical shift of the contact-shifted proximal histidine resonance exhibits a strong dependence on temperature. During ischemia, when blood flow is interrupted, tissue temperature changes; hence, the proximal histidine resonance of Mb-δ shifts. Under partial coronary occlusion (Fig. 1G), the ischemic myocardial blood flow was 0.78, 0.54, and 0.33 ml-min⁻¹·g⁻¹ for Epi, Mid, and Endo, respectively, compared with basal values of 1.10, 1.16, and 1.23, respectively. The blood flow gradient leads a corresponding temperature gradient as well. In turn, these gradients are expected to affect both the average (i.e., "peak") resonance frequency and the distribution of that frequency for the Mb-δ proximal histidine resonance. In contrast to partial occlusion, total occlusion (Fig. 1H) resulted in severe and relatively uniform reduction in blood flow (the myocardial blood flow decreased to 0.03, 0.00, and 0.00 for Epi, Mid, and Endo, respectively). In this case, the line width was narrower and the "peak" frequency tended to shift further away from the water resonance.

In one animal we performed a partial coronary occlusion during the catecholamine-induced high work state (data not shown). This was done to prove that the higher heart rates during the high work states did not limit our ability to detect the Mb-δ resonance with 1H NMR spectroscopy. A large Mb-δ resonance was detected during partial LAD occlusion performed during the catecholamine infusion.

DISCUSSION

This study tested the hypothesis that the reduction of the myocardial HEP and the appearance of P1 observed during high cardiac work states are not caused by inadequate intracellular oxygenation. The data support this hypothesis because the HEP reductions at high work states were not associated with detectable myoglobin desaturation. In contrast, when reductions of HEP comparable to those during catecholamine stimulation were induced by moderate blood flow restriction, myoglobin desaturation was present (the expected result), and total occlusion of the coronary artery was associated with a much larger Mb-δ signal. Taken together, the data indicate that in normal canine
myocardium neither blood flow (i.e., convective $O_2$ delivery) nor $O_2$ diffusion from the red blood cell into the cytosol limits mitochondrial $O_2$ availability at moderately high workloads.

Myocyte oxygenation measurements: comparison with previous data. Transmission and reflectance optical spectroscopic techniques have been used to evaluate myocardial myoglobin and cytochrome oxidase $O_2$ saturations in crystalloid-perfused hearts (15, 27, 39, 46). Such studies have reported no or moderate myoglobin desaturation under baseline conditions, and all demonstrated myoglobin desaturation when perfusate flow was reduced. In several of these studies, mild to moderate increases of work state were not associated with additional myoglobin desaturation, even if some desaturation was present at baseline. However, the relevance of these studies to the in vivo state has not been clear because perfusate composition, flow, and the workloads attained in the ex vivo hearts differ significantly from in vivo conditions. In the present study, as well as in an earlier report (6), we were unable to detect Mb- of in vivo canine myocardium under baseline conditions, although it was readily observed when blood flow was reduced. Several preliminary reports published in abstract form are also relevant to the present data. In one study (2), reflectance spectroscopy was used to determine myocardial myoglobin desaturation in the epicardium of the in vivo dog heart during baseline and catecholamine-stimulated states. No myoglobin desaturation was observed during either condition. In another preliminary study in which Mb- in the in vivo rat heart was assessed with $^1$H MRS (25), the investigators were unable to detect Mb- under baseline conditions or with dobutamine stimulation sufficient to double the RPP. However, resonances from both Mb- and deoxyhemoglobin were detected subsequent to hypoxia.

Myocardial myoglobin saturation and $P_{O_2}$ measurements: methodological considerations. Because absolute $P_{O_2}$ values cannot be extrapolated from the $^1$H MRS Mb- measurements obtained in this report, a brief discussion of the physiological boundaries that apply to our data is warranted. The Mb- resonances obtained during partial coronary occlusion were normalized to the Mb- resonance during total coronary occlusion, which was taken to represent 100% myoglobin desaturation. However, because of continuing collateral blood flow (representing ~5% of mean baseline flow), it is clear that the Mb- resonance during total occlusion was smaller than would have been the case if there were 100% myoglobin desaturation. Because of collateral blood flow, it is likely that the Mb- resonance obtained during complete LAD occlusion corresponded...
to <95% of the total myoglobin content (corresponding to an equilibrating PO2 value <0.13 mmHg according to the Hill equation). Similarly, myocyte PO2 values during basal and catecholamine-stimulated conditions are also indeterminate. However, from the myoglobin-PO2 relationship (assuming a P50 value for O2 of 2.39 mmHg), it is likely that myoglobin was at least ~10% desaturated during basal and elevated work states for the following reasons (37). Coronary venous blood has a PO2 of ~30 mmHg in this open chest model under both basal work state conditions and catecholamine stimulation. This presumably reflects a mean capillary PO2 that is higher; i.e., ~40 mmHg (this estimate is based on published estimates in in vivo skeletal muscle working at ~30–50% of maximum O2 consumption) (33, 34). If a 20- to 30-mmHg PO2 gradient exists between the capillary and the cytosol (33, 34), then the myoglobin desaturation curve would predict that myoglobin will be between 10 and 20% desaturated (corresponding intramyocyte PO2 values between 21 and 10 mmHg). A resonance reflecting ~10% desaturation would be difficult to detect in our 1H spectra. This limitation reflects the fact the myoglobin resonance is very broad, and spectral baselines are not totally flat. Consequently, it is difficult to assign with certainty the peaks seen in the appropriate chemical shift region to the Mb-d resonance when deoxymyoglobin desaturation is <10%. At higher levels of desaturation, the peak can be confidently identified, and the accuracy of the measurement based on the signal-to-noise ratio is better than 10%.

An additional consideration is that the sensitivity for Mb-d detection is greatest in the outer myocardial layers that are closest to the surface coil. This is of special concern when coronary blood flow is limited by a stenosis, because the flow deficit and HEP reductions occur primarily in the subendocardium (32). In this situation Mb-d spectra might underestimate the degree of desaturation present in the inner layers where ischemia is most severe. Nevertheless, the coronary stenosis was associated with a prominent Mb-d peak. The effect of proximity to the surface coil is less of a consideration during the catecholamine infusion protocol, because the HEP alterations during catecholamine infusion are essentially uniform across the LV wall (45). Furthermore, when 31P and 1H spectra are compared, differential sensitivity across the wall is not a concern because both data sets have similar sensitivity profiles as a function of distance from the surface coil. Both 31P and 1H spectra represent signals originating from the entire wall under the coil without transmural spatial differentiation. The 31P spectra were obtained using an ISIS column selection perpendicular to the LV; the dimension of this ISIS localization was 2.3 × 2.3 cm², whereas the coil diameter was 2.8 cm. Thus the ISIS voxel cross section was large relative to the coil dimensions. Therefore, in the plane perpendicular to the coil axis (i.e., parallel to the surface of the LV wall under the coil), the 31P signal covered ~60–70% of the area seen in the 1H studies. The two volumes, however, are concentric. Perpendicular to the coil axis, across the LV wall, both 1H and 31P spectra cover the same volume, so that any partial volume effect resulting from the sensitive volume extending into cavitary blood would be similar for the two spectra. A final consideration concerns the difference in line width for 1H spectra observed during partial and total coronary artery occlusion (Fig. 1, G and H, respectively). The broader line width during partial coronary occlusion is likely the result of a temperature gradient across the LV wall. The chemical shift of the contact-shifted proximal histidine resonance exhibits a strong dependence on temperature (23). A coronary stenosis that decreases arterial inflow results in marked heterogeneity of tissue perfusion, with flows lowest in the subendocardium. It is likely that a corresponding gradient of myocardial temperature occurs, which would result in a variable shift in the proximal histidine resonance, thereby broadening the spectrum. In contrast, during total occlusion, blood flow is markedly decreased across the entire LV wall; in this case, the peak shift of the proximal histidine resonance away from the water resonance would be greater than the average shift during a partial coronary stenosis, but the temperature-induced shift would be more uniform across the LV wall, resulting in a narrower resonance.

Myocytotoxic oxygenation measurements: physiological implications. It is of interest to compare the present data obtained during moderately high workloads in normal myocardium with previous data from working skeletal muscle. No Mb-d resonance is detected in resting skeletal muscle (28, 31, 34). However, in an 1H MRS study of exercised human quadriceps muscle, myoglobin saturation decreased to ~50% with half-maximal work and then remained essentially constant as the load was further increased to achieve a maximal work state (34). Wittenberg (43) pointed out that maximal facilitation of O2 transport (by cytosolic myoglobin) occurs only when myoglobin is significantly desaturated. The findings in skeletal muscle suggest that myoglobin plays a role in facilitating O2 transport to the mitochondria over a broad range of workloads. It is of interest that skeletal muscle PO2 levels and maximal exercise capacity are reduced during hypoxia (34), while hyperoxia (induced by breathing 100% O2) increased maximal skeletal muscle O2 utilization (22). Taken together, these data imply that maximal O2 delivery to the mitochondria is the rate-limiting step for oxidative phosphorylation in oxidative skeletal muscle. In the heart, mitochondrial concentration and capillary density are more than twice as great as in skeletal muscle. Thus, in normal porcine, canine and human myocardium mitochondria comprise ~25% of cell volume, whereas in highly oxidative skeletal muscle mitochondrial volume is 4–9% (3, 17, 37). O2 consumption rates in human maximal single leg exercise are reported to be as high as ~60 ml·min⁻¹·100 g⁻¹ (33, 34), which translates to 8–10 ml·min⁻¹·ml of mitochondria⁻¹ (38). Because mitochondrial volume and capillarity are strongly correlated with muscle oxidative capacity (Vmax) (17, 38), the greater capillarity and mitochondrial content of myocardium suggest that the
O₂-delivery capacity of the heart should be far higher than the peak MV̇O₂ values attained in our anesthetized animals (−30 ml·min⁻¹·100 g⁻¹). Exercise-recruitable blood flow is greater in cardiac muscle than in oxidative skeletal muscle (18, 33, 34), and the functional capillary diffusion surface area increases proportionally with blood flow (4). Consistent with a large O₂ delivery and diffusion capacity, the current observations suggest that substantial myoglobin desaturation is not required to facilitate O₂ conductance at MV̇O₂ values that approximate 35–50% of those achieved in heavily exercising animals. For example, if 10% (or even 20%) desaturation is assumed, the carrier function of myoglobin would be expected to be modest (see Ref. 43 for discussion). Recent data obtained in a transgenic mouse model support the concept that myoglobin facilitation of O₂ transport in the heart is not essential at moderately high cardiac workloads. Mice without skeletal or cardiac muscle myoglobin exhibited normal exercise and O₂ consumption capacities during treadmill testing (10). Furthermore, perfused hearts isolated from these animals performed similarly to normal hearts across a considerable range of workloads (10). However, these data do not exclude the possibility that myoglobin facilitation of O₂ conductance would be required at the higher MV̇O₂ levels achievable during exercise. During heavy treadmill exercise in the dog, coronary venous PO₂ can fall to ~15 mmHg (18); under these conditions myocardial myoglobin desaturation sufficient to facilitate O₂ transport would not be unexpected.

HEP responses during catecholamine infusion. During catecholamine infusion, the CP/ATP fell and ΔPi/CP rose significantly in all transmural myocardial layers (transmural layer data not shown). These HEP changes were not associated with detectable myoglobin desaturation, indicating that the cytosolic O₂ concentration remained high during this period of markedly increased ATP synthesis. The mechanisms of the HEP and P̄ alterations during catecholamine infusion are at present unknown. However, in preliminary studies we observed that pyruvate infusion can correct the CP/ATP abnormalities present during catecholamine infusion without causing MV̇O₂ to increase (7). This suggests that the HEP reductions may be mediated, at least in part, by alterations in the carbon substrate utilization pattern without either carbon substrate or O₂ delivery being the rate-limiting step. It has previously been documented both in vitro and in vivo that the type of carbon substrate utilized affects the HEP and P̄ levels at any given steady-state O₂ consumption rate (9, 21, 40). This is thought to be a consequence of substrate-induced alterations of mitochondrial NADH levels that affect cytosolic ADP levels (9, 40). Thus a relatively higher glucose + glycogen-to-fatty acid utilization ratio during catecholamine infusion would be consistent with increased ADP and ΔP̄, as observed at high workloads in the canine myocardium. Because myocardial creatine kinase is near equilibrium, CP levels would fall as ADP levels rose (41). Clearly, more work will be required to fully identify the mechanisms that contribute to the observed HEP changes during high cardiac workloads.

In conclusion, the current data support the hypothesis that the decreases of myocardial CP/ATP, CP, and ATP and the elevation of P̄ observed at moderately high levels of MV̇O₂ in normal canine myocardium are not a consequence of inadequate intracellular oxygenation. Thus neither blood flow nor O₂ diffusion capacity restrict mitochondrial O₂ availability at the high workloads achieved in the present study. The data demonstrate the value of myocyte oxygenation measurements when interpreting the mechanism of myocardial HEP changes resulting from pharmacological or physiological interventions.

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