Myoglobin desaturation with exercise intensity in human gastrocnemius muscle

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Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R173–R180, 1999.—The present study evaluated whether intracellular partial pressure of O2 (PO2) modulates the muscle O2 uptake (V02) as exercise intensity increased. Indirect calorimetry followed V02, whereas nuclear magnetic resonance (NMR) monitored the high-energy phosphate levels, intracellular pH, and intracellular PO2 in the gastrocnemius muscle of four untrained subjects at rest, during plantar flexion exercise with a constant load at a repetition rate of 0.75, 0.92, and 1.17 Hz, and during postexercise recovery. V02 increased linearly with exercise intensity and peaked at 1.17 Hz (15.1 ± 0.37 watts), when the subjects could maintain the exercise for only 3 min. V02 reached a peak value of 13.0 ± 1.59 ml O2·min⁻¹·100 ml leg volume⁻¹. The 31P spectra indicated that phosphocreatine decreased to 32% of its resting value, whereas intracellular pH decreased linearly with power output, reaching 6.86. Muscle ATP concentration, however, remained constant throughout the exercise protocol. The 1H NMR deoxymyoglobin signal, reflecting the cellular PO2, decreased in proportion to increments in power output and V02. At the highest exercise intensity and peak V02, myoglobin was ~50% desaturated. These findings, taken together, suggest that the O2 gradient from hemoglobin to the mitochondria can modulate the O2 flux to meet the increased V02 in exercising muscle, but declining cellular PO2 during enhanced mitochondrial respiration suggests that O2 availability is not limiting V02 during exercise.

Bioenergetics; oxygen; nuclear magnetic resonance; metabolism

During exercise, the ability of the working muscle in healthy individuals to increase its O2 uptake (V02) is limited by either a central or a peripheral mechanism (37). The central mechanism represents the systemic increase in O2 delivery to the muscle, which includes enhanced blood flow through the capillaries. The peripheral mechanism focuses on the diffusion of O2 from hemoglobin (Hb) to the mitochondria and the metabolic regulation of oxidative phosphorylation. Proponents of a central mechanism point out a tight correlation between V02 and blood flow (Q) while others have countered that such a mechanism cannot account for the large residual venous PO2 at maximal O2 uptake (V02max; see Refs. 13–15, 40). In the latter group’s view, convective control is only secondary to diffusion control. Despite numerous studies, the limiting mechanism for V02 is still under debate.

Even though both models entail an O2 gradient role, the diffusion model emphasizes the gradient from the capillary to the mitochondria, which is a driving force of O2 transport. Analysis of the role of gradients, however, requires a measurement of the mean end-capillary and the cellular partial pressure of O2 (PO2). For the cellular PO2 studies have assumed that the mitochondrial PO2 is approximately zero. Such an assumption is dictated largely by the current limitation in observing quantitatively the cellular PO2 in muscle during exercise. Honig et al. (16) have already demonstrated in cryosection experiments that, even though the intracellular PO2 is quite low, it does decrease with exercise intensity (16). The observation implies that the cell can modulate its O2 gradient and suggests that myoglobin (Mb)-facilitated diffusion, which becomes increasingly pronounced as the cellular PO2 falls, contributes significantly to O2 transport to the mitochondria (11, 46).

The intracellular PO2 during exercise is then a key variable in helping to determine the regulatory mechanism of V02 in exercising muscle. In addition, the intracellular PO2 response during exercise can help clarify the hypothesis that O2 supply limits V02max. If the critical PO2 in the cell is less than ~2.9 mmHg (Mb p50 at 39°C), then the PO2 at the mitochondria is estimated to be at the Michaelis constant of the cytochrome oxidase activity (3, 43). The cellular O2 supply can then limit the aerobic capacity of working muscles without metabolic limitation, which can modulate the phosphorylation potential and redox state (8, 45). However, if the critical intracellular PO2 is well above 2.9 mmHg, then cytochrome oxidase is saturated even at V02max, suggesting that O2 alone is not modulating mitochondrial respiration (25). Unfortunately, measurement of intracellular PO2 in human skeletal muscle during intense exercise poses a formidable technical challenge.

In recent years, 1H NMR has presented an approach to measure the intracellular PO2 with the Mb signals, first in myocardium and subsequently in skeletal muscle (19, 21, 42). In fact, one study has now shown that Mb desaturates rapidly to 51 and 60% of control under normoxic and hypoxic exercise conditions, respectively; yet, surprisingly, Mb desaturation is not proportional to increased work output (32). The results are provocative and have significant impact on the current view of respiratory control in muscle. They compel us to reexamine the relationship between work output and Mb desaturation in a plantar flexion exercise protocol.
which is less prone to motional artifact than the reported quadriceps exercise. As a result, the NMR data are much improved and show that both the $^3$H-deoxy-Mb and $^{31}$P high-energy phosphate signal intensities change in proportion to power output or VO$_2$. At VO$_{2\text{max}}$, Mb is 48% desaturated. The present study’s results indicate that the cellular PO$_2$ can modulate the O$_2$ gradient and that Mb-facilitated diffusion may play a significant role in regulating VO$_2$. Moreover, O$_2$ availability does not appear to limit VO$_2$ during exercise.

**MATERIALS AND METHODS**

Experimental design. The protocol for this study was reviewed and approved by the Human Subjects Welfare Committee of the University of California, Davis. Four young adult men (Table I) were recruited from the student body of the university. All were untrained volunteers, who gave written consent to participate in this study. They were informed of the procedures, requirements, and risks. Each subject reported to the laboratory several times to practice plantar flexion exercise and to become familiar with breathing through the respiratory apparatus. During this time, the exercise intensity was adjusted so that the subject could reach peak O$_2$ uptake (VO$_{2\text{peak}}$).

Two sessions were required to complete this study. Session I was conducted in the Human Performance Laboratory and involved characterizing each subject’s body composition, steady-state VO$_2$ at several intensities of plantar flexion exercise, and VO$_{2\text{peak}}$ for plantar flexion exercise. A fiberglass (ScotchCast; 3M) cast was made from the subject’s lower leg from below the knee to the ankle. The cast was used as another means to calibrate the percent desaturation of the deoxy-Mb signal and to estimate the lower leg volume, which was then used to normalize the net change in VO$_2$. Session II was performed at the nuclear magnetic resonance (NMR) Research Facility at General Electric (GE) Medical Systems (Fremont, CA) and involved duplicate protocols for plantar flexion exercise for determination of metabolic phosphates by $^{31}$P NMR and deoxy-Mb by $^3$H NMR. Both protocols corresponded to the protocol performed in session I.

Body composition. Air displacement plethysmography was employed to determine body composition with the BOD POD instrument (LMI, Concord, CA). The method has been described in detail elsewhere (26). Each subject was weighed to the nearest gram, and the height was measured to the nearest centimeter. The raw body volume was determined in duplicate and averaged. Next, thoracic gas volume was then measured in the BOD POD. Body density was calculated as the ratio of body mass to corrected body volume. Percent body fat was then calculated from body density using the Siri (35) formula.

Plantar flexion ergometer. The same ergometer was employed to assess the mechanical power, energy transfer rate by whole body indirect calorimetry, the muscle concentrations of P$_i$, phosphocreatinine (PCr), ATP, and intracellular pH by $^{31}$P NMR, and deoxy-Mb by $^3$H NMR during plantar flexion exercise of the calf muscles. The ergometer consists of a three-sided box with dimensions of 25.4 cm wide $\times$ 25.4 cm high $\times$ 91.4 cm long with a foot pedal on an axle at one end and a moveable back plate at the other end of the box. An aluminum bar served as an end stop for the pedal arc during plantar flexion exercise. Latex rubber tubing (1.3 cm diameter and 34.3 cm length) with a Hooke’s constant of 31.12 N/cm length change was attached to the back plate and the axle of the foot pedal. Resistance to plantar flexion can be varied by the number of tubes used and/or by changing the stretch of tubing between the axle and back plate. Mechanical work of plantar flexion involved moving the pedal against a specified resistance through an arc of 3.8 cm. The pedal movement was controlled by stops for forward and reverse movements with plantar flexion and relaxation. In this study, power was incremented by varying the contraction frequency from 30 to 70 contractions/min (0.5–1.17 Hz) with the resistance and plantar flexion arc held constant. Contraction frequency was not measured but was controlled by requiring the subject to follow a metronome beat, with verbal assistance from a technician for added control. The technician monitored the exercise protocol, which required the subject to push the pedal until it stops at the aluminum bar. Any learning effect was minimized by having the subject practice the exercise procedures several times before the experiments.

Steady-state VO$_2$ and VO$_{2\text{peak}}$ for plantar flexion exercise. Each subject performed three to five intensities of plantar flexion exercise of the dominant leg, each for 3 min with a 5-min recovery period after each bout. Energy expenditure was determined continuously by indirect calorimetry. The sequence was as follows: after resting for 10 min in a supine position, the subject breathed for 5 min through a mouthpiece and tubing connected to a SensorMedics metabolic cart (model 2900) for breath-by-breath measurements of resting VO$_2$ and CO$_2$ production (VCO$_2$). Next, the subject performed a series of three to five exercise bouts at progressively higher intensities by varying the frequency from 30 to 70 contractions/min (0.5–1.17 Hz) on the foot ergometer, with the resistance held constant. Each bout lasted 3 min and was followed by 5 min of resting recovery. The calculation time (3 s) during the last 30 s of each bout was used to characterize the VO$_2$, VCO$_2$, and respiratory exchange ratio for the bout.

After a rest period of 10–15 min, the subject’s VO$_{2\text{peak}}$ was determined by holding the resistance constant and progressively increasing the contraction frequency each minute until the subject could no longer maintain the required cadence. VO$_2$ and VCO$_2$ were determined throughout the test as described above. VO$_2$ was averaged over each 15-s interval. The highest VO$_2$ value was designated as the subject’s VO$_{2\text{peak}}$.

NMR. NMR measurements were performed on a 1-m bore diameter GE Signa scanner at 1.5 T. $^{1}$H (63.86 MHz) NMR signal acquisition utilized a body coil transmit/surface coil (5-in. diameter)-received configuration. Magnetic field shimming used a three-point Dixon method to improve the field homogeneity, yielding a water line width of $\pm$ 40 Hz (34). A selective excitation pulse sequence was optimized to excite the deoxy-Mb and deoxy-Hb histidyl-F8 N$^\ddagger$H signals, $\pm$ 4.6 kHz from the water resonance (28). Numerical simulation and experimental data verified that the experimental pulse length of 800 µs had a full width at half-maximum excitation of 2 kHz. At an offset of 800 Hz or 13 ppm from the excitation maximum, the pulse power dropped by 25%. Each data block was comprised of 200 transients or 45 s signal averaging time. The repetition time was 160 ms. The spectral width was 16 kHz, and the data block size was 512. All spectra were

<table>
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<th>Body Fat, %</th>
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<td>63.7 ± 4.49</td>
<td>11.6 ± 1.20</td>
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**Table 1. Subject characteristics**
MYOGLOBIN DESATURATION WITH EXERCISE IN MUSCLE

RESULTS

Selected characteristics of the subjects are presented in Table 1 and show that these young, untrained adult men are relatively lean for their age and gender.

A representative example of a stack plot of $^1$H NMR deoxy-Mb for subject A is illustrated in Fig. 1. Figure 1A shows no detectable deoxy-Mb at rest where Mb presumably is fully saturated with $O_2$. Figure 1, B-D, shows that the magnitude of the deoxy-Mb peak grows with power outputs of 9.4, 11.5, and 14.7 watts, respectively. Within 5 min of recovery from exercise at 14.7 watts, the deoxy-Mb signal disappears into the noise (Fig. 1E), indicating that Mb has become saturated with $O_2$.

The steady-state ATP, PCr, $P_i$, and intracellular pH results at rest and with variations in exercise intensity are presented in Table 2 and Fig. 2. Muscle ATP does not change relative to rest when power output is varied from 7.8 to 15.1 watts for plantar flexion exercise. In contrast, muscle PCr decreases, whereas $P_i$ increases linearly with power output (Fig. 2, B-D). $P_i$ falls to 33%, and PCr rises to 81% of the resting PCr level at the highest intensity of exercise, which elicits $\dot{V}O_2$peak.

Figure 3 shows the graph of ATP and PCr level as a function of plantar flexion work output. ATP is set at 8.2 mM and is the normalizing value for the PCr and $P_i$. Clearly, the ATP level remains constant throughout the exercise protocol (Fig. 3A). PCr falls 4.5%/watt output. The corresponding graphs for $P_i$ and pH are shown in Fig. 4. $P_i$ increases with exercise at a rate of 4.1%/work output.

The relationship between normalized net $\dot{V}O_2$ and plantar flexion power is shown in Fig. 5. As exercise intensity increases, the $\dot{V}O_2$ also increases linearly. Because the net $\dot{V}O_2$ of 13.2 ± 2.1 and 12.8 ± 1.2 ml·min⁻¹·100 ml leg volume⁻¹ for the last two power outputs of 15.1 ± 0.3 and 17.6 ± 1.0 watts are not significantly different ($P > 0.05$, paired t-test), these values are averaged to yield a net $\dot{V}O_2$peak of 13.0 ± 1.6 ml·min⁻¹·100 ml⁻¹. This $\dot{V}O_2$peak satisfies the criterion for $\dot{V}O_2$max for leg muscles performing plantar flexion exercise.

The relationship between deoxy-Mb signal and exercise power and net $\dot{V}O_2$ is presented in Fig. 6. A distinct linear relationship is found between the percent Mb desaturation and power output (Fig. 6A) and between percent Mb desaturation and net $\dot{V}O_2$ (Fig. 6B). Mb desaturates progressively as work output or $\dot{V}O_2$ increases. At $\dot{V}O_2$peak, Mb desaturates to 48.4%. A similar relationship is apparent with PCr. As Mb desaturates, the PCr level falls linearly as does pH (data not shown).
DISCUSSION

Resting state intracellular Po2. The proximal histidyl N\textsubscript{H} signal of deoxy-Mb reflects the degree of tissue oxygenation during rest and exercise. At rest, no signal is detected, whereas during exercise the signal appears. Given the excellent signal to noise of the deoxy-Mb peak under the normalization condition of the cuffed leg spectra, a 10–20% deoxy-Mb saturation would certainly reveal an observable signal above the noise. Because none is detected, even after the addition of several reference spectra (data not shown), the resting Po2 of skeletal muscle must be sufficient to saturate Mb \textsubscript{O2} >80%, which reflects a resting cellular Po2 >10 Torr (given a Mb p50 of 2.9 at 39°C), and is consistent with muscle cryosection data (10, 11).

Mb de saturation with work output. As muscle work output increases, O\textsubscript{2} flux must also increase to match the enhanced \textit{V}O\textsubscript{2}. Such an enhanced O\textsubscript{2} flux is governed phenomenologically by Fick’s law of diffusion

\[ \textit{V}O_2 = D_0[\text{Po}_2\text{cap} - \text{Po}_2\text{mito}] \]

where D\textsubscript{O} is the lumped conductance for O\textsubscript{2} diffusion transport in tissue and (Po2\textsubscript{cap}) and (Po2\textsubscript{mito}) are the partial pressures of O\textsubscript{2} at the capillary and at the mitochondria, respectively.

A driving force for O\textsubscript{2} flux involves then a gradient from the capillary to the mitochondria. Because the mitochondrial Po2 is assumed to be approximately zero, only the capillary Po2 is often used to determine the interaction between the O\textsubscript{2} gradient and V\textsubscript{O2}. However, according to Honig et al. (16), the cellular Po2 can also modulate the O\textsubscript{2} flux. Intracellular Po2 is low but not zero. As work intensity increases, the cellular Po2 can decline, as reflected by Mb desaturation, and therefore enhances the gradient driving force for O\textsubscript{2} flux (11). Even though V\textsubscript{O2} has increased, the intracellular Po2 has fallen. A rise in V\textsubscript{O2} in face of a drop in intracellular Po2 is consistent with an enhanced role of Mb in facilitating O\textsubscript{2} diffusion to the mitochondria during exercise (44, 46).

Validation of the Honig hypothesis in a dynamic model has presented a formidable challenge, since experimental measurements must assess the intracellular Po2 during exercise. With \textsuperscript{1}H NMR, a strategy has emerged to observe the intracellular Po2 with the signals of Mb (22). Indeed, the deoxy-Mb proximal histidyl N\textsubscript{H} signal in our study demonstrates clearly that intracellular Po2 in exercising skeletal muscle falls progressively with work output. As the work output varies from 7.8 ± 0.32 to 15.1 ± 0.3 watts, Mb desaturates from 30.2 ± 2.4 to 48.4 ± 5.1%. Both the V\textsubscript{O2} and work output form linear relationships with Mb desaturation.

These results are in contrast with a previous NMR report, which shows that Mb desaturates rapidly to a constant 51% under exercise intensity >50% of V\textsubscript{O2\textsubscript{max}} (32). Below 50% of V\textsubscript{O2\textsubscript{max}}, a dubious experimental point is slightly <50% and suggests the presence of a linear response region at low work output. In the present study, the exercise protocol also elicits V\textsubscript{O2} that spans the range above 50% of V\textsubscript{O2\textsubscript{max}}. At the highest level of exercise performance, the subjects reach V\textsubscript{O2\textsubscript{peak}}.
The observed pH and P_i/PCr change also supports the interpretation that our subjects are exercising above 50% VO\textsubscript{2}max. At the highest work output of 15 watts, the cellular pH is 6.87 ± 0.16, which is consistent with the value of 6.554 ± 0.325 observed by Richardson et al. (32) during exercise at 95% of VO\textsubscript{2}max. Because calf muscle has heterogeneous fibers, the pH values during exercise can range from 6.2 to 7.1 (27, 39). The pH observation during exercise is then consistent with a high work output.

Moreover, the P\textsubscript{i}-to-PCr ratio also supports a high VO\textsubscript{2} exercise. Studies have reported values ranging from 1.3 to 4.1 during maximal exercise (4, 31, 39). At the highest work output, the P\textsubscript{i}-to-PCr ratio is 2.4. Although the study by Richardson et al. (32) reports P\textsubscript{i}-to-PCr ratios approaching 10, the unacceptably large SE precludes any comparative analysis. In that study, the reported PCr-to-P\textsubscript{i} ratio is 0.1 ± 0.2 during exercise at 95% of VO\textsubscript{2}max.

Clearly, the discrepancy in the two reports requires further study and may originate from differences in the interrogated muscle groups (quadiceps vs. gastrocnemius), the subject’s athletic training, and NMR acquisition/processing methodology.

Diffusional conductance. Because the Mb is desaturating as exercise intensity increases, the diffusion equation (Eq. 1) would imply that O\textsubscript{2} gradient from the capillary to the cell is indeed modulating O\textsubscript{2} delivery to match the VO\textsubscript{2}. To determine the specific relationship requires the assessment of the VO\textsubscript{2} as a function of exercise intensity. Even though the present study has utilized whole body VO\textsubscript{2}, both theoretical and empirical studies have shown that the kinetics of VO\textsubscript{2} of working muscle is the same as pulmonary VO\textsubscript{2} in phases 2 (metabolic) and 3 (steady state; see Refs. 5 and 6). A number of other studies have demonstrated that the increase in leg VO\textsubscript{2} accounts for >57% (ranging from 57 to 93%) of the increment in whole body VO\textsubscript{2} during leg exercise (1, 2, 18, 20, 30, 36, 41).

The experimentally determined change in (\Delta) VO\textsubscript{2} from exercise level 1 to 3 is 7 ml·min\textsuperscript{-1}·100 ml leg volume\textsuperscript{-1}, whereas the ([Po\textsubscript{2}]\textsubscript{cap} - [Po\textsubscript{2}]\textsubscript{mito}) rises from
31.4 (40–8.6) to 36.1 (40–3.9) Torr, assuming a constant mean end-capillary PO2 of 40 Torr. Even though the V̇O2 has increased by a factor of 2, the ΔPO2 has only increased by a factor of 1.15. If the mean end-capillary PO2 is 13 Torr, then the ΔPO2 is now altered to 4.4 (13–8.6) and 8.1 (13–3.9), respectively. The change would imply that the O2 gradient is sufficient to match the enhanced V̇O2 and that ΔO2 is relatively constant. Because ΔO2 is a lumped constant, which includes aggregate capillary surface area and capillary to cell distance, a relatively constant ΔO2 would diminish the contribution of diffusion-controlled regulation of O2 flux during exercise.

Nevertheless, ΔO2 is multifactorial and nonlinear. Relatively small changes can enhance O2 flux (9, 17). The extent of ΔO2 modulation is unclear from the present experimental data, since, in part, the NMR observes only a spatially averaged signal and cannot discriminate any heterogeneity, which can complicate the interpretation (29). The specific heterogeneity in question, V̇O2/Q̇, is difficult to assess, since at present no definitive measurements can resolve the V̇O2/Q̇ heterogeneity contribution in exercising muscle. Researchers have argued reasonably against any significant contribution, which is an underlying assumption in the above analysis (32).

Determinant of respiration during exercise. Even with the modulation of O2 delivery, the O2 supply does not appear to be sufficient to meet the O2 demand during exercise without metabolic adaptation. Because oxidative phosphorylation depends on the phosphorylation potential or charge, redox state, ADP, and carbon substrate availability, the associated metabolite levels can also regulate respiration. Indeed, as V̇O2 increases, the 31P-PCr signal declines, whereas the P_i signal increases. The P_i-to-PCr ratio, which reflects the ADP concentration, shifts from 0.19 to 2.7 and is consistent with the linear relationship between percent peak power vs. ADP (4).

Fig. 5. Net O2 uptake (V̇O2) as a function of plantar flexion power output in 4 untrained men. As plantar flexion power increases, so does V̇O2. The values reflect the difference between the average V̇O2 of the last 30 s of exercise and 5 min of rest and are expressed as means ± SE. Values are normalized for each individual's lower leg volume, which was obtained from the cast of the lower leg. V̇O2 values for the last two exercise intensities were not significantly different (paired t-test, P < 0.05), indicating that V̇O2 peaked at 15.1 watts, the highest intensity employed for the NMR experiments. The regression line is based on the mean values (n = 4). Differences between V̇O2 for the 3 exercise intensities are significant (P = 0.000883), with Bonferroni’s post hoc test showing that the results from the 45 vs. 70 rpm experiments are significant. Regression of V̇O2 vs. power (without the 0,0 points) for each subject shows that the slopes for each subject are significant. Slopes, SE estimate, and P values for subjects A, B, C, and D are (1.17, 0.387, 0.05), (0.692, 0.0329, 0.0002), (0.629, 0.130, 0.017), and (0.885, 0.129, 0.0064), respectively. Using the mean of the slopes as a measure of the change in V̇O2 per unit change in power (3.37) and the pooled SE estimate (0.931), the power is 0.994. A sample size, n = 4, is needed to detect a difference at P = 0.05.

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Fig. 6. Graph of the percent deoxymyoglobin (deoxy-Mb) intensity as a function of power output (A) and net V̇O2 (B). As the V̇O2 increases with muscle power output, the intracellular PO2, as reflected in the deoxy-Mb proximal histidyl N̄D signal, declines linearly. The regression lines are based on mean values (n = 4). Repeated-measures ANOVA on deoxy-Mb also shows significance (P = 0.00632), with 45 vs. 70 and 55 vs. 70 (P < 0.05). Regression analysis of deoxy-Mb vs. power (without the 0,0 points) for each subject shows that the slopes are significant for each subject. The slope, SE estimate, and P values for subjects A, B, C, and D are (5.55, 0.864, 0.010), (2.702, 0.199, 0.0054), (3.02, 0.248, 0.0067), and (2.81, 0.464, 0.0261), respectively. Using the mean of the slopes (3.53) as a measure of the change in deoxy-Mb per unit change in power and the pooled SE estimate (0.516), the power is 0.99. A sample size, n = 3, is needed to detect a difference at P = 0.05. Dotted line corresponds to peak V̇O2 at 15.1-W output.
Although the O₂ gradient increases with exercise intensity and therefore enhances the driving force for O₂ transport, the intracellular \( \text{PO}_2 \) nevertheless drops and suggests that an additional O₂ diffusion route to the mitochondria becomes increasingly significant. Such a role is postulated for Mb. Even the enhanced driving force for O₂ flux, however, does not preclude an apparent ADP-dependent stimulation of respiration or any modulation from the lumped O₂ conductance factor, which includes O₂ unloading and aggregate capillary surface area. Respiratory control does not appear to depend solely on the regulation of O₂ delivery or supply. Nevertheless, the study has demonstrated that the O₂ gradient from the vasculature to the cell is limiting respiration as the muscle activity approaches a \( \dot{V}_\text{O}_2 \text{max} \) level. Propionate effects of increased Hb-0₂ on \( \dot{V}_\text{O}_2 \text{max} \) at constant O₂ delivery in dog muscle in situ. J. Appl. Physiol. 70: 2656–2662, 1991.


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