Effect of varied extracellular $\text{PO}_2$ on muscle performance in Xenopus single skeletal muscle fibers

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Stary, Creed M., and Michael C. Hogan. Effect of varied extracellular $\text{PO}_2$ on muscle performance in Xenopus single skeletal muscle fibers. J. Appl. Physiol. 86(6): 1812–1816, 1999.—The purpose of this study was to examine the development of fatigue in isolated, single skeletal muscle fibers when $\text{O}_2$ availability was reduced but not to levels considered rate limiting to mitochondrial respiration. Tetanic force was measured as 75% of initial maximum force) was measured during three separate work bouts (with 45 min of rest between) as the perfusate $\text{PO}_2$ was switched between values of 30, 6, or 159 Torr in a blocked-order design. No significant differences were found in the initial peak tensions between the high-, intermediate-, and low-$\text{PO}_2$ treatments (323 ± 22, 298 ± 27, and 331 ± 24 kPa, respectively). The time to fatigue was reached significantly sooner ($P < 0.05$) during the 30-Torr treatment (233 ± 39 s) compared with the 76- (385 ± 62 s) or 159-Torr (416 ± 65 s) treatments. The calculated critical extracellular $\text{PO}_2$ necessary to develop an anoxic core within these fibers was 13 ± 1 Torr, indicating that the extracellular $\text{PO}_2$ of 30 Torr should not have been rate limiting to mitochondrial respiration. The magnitude of an unstripped layer (243 ± 64 μm) or an intracellular $\text{O}_2$ diffusion coefficient (0.45 ± 0.04 × 10⁻⁵ cm²/s) necessary to develop an anoxic core under the conditions of the study was unlikely. The earlier initiation of fatigue during the lowest extracellular $\text{PO}_2$ condition, at physiologically high intracellular $\text{PO}_2$ levels, suggests that muscle performance may be $\text{O}_2$ dependent even when mitochondrial respiration is not necessarily compromised.

mitochondria; respiration; oxidative phosphorylation; fatigue; oxygen consumption

IT HAS BEEN SUGGESTED (21) that, during high-intensity work, fatigue occurs when an imbalance develops between the ATP demand of the ATPases and the ATP production by oxidative phosphorylation and substrate-level phosphorylation [glycolysis and phosphocreatine (PCr) hydrolysis]. Whether this ATP supply/demand imbalance is the result of inadequate mitochondrial concentration, substrate limitation (NADH, P, ADP) to the working mitochondria, or a result of inadequate availability of $\text{O}_2$ as the electron acceptor at the terminal end of oxidative phosphorylation is unclear and likely variable. Although in isolated mitochondria the rate of ADP rephosphorylation only becomes limited when the $\text{PO}_2$ is as low as 0.5 Torr (3), ischemic and hypoxic hypoxia have been shown to contribute to an early onset of fatigue in working whole muscle even when the extracellular $\text{O}_2$ tension is high (8, 11, 12).

When the concentration of $\text{O}_2$ ($[\text{O}_2]$) is rate limiting within a working skeletal muscle cell, muscle performance can be directly inhibited by inadequate ADP rephosphorylation. However, our laboratory has demonstrated that modulation of oxidative phosphorylation substrates may occur in whole muscle at similar rates of respiration when tissue oxygenation is altered (6, 8, 12, 13). These changes may have subsequently affected muscle function, leading to an earlier onset of fatigue even though the $[\text{O}_2]$ was not limiting to oxidative phosphorylation. However, blood flow and fiber type heterogeneity in whole muscle experiments make an exact determination of a specific limitation threshold difficult. Therefore, the extracellular ($\text{PO}_2$) and intracellular $\text{PO}_2$ ($\text{PO}_2$) at which single muscle fibers become compromised, and performance attenuated, are unknown.

In the present study, we used an isolated, working single skeletal muscle cell model to avoid some of these confounding factors associated with whole muscle experiments. $\text{PO}_2$ was homogeneous and easily determined, and the mitochondria respired in their normal environment. The purpose of the present experiments was to examine single muscle fiber performance at three extracellular $\text{O}_2$ tensions that were well above that calculated to be rate limiting to mitochondrial respiration.

METHODS

Adult female Xenopus laevis were doubly pithed and decapitated. Lumbrical muscles were removed, and single living muscle fibers (n = 6) were microdissected from the muscle. After isolation, myocytes were fiber typed according to cross-sectional area and appearance under dark-field illumination (20). Platinum clips were attached to the tendons, and the fibers were mounted in a glass chamber and continually perfused with Ringer solution (in mM: 112 NaCl, 1.8 KCl, 0.82 CaCl₂, 2.38 NaHCO₃, 0.07 NaH₂PO₄) at 20°C and 7.0 pH. Before each contraction period, the resting fiber was passively stretched until the force produced by a single tetanic contraction was maximal ($P_o$).

Tetanic contractions were induced by direct stimulation (50 impulses/s of 1-ms duration at 9 V, with a train duration of 200 ms) with platinum conducting electrodes on either side of the fiber, by using a Grass (model 548, Grass Instruments, Quincy, MA) stimulator. Force development was measured with a 5-g force transducer system (model 400A, Aurora Scientific, Aurora, Ontario) and is reported in newtons and kilopascals (kPa = N/m²). Waveforms were recorded and measured on a Gould flatbed chart recorder (model 220, Gould, Cleveland, OH).
Experimental protocol. Each fiber had its rate of fatigue development measured during three separate work bouts (with 45 min of rest between) with the perfusate PO2 being switched between values of 30 ± 2, 76 ± 3, or 159 Torr in a blocked-order design, which incorporated each possible order of oxygenation. Fibers were stimulated for each of the three work bouts at increasing contraction rates (0.25, 0.33, 0.5, and 1 Hz) in a sequential manner with each stimulation frequency lasting 2 min. Electrical stimulation was terminated when force fell to 50–60% of maximal. Low-PO2 fiber was generated by N2 aeration and checked with a Clark-style electrode to ensure proper deoxygenation. The PO2 of the perfusate in the chamber was monitored with a Clark-style electrode (Diamond General, Ann Arbor, MI) placed adjacent to the working fiber. Individual peak tensions were compared with the highest peak tension within that run (P0). Fatigue was determined as the time point at which the development of force (P) had declined to 75% of the initial maximum tension (P/P0 = 0.75). Calculations. The relationship between the diffusion of O2 and its consumption (V˙O2) in a muscle fiber that does not contain myoglobin is described by the Hill equation (7)

\[ \text{PO2extra} - \text{PO2intra} = \left( \frac{\text{V˙O2}}{0.44 \cdot \text{D} \cdot \text{O2}} \right) (\text{cross-sectional area}) \]  

(1)

where D is the O2 diffusion coefficient, the cross-sectional area is the diffusion path length for O2 (dependent on the radius of the cell), and PO2intra is the PO2 at the center of the cell. The Hill equation assumes a uniform distribution of O2 surrounding a cylindrical muscle fiber and an absence of any unstirred layers of medium. This equation can also be used, for a particular V˙O2, to calculate any PO2intra for any given PO2extra.

Equation 1 has been extended to calculate the threshold for O2 limitation (PO2critical) in single muscle fibers (4) by assuming that oxidative phosphorylation is limited when an anoxic core develops in the center of the cell at maximal rates of respiration. This threshold is represented by PO2extra when PO2intra is 0 Torr at the highest rate of VO2 (VO2max). This is summarized by the following equation

\[ \text{PO2critical} = \left( \frac{\text{V˙O2}}{0.44 \cdot \text{D} \cdot \text{O2}} \right) (\text{cross-sectional area}) \]  

(2)

A value of 1.01 × 10^{-3} cm/s for D (16) and VO2max values (average = 0.06 nmol O2 mm^{-3} s^{-1}) for Xenopus single fibers working maximally were used (10). Cross-sectional area was determined by measuring and averaging the three largest and smallest diameters with an optical reticle. Muscle performance was compromised under PO2extra conditions not calculated to induce any intracellular anoxic core, Eq. 2 could be used to calculate the different DO2 necessary to account for the formation of an anoxic core.

The existence of unstirred layers could also account for a higher than predicted rate-limiting PO2extra and can be calculated from the following equation (9)

\[ R_e + R_t = \left( \frac{\text{K} \cdot \text{P} \cdot \text{O2}}{\text{V˙O2} \cdot \text{R} \cdot \text{P} \cdot \text{O2}} \right) \]  

(3)

where Re and Rt are the radii of the extracellular fluid and muscle fiber, respectively, ΔPO2 is the PO2 gradient, and Ke and Kt are the Krogh coefficients for extracellular fluid (2.9 × 10^{-6} nmol O2 mm^{-3} s^{-1} mmHg^{-1}) and muscle tissue (2.3 × 10^{-6} O2 mm^{-3} s^{-1} mmHg^{-1}), respectively (15, 16).

Statistics. Two-way repeated-measures analysis of variance was used for the statistical analysis in all analyses, the 0.05 level of significance was used.

RESULTS

The PO2 was maintained at 30 ± 2 Torr during the duration of the low-PO2 fatigue run, 76 ± 3 Torr during the intermediate, and 159 Torr during the high.

No significant differences were found in the initial peak tensions (P0) between the high- (2.65 ± 0.6 × 10^{-3} N = 323 ± 22 kPa), intermediate- (2.44 ± 0.7 × 10^{-3} N = 298 ± 27 kPa), and low-PO2 (2.71 ± 0.6 × 10^{-3} N = 331 ± 24 kPa) treatments.

Figure 1 compares the time to fatigue for the low-, intermediate-, and high-PO2 treatments. Fatigue (P0 = 0.75) was reached significantly sooner (P < 0.05) during the low-PO2 treatment (233 ± 39 s) than during both the intermediate- (385 ± 62 s) and the high-PO2 treatment (416 ± 65 s). No significant difference in time to fatigue was found between the intermediate- and high-PO2 treatments. The large SE for each PO2 condition was a result of differences in fatigability among the fibers.

The mean cross-sectional area for these single skeletal muscle fibers was 8 ± 1 × 10^{-3} mm². Fibers were of type I (fast twitch: n = 3) and type II (intermediate: n = 3). With the use of the Hill equation (Eq. 1), DO2 (16) for single amphibian muscle fibers, and previously published mean values for VO2max corresponding to fiber type (10), the mean core PO2intra was 17 ± 1 Torr at a PO2extra of 30 Torr in these maximally contracting muscle fibers. By using Eq. 2, the calculated extracellular PO2critical for these fibers was 13 ± 1 Torr. Because it appears that the level of oxygenation was not rate limiting, yet force production was compromised, Eq. 2 was used to calculate an DO2 in the cell cytoplasm (0.45 ± 0.04 × 10^{-5} cm²/s) that would have been necessary to produce an anoxic core at a PO2extra of 30 Torr. Equation 3 was used to calculate an unstirred

![Fig. 1. Fatigue rates of single fibers (n = 6) subjected to identical stimulation protocols while being exposed to extracellular PO2 of either 159, 76, or 30 Torr. Values are means ± SE. Solid symbols represent mean time to fatigue (force P0 = 0.75) of the maximum tetanic contractile force (P0) for each PO2 treatment. *Significantly faster (P < 0.05) time to fall to 75% of P0 (impaired performance) during 30-Torr extracellular PO2 condition compared with other 2 extracellular PO2 conditions.](image)
layer of medium (242 ± 64 μm), which would account for a rate-limiting PO_{2extra} of 30 Torr.

**DISCUSSION**

These results demonstrated that isolated muscle cells fatigue sooner during high-intensity work at a PO_{2extra} of 30 Torr, compared with either 76 or 159 Torr, even though the calculated intracellular O_{2} availability was always higher than that necessary to inhibit the maximal rate of mitochondrial respiration.

Direct effects of O_{2} limitation. The absence of O_{2} as the terminal electron acceptor in mitochondrial electron transport will halt oxidative phosphorylation, driving the cell to substrate-level phosphorylation (PCr hydrolysis and anaerobic glycolysis). Work can only be maintained transiently once this occurs, and, in this respect, an O_{2} limitation would be directly work inhibiting. Studies using isolated mitochondria have provided evidence that a PO_{2} of 0.5 Torr is necessary to limit isolated mitochondrial oxidative capacity (3). The possibility exists, however, that isolated mitochrondria function differently than when in an intact cellular environment. It has been shown that mitochondria form an interconnected tubular network within the cell (1, 17), and it is possible that the isolation method disrupts this interaction, compromising mitochondrial function (19).

In addition, it is possible that the in vivo PO_{2intra} that is rate limiting for maximal respiration is higher than in isolated mitochondrial preparations. The intact, single skeletal muscle fiber model used in the present investigation provides a cellular system composed of an intact intracellular mitochondrial matrix surrounded by an extracellular medium with an O_{2} tension homogeneous and easily quantifiable. Because these fibers lack myoglobin, O_{2} diffusion into the cell can be accurately calculated during the duration of the experiments.

In single fibers, the level of extracellular oxygenation necessary to reduce O_{2} tension at the mitochondria to predicted rate-limiting levels (PO_{2critical}) can be calculated from an extension of the Hill equation (Eq. 2).

This calculation is dependent on the VO_{2max} of the cell, the path length of the diffusion of O_{2} into the cell, and a diffusion constant of O_{2} through tissue (D_{O_{2}}). In the Hill model of O_{2} diffusion into muscle, the distribution of O_{2} surrounding the cell is homogenous and diffuses radially inward along the concentration gradient developed from the working mitochondria. When PO_{2critical} is reached during maximal rates of respiration, the supply of O_{2} to mitochondria is reduced to a rate-limiting level and the phosphorylation of ADP cannot keep pace with the demand of the ATPases, thus compromising cell performance. By using an O_{2} diffusion constant calculated by Mahler et al. \((D_{O_{2}} = 1.011 \times 10^{-5} \text{ cm}^2/\text{s})\) (16) and published values of VO_{2max} of single fibers similar to those used in the present study (10), the mean calculated value for PO_{2extra} that would be necessary to develop an anoxic core within the maximally working single Xenopus fibers in the present study was 13 Torr. The results of this study indicate that force production became limited at a significantly higher PO_{2extra}.

If the reduction in force were due to an insufficient O_{2} supply, the following explanations for a higher than predicted rate-limiting PO_{2extra} would be possible: 1) the DO_{2} in cytoplasm is inaccurate; 2) even in a well-stirred or well-perfused system an unstirred layer exists in the surrounding medium adjacent to the cell; 3) the PO_{2intra} that inhibits the maximal rate of mitochondrial respiration in vivo is significantly higher than that in isolated mitochondrial preparations; and 4) performance of the cell becomes limited before any true anoxic core develops.

The diffusion coefficient presented by Mahler et al. (16) has been considered the most practical for single skeletal muscle fiber diffusion considerations. It was developed from studies using frog tissue at temperatures similar to our preparation. If we assume that there is a definite, direct O_{2} limitation at a PO_{2extra} of 30 Torr in the present study, and that the maximal rate of mitochondrial respiration was similar to published values, it is possible to extrapolate a diffusion coefficient by using Eq. 2. The diffusion coefficient calculated is \(0.45 \times 10^{-5} \text{ cm}^2/\text{s}\), which is a value significantly lower than related published values (see Ref. 2) and thereby unlikely.

It is apparent from the Hill equation (Eq. 1) that a strong relationship exists between the diffusion rate and the path length for O_{2} diffusion into the cell. A higher rate-limiting PO_{2extra} than predicted could be accounted for by the magnitude of unstirred layers of medium surrounding the cell. The effect of an unstirred layer would be an increase in the path length for O_{2} diffusion. Equation 3 was used to calculate the unstirred layer necessary to preserve Mahler’s diffusion coefficient and induce an anoxic core under the experimental conditions of the present study. This calculated, unstirred layer value would be 243 μm, significantly larger than what is presently believed to be the thickness of the unstirred layer surrounding a cell (2) and quite unlikely, considering that these cells were constantly perfused and contracting.

Another possibility is that the O_{2}-dependent initiation of an earlier fatigue development at a PO_{2extra} of 30 Torr may have been the result of mitochondrial respiration inhibition at a higher PO_{2} than that found in isolated mitochondrial models. At a PO_{2extra} of 30 Torr, the calculated PO_{2} at mitochondria in the center of the cell was 17 Torr, well above the rate-limiting PO_{2} of 0.5 Torr in isolated in vitro mitochondrial models (3). In support of a higher inhibiting PO_{2} in in vivo mitochondrial preparations than in isolated mitochondrial models, it was previously shown (10) that VO_{2max} of Xenopus single fibers began to be significantly reduced at a PO_{2extra} of 90 Torr. At this time, whether the rate-limiting PO_{2} for the maximum mitochondrial respiration in vivo is different than that in isolated mitochondrial preparations is unknown.

The final possible explanation for the attenuation of force by an O_{2} level higher than PO_{2critical} is that an anoxic core failed to develop and that the cell was not compromised directly by O_{2} availability. In this case, an alternate cellular mechanism resulting from the re-
duced PO_{extra} might have been initiated, independent of any rate limitation of oxidative phosphorylation.

Indirect effects of reduced O_2 availability. The amount of O_2 utilized by the cell is described by Fick’s law

\[ \dot{V}_O_2 = D_O_2 (P_O_2^{extra} - P_O_2^{intra}) \]

which demonstrates the relationship between \( \dot{V}_O_2 \), the diffusive capacity of O_2 (D_O_2), and P_O_2^{intra} and P_O_2^{extra}. Reducing P_O_2^{intra} provides the only means to increase the O_2 flux into the cell, as P_O_2^{extra} and D_O_2 remain constant. Rumsey et al. (18) and Wilson et al. (23) have demonstrated, using isolated mitochondria and single-cell models, that a wide range of O_2 values can influence the metabolic state of the cell. Our laboratory has shown previously in isolated whole muscle that the concentration of some intracellular metabolites (H^+, P_i, PCr, and lactate) can be altered by O_2 availability, even when the rate of respiration is not altered (8, 12). In addition, our laboratory has shown that, in humans, inspiring a reduced fraction of inspired O_2 during exercise results in an alteration of PCr hydrolysis and intracellular H^+ concentration at steady-state levels of V_O_2 (6, 13). This intracellular adjustment was accompanied by an earlier onset of fatigue (13), which we have postulated was a result of mechanisms induced by a lowered P_O_2^{intra}. There is evidence that low, but not limiting, levels of O_2 as substrate for oxidative phosphorylation may drive the cell toward the glycolytic state (8, 12, 14). The association between increased intracellular metabolites and the development of fatigue has been well established, and it is possible that increases in intracellular metabolites directly inhibit contractility and Ca^{2+} metabolism (see Refs. 5, 22).

This relationship between O_2 levels and intracellular metabolic intermediates offers the possibility of an alternate role in the attenuation of force production. It is possible that the O_2 limitation imposed on the cell in the present study was sufficient to disrupt the intracellular metabolite concentrations, leading to an attenuation of force and an earlier onset of fatigue, yet remain high enough to maintain respiration. If this is correct, it is possible that the reduction in V_O_2^{max} observed previously (10) on reduced O_2 availability was secondary to an attenuation of force production. This reduction in V_O_2^{max} would, therefore, be caused primarily by an intracellular disruption in metabolite concentration, inhibiting contractility and decreasing the ATP demand. Whether the reductions in force production and respiration observed in this and the previous study (10) were a primary effect because of reduced O_2 utilization or secondary due to metabolic inhibition of force remains to be determined.

In summary, the results of this study indicate that a P_O_2^{extra} of 30 Torr is sufficient to induce an earlier onset of fatigue in working, isolated single muscle fibers. This P_O_2^{extra} is significantly above that predicted necessary to produce an intracellular O_2 tension low enough to be rate limiting to mitochondria, even at the highest rates of respiration. This suggests that, in working single skeletal muscle fibers, force generation may be affected by a P_O_2^{intra} above that which limits mitochondrial respiration, possibly mediated through secondary effects of [O_2] on other cellular processes.

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