Opposite effects of hyperoxia on mitochondrial and contractile efficiency in human quadriceps muscles

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Submitted 10 November 2014; accepted in final form 10 February 2015

Layec G, Bringard A, Le Fur Y, Micallef J, Vilmen C, Perrey S, Cozzone PJ, Bendahan D. Opposite effects of hyperoxia on mitochondrial and contractile efficiency in human quadriceps muscles. Am J Physiol Regul Integr Comp Physiol 308: R724–R733, 2015. First published February 18, 2015; doi:10.1152/ajpregu.00461.2014.—Exercise efficiency is an important determinant of exercise capacity. However, little is known about the physiological factors that can modulate muscle efficiency during exercise. We examined whether improved O2 availability would 1) impair mitochondrial efficiency and shift the energy production toward aerobic ATP synthesis and 2) reduce the ATP cost of dynamic contraction owing to an improved neuromuscular efficiency, such that 3) whole body O2 cost would remain unchanged. We used 31P-magnetic resonance spectroscopy, surface electromyography, and pulmonary O2 consumption (V˙O2p) measurements in eight active subjects during 6 min of dynamic knee-extension exercise under different fractions of inspired O2 (FIO2, 0.21 in normoxia and 1.0 in hyperoxia). V˙O2p (755 ± 111 ml/min in normoxia and 799 ± 188 ml/min in hyperoxia, P > 0.05) and O2 cost (P > 0.05) were not significantly different between normoxia and hyperoxia. In contrast, the total ATP synthesis rate and the ATP cost of dynamic contraction were significantly lower in hyperoxia than normoxia (P < 0.05). As a result, the ratio of the rate of oxidative ATP synthesis from the quadriceps to VO2p was lower in hyperoxia than normoxia but did not reach statistical significance (16 ± 3 mM/ml in normoxia and 12 ± 5 mM/ml in hyperoxia, P = 0.07). Together, these findings reveal dynamic and independent regulations of mitochondrial and contractile efficiency as a consequence of O2 availability in young active individuals. Furthermore, muscle efficiency appears to be already optimized in normoxia and is unlikely to contribute to the well-established improvement in exercise capacity induced by hyperoxia.

31P-magnetic resonance spectroscopy; mitochondria; muscle efficiency; muscle energetics; O2 availability

EXERCISE EFFICIENCY, defined as the ratio of mechanical work performed to energy expended (65), plays a key role in exercise tolerance, since even a small improvement in efficiency brings about major increases in exercise capacity (22). Conversely, lower muscle efficiency significantly contributes to the reduced mobility and exercise intolerance associated with some debilitating disorders (30, 43, 51, 67). Conceptually, muscle efficiency is determined to a similar extent by mitochondrial (conversion of chemical energy to ATP) and contractile (conversion of ATP to mechanical work, i.e., the cost of muscle contraction) efficiency (65). While the latter is commonly quantified using 31P-magnetic resonance (MR) spectroscopy (MRS) (26), the measurement of mitochondrial efficiency in vivo is technically more challenging, as it requires the simultaneous measurement of O2 consumption (V˙O2) and ATP production. Consequently, it has actually been assessed very rarely in humans and only in resting muscle (1, 46). Interestingly, we recently developed an experimental setup allowing the simultaneous measurement of pulmonary VO2 (V˙O2p) (7) and oxidative ATP synthesis rate during knee-extension exercise (38) that can shed some light on mitochondrial efficiency in exercising muscle.

Several physiological factors (e.g., fiber type, substrates, and uncoupling protein content) can affect exercise efficiency (5, 11, 44). Among these factors, O2 availability is often advocated to play an important role in modulating muscle efficiency. In isolated mitochondria, it has been consistently demonstrated that the phosphate-to-oxygen (P/O) ratio, i.e., mitochondrial efficiency, is inversely related to the level of O2 surrounding the mitochondria (12, 54). In other words, increased O2 availability appears to be associated with diminished mitochondrial efficiency. Interestingly, these in vitro results are consistent with the finding that breathing a hyperoxic gas mixture [inspired O2 fraction (FIO2) = 0.3] during cycling exercise increases whole body O2 cost (V˙O2/power output), i.e., decreases exercise efficiency, compared with normoxia (47). However, conflicting results have been reported regarding the interaction between O2 availability and muscle efficiency in humans. For instance, other studies have reported constant O2 cost during cycling exercise with FIO2 = 0.12–1.0 (3, 34, 37, 63, 66) and even a decreased exercise efficiency during cycling exercise in hypoxia (54).

The major caveat to most of the above-mentioned studies is the exclusive assessment of aerobic energy production using gas exchange measurements, from the lung or the systemic circulation, to assess muscle efficiency, thereby ignoring potential changes in anaerobic energy contribution [glycolysis and phosphocreatine (PCr)] to meet muscle ATP demand. Yet changes in these pathways have been largely acknowledged to occur when O2 availability is altered (14, 15, 18, 19, 42) and, therefore, influence estimation of the total energy expenditure.

The purpose of the present study was to examine the effects of O2 availability on exercise efficiency, specifically mitochondrial...
drial and contractile efficiency, as well as the interplay between the energy pathways of ATP synthesis. Using an integrative approach combining 31P-MRS, surface electromyography (EMG), and VO2p measurements, we examined whether, compared with normoxia (FIO2 = 0.21), breathing hyperoxic gas (FiO2 = 1.0) would affect pulmonary O2 cost, the ATP cost of dynamic contraction, and the ATP synthesis rates through oxidative phosphorylation, glycolysis, and creatine kinase (CK) in the knee-extensor muscles of young active subjects. We hypothesized that an increased O2 availability 1) would impair mitochondrial efficiency and shift the energy production toward aerobic ATP synthesis, which 2) would be concomitant to a reduction in the ATP cost of dynamic contraction, such that 3) whole body O2 cost would remain unchanged.

**METHODS**

Subjects

After providing informed consent, eight active subjects [1 woman and 7 men; mean ± SD: 65 ± 11 kg body wt, 174 ± 6 cm height, 32 ± 5 yr of age, 59 ± 15 ml·min⁻¹·kg⁻¹ maximal VO2 (VO2max)] participated in the study. All experimental procedures were undertaken with the approval of the Ethics Committee of Timone University Hospital (Marseille, France). In the 24-h period before all visits, subjects abstained from intense physical activity and caffeine and alcohol consumption.

Experimental Protocol

Cardiopulmonary assessment. Subjects initially performed an incremental cycling exercise test to exhaustion to determine VO2max. Throughout this exercise trial, expired gases were collected breath-by-breath (ZAN 680, ZAN Messgeräte, Oberschüla, Germany). Baseline exercise intensity was 60 W, and after a 3-min warm-up period, intensity was increased by 30 W every minute until volitional exhaustion, which was evaluated in terms of maximal heart rate (≥90% age-predicted (52)), respiratory exchange ratio (≥1.15), and VO2 plateau occurrence. The pedaling rate was kept constant at 70 rpm throughout the exercise period. VO2max was averaged over the last 30 s of exercise.

Metabolic assessment. After a familiarization session, voluntary maximum isometric force [maximal voluntary contraction (MVC)] was determined during three repeated isometric leg extensions of each leg using a dedicated dynamometric ergometer (40). Subjects lay prone with the knee flexed at ~40°. Each contraction was maintained for 3 s and was repeated after a resting period of ~30 s. The MVC was defined as the average of the three peak measurements. If the contraction intensity was ±10% different from the previous contraction, subjects were asked to complete an additional contraction. Leg weight, which was determined as the weight measured when the leg was attached to the ergometer, was subtracted from the MVC measurement.

On separate days, subjects performed constant-load submaximal double-leg alternate knee extension-flexion at ~25% of MVC (rate of 1 Hz) in normoxia (FiO2 = 0.21) or hyperoxia (FiO2 = 1.0) in the Vision Plus whole body 1.5-T MRI system (Siemens, Erlangen, Germany), as previously described (40). Specifically, after 3 min of rest, subjects exercised for 6 min and then were allowed to recover for 6 min. Repeated leg extensions were gated to the MR recordings using an audible signal, so that MR spectra were always recorded at the same time of the contraction-relaxation process. The sequence of the two conditions was balanced to minimize any potential ordering effects. Each trial was separated by ≥2 days.

31P-MRS

31P-MRS data were recorded with a dual 31P-1H surface coil with linear polarization (Rapid Biomedical, Rimpar, Germany) positioned under the right quadriceps muscle. The 140-mm-diameter 31P single-loop coil was surrounded by a 270-mm 1H coil loop. An automatic localized map-shimming procedure was initially performed. Then a set of multislice fast proton MR images was acquired to determine the position of the leg with respect to the surface coil. Before each experiment, three relaxed spectra were acquired at rest, with four averages per spectrum and a repetition time (TR) of 10 s. Then MRS data were acquired throughout the rest-exercise-recovery protocol using a free-induction-decay sequence with 500-μs radio-frequency hard pulse and the following parameters: 2-s TR, 32-kHz sweep width, 4,096 data points, and 4 averages per spectrum. We quantified saturation factors from the comparison of relaxed (TR = 10 s) and partially relaxed (TR = 2 s) spectra.

As previously described (40), relative concentrations of PCr ([PCr]), inorganic phosphate ([Pi]), and ATP ([ATP]) were obtained by a time-domain fitting routine using the AMARES algorithm (61) incorporated into the CSIAP software (41). Intracellular pH was calculated from the chemical shift difference between the P, and PCr signals. The free cytosolic ADP concentration ([ADP]) was calculated from [PCr] and pH using the CK equilibrium constant (KCK = 1.66 × 10⁹ M⁻¹) and assuming that PCr represents 85% of the total creatine content (27). The resting concentrations were calculated from the average peak areas of the relaxed spectra recorded at rest and with the assumption that [ATP] was 8.2 mM. The cytoplasmic free energy of ATP hydrolysis (ΔG ATP) was calculated using the following equation and constants according to Kemp et al. (27)

\[ \Delta G_{ATP} = \Delta G_{ATP}^0 + RT \ln \left( \frac{[ADP]}{[Pi]} \right) \]

\[ \Delta G_{ATP}^0 = RT \left( \ln \left( \frac{f_{ATP}}{f_{ADP} \cdot f_{Pi} \cdot f_{H^+}} \right) \right) \]

where K^PCr = 0.722 (13), R = 8.3145 K⁻¹·M⁻¹·mol⁻¹, T = 310 K, and f terms can be derived for any assumed set of ionic dissociations for each reactant.

Changes in pH and in the concentration of phosphorus metabolites during contraction and recovery phases were used to calculate the ATP cost of dynamic contraction, as previously described (4, 31). The calculations were performed for each 8-s spectrum and then averaged over the last minute of the exercise for comparison with VO2 and EMG measurements. Total ATP synthesis generated from aerobic and anaerobic pathways was scaled to the power output (in W) from the leg investigated by the MR coil. Methodological considerations addressing the validity and the reproducibility of the calculation of ATP synthesis rates have been previously addressed by us and others (25, 29, 31, 33, 38, 39). The ratio of the rate of oxidative ATP synthesis to VO2max was also calculated as an index of mitochondrial efficiency (ATP/VO2).

Model variables for total proton disappearance and PCR offset kinetics were determined with an iterative process by minimizing the sum of squared residuals between the monoexponential fitted function and the observed values. Goodness of fit was assessed by visual inspection of the residual plot and the frequency plot distribution of the residuals, χ² values, and the coefficient of determination (r²) calculated as follows (45)

\[ r^2 = 1 - \frac{(SS_{res}/SS_{tot})}{SS_{res}} \]

where SS_{res} is the sum of squares of the residuals from the fit and SS_{tot} is the sum of squares of the residuals from the mean.
Measurements of $V_{O_{2p}}$

$V_{O_{2p}}$ during exercise was measured on a breath-by-breath basis (ZAN 680). Subjects breathed through a low-dead-space (40 ml) low-resistance mouthpiece-and-flow sensor assembly. Gases were continuously drawn from the mouthpiece through a 6.5-m capillary line (2 mm diameter), and analyzed for O$_2$ and CO$_2$ concentrations by electrochemical and infrared absorption analyzers, respectively. In a preliminary set of experiments, we checked successfully that the synchronization between flow and gas concentration measurements during moderate-intensity knee-extension exercise was not affected by the length of the sampling line (7). Expiratory volume was determined by a flow sensor calibrated before each test using a known-volume (1-liter) syringe (ZAN 680). Gas analyzers were calibrated before each test from the ambient air (20.93% O$_2$-0.03% CO$_2$) and a gas mixture of known composition (16% O$_2$-5% CO$_2$ in normoxia and 95% O$_2$-5% CO$_2$ in hyperoxia). $V_{O_{2p}}$ was determined inside the MRI system simultaneously with the MR measurements, as previously described (7). Given the methodological difficulties associated with the direct measurements of $V_{O_{2p}}$ from O$_2$ analyzers during hyperoxic gas breathing, we have chosen to estimate $V_{O_{2p}}$ according to the following equation previously validated under hyperoxic conditions (64)

$$V_{O_{2p}} = V_{insp} - V_{exp} + V_{CO_{2}}$$

where $V_{insp}$ and $V_{exp}$ indicate inspired and expired volumes and $V_{CO_{2}}$ indicates CO$_2$ production. This equation only relies on the accuracy of volume measurements and $V_{CO_{2}}$, which have been demonstrated to be unaffected by hyperoxia (64). For technical reasons, the data collected in one subject were not included in the analysis.

Surface EMG Measurements

Bipolar (circular, 20 mm center-to-center) Ag/AgCl surface electrodes (product no. 2700-030, Commed, Utica, NY) were used to measure EMG changes in the left vastus lateralis muscle. As recommended by SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles), the electrodes were placed on the muscle belly, over the lateral portion of the muscle (16). To minimize interelectrode resistance, the skin surface was shaved and thoroughly cleaned with alcohol. The reference electrode was secured over the tibia head. The EMG cable was strapped to the leg and wrapped to minimize disruption during leg movements. The EMG signal was preamplified (gain 1,000) and filtered (20–500 Hz) using a custom-made device (common mode rejection ratio = 85 db). The analog signal was sampled at a rate of 1,000 Hz, and the digital signal was stored on a computer disk for later analysis. The EMG signal was analyzed using commercially available software (WinATS version 6.5, Sysma). The integrated EMG (iEMG), a global measure of muscle activity, was calculated for each contraction from the raw EMG signal. After it was rectified, the EMG signal was integrated over the entire duration of the contraction as measured by the potentiometer from the displacement of the weight. Then iEMG was normalized with respect to power output as an index of the neuromuscular efficiency (55).

Statistics Analyses

Differences between normoxic and hyperoxic conditions were assessed with two-tailed paired $t$-tests or nonparametric Wilcoxon tests, where appropriate (Statistica version 5.5, Statsoft, Tulsa, OK). In addition, effect size ($d$) statistics were calculated using a pooled standard deviation of normoxic and hyperoxic conditions. Statistical significance was accepted at $P < 0.05$. Values are means ± SD in Tables 1 and 2 and means ± SE in Figs. 1–4 for the sake of clarity.
High-Energy Phosphate Compounds and Intracellular pH

Table 1 summarizes intracellular metabolite concentrations and pH at rest and during the last minute of the dynamic contractions in normoxia and hyperoxia. The group mean changes in PCr, pH, P, and ATP during these two exercise conditions are illustrated in Fig. 3. Specifically, there was no significant difference between normoxia and hyperoxic conditions for all the variables.

Rates of ATP Synthesis During Exercise

The rates of ATP synthesis from glycolysis, the CK reaction, and oxidative phosphorylation during the last minute of the knee-extension exercises are presented in Table 2. The relative contribution from oxidative phosphorylation to total ATP synthesis was not significantly different between conditions (61 ± 7% in normoxia and 63 ± 6% in hyperoxia, P = 0.31, d = 0.49). At the end of exercise, the total ATP synthesis rate and the ATP cost of dynamic contraction (P = 0.042, d = 1.1) were significantly lower in hyperoxia than normoxia (Fig. 1).

\( \dot{V}_{O_2p} \) During Exercise

\( \dot{V}_{O_2p} \) (755 ± 111 ml/min in normoxia and 799 ± 188 ml/min in hyperoxia, P = 0.64, d = 0.29; Fig. 4) and the \( \dot{V}_{O_2} \) level of \( \dot{V}_{O_2p} \) during exercise were not significantly different between normoxia and hyperoxia (Fig. 1).

Using an integrative approach combining \( ^3P-MRS \), surface EMG, and \( \dot{V}_{O_2p} \) measurements, we sought to examine in the knee extensor muscles of young active subjects whether increased \( O_2 \) availability 1) modulates mitochondrial efficiency and the cost of dynamic muscle contraction and 2) modifies the relative contribution from the main energy-generating pathways to ATP production. Consistent with our hypotheses, the decreased cost of dynamic muscle contraction in the quadriceps muscles in hyperoxia was likely concomitant to a reduction in mitochondrial efficiency, such that the whole body \( O_2 \) cost was not different between conditions. Interestingly, and contrary to our hypothesis, the improved \( O_2 \) availability did not affect the interplay between the main energy pathways generating ATP (oxidative phosphorylation, glycogen, and CK reaction) during low-intensity dynamic knee extension. Together, these findings reveal dynamic and independent regulations of mitochondrial and contractile efficiency as a consequence of \( O_2 \) availability in young active individuals.

Evidence of a Decreased Cost of Muscle Contraction in Hyperoxia

A major and novel finding of this study is the documentation of a substantial reduction in the ATP cost of contraction during dynamic knee-extension exercise in hyperoxia compared with normoxia (Fig. 1). Interestingly, the <25% reduction in total ATP synthesis in hyperoxia was accompanied by an unaltered level of \( \Delta G_{ATP} \) and a <40% lower iEMG/power output ratio (Fig. 2). Together, these findings imply that the hyperoxia-induced reduction in the ATP cost of dynamic contraction likely stemmed from a decrease in the ATP demand, rather than a more favorable exergonic potential for ATP hydrolysis. In other words, the energy released in skeletal muscle during the hydrolysis of ATP was unaffected by \( O_2 \) availability, thereby indicating that the change in the ATP cost of dynamic contraction was, instead, driven by a lower energy demand from contractile and/or noncontractile processes. In this regard, it is noteworthy that the iEMG/power output ratio was substantially lower in hyperoxia, suggestive of improved neuromuscular efficiency. Interestingly, hyperoxia has recently been reported to facilitate muscle membrane excitability (6). Therefore, one could speculate that the greater membrane excitability in hyperoxia may have decreased the metabolic demand associated with noncontractile processes of ion transport, such as \( Na^-\text{-}K^+\text{-}ATPase. \) Considering that up to 50% of the total metabolic demand can be attributed to noncontractile processes (2, 59), this mechanism could account, at least partly, for the reduced ATP cost of dynamic contraction observed here. Further studies are therefore warranted to determine the relative contribution from cross-bridge cycling and ion pumping to the decreased metabolic demand in hyperoxia.

### Table 1. Metabolite concentrations at rest and during dynamic contractions of knee extensors in normoxia and hyperoxia

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hyperoxia</th>
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<tr>
<td><strong>Rest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr, mM</td>
<td>34 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>P, mM</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>ADP, μM</td>
<td>9.2 ± 0.4</td>
<td>9.1 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.02 ± 0.02</td>
<td>7.02 ± 0.04</td>
</tr>
<tr>
<td>( \Delta G_{ATP} ), kJ/mol</td>
<td>-61 ± 1</td>
<td>-61 ± 1</td>
</tr>
<tr>
<td><strong>Last minute of exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr, mM</td>
<td>22 ± 3</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>P, mM</td>
<td>10 ± 3</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>ADP, μM</td>
<td>38 ± 11</td>
<td>37 ± 12</td>
</tr>
<tr>
<td>pH</td>
<td>6.98 ± 0.04</td>
<td>6.97 ± 0.04</td>
</tr>
<tr>
<td>( \Delta G_{ATP} ), kJ/mol</td>
<td>-58 ± 1</td>
<td>-58 ± 1</td>
</tr>
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Values are means ± SD. PCr, phosphocreatine; \( \Delta G_{ATP} \), cytoplasmic free energy of ATP hydrolysis.
The substantial decrease in the iEMG/power output ratio in hyperoxia compared with normoxia is suggestive of a reduced muscle activity. It has previously been documented that muscle activation of the quadriceps assessed by iEMG was inversely related to the level of O2 availability during cycling exercise (60). However, the mechanism whereby hyperoxia would diminish the muscle activation for a given power output and lower the ATP demand remains unclear.

Acute exposure to a high level of O2 is well recognized to increase the level of reactive oxygen species throughout the body (20). Interestingly, the redox balance plays an important role in modulating the muscle force-generating capacity (49). According to the biphasic model depicting the effects of cellular redox state on skeletal muscle contractile properties (49), it can be speculated that modulation of the skeletal muscle redox balance in hyperoxia during repeated contractions of the knee extensor might have positioned this muscle in a more favorable redox state, thereby improving the force-generating capacity per ATP and decreasing the cost of muscle contraction. However, this hypothesis does not appear to be supported by experimental evidence from human studies. Indeed, it has been previously documented that the contractile properties of the muscle fibers, as measured by the maximal rate of force development and the maximal twitch force electrically evoked, are independent of O2 availability at rest (24, 58). This would suggest that the lower recruitment of muscle fibers in the quadriceps during hyperoxia cannot be attributed to a greater force-generating capacity per ATP. However, in these studies (24, 58), the contractile properties were assessed at rest, and results from these studies might differ from results from the present study, where repeated contractions occur. Further studies are therefore warranted to elucidate the effects of hyperoxia on skeletal muscle contractile properties during dynamic exercise.

Evidence of Unchanged Whole Body Exercise Efficiency and Reduced Mitochondrial Efficiency in Hyperoxia

Another important observation from the present study is the constant whole body O2 cost during knee-extension exercise with different FIO2 levels (Fig. 4). Consistent with this finding, several studies reported unchanged pulmonary O2 cost during cycling exercise with FIO2 = 0.12–1.0 (3, 37, 66). Further supporting these results, using the direct Fick method, Knight et al. (34) documented similar slopes in the relationship between leg VO2 and power output in the first stages of an incremental cycling exercise in conditions ranging from hypoxia (FIO2 = 0.12) to hyperoxia (FIO2 = 1.0). Similarly, leg VO2 in hyperoxia (FIO2 = 1.0) was akin to normoxia during constant-load submaximal cycling exercise (63). Together, these results suggest that whole body O2 cost appears to be independent of O2 availability. However, inconsistent results have been reported on this topic. For instance, gas exchange measurements from the lung have shown impaired whole body exercise efficiency in moderate hyperoxia (FIO2 = 0.30) and hypoxia (FIO2 = 0.16) (47). The discrepancy between these studies and the aforementioned results are unclear. However, these two recent studies were performed using moderate alter-

Table 2. ATP synthesis rates at the end of knee-extension exercise in normoxia and hyperoxia

<table>
<thead>
<tr>
<th>ATP synthesis, mM/min</th>
<th>Normoxia</th>
<th>Hyperoxia</th>
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<tr>
<td>Glycolysis</td>
<td>5.8 ± 2.0</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>1.9 ± 0.9</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>11.8 ± 2.2</td>
<td>10.1 ± 2.4*</td>
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Values are means ± SD. *P < 0.05 vs. normoxia (by Wilcoxon’s test).
O2 availability in severe hypoxia (12) or within physiological conditions (Po2 = 2–75 mmHg) (54). Therefore, the present results extend these findings by revealing that increased tissue O2 availability induced by acute exposure to hyperoxia appears to modulate mitochondrial efficiency in vivo in the knee extensor of active individuals. The relative excess capacity of the electron transport chain compared with the maximum phosphorylation rate of ATP synthase, i.e., mitochondrial complex V (35), might account for the decline in efficiency with higher O2 pressure (Po2). Indeed, at high Po2, cytochrome-c oxidase capacity (mitochondrial complex IV) largely outstrips maximal phosphorylation flux from ATP synthase, resulting in occasional slipping of the proton pumps in the respiratory chain, which decreases the H+/e− stoichiometry of the proton pumps (23) and lessens mitochondrial efficiency for ATP production. This interpretation is further supported by the fact that partial inhibition of cytochrome-c oxidase by nitric oxide, which curtails slipping of the proton pumps, increases mitochondrial efficiency in isolated mitochondria (8).

As for the cost of muscle contraction, hyperoxia-induced change in skeletal muscle redox balance (20) is another possible mechanism by which mitochondrial efficiency can be affected. Indeed, there is now a better appreciation for a subtle role for oxidative stress in regulating adaptive responses of mitochondria to a physiological stress (i.e., redox signaling) and the existence of a dynamic interaction between the cell environment and mitochondria (56, 57). Accordingly, in the absence of direct measurement of the redox state, it cannot be ruled out that the impairment of mitochondrial efficiency in hyperoxia, as suggested by our data, was the consequence of a prooxidizing shift in the redox balance of the skeletal muscle cells.

**Effect of Hyperoxia on Energy Pathways**

It has been previously documented that breathing hyperoxic gas was associated with a reduced lactate accumulation and PCr breakdown under hyperoxic conditions (15, 18, 19, 42). Also, it has been proposed that altering FIO2 can modulate the absolute work rate at which the lactate threshold is reached during an incremental exercise (62). Lending support to this suggestion, Haseler et al. (14) reported that an FIO2 of 1.0 ablated the slow component of PCr consumption, which was attributed to a reduction in the relative exercise intensity. Although we observed a trend toward a reduction in glycolytic flux (Table 2; P = 0.10), which is somewhat in agreement with these previous findings, the relative contribution from the three main pathways of ATP synthesis (oxidative phosphorylation, glycolysis, and CK reaction) was not significantly different between conditions here. However, in the current study, restriction to low-intensity exercise, as suggested by the limited changes in pH (end-exercise ∼6.97) and PCr consumption (30–35%), may have hindered our ability to observe relatively subtle changes in the interplay between the energy pathways. Further studies using higher exercise intensities are therefore warranted to address this issue.

**Methodological Consideration**

We acknowledge that this preliminary investigation is limited by the small sample size and that care should be taken in extrapolating the current findings. Further studies with a larger
sample size are therefore warranted to confirm that changes in neuromuscular and mitochondrial efficiencies are a common response to hyperoxia.

Despite the use of a relatively standardized (fixed-weight) exercise (40), it is interesting to note that a small, but significant, increase in mean power output resulted from an augmented displacement of the weight throughout the exercise. Indeed, we previously documented that our setup exhibits an inherent variability (~15%) in the power output produced because of the subjects’ difficulty with fine control of the range of motion throughout a 6-min exercise (39). One can argue that this power output change may confound the results of the present study. However, given that all the main outcome variables were scaled to the power output or to the total ATP synthesis rates, it is unlikely that this factor interfered with interpretation of the present findings. In addition, the changes in power output were relatively minimal and, therefore, would not lead to a substantial alteration in muscle recruitment pattern, which, if anything, would increase, not decrease, the energy cost in hyperoxia owing to the recruitment of less efficient type II fibers.

The measurement of mitochondrial efficiency necessitates the simultaneous measurement of VO2 and ATP synthesis and, therefore, represents a technical challenge in vivo that has been undertaken by only a few groups in humans (1, 46). In the present study the ratio of the local rate of oxidative ATP synthesis to VO2p was employed as an index of mitochondrial efficiency. Although we acknowledge the limitation of such an approach, most notably the comparison between a systemic measurement of VO2 and a local assessment of mitochondrial phosphorylation, the validity of this method appears sound in the present experimental conditions. Indeed, it has been demonstrated that, for similar exercise conditions, VO2p primarily reflected muscle VO2 from the active muscle (53). Also, given that hyperoxia has been associated with diminished ventilation and heart rate (63), one could have expected a reduced systemic VO2 due to respiratory or cardiac muscles in the present experimental condition. Interestingly, VO2p and O2 cost were similar for both conditions, whereas we quantified a reduced oxidative ATP synthesis in hyperoxia. Together, these findings suggest that the nonsignificant 23% reduction in ATP/VO2 observed in hyperoxia likely underestimated the actual change in mitochondrial efficiency, which further supports our conclusion that mitochondrial efficiency was diminished in hyperoxia. Accordingly, from the combined 31P-MRS and gas exchange measurements, it was estimated that the P/O ratio decreased from ~2.0 in normoxia to ~1.58 in hyperoxia using the following assumptions: 1 mol of O2 = 25.5 liter and quadriceps muscle mass = 2.292 liters (48).

Although quite large (270 mm), the dimension of the 1H coil was, rather, an advantage, in that it enabled us to perform the shimming procedure over a large field of view and to ensure a correct and reproducible position of the leg over the two conditions. Regarding the 31P coil, one should keep in mind the selectivity and the inhomogeneous sampling volume of a surface coil, which samples the MR signal over a muscle volume proportional to the surface coil radius, making this signal the weighted average of the muscle fibers within the sampling volume (~7 cm deep). It is also important to note that the sensitivity of the reception probe is greater in the region close to the coil, i.e., in the vastus (lateralis and medialis) and rectus femoris, the muscles predominantly recruited during knee-extension exercise (48, 50). Therefore, in the present study, care was taken to sample exercising muscles, and the MR signal recorded with the present experimental setup was not affected by potential extraneous sources.

**Perspective and Significance**

The effect of breathing a hyperoxic gas mixture with varied FIO2, on muscle mitochondrial respiration, metabolism, and fatigue during exercise has been extensively studied. However, the present study emphasizes the additional role of O2 availability on ATP demand during exercise by altering the cost of dynamic muscle contraction and, possibly, mitochondrial efficiency. Such findings expand our understanding of the interplay between muscle metabolism, efficiency, and performance under different environmental challenges. The results documented here have potential implications for future studies designed to better understand normal and pathological muscle function when O2 availability is altered.

**Conclusion**

In summary, we have demonstrated in the present study that increased O2 availability was associated with a decreased ATP cost of muscle dynamic contraction and a concomitant reduction in mitochondrial efficiency during low-intensity knee-extension exercise. As a result of these opposed effects on the main components of muscle efficiency, the whole body O2 cost of these young healthy active subjects was independent of O2 availability. Together, these findings reveal dynamic and independent regulations of mitochondrial and contractile efficiency as a consequence of altered O2 availability in young active individuals. In addition, muscle efficiency appears to be optimally regulated in normoxia, such that any change in tissue O2 availability appears to be detrimental to this process. The present findings also suggest that changes in exercise efficiency do not appear to contribute to the well-documented improvement in exercise capacity induced by hyperoxia.

**APPENDIX**

### ATP production from PCr breakdown (ATPCr).

The rate of ATP production from PCr breakdown through the CK reaction (D, mM/min) was calculated from the change in PCr for each time point of the exercise period (26)

\[
\text{ATPCr} = \frac{d\text{PCr}}{dt} \quad (A1)
\]

### ATP production from oxidative phosphorylation (ATPox).

The rate of oxidative ATP production (ATPox, mM/min) and [ADP], the rate of mitochondrial ATP production was calculated as

\[
\text{ATPox} = \frac{V_{max}}{1 + \left(\frac{K_m}{[\text{ADP}]}\right)^2} \quad (A2)
\]

where K_m ([ADP] at half-maximal oxidation rate) is ~30 μM in mitochondrial function (21), and V_{max} is the inferred peak rate of mitochondrial respiration in vivo.

\[
V_{\text{max}} \quad (\text{mM/min}) \text{ was calculated using the initial rate of PCr synthesis (V_{Pcstr}) during the recovery period, and [ADP] was measured at the end of exercise}
\]

\[
V_{\text{max}} = \frac{V_{\text{Pcstr}}}{1 + \left(\frac{K_m}{[\text{ADP}]_{\text{end}}}\right)^2} \quad (A3)
\]

V_{Pcstr} was calculated from the derivative of Eq. A5 at time 0

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where $\Delta [\text{PCr}]$ represents the amount of PCr resynthesized during the recovery and the rate constant $k$ is $1/r$ (26). The first-order PCr recovery rate constant ($k$) was determined from a fitting of the PCr time-dependent changes during the recovery period to a single-exponential curve described as

$$Y(t) = Y_{\text{end}} + Y_{\text{res}} \left[ 1 - e^{(-T-\gamma)} \right]$$

where $Y_{\text{end}}$ is [PCr] measured at the end of exercise, $Y_{\text{res}}$ is the amount of PCr resynthesized during the recovery, and TD is the time delay before PCr increases.

**ATP production from anaerobic glycolysis (ATP$_{\text{gly}}$).** Throughout the exercise period, glycogen breakdown to pyruvate and lactate, proton efflux, buffering capacity, protons produced by oxidative phosphorylation, and consumption of protons by the CK reaction lead to changes in intramuscular pH (26). With the assumption that the glycolytic production of 1 mol of H$^+$, when coupled to ATP hydrolysis, yields 1.5 mol of ATP, ATP$_{\text{gly}}$ can be deduced from the total number of protons produced throughout exercise (17)

$$P = H_{\text{CK}}^0 + H_{\text{aux}}^0 + H_{\text{efflux}}^0$$

$H_{\text{CK}}^0$ (in mM/min) was calculated from the time-dependent changes in [PCr] and from the stoichiometric coefficient ($\gamma$)

$$H_{\text{CK}}^0 = -\gamma \cdot \text{ATP}_{\text{CK}}$$

where $\gamma$ is the proton stoichiometric coefficient of the coupled Lohmann reaction, as described previously (36).

$H_{\text{aux}}^0$ (in mM/min) was calculated from the apparent buffering capacity $\beta_{\text{total}}$ [in Slykes, i.e., mmol acid added/unit change in intracellular pH (pH$_{\text{i}}$)] and from the rate of pH changes

$$H_{\text{aux}}^0 = -\beta_{\text{total}} \cdot \text{d pH}/\text{d}t$$

where

$$\beta_{\text{total}} = \beta_{\text{nonbicarbonate}} - \beta_{\text{Pi}} + \beta_{\text{PME}} + \beta_{\text{bicarbonate}}$$

where $\beta_{\text{nonbicarbonate}}$ was determined from the initial change in PCr ($\Delta$PCr$_{\text{i}}$) and alkalization of pH ($\Delta$Hi$_{\text{i}}$) (9)

$$\beta_{\text{a}} = \gamma \cdot \text{ATP}_{\text{ox}} \cdot \text{d pH}/\text{d}t$$

P$_{\text{i}}$ and phosphomonoester (PME) buffering capacities ($\beta_{\text{Pi}}$ and $\beta_{\text{PME}}$) were determined on the basis of the dissociation constant of the buffer ($K$) according to the standard formula (10)

$$\beta_{\text{a}} = (2.303 \cdot H^+ \cdot K \cdot X)/K \cdot (H^+)^2$$

where $X$ is P$_{\text{i}}$ or PME and $K = 1.77 \times 10^{-7}$ for P$_{\text{i}}$ and $6.3 \times 10^{-7}$ for PME.

In agreement with previous studies and with the assumption that muscle is a closed system during exercise (10, 28), $\beta_{\text{bicarbonate}}$ was set to zero.

$H_{\text{oxy}}^0$ (in mM/min) was calculated from the factor $m = 0.16/[1 + 10^{-6.1 - \text{pH}}]$, which accounts for the amount of protons produced through oxidative ATP production

$$H_{\text{oxy}}^0 = m \cdot \text{ATP}_{\text{ox}}$$

$H_{\text{efflux}}^0$ (in mM/min) was calculated for each time point of exercise using the proportionality constant $\lambda$, relating proton efflux rate to pH$_{\text{i}}$

$$H_{\text{efflux}}^0 = -\lambda \cdot \text{d pH}/\text{d}t$$

The proportionality constant $\lambda$ (in mM-min$^{-1}$·pH unit$^{-1}$) was calculated during the recovery period

$$\lambda = -V_{\text{eff}}/\text{d pH}/\text{d}t$$

where $V_{\text{eff}}$ is efflux rate. During this period, PCr is regenerated throughout the CK reaction as the consequence of oxidative ATP production in mitochondria. Thus, $H_{\text{oxy}}^0$ can be calculated from the rates of proton production from the CK reaction ($H_{\text{CK}}^0$, in mM/min) and mitochondrial ATP production (H$_{\text{ox}}^0$, in mM/min) on one side and the rate of pH changes on the other side. At this time, ATP production is exclusively aerobic, and lactate production is considered negligible

$$V_{\text{eff}} = \beta_{\text{total}} \cdot \text{d pH}/\text{d}t + \gamma \cdot \text{PCr} + m \cdot \text{ATP}_{\text{ox}}$$

To improve precision, we use a modified version of this calculation (32) in which the total proton disappearance (i.e., $[Edt]$) is estimated cumulatively from the start of recovery and then fitted to an exponential function to obtain the initial recovery rate $E$.

**Total ATPase rate.** The total ATPase rate (ATP$_{\text{ox}}$, in mM/min) was calculated for each time point as

$$\text{ATP}_{\text{tot}} = \text{ATP}_{\text{ox}} + \text{ATP}_{\text{CK}} + \text{ATP}_{\text{gly}}$$

The ATP cost of contraction (in mM-min$^{-1}$·W$^{-1}$) was calculated as the ratio of total ATP production (ATP$_{\text{ox}}$ + ATP$_{\text{CK}}$ + ATP$_{\text{gly}}$) to power output.

**GRANTS**

This work was supported by a PhD fellowship from the Association Française contre les Myopathies and Association pour le Développement des Recherches Biologiques et Médicales.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

G.L., A.B., Y.L.F., J.-P.M., C.V., S.P., P.J.C., and D.B. developed the concept and designed the research; G.L., A.B., Y.L.F., J.-P.M., and D.B. performed the experiments; G.L. and A.B. analyzed the data; G.L. and D.B. interpreted the results of the experiments; G.L. prepared the figures; G.L. drafted the manuscript; G.L., A.B., Y.L.F., J.-P.M., C.V., S.P., P.J.C., and D.B. edited and revised the manuscript; G.L., A.B., Y.L.F., J.-P.M., C.V., S.P., P.J.C., and D.B. approved the final version of the manuscript.

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