Impairment of maximal aerobic power with moderate hypoxia in endurance athletes: do skeletal muscle mitochondria play a role?

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1Service de Physiologie et d’Explorations Fonctionnelles, Hôpital Civil et Département de Physiologie, UPRES E.A. 3072, Faculté de Médecine, Strasbourg, France; 2School of Health and Medical Sciences, Örebro University, Örebro, Sweden; 3UFR STAPS, Université de Strasbourg, Strasbourg, France; 4Service de Cardiologie, Hôpitaux Civils de Colmar, Colmar, France; and 5U-769 INSERM, Faculté de Pharmacie, Châtenay-Malabry, France

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Ponsot E, Dufour SP, Doutreleau S, Lonsdorfer-Wolf E, Lampert E, Piquard F, Geny B, Mettauer B, Ventura-Clapier R, Richard R. Impairment of maximal aerobic power with moderate hypoxia in endurance athletes: do skeletal muscle mitochondria play a role? Am J Physiol Regul Integr Comp Physiol 298: R558 –R566, 2010. First published December 9, 2009; doi:10.1152/ajpregu.00216.2009.—This study investigates the role of central vs. peripheral factors in the limitation of maximal oxygen uptake (V̇O2max) with moderate hypoxia [inspired fraction (FiO2) = 0.145%]. Fifteen endurance-trained athletes performed maximal cycle incremental tests to assess V̇O2max, maximal cardiac output (Q̇max), and maximal arteriovenous oxygen (a-vO2) difference in normoxia and hypoxia. Muscle biopsies of vastus lateralis were taken 1 wk before the cycling tests to evaluate maximal muscle oxidative capacity (V̇O2max) and sensitivity of mitochondrial respiration to ADP (Km) on permeabilized muscle fibers in situ. Those athletes exhibiting the largest reduction of V̇O2max in moderate hypoxia (Severe Loss group: −18 ± 2%) suffered from significant reductions in Q̇max (−4 ± 1%) and maximal a-vO2 difference (−12 ± 2%). Athletes who well tolerated hypoxia, as attested by a significantly smaller drop of V̇O2max with hypoxia (Moderate Loss group: −7 ± 1%), also display a blunted Q̇max (−9 ± 2%) but, conversely, were able to maintain maximal a-vO2 difference (+1 ± 2%). Though V̇O2max was similar in the two experimental groups, the smallest reduction of V̇O2max with moderate hypoxia was observed in those athletes presenting the lowest apparent Km for ADP in the presence of creatine (Km+Cr). In already-trained athletes with high muscular oxidative capacities, the qualitative, rather than quantitative, aspects of the mitochondrial function may constitute a limiting factor to aerobic ATP turnover when exercising at low FiO2, presumably through the functional coupling between the mitochondrial creatine kinase and ATP production. This study suggests a potential role for peripheral factors, including the alteration of cellular homeostasis in active muscles, in determining the tolerance to hypoxia in maximally exercising endurance-trained athletes.

HYPOXIC ENVIRONMENTS HAVE repeatedly been observed to depress maximal oxygen uptake (V̇O2max) during whole body exercise in humans, and the magnitude of this reduction is thought to be proportional to the hypoxia-induced diminution of arterial oxygen content (15). Although this phenomenon is well described, a wide interindividual variability in the magnitude of reduction of V̇O2max at a given level of hypoxia has been reported by several laborato-

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CHONDRIA TO COMMUNICATE WITH THEIR SUBCELLULAR ENVIRONMENT. A LOW SENSITIVITY OF MITOCHONDRIAL RESPIRATION TO CYTOSOLIC ADP AND A HIGH CONTROL OF RESPIRATION BY THE CREATINE KINASE (CK) SHUTTLE SYSTEM, WITH MITOCHONDRIAL CK (mi-CK) AS THE ULTIMATE ELEMENT, ARE NOW CLEARLY ESTABLISHED AS A HALLMARK OF FATIGUE-RESISTANT OXIDATIVE MUSCLES (27, 43). IN THIS CONDITION, MITOCHONDRIAL RESPIRATION IS NO MORE SET BY CYTOSOLIC ADP LEVELS BUT BY THE LOCAL CREATEINE PHOSPHATE/CREATINE (PCr/Cr) RATIOS (45). INCREASING AEROBIC PERFORMANCE IS THEN ASSOCIATED WITH A REORGANIZATION OF THE MUSCLE FIBERS’ CYTOARCHITECTURE, INCLUDING QUANTITATIVE AND QUALITATIVE MITOCHONDRIAL ADAPTATIONS (52, 62, 63). SUCH CHANGES ARE EXPECTED TO LIMIT PERTURBATIONS OF CELLULAR HOMEOSTASIS LIKE THE DECREASE OF THE ATP/ADP RATIO AND TO DELAY THE CONTRIBUTION OF ANAEROBIC GLYCOLYSIS TO ENERGY SUPPLY. WHILE A GREATER HOMEOSTASIS DISTURBANCE HAS BEEN SHOWN TO OCCUR DURING HYPOXIC VS. NORMOXIC EXERCISE (22, 24, 49), LITTLE IS KNOWN ABOUT THE POTENTIAL CONTRIBUTION OF QUANTITATIVE AND QUALITATIVE PROPERTIES OF THE SKELETAL MUSCLE MITOCHONDRIAL FUNCTION IN THE TOLERANCE TO HYPOXIA DURING MAXIMAL EXERCISE (I.E., \( \dot{V}O_2 \text{max} \)) IN ENDURANCE-TRAINED ATHLETES.

USING A CROSS-SECTIONAL DESIGN, THE PRESENT STUDY INVESTIGATES THE ROLE OF MITOCHONDRIAL FUNCTION IN THE HYPOXIA-INDUCED LIMITATION OF \( \dot{V}O_2 \text{max} \) IN A GROUP OF HOMOGENOUS ENDURANCE-TRAINED ATHLETES. WE HYPOTHESIZED THAT THE ATTENUATION OF \( \dot{V}O_2 \text{max} \) WITH MODERATE HYPOXIA IN ENDURANCE-TRAINED ATHLETES IS RELATED TO THEIR ABILITY TO MAINTAIN MAXIMAL A-V\( O_2 \) DIFFERENCE AND IS ASSOCIATED WITH QUANTITATIVE AND QUALITATIVE PROPERTIES OF THEIR SKELETAL MUSCLE FUNCTION.

MATERIAL AND METHODS

Ethical Approval

All subjects gave their written consent to participate in the study and were fully informed about its potential risks. The study conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee for Strasbourg’s Hôpital Civil, in accordance with French law.

Subjects

Fifteen male endurance runners and triathletes (age: 31 ± 2 yr; height: 179 ± 1 cm; weight: 69.8 ± 1.3 kg; \( \dot{V}O_2 \text{max} \): 58.6 ± 1.7 ml·min\(^{-1}\)·kg\(^{-1}\)) completed the study (Table 1). All were highly motivated to participate in the study and engaged in competitive endurance running events for > 5 yr, with the current 10,000 m or equivalent personal best times of < 35:00 (min/s).

Study Design

Basal examination. Two weeks before the experiment, each subject came to the laboratory for anthropometric measurements, physical examination, forearm venous blood sampling, resting electrocardiography, and echocardiography recordings. All results were within normal limits.

Cycling incremental test to exhaustion. The exercise tests were performed in the upright position on an electronically braked cycle ergometer (Medifit 1000S). Incremental tests to exhaustion were performed by each subject to determine the ventilatory thresholds ( VTs) and \( \dot{V}O_2 \text{max} \) in two experimental conditions: 1) at sea level (FIO\(_2\) = 21%) and 2) at simulated altitude (FIO\(_2\) = 14.5%; ~3,000 m). The tests were randomly balanced and separated by 24-h rest.

Skeletal muscle biopsy. Vastus lateralis muscle biopsies were taken by the percutaneous Bergström technique under local anesthesia (lidocaine/lignocaine) within 1 wk prior to the cycling tests. No complications followed biopsies in any subject.

Table 1. Group anthropometric data and performance measures in normoxia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Severe Loss Group</th>
<th>Moderate Loss Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30 ± 2</td>
<td>33 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.9 ± 1.7</td>
<td>70.6 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180 ± 2</td>
<td>178 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>149 ± 5</td>
<td>151 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>44 ± 2</td>
<td>45 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>P(_{\text{peak}}), W</td>
<td>342 ± 14</td>
<td>325 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{max} ): ml·min(^{-1})·kg(^{-1})</td>
<td>61.4 ± 1.7</td>
<td>56.2 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>VT1, % ( \dot{V}O_2 \text{max} )</td>
<td>61.7 ± 3.1</td>
<td>61.4 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>VT2, % ( \dot{V}O_2 \text{max} )</td>
<td>81.4 ± 3.3</td>
<td>86.9 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>( Q_{\text{max}} ) : l/min</td>
<td>27.3 ± 1.1</td>
<td>25.0 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Maximal a-V( O_2 ) difference, ml O(_2) l</td>
<td>156 ± 6</td>
<td>160 ± 6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Severe Loss group, group of subjects with \( \Delta \dot{V}O_2 \text{max} \) superior to 8 ml·min\(^{-1}\)·kg\(^{-1}\); Moderate Loss group, group of subjects with \( \Delta \dot{V}O_2 \text{max} \) inferior to 8 ml·min\(^{-1}\)·kg\(^{-1}\); \( \dot{V}O_2 \text{max} \), maximal oxygen uptake determined in the normoxic test; P\(_{\text{peak}}\), peak pressure; \( Q_{\text{max}} \), maximal cardiac output; a-V\( O_2 \), arteriovenous oxygen; NS, not significant. Ventilatory threshold-1 ( VT1) and VT2 were determined during the normoxic test.

Procedures

Exercise tests. Incremental tests to exhaustion began at 80 W, and the power output was subsequently increased by 40 W every 2 min until exhaustion. Pedal frequency was maintained constant at 60-70 rpm during the test and each subject was encouraged to give a maximum effort.

VTs were assessed using established criteria (7, 48). VT1 corresponds to the breakpoint in the plot of carbon dioxide output ( \( \dot{V}CO_2 \)) as a function of \( \dot{V}O_2 \). At that point, the ventilatory equivalent for O\(_2\) (\( V_{E/O_2} \)) increases without an increase in the ventilatory equivalent for CO\(_2\) (\( V_{E/CO_2} \)). VT2 was located between VT1 and \( \dot{V}O_2 \text{max} \), when \( V_{E/O_2} \) increases and \( V_{E/CO_2} \) decreases. \( \dot{V}O_2 \text{max} \) was defined as the highest 30-s averaged \( \dot{V}O_2 \) value. Most subjects had a 1-min or more \( \dot{V}O_2 \) leveling off at maximal exercise (Table 2) as criteria for \( \dot{V}O_2 \text{max} \) and all attained at least two of the following criteria: 1) a maximal heart rate > 90% of the predicted maximal heart rate, 2) a lactate level at maximal exercise of > 8 mmol/L, or 3) a respiratory exchange ratio at peak exercise > 1.10.

Gas exchange measurements. \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were measured breath-by-breath with an open-circuit metabolic cart (Sensor Medics MSE, Yorba Linda, CA). The pneumotachograph used a hot-wire technology and was calibrated before each test with several strokes given by a 3-liter calibration syringe. The gas analyzers were calibrated using reference gases with known O\(_2\) and CO\(_2\) concentrations (12% O\(_2\)-5% CO\(_2\)). The accuracy of gas analyzers were verified monthly against a metabolic simulator.

Cardiovascular measurements. Stroke volume was measured by bioimpedance, whereas heart rate was simultaneously estimated from the electrocardiogram first derivative (Manatec type PF051L; Physio Flow, Paris, France). The accuracy of this bioimpedance device has previously been established against the direct Fick method during maximal incremental exercise in healthy subjects (36). Calibration of the impedance device was done using a procedure based on 24 consecutive heartbeats recorded with the subject resting on the ergometer (10). Arterial oxygen saturation was monitored by pulse oximetry at the earlobe; a-V\( O_2 \) was calculated as the \( V_{O2/CO2} \) ratio.

Blood analyses. Blood samples were obtained as part of the basal examination, forearm venous blood sampling, resting electrocardiography, and echocardiography recordings. All results were within normal limits.

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Table 2. Absolute and delta values at maximal exercise for $V_{O2}$, $Q_{max}$, and a-v$O_2$ difference under hypoxic and normoxic conditions

<table>
<thead>
<tr>
<th>Variables</th>
<th>Severe Loss Group</th>
<th>Moderate Loss Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic $V_{O2 max}$, ml/min</td>
<td>4220 ± 135</td>
<td>3948 ± 174</td>
</tr>
<tr>
<td>Leveling off $V_{O2}$ in normoxia, yes/no</td>
<td>4/3</td>
<td>5/3</td>
</tr>
<tr>
<td>Hypoxic $V_{O2 max}$, ml/min</td>
<td>3473 ± 121*</td>
<td>3665 ± 148*</td>
</tr>
<tr>
<td>Leveling off $V_{O2}$ in hypoxia, yes/no</td>
<td>4/3</td>
<td>7/1</td>
</tr>
<tr>
<td>$\Delta V_{O2 max}$, ml/min</td>
<td>747 ± 100</td>
<td>283 ± 56*</td>
</tr>
<tr>
<td>Normoxic $Q_{max}$, l/min</td>
<td>27.3 ± 1.1</td>
<td>25.0 ± 1.7</td>
</tr>
<tr>
<td>Hypoxic $Q_{max}$, l/min</td>
<td>26.2 ± 1.0*</td>
<td>23.0 ± 1.7*</td>
</tr>
<tr>
<td>$\Delta Q_{max}$, l/min</td>
<td>1.1 ± 0.3</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Normoxic $SaO_2$, %</td>
<td>95 ± 1</td>
<td>95 ± 1</td>
</tr>
<tr>
<td>Hypoxic $SaO_2$, %</td>
<td>81 ± 2*</td>
<td>83 ± 2*</td>
</tr>
<tr>
<td>$\Delta SaO_2$, ml/l</td>
<td>15 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Normoxic maximal a-v$O_2$ difference, ml/l</td>
<td>156 ± 6</td>
<td>163 ± 6</td>
</tr>
<tr>
<td>Hypoxic maximal a-v$O_2$ difference, ml/l</td>
<td>134 ± 6</td>
<td>165 ± 6</td>
</tr>
<tr>
<td>$\Delta Maximal$ a-v$O_2$ difference, ml/l</td>
<td>22 ± 4</td>
<td>-2 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. $\Delta V_{O2 max} = V_{O2 max}$ measured during the normoxic test $- V_{O2 max}$ measured during the hypoxic test. $\Delta Maximal$ a-v$O_2$ difference, maximal a-v$O_2$ difference measured during the normoxic test $- \Delta Maximal$ a-v$O_2$ difference measured during the hypoxic test. *Significantly different from normoxia ($P < 0.05$). †Significantly different from the Severe Loss group ($P < 0.05$).

In situ study of mitochondrial respiration. The mitochondrial respiration was studied in situ in a bundle of separated saponin skinned fibers as previously described (39, 44). Briefly, fibers were separated under binocular microscope in solution S at 4°C (see below) and permeabilized in solution S with 50 μg/ml of saponin for 30 min. After being placed 10 min in solution R (see below) to wash out adenine nucleotides and PCr, skinned separated fibers were transferred into a 3-ml water-jacketed oxygraphic cell (Strathkelvin Instruments, Glasgow, Scotland) equipped with a Clark electrode as previously described (33). Fiber respiration rates were measured at 22°C under continuous stirring. ADP-stimulated respiration ($V_{ADP}$) above $V_0$ was measured by stepwise addition of ADP as phosphate acceptor (from 10 to 2,000 μM) with or without Cr (20 mM). The decrease in $O_2$ concentration vs. time within the oxygraphic cell gave a measure of the respiration rate in micromoles $O_2$ per minute, subsequently expressed per gram of dry weight muscle tissue. The apparent $K_m$ values for ADP were calculated by using a nonlinear monoexponential fitting of the Michaelis-Menten equation ($K_m = [Cr]$ in the oxygraphic cell; $K_{Cr+Cr} = 20$ mM Cr in the oxygraphic cell). Nonlinear fitting (Microsoft Excel, Redmond, WA) for $K_m$ assessment in skinned muscle fibers is an already established fitting method, giving consistent results and yielding correlation coefficients $\geq 0.99$ for each measurement. Moreover, it gives an equal weight to each experimental measurement, avoiding the disadvantages of linear fitting that overweight the extreme points compared with the others. Maximal respiration rate ($V_{max}$) was calculated as ($V_{ADP}$ + $V_0$). The acceptor control ratio (ACR) was calculated as $V_{max}/V_0$.

Both solutions R and S contained: 2.77 mM CaK$_2$ EGTA, 7.23 mM K$_2$ EGTA (100 mM free Ca$^{2+}$), 6.56 mM MgCl$_2$ (1 mM free Mg$^{2+}$), 20 mM taurine, 0.5 mM DTT, 50 mM K-methane sulfonate (160 mM ionic strength), 20 mM imidazole (pH 7.1). Solution S also contained 5.7 mM Na$_2$ATP and 15 mM PCr. Solution R contained 3 mM phosphate, 2 mg/ml fatty acid-free bovine serum albumin, 2 mM malate, and 5 mM glutamate. After the experiments, fibers were harvested, dried, and weighed to express respiration rates as micromole $O_2$ per minute per gram dry weight.

Group Assignment

For each subject, the difference between the $V_{O2 max}$ values measured in normoxia and hypoxia was calculated ($\Delta V_{O2 max}$). In the whole population, the mean $\Delta V_{O2 max}$ was 7.2 ± 1.1 ml·min$^{-1}$·kg$^{-1}$. The subjects with a $\Delta V_{O2 max}$ $> 8$ ml·min$^{-1}$·kg$^{-1}$ were assigned to the Severe Loss group (SL, $n = 7$, $\Delta V_{O2 max}$ range: 8.1 to 16.7 ml·min$^{-1}$·kg$^{-1}$), whereas the remaining subjects were assigned to the Moderate Loss group (ML, $n = 8$, $\Delta V_{O2 max}$ range: 0.2 to 6.3 ml·min$^{-1}$·kg$^{-1}$).

Statistics

Statistical analyses were performed using Sigma Stat for Windows (version 3.0; SPSS, Chicago, IL). The differences between groups (SL vs. ML) were tested using Student’s t-test (mitochondrial parameters) and two-way ANOVA for repeated measures followed by a Student-Newman-Keuls post hoc procedure (exercise data). Spearman analysis was used to determine any potential relationship between variables. Data are means ± SE. Differences were considered to be significant for $P \leq 0.05$.

RESULTS

As shown in Table 1, no difference was observed between groups for the anthropometric characteristics blood hemoglobin and hematocrit. Normoxic aerobic function was also similar, with equivalent $V_{O2 max}$, $Q_{max}$, and maximal a-v$O_2$ difference; and no differences were observed between groups at VT$_1$ and VT$_2$.

Effect of Hypoxia on Metabolic and Cardiovascular Responses to Maximal Exercise

By design, the hypoxia-induced limitation of $V_{O2 max}$ was greater in the SL vs. the ML group, with reductions of $-18 \pm 2$% vs. $-7 \pm 1\%$, respectively ($P < 0.05$; Fig. 1 and Table 2). No significant correlation was found between the absolute reduction of $V_{O2 max}$ and $Q_{max}$ measured in normoxia ($r = 0.37; P = 0.17$). Both groups displayed similar levels of arterial oxygen desaturation with hypoxia ($-15\%$, $P < 0.05$). $Q_{max}$ was attenuated by hypoxia, with the SL group tending to exhibit a smaller decrease than the ML group ($-4 \pm 1\%$ vs. $-9 \pm 2\%$, respectively; $P = 0.08$). As a mirror image, maximal a-v$O_2$ difference was impaired by $-14 \pm 2\%$ with hypoxia in the SL group but was maintained in the ML group ($-1 \pm 2\%$; $P < 0.05$ between groups). Although significant in the SL group only, hypoxia tended to reduce maximal heart rate, whereas maximal stroke volume was never significantly altered (Fig. 2). An overview of these results is given in Fig. 3. Maximal blood lactate concentration was not different between groups either in normoxia (SL: $13.6 \pm 1.8$ vs. ML: $12.1 \pm 0.8$ mmol/l; not significant) or in hypoxia (SL: $12.9 \pm 1.2$ vs. ML: $11.2 \pm 1.1$ mmol/l; not significant).

Effect of Hypoxia on Metabolic and Cardiovascular Responses to Submaximal Exercise

As shown in Fig. 1, $V_O2$ was similar during submaximal exercise in both conditions of oxygen availability. $Q$ was not modified by hypoxia at submaximal exercise in the ML group, whereas the SL group displayed an enhanced $Q$ response in hypoxia at any given workload. A greater heart rate appears to be the main mechanism underlying the enhanced $Q$ response observed in the SL group. By contrast, stroke volume was similar in normoxia and hypoxia in both groups (Fig. 2). As a result of the greater hypoxic $Q$ response in the SL group, a-v$O_2$ difference was lower at any given workload. Of note, a-v$O_2$ difference tended to be higher in hypoxia in the ML group (Fig. 1).
Mitochondrial Function

As indicated in Table 3, the quantitative parameters of mitochondrial function (V_o2, V_max, ACR) were not significantly different between groups. V_max was neither correlated to V_o2max measured in normoxia (r = 0.41; P = 0.12) nor to ΔV_o2max (r = 0.36; P = 0.18). Conversely, significant differences appeared for the qualitative properties, reflecting the role of mi-CK in oxidative phosphorylation coupling. Indeed, K_m/Cr was lower in ML compared with SL, whereas the K_m/K_m+Cr ratio was higher. K_m for ADP without Cr was not different between groups. When all subjects were pooled together, K_m+Cr was positively correlated to Δmaximal a-vO2 difference (r = 0.52; P = 0.05) (Fig. 4). The K_m+Cr ratio was negatively correlated to ΔV_o2max (r = −0.53; P = 0.03) and Δmaximal a-vO2 difference (r = −0.55; P = 0.03) (Fig. 4).

DISCUSSION

Major Findings

This study suggests that the individual magnitude of the hypoxia-induced reduction of V_o2max in a homogenous group of endurance-trained athletes is accounted for by specific responses of central and peripheral factors involved in O2 delivery, extraction, and processing at the cellular level. Those athletes exhibiting the largest reduction of V_o2max in moderate hypoxia suffer from both significant reductions in Q_max and maximal a-vO2 difference together with a lower degree of coupling between CK shuttle and oxidative phosphorylation at the mitochondrial level. Conversely, athletes presenting the smallest attenuation of V_o2max with moderate hypoxia are able to maintain maximal a-vO2 difference despite a blunted Q response and reveal a higher coupling of the CK shuttle.
suggests that the functional coupling between ATP production and the CK shuttle appears to play a role in the maintenance of V\textsuperscript{O}2\textsubscript{max} as close as possible to its normoxic level when exercising in moderate hypoxia.

**Attenuation of oxygen uptake and extraction during maximal exercise in moderate hypoxia.** Both groups present a similar V\textsuperscript{O}2\textsubscript{max} in normoxia (P > 0.25), and exercise in moderate hypoxia (i.e., F\textsubscript{IO}2 = 14.5% or ~3,000 m) reduced the normoxic V\textsuperscript{O}2\textsubscript{max} by ~12 ± 2% in the whole population. This value falls within the range of previously published values reporting attenuations of V\textsuperscript{O}2\textsubscript{max} between ~9 and ~20% in this setting (6, 8, 26, 31, 37). A closer analysis of our data demonstrates quite large interindividual variability in the V\textsuperscript{O}2\textsubscript{max} response to hypoxia, with ΔV\textsuperscript{O}2\textsubscript{max} presenting a coefficient of variation of 36% between subjects. Numerous factors have been proposed to account for the reduction of V\textsuperscript{O}2\textsubscript{max} with hypoxia and its variability (6), and their integrated effects can be approached by focusing on the convection (i.e., Q\textsuperscript{\text{\bullet}}).

![Fig. 2](http://ajpregu.physiology.org/)

**Fig. 2.** Group responses of heart rate and stroke volume to incremental exercise in normoxia and moderate hypoxia. The last 2 points represent data at maximal exercise. *P < 0.05 vs. hypoxia; $P < 0.001$ vs. moderate hypoxia. Due to technical difficulties, stroke volume is missing for 1 subject. Therefore, these data are presented for n = 14 subjects.

![Fig. 3](http://ajpregu.physiology.org/)

**Fig. 3.** Relative changes of VO\textsubscript{2}, Q\textsuperscript{\text{\bullet}}, and a-\textit{V}\textsubscript{O}2\textsubscript{2} difference at maximal exercise with moderate hypoxia in the Severe Loss and the Moderate Loss groups. Levels of significance are for between-group comparisons.

**Table 3. Mitochondrial function**

<table>
<thead>
<tr>
<th></th>
<th>Severe Loss Group</th>
<th>Moderate Loss Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V\textsubscript{b}, μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>V\textsubscript{max}, μmol O\textsubscript{2}·min\textsuperscript{-1}·g dry wt\textsuperscript{-1}</td>
<td>8.2 ± 0.5</td>
<td>7.4 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>ACR</td>
<td>6.3 ± 0.9</td>
<td>4.5 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>K\textsubscript{m}, μM</td>
<td>481 ± 34</td>
<td>421 ± 68</td>
<td>NS</td>
</tr>
<tr>
<td>K\textsubscript{m}+Cr, μM</td>
<td>166 ± 25</td>
<td>86 ± 25</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>K\textsubscript{m}/K\textsubscript{m}+Cr</td>
<td>3.3 ± 0.5</td>
<td>6.6 ± 1.0</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Severe Loss group of subjects had V\textsuperscript{O}2\textsubscript{max} superior to 8 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}; the Moderate Loss group of subjects had ΔV\textsuperscript{O}2\textsubscript{max} inferior to 8 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}. V\textsubscript{b}, basal respiration rate. V\textsubscript{max}, maximal respiration rate; ACR, acceptor control ratio calculated as V\textsubscript{max}/V\textsubscript{b}. K\textsubscript{m}, apparent K\textsubscript{m} for ADP. K\textsubscript{m}+Cr, apparent K\textsubscript{m} for ADP with creatine.
The present results show that moderate hypoxia blunted both $Q_{\text{max}}$ and maximal a-$\text{V}_0$-$\text{O}_2$ difference in the ML group, ultimately reducing normoxic $V_{\text{O}2\text{max}}$ by ~18%. Although a similar decrease in $Q_{\text{max}}$ was also observed in the ML group, a new finding of the present study is that maximal a-$\text{V}_0$-$\text{O}_2$ difference was better maintained in this group, allowing $V_{\text{O}2\text{max}}$ to be only 7% lower under moderate hypoxia compared with its normoxic value. Such specific group responses were also identified at submaximal exercise, where athletes of the SL group displayed the well-known elevation of heart rate and $Q$ at any given power output to compensate for the reduction in a-$\text{V}_0$-$\text{O}_2$ difference (41) imposed by hypoxia. Conversely, athletes from the ML group tend to have a lower cardiovascular response (i.e., $Q$, heart rate, and stroke volume) together with an increased a-$\text{V}_0$-$\text{O}_2$ difference, allowing them to maintain aerobic energy turnover at submaximal exercise. Such compensatory adjustments between $Q$ and maximal a-$\text{V}_0$-$\text{O}_2$ difference have already been reported after $\beta$-adrenergic blockade in normoxia but also in severe hypoxia (4,300 m or $F_{\text{O}_2} = \sim 12.5\%$), where the drug-induced limitation in heart rate and $Q$ were compensated for by elevations in a-$\text{V}_0$-$\text{O}_2$ difference and $O_2$ extraction at submaximal (50) and maximal exercise (28), allowing maintenance of oxygen uptake. Taken together, these results suggest that factors improving systemic $O_2$ extraction, either directly or more likely by reflex modification of blood flow redistribution, could compensate for the reduced systemic $O_2$ delivery. In support of this, we observed that $\Delta V_{\text{O}2\text{max}}$ was significantly correlated with $\Delta$maximal a-$\text{V}_0$-$\text{O}_2$ difference but not with $\Delta Q_{\text{max}}$ nor with $\Delta S_{\text{aO}_2}$. The central components of systemic $O_2$ delivery then appear unlikely to have played a major role in setting the individual tolerance to moderate hypoxia in our athletes (also displaying similar blood hemoglobin content), further pointing to a key role of downstream factors, within the active locomotor muscles.

$O_2$ extraction is already known to be higher at maximal exercise in hypoxia vs. normoxia in an attempt to compensate for the reduction in arterial $O_2$ content (46). However, maximal a-$\text{V}_0$-$\text{O}_2$ difference is usually reduced with hypoxia (31), mainly because of its critical dependence on arterial $O_2$ content. Therefore, the unique result of an unchanged maximal a-$\text{V}_0$-$\text{O}_2$ difference in the ML group may be specific to moderate hypoxia, where venous $O_2$ content can be further lowered to compensate for the modest reduction in arterial $O_2$ content. The ability to enhance $O_2$ extraction with hypoxia has been advanced as a plausible mechanism explaining part of the better tolerance to altitude observed in untrained vs. trained subjects (35), but the underlying mechanisms remain unclear. A preferential redistribution of blood flow toward locomotor muscles, either by increasing red blood cell flow in already perfused capillaries and/or recruiting previously nonperfused capillaries (9, 35), together with the properties of the skeletal muscle mitochondrial function, might contribute to improving capillary-myocyte $O_2$ exchange with moderate hypoxia.

**Maintenance of $V_{\text{O}2\text{max}}$ and maximal a-$\text{V}_0$-$\text{O}_2$ difference in hypoxia is not linked to quantitative aspects of mitochondrial function.** In conditions where $O_2$ delivery is reduced, the question of what is/are the factor(s) involved in $O_2$ extraction is still open, and the role of the mitochondria in this setting remains unclear. Some studies suggest that the major cause for the reduced muscle $O_2$ consumption in hypoxia is $O_2$ availability in the surroundings of the mitochondria rather than skeletal muscle oxidative capacities (21, 27, 44, 56). Other studies suggest that both oxygen supply and mitochondrial oxidative capacities interact to determine $V_{\text{O}2\text{max}}$ (23, 25). Interestingly, our measures of mitochondrial respiration in situ ($V_{\text{max}}$) showed that high maximal muscle oxidative capacity, which is likely to reflect the mitochondrial density, might not be critical to attenuate the hypoxia-related reduction of $V_{\text{O}2\text{max}}$ and maximal a-$\text{V}_0$-$\text{O}_2$ difference in already endurance-trained athletes. In a previous study, we showed that $V_{\text{max}}$ was positively correlated to $V_{\text{O}2\text{max}}$ when the population ranges from sedentary ($V_{\text{O}2\text{max}} = 28.5$ ml·kg$^{-1}$·min$^{-1}$) to moderately

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**Fig. 4. Relationship between mitochondrial function and differences in maximal $O_2$ uptake ($\Delta V_{\text{O}2\text{max}}$) and maximal a-$\text{V}_0$-$\text{O}_2$ difference measured in normoxia and moderate hypoxia.**

**A:** $K_m/K_{\text{m+Cr}}$ was negatively correlated to $\Delta V_{\text{O}2\text{max}}$.

**B:** $K_m/K_{\text{m+Cr}}$ was negatively correlated to $\Delta$maximal a-$\text{V}_0$-$\text{O}_2$ difference.

**C:** $K_{\text{m+Cr}}$ was positively correlated to $\Delta$maximal a-$\text{V}_0$-$\text{O}_2$ difference.

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{oxygen content} and diffusion steps (i.e., alveolar to arterial, capillary to myocytes) involved in $O_2$ delivery and $O_2$ extraction.
trained subjects ($\dot{V}_{O_2\text{max}} = 50 \text{ ml·kg}^{-1} \cdot \text{min}^{-1}$) (51). However, in subsequent studies, endurance athletes with higher $\dot{V}_{O_2\text{max}}$ (~60 ml·kg$^{-1}$·min$^{-1}$) were shown to have similar $V_{\max}$ values compared with their moderately trained counterparts (12, 34), suggesting that the quantitative aspects of tissue oxidative capacity levels off before the highest levels of endurance training are reached. Moreover, the higher $\dot{V}_{O_2\text{max}}$ demonstrated by endurance-trained athletes was not accompanied by any increase in enzymatic markers of quantitative aspects of mitochondrial function (34). In the present study, the ACR was similar between groups, suggesting that the degree of coupling between electron transport and phosphorylation does not explain the difference in the ability to maintain the normoxic $\dot{V}_{O_2\text{max}}$ in moderate hypoxia.

Intrinsic qualitative mitochondrial properties correlate with a better maintenance of $\dot{V}_{O_2\text{max}}$ and maximal a-$\text{VO}_2$ difference during exercise in moderate hypoxia. The combination of increased mitochondrial content and oxidative capacities together with specific reorganization of intracellular energy fluxes is known to be an integral part of the adaptation to endurance training (40, 55, 58, 63). Accordingly, the apparent $K_m$ for ADP (inversely proportional to the apparent affinity of mitochondria for ADP) was high in all our athletes, regardless of the magnitude of their drop in $\dot{V}_{O_2\text{max}}$ with moderate hypoxia, suggesting that the sensitivity to cytosolic ADP is not a key factor involved in the hypoxia tolerance in athletes with already high $K_m$ values. Nevertheless, high $K_m$ values reflect a low sensitivity of mitochondrial respiration to cytosolic ADP, as expected in endurance-trained subjects (34, 47, 51) with skeletal muscles featuring high oxidative capacities (8, 31, 48, 53, 55, 62).

However, other qualitative properties of the muscular mitochondria may contribute to maintain $\dot{V}_{O_2\text{max}}$ close to its normoxic value in athletes exercising in moderate hypoxia. In support for this contention, we report for the first time that the ability to keep high levels of $\dot{V}_{O_2\text{max}}$ and maximal a-$\text{VO}_2$ difference during moderate hypoxic exercise is correlated with the decrease of the $K_m$ for ADP in the presence of Cr (lower $K_m$+Cr, higher $K_m$/($K_m$+Cr) Ration). The coupling between mi-CK and oxidative phosphorylation might then play a role in the limitation of $\dot{V}_{O_2\text{max}}$ with hypoxia. In that way, the phosphate moiety is transferred more efficiently to PCR, and ADP is more rapidly recycled toward oxidative phosphorylation, so that a smaller cellular ADP signal is necessary to stimulate mitochondrial $O_2$ uptake and muscle $O_2$ extraction. In intact muscle, where mi-CK is active and coupled, it is the PCR/CR ratio that triggers mitochondrial respiration rather than the ADP/ATP ratio. It has already been suggested in cardiomyocytes that mitochondrial intrinsic regulation can compensate for relatively slow oxygen diffusion (42). Skeletal muscles with higher coupling between mi-CK and ADP rephosphorylation produce energy on a “pay as you go” basis and therefore may be able to maintain longer the cellular homeostasis in conditions of lower $O_2$ availability during an incremental test. This may delay the use of anaerobic energy production while exercising at low $\text{Fi}_O_2$, thereby maintaining a more favorable intracellular redox potential and producing less metabolite by-products until a final level of peripheral muscle fatigue develops and exhaustion occurs (4, 5, 38). Because $\dot{V}_{O_2\text{max}}$ in normoxia and hypoxia ultimately represents an $O_2$ flow, in part driven by the potential to extract $O_2$ from capillary blood, the link between the smaller attenuation of $\dot{V}_{O_2\text{max}}$ and the ability to maintain the cellular redox potential under moderate hypoxia remains unclear at present.

With hypoxia, the well-known greater metabolic accumulation ($H^+$, $P_i$, lactate, ADP, adenosine, ATP, bradykinin, etc.) in active muscles and their interstitial surroundings (11) has been proposed to exacerbate the stimulation of group III and IV muscle afferents ultimately resulting in a cessation of central motor output and exhaustion (29). How exactly the reduced central motor output occurs is still a matter of debate. Higher plasma catecholamine and muscle sympathetic nerve activity have been documented with hypoxia (17, 18), triggering peripheral sympathetic vasoconstrictor activity that overrides vasodilatory stimuli generated within the active limbs, particularly at maximal exercise. In support of this mechanism, exhaustion during maximal incremental exercise in normoxia is associated with increasing mean arterial pressure and falling systemic and leg vascular conductance (32). Of note, intravascular limb ATP injection reduces mean arterial pressure in hypoxia but not in normoxia during maximal cycle exercise, suggesting the prevalence of a higher level of vasoconstriction in hypoxia (30). Moreover, experimental evidence for a critical role of somatosensory feedback in fatigue during maximal exercise has accumulated recently (1–3), further pointing to a key role of cellular homeostasis disturbance in limiting aerobic exercise capacity. In the present study, a lower disturbance in intracellular energetics in muscles with highly coupled mitochondria may delay muscle afferent activation involved in sympathetic activation and local vasoconstriction. The lower heart rate for a given power output in the ML group also fits with this hypothesis.

Limitations of the Study. The SL group exhibited a somewhat greater normoxic $\dot{V}_{O_2\text{max}}$ compared with the ML group, suggesting a risk for a type 2 error with our small number of subjects. Despite $P$ values always > 0.27, significant differences would not have changed the main conclusions of this study, which, for the first time, provides evidence for a role of skeletal muscle mitochondria in the tolerance to moderate hypoxia in endurance-trained athletes. Moreover, whatever the level of statistical significance for mitochondrial $V_{\max}$ between the two groups, the magnitude of the difference clearly points to qualitative ($K_m = 9\%$; $K_m$+Cr = 48\%; $K_m$ ratio = 100\%), rather than quantitative, adaptations ($V_{\max} = 9\%$) as major mitochondrial properties involved in hypoxia tolerance. Nevertheless, the present data do not allow further insights into the mechanisms linking mitochondrial properties to the hypoxia-mediated reduction in $\dot{V}_{O_2\text{max}}$. Another limitation of the present study is the use of cardiac impedance to estimate stroke volume and calculate $Q$. Nevertheless, this noninvasive methodology has been validated by our and other laboratories and is currently largely used in exercise physiology, especially in trained and lean subjects where good thoracic impedance signals can be collected. The same technique has been applied to all subjects in the present study, and a closer analysis of our hemodynamic data reveals that the main difference between the SL and the ML groups are heart rate mediated and therefore do not rely on the stroke volume measurement. Therefore, the conclusions reported in the present report are not likely to be methodology dependent.
Perspective and Significance

The individual tolerance to moderate hypoxia is quite variable from one subject to another, even among endurance-trained athletes with similar VO2max. A better knowledge of the mechanisms involved in the hypoxia-induced limitation of VO2max could help in setting optimal support for mountaineers or competitive athletes preparing for exercises at a moderate altitude. Whereas the blunting of Qmax seems a universal response to moderate hypoxia, we observed a smaller hypoxia-induced attenuation of VO2max in those athletes able to maintain their maximal a-vO2 difference. Additionally, high qualitative (i.e., intrinsic regulation) rather than quantitative (i.e., maximal oxidative capacity) properties of skeletal muscle mitochondria are characteristics of endurance-trained athletes, featuring the better tolerance to moderate hypoxia. Future studies will need to explore how a greater functional coupling between the mi-CK and ATP production, and therefore, a better control of oxidative capacity) properties of skeletal muscle mitochondria may contribute to the individual tolerance to moderate hypoxia in endurance-trained athletes.

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DISCLOSURES

The authors declare that they have no conflict of interest.

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