Skeletal muscle oxidative metabolism in sedentary humans:

$^{31}$P-MRS assessment of $O_2$ supply and demand limitations

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The supply of $O_2$ and its utilization by skeletal muscle is a tightly interwoven process that cannot easily be assessed by a single measurement. Although $^{31}$P-magnetic resonance spectroscopy (MRS) measurements of phosphocreatine (PCr) recovery provide an accurate measure of skeletal muscle oxidative metabolism (2, 11, 15, 17), alone this methodology is unable to discern limitation caused by $O_2$ supply from that of mitochondrial capacity and not $O_2$ availability in normoxia. Additionally, the significant elongation of PCr recovery in these subjects in hypoxia illustrates the reliance on $O_2$ supply at the other end of the $O_2$ availability spectrum in both sedentary and active populations.

Oxidative capacity; mitochondria; exercise; intracellular oxygenation; $^{31}$-phosphorous-magnetic resonance spectroscopy

There is a growing appreciation of the concept that $O_2$ supply and demand limitations to maximal metabolic rate are dependent on the population studied (4, 26, 31). However, it should be noted that the preponderance of data have been collected from physically active or endurance-trained subjects who reveal $O_2$ supply dependence at maximal $O_2$ uptake ($V_{O_2\ max}$). The supply of $O_2$ and its utilization by skeletal muscle is a tightly interwoven process that cannot easily be assessed by a single measurement. Although $^{31}$P-magnetic resonance spectroscopy (MRS) measurements of phosphocreatine (PCr) recovery provide an accurate measure of skeletal muscle oxidative metabolism (2, 11, 15, 17), alone this methodology is unable to discern limitation caused by $O_2$ supply from that of mitochondrial $O_2$ demand.

However, the combination of PCr recovery measurement under conditions of altered $O_2$ availability, manipulated by varying the inspired $O_2$ fraction ($F_{O_2}$), has been utilized to demonstrate that, in exercise-trained humans, under normoxic conditions, $O_2$ availability limits maximal oxidative rate (7). The practical implication of these data is that PCr recovery measurements alone should be interpreted with caution because differences in PCr recovery between subjects may not be due to metabolic limitations (1, 5, 19) but rather to limitations in $O_2$ supply (30). To solidify this unique approach of combining manipulations in $O_2$ availability with PCr recovery, it is important to demonstrate that this methodology is able to determine the difference between mitochondrial limitation vs. $O_2$ supply limitation across subject populations with quite different skeletal muscle oxidative capacities.

To achieve this goal, we again used $^{31}$P-MRS to study the exercising human gastrocnemius muscle under conditions of varied $F_{O_2}$, but we now turned our attention to sedentary subjects and compared the data obtained in the present investigation with that obtained previously in exercise-trained subjects under identical conditions (7). We hypothesized that 1) PCr recovery would not be enhanced by increased $O_2$ availability in sedentary subjects because they are limited by mitochondrial capacity and 2) PCr recovery would be unaffected by a reduction in $O_2$ availability in sedentary subjects due to limited mitochondrial capacity. Such unique observations would demonstrate that PCr recovery data coupled with manipulations in $O_2$ availability could noninvasively distinguish between $O_2$ supply limitations and mitochondrial limitations across populations, providing a powerful addition to the study of muscle pathophysiology.

Methods

Subjects. Six sedentary subjects (3 men, 3 women, 24.3 ± 1.1 yr, 75.0 ± 7.0 kg, 174 ± 5.2 cm) volunteered to participate in this study and gave written, informed consent. The study was approved by the University of California, San Diego, Human Subjects Protection Program. The subjects were screened to assess physical activity level by using a modified Minnesota Leisure Time Physical Activity questionnaire that correlates well with exercise testing (6).

Exercise protocol. Subjects were familiarized with plantar flexion exercise in the confines of a whole body magnetic resonance imaging system. At this time, a level of work was determined for each subject that would result in a depletion of PCr of between 30 and 40% under normoxic conditions. Subjects performed constant-load submaximal plantar flexion at this intensity (frequency of 1 Hz, maintained with the aid of an electronic metronome) while lying supine in a superconducting 1.5-T magnet. In each $F_{O_2}$ treatment (0.1, 0.21, and 1.00), subjects performed a 5-min warm-up period followed by 5 min of rest, and then they performed 6 min of exercise followed by 5 min of recovery. Subjects were allowed 30 min of rest between each com-

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plete exercise bout. MRS data were acquired continuously for 2 min before exercise, for the 6 min of exercise, and for the 5 min of recovery. The order of the three treatments was varied to minimize any ordering effects. The treatment order was not disclosed to the subjects.

Heart rate, arterial saturation, and arterial Po2. Throughout each exercise bout, subjects breathed through a low-resistance two-way breathing valve (model 2700, Hans-Rudolph, Kansas City, MO). Heart rate and arterial O2 saturation were monitored continuously throughout the experiment with a finger probe oximeter (Omni-Trak, In Vivo Research). During previous studies (7, 8), a high correlation between arterial O2 saturation calculated from end-tidal O2 gas measurement and that measured with this oximeter system was observed. These saturations were then used to calculate arterial Po2 by using the Hill equation, assuming a normal O2 half-saturation pressure.

31P-MRS. MRS was performed by using a clinical 1.5-T General Electric Signa system (5.4.2 version) operating at 25.86 MHz for 31P. 31P-MRS data were acquired with a dual-frequency flexible-array spectroscopy coil (Medical Advances) placed around the calf at its maximum diameter. The phosphorus coil was an 11.5-cm square centered between two 14 × 15.5-cm Helmholtz-type proton coils. The centering of the coil around the leg was confirmed by longitudinal relaxation time-weighted 1H Helmholtz-type proton coils. The magnetic field homogeneity was optimized by shimming on the proton signal from tissue water and the 31P-MRS signal was optimized by prescan transmitter gain adjustment. A 500-μs hard pulse was used for signal excitation. The signal-to-noise ratios between the active and sedentary groups were similar. The spectral width was 2,500 Hz, and data were acquired continuously for 13 min with a single free induction decay (FID) acquired every 4 s. Thus 195 FIDs were acquired during the 2-min rest period, 6 min of exercise, and 5 min of recovery. As a result, the data were expressed with a time resolution of 4 s.

Data analysis. Data were processed by using SAGE/IDL software on a Silicon Graphics INDIGO workstation. Each FID consisted of 1,024 complex points and was processed with 5-Hz exponential line broadening before zero filling and Fourier transformation. All spectra were manually phased by using zero and first-order phase corrections. There were no phase variations between rest, exercise, and recovery during the experiment. The levels of PCr determined from the intensity of that peak were normalized to 100% using the average value obtained from the last 40 s of rest acquired for each subject as a reference. Muscle intracellular pH was calculated from the chemical shift difference (δ) of the P; peak relative to the PCr peak by using the following equation: pH = 6.75 + log[(δ − 3.27)/(5.69 − δ)] (29).

RESULTS

On the basis of the physical activity assessment questionnaire, all subjects were categorized as sedentary with no previous history of physical training or recreational sport participation and no regular or occasional physical exercise above that required for daily activities. For comparison of the physical characteristics of the sedentary subjects with those of the exercise-trained subjects from the previous study (7), Table 1 contains the age, weight, and BMI of both subject groups.

For calculation of PCr recovery τ, the monoeponential function was fitted to the unaveraged data set (temporal resolution of 4 s), in a manner similar to that previously published (7), because sufficient signal-to-noise ratio of ~30:1 for the PCr resonance was obtained. The effect of FiO2 on PCr recovery τ for these sedentary subjects is shown in Fig. 1. Because of technical difficulties, there were no hypoxia data acquired for a single sedentary subject. The data obtained under the normoxic and hyperoxic conditions from this subject were typical and had no impact on the conclusions of this study, whether included or excluded from the analysis. For these sedentary subjects, τ was significantly longer in hypoxia (47.0 ± 3.2 s; P ≤ 0.05), but there was no difference between normoxia (30.0 ± 2.1 s) and hyperoxia (31.8 ± 2.0 s; Fig. 1). A direct statistical analysis of the change in PCr recovery τ between normoxia and hyperoxia for these sedentary subjects compared with that from the exercise-trained subjects obtained previously (7) revealed a P value of 0.0063, indicating that the two subject groups exhibited different behavior to the increase in FiO2.

For each FiO2 treatment, the end-exercise pH values, PCr recovery rate constants (1/τ), arterial O2 saturations, arterial Po2, and end exercise levels of PCr (expressed as a percentage of resting levels) are shown in Table 2. The FiO2 breathed had no effect on the resting levels of PCr or the end-exercise levels of PCr. The mean work rate in each exercise bout under the different FiO2 treatments was kept constant (5.5 ± 0.5 W).

A reduced arterial hemoglobin saturation and Po2, the result of breathing the hypoxic gas, lowered the PCr recovery rate constants (1/τ) of these sedentary subjects. The most significant effect of hyperoxia is to raise the blood Po2 because hemoglobin saturation is already close to its ceiling in normoxia. However, in these sedentary subjects, an increased arterial Po2 had no effect on the PCr recovery rate constants.

DISCUSSION

Previously, our laboratory has demonstrated that PCr recovery in exercise-trained humans is altered by manipulations in FiO2 (7). The observed increase in τ with hypoxia and decrease in τ with hyperoxia suggested limited metabolic function due to fatigue.

Table 1. Physical characteristics of the sedentary subjects and those of the exercise-trained subjects used in the previous study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sedentary</th>
<th>Trained (Ref. 7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.3 ± 0.6</td>
<td>31.8 ± 2.6</td>
<td>0.023*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.0 ± 7.6</td>
<td>79.2 ± 3.06</td>
<td>0.595</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174.0 ± 5.5</td>
<td>182.2 ± 2.1</td>
<td>0.172</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.5 ± 1.4</td>
<td>23.8 ± 0.6</td>
<td>0.650</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index. *Significantly different.
to O₂ availability during recovery from exercise, in agreement with previous findings using very different methodologies in similarly athletically trained subjects at maximal exercise (22, 25). The results of the present study reveal that increased FİO₂ (100%) did not result in an enhanced PCr recovery, suggesting that in normoxia the maximal oxidative rate of these sedentary subjects is limited by mitochondrial capacity and not O₂ availability. Additionally, the elongated PCr recovery τ in sedentary subjects observed in this study and recorded previously in exercise-trained subjects (7) while breathing the hypoxic gas (10% O₂) highlights the observation that reductions in O₂ supply soon exceed even a low mitochondrial capacity (sedentary subjects) as a limitation to maximal oxidative rate.

**PCr recovery to differentiate O₂ supply vs. O₂ utilization limitations.** PCr recovery is a useful measure of skeletal muscle oxidative capacity and is dependent on mitochondrial respiratory function (3, 11). A further advantage of the measure is that PCr recovery does not require a correction for active muscle mass (10, 16) and is independent of the work level (17), provided that muscle intracellular pH does not fall severely (2). Thus the measurement of PCr recovery has proven useful in determining the oxidative capacity of skeletal muscle in a variety of conditions (12, 15, 30, 32). However, although providing an accurate index of skeletal muscle oxidative metabolism, this measurement does not allow the differentiation between limitations caused by O₂ supply and those resulting from O₂ demand.

In a previous study, our laboratory observed an O₂ supply dependence of PCr recovery in exercise-trained subjects (7). The normoxic PCr τ values from that study (25.0 ± 2.7 s) are shorter than those reported here for the sedentary subjects (30.0 ± 2.1 s). This is consistent with previous studies showing shorter PCr τ values in exercise-trained individuals due to the increased oxidative capacity of the subjects (14, 16, 32). The assessment of an individual’s response to the manipulations in O₂ availability used here and previously (7) helps to move the PCr τ measurement from a subjective to an objective evaluation of the O₂ supply and demand limitations to metabolic capacity.

**"Titration" of metabolic capacity using PCr recovery.** Both the rate constant for PCr recovery (1/τ) and VO₂ max are indexes of the maximal rate of oxidative ATP synthesis and are traditionally considered to be linearly dependent on muscle oxidative capacity (9, 18). Thus, as discussed previously (7), PCr recovery data are clearly indicative of both the maximal rate of oxidative ATP synthesis and muscle oxidative capacity. Several earlier studies have illustrated a strong relationship between O₂ supply and skeletal muscle oxidative capacity during maximal exercise in exercise-trained subjects (13, 21). However, the limited research performed in sedentary subjects suggests that maximal oxidative rate appears to be determined by mitochondrial capacity and not O₂ supply (4). The data presented here in Fig. 2 illustrate the O₂ supply dependence of the rate constant for PCr recovery in exercise-trained subjects (from Ref. 7), but not the sedentary subjects of the present study, due to the increased arterial P O₂ that results from breathing a hyperoxic gas, consistent with the above concept. The rate constants of both the sedentary and exercise-trained subjects were lowered while breathing 10% O₂, suggesting that the reduced arterial P O₂ has influenced the rate of PCr recovery in each population. However, the data of Richardson et al. (23) showed that, when sedentary subjects, with a similar activity profile to those examined in this study, breathed 12% O₂, no effect on maximal oxidative rate was observed. This suggests that, for the sedentary subjects, a “critical P O₂” may exist between breathing 10 and 12% O₂ where PCr recovery (and VO₂ max) would be unaffected by P O₂. This critical P O₂ would depend on mitochondrial capacity. Whereas in sedentary sub-

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### Table 2. Summary of data collected at the end of the submaximal exercise period

<table>
<thead>
<tr>
<th>FİO₂</th>
<th>0.1</th>
<th>0.21</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-exercise PCr, % of rest</td>
<td>63.0±7.7</td>
<td>66.9±6.0</td>
<td>70.5±6.1</td>
</tr>
<tr>
<td>End-exercise pH</td>
<td>6.97±0.03</td>
<td>6.98±0.03</td>
<td>7.03±0.3</td>
</tr>
<tr>
<td>Arterial saturation, %</td>
<td>68.6±1.2</td>
<td>96.8±0.3</td>
<td>99.0±0.3</td>
</tr>
<tr>
<td>Arterial P O₂, Torr</td>
<td>40.8±0.9</td>
<td>106.2±2.9</td>
<td>640.0±9.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data represent an average of the 40 s before recovery. FİO₂, inspired O₂ fraction; PCr, phosphocreatine.
jects evidence is accumulating to suggest that a critical \( \text{PO}_2 \) may be achieved by inspiring 10–12% \( \text{O}_2 \), exercise-trained subjects may experience supply limitation far earlier, even at the reduced \( \text{O}_2 \) availability invoked by breathing 15% \( \text{O}_2 \) gas (27). At the other end of the spectrum, a person with a severe mitochondrial myopathy may not demonstrate an effect of reduced \( \text{Fi}_2 \) before safety prohibits a further reduction (e.g., below 10% \( \text{O}_2 \)). Hence the technique used in the present study (\( ^{31} \text{P}-\text{MRS} \) coupled with altered \( \text{O}_2 \) availability) appears useful to distinguish between \( \text{O}_2 \) supply and demand limitations in the study of muscle pathophysiology, providing a differentiation between populations with reduced oxidative capacity on the basis of the sensitivity of \( \text{PCR} \tau \) measurements to reduced and elevated \( \text{O}_2 \) availability.

Although not a focus of the present research, it is interesting to note that these sedentary subjects revealed a greater insensitivity to changes in \( \text{O}_2 \) availability even during submaximal exercise. Specifically, unlike previously studied exercise-trained subjects (8), these sedentary subjects revealed very little variation in percent \( \text{PCR} \) depletion under conditions of varied \( \text{Fi}_2 \) breathing (Table 2). This may have implications for the role of \( \text{O}_2 \) sensing in exercising muscle and the effect of regular activity on this phenomenon.

As cited above, previous studies of sedentary subjects were invasive in nature with vascular catheters in place, facilitating the measurement of blood flow and arterial and venous blood gases to allow calculation of muscle \( \text{O}_2 \) uptake, with the subjects required to exercise to maximum via cycle (4) or knee-extension (23) ergometry. A clear advantage of using \( \text{PCR} \) recovery to assess maximal oxidative capacity is that subjects can perform submaximal exercise, so there is no need for attainment of a true maximum or peak exercise level, which may be practically difficult to obtain in certain disease conditions such as chronic heart failure or chronic obstructive pulmonary disease. Additionally, this technique is noninvasive.

It is interesting to note that, in the present study, we may have underestimated the mitochondrial capacity of the sedentary subjects by selecting a hypoxic gas of 10% \( \text{O}_2 \) because this had a somewhat unexpected negative effect on \( \text{PCR} \tau \). However, as indicated above, this result sets the scene for measuring the contributions of \( \text{O}_2 \) supply and mitochondrial capacity to maximal oxidative rate in pathophysiological conditions where mitochondrial myopathy is suspected. In such a patient, a similar reduction in \( \text{O}_2 \) availability with no effect on \( \text{PCR} \tau \) would imply a reduced mitochondrial function (below that of a sedentary lifestyle) independent of \( \text{O}_2 \) supply.

\( \text{O}_2 \) tension and implications for diffusion limitation. There is prior evidence in subjects performing small-muscle-mass submaximal exercise that suggests the effect of varying \( \text{Fi}_2 \) on \( \text{O}_2 \) delivery is dampened by an alteration in muscle \( \text{blood} \) flow (28). Specifically, it has been shown that across a range of submaximal work intensities muscle \( \text{blood} \) flow is elevated to compensate for the reduced arterial \( \text{O}_2 \) content in hypoxia and decreased in hyperoxia, thus either partially or totally compensating, in terms of \( \text{O}_2 \) delivery, for changes in arterial \( \text{O}_2 \) content (20, 24). Given this scenario, convective \( \text{O}_2 \) delivery would remain relatively constant while the diffusive component of \( \text{O}_2 \) transport would be altered. This situation has been reported previously, where the convective delivery of \( \text{O}_2 \) and the \( \text{O}_2 \) diffusing capacity (\( \text{DO}_2 \)) were unchanged between hypoxia and normoxia, but the \( \text{PO}_2 \) gradient from capillary (\( \text{Pcap}_2 \)) to tissue (\( \text{Ptio}_2 \)) was significantly reduced, decreasing both \( \text{V} \text{O}_{2\text{max}} \) and \( \text{Ptio}_2 \) \( [\text{V} \text{O}_{2\text{max}} = D \text{O}_2 (\text{Pcap}_2 - \text{Ptio}_2)] \) (24).

In our laboratory’s previous study (7), the increase in the \( \text{PCR} \) recovery rate constant in hyperoxia and decrease in hypoxia for the physically active group is also suggestive of this scenario because the most significant effect of hyperoxia is to raise \( \text{blood} \) \( \text{P} \text{O}_2 \) because hemoglobin saturation is already close to its ceiling in normoxia (and muscle blood flow tends to fall in hyperoxia). Hence, it is suggested that in hyperoxia the driving gradient from blood to muscle was enhanced, resulting in an elevated intracellular \( \text{PO}_2 \), facilitating increased oxidative metabolism, and ultimately increased \( \text{PCR} \) recovery rate constants. Conversely, hypoxia may result in elevated blood flows but in a reduced \( \text{O}_2 \) driving gradient and slower \( \text{PCR} \) recovery rate constants (Fig. 2). Thus our laboratory’s previous data indicate that under normoxic conditions the rate constant for \( \text{PCR} \) recovery is limited by \( \text{O}_2 \) availability (probably the diffusive component, rather than convective component) in the exercise-trained subjects (7). However, breathing the hyperoxic gas (100% \( \text{O}_2 \)) did not result in faster \( \text{PCR} \) recovery rate constants for the sedentary subjects (Fig. 2). This suggests that the sedentary group is not limited by \( \text{O}_2 \) availability (particularly \( \text{Ptio}_2 \), but rather by their reduced metabolic capacity, even in the face of an increased driving gradient for \( \text{O}_2 \) from blood to muscle. In both groups, the \( \text{PCR} \) recovery rate constants were slower when breathing the hypoxic gas (10% \( \text{O}_2 \)) and most probably under conditions of elevated muscle \( \text{blood} \) flow which compensates somewhat for reduced \( \text{blood} \) \( \text{O}_2 \) content, again highlighting the importance of the diffusive component of \( \text{O}_2 \) transport in conditions of reduced \( \text{O}_2 \) availability.

Summary. The results presented here demonstrate that \( ^{31} \text{P}-\text{MRS} \) measurements of skeletal muscle \( \text{PCR} \) recovery coupled with variations in \( \text{O}_2 \) availability can distinguish the capacity of muscle to utilize \( \text{O}_2 \) from limitations to \( \text{O}_2 \) supply across sedentary and exercise-trained populations. The finding that \( \text{PCR} \) recovery in sedentary subjects was not enhanced while breathing a hyperoxic gas suggests that the maximal oxidative capacity of these sedentary subjects, unlike their exercise-trained counterparts (7), is limited by mitochondrial capacity and not \( \text{O}_2 \) availability. However, the slowed \( \text{PCR} \) recovery while breathing a hypoxic gas continues to highlight the observation that severe reductions in \( \text{O}_2 \) supply will ultimately exceed even the lowest of mitochondrial capacities as a limitation to maximal oxidative rate. As a whole, these observations are an important step in the continued development and validation of methodologies to distinguish metabolic limitations from \( \text{O}_2 \) supply limitations, which may ultimately become a powerful addition to the study and diagnosis of skeletal muscle pathophysiology and etiologies such as inactivity and aging.

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