Effects of hyperoxia on skeletal muscle carbohydrate metabolism during transient and steady-state exercise

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Effects of hyperoxia on skeletal muscle carbohydrate metabolism during transient and steady-state exercise. J Appl Physiol 98: 250–256, 2005. First published September 17, 2004; doi:10.1152/japplphysiol.00897.2004.—This study compared the effects of inspir- ing either a hyperoxic (60% O2) or normoxic gas (21% O2) while cycling at 70% peak O2 uptake on 1) the ATP derived from substrate phosphorylation during the initial minute of exercise, as estimated from phosphocreatine degradation and lactate accumulation, and 2) the reliance on carbohydrate utilization and oxidation during steady-state cycling, as estimated from net muscle glycogen use and the activity of pyruvate dehydrogenase (PDH) in the active form (PDHa), respectively. We hypothesized that 60% O2 would decrease substrate phosphorylation at the onset of exercise and that it would not affect steady-state exercise PDH activity, and therefore muscle carbohydrate oxidation would be unaltered. Ten active male subjects cycled for 15 min on two occasions while inspiring 21% or 60% O2, balance N2. Blood was obtained throughout and skeletal muscle biopsies were sampled at rest and 1 and 15 min of exercise in each trial. The ATP derived from substrate-level phosphorylation during the initial minute of exercise was unaffected by hyperoxia (21%: ±2.2 ± 11.1; 60%: ±5.4.0 ± 9.5 mmol ATP/kg dry wt). Net glycogen breakdown during 15 min of cycling was reduced during the 60% O2 trial vs. 21% O2 (197.7 ± 25.3 vs. 138.6 ± 16.8 mmol glycosyl units/kg dry wt). Hyperoxia had no effect on PDHa, because it was similar to the 21% O2 trial at rest and during exercise (21%: ±2.2 ± 0.26; 60%: ±2.25 ± 0.30 mmol/kg wet wt −1 min−1). Blood lactate was lower (6.4 ± 1.0 vs. 8.9 ± 1.0 mM) at 15 min of exercise and net muscle lactate accumulation was reduced from 1 to 15 min of exercise in the 60% O2 trial compared with 21% (8.6 ± 5.1 vs. 27.3 ± 5.8 mmol/kg dry wt). We concluded that O2 availability did not limit oxidative phosphorylation in the initial minute of the normoxic trial, because substrate phosphorylation was unaffected by hyperoxia. Muscle glycogenolysis was reduced by hyperoxia during steady-state exercise, but carbohydrate oxidation (PDHa) was unaffected. This closer match between pyruvate production and oxidation during hyperoxia resulted in decreased muscle and blood lactate accumulation. The mechanism responsible for the decreased muscle glycogenolysis during hyperoxia in the present study is not clear.

oxidative and substrate phosphorylation; pyruvate dehydrogenase activity; carbohydrate oxidation; lactate; glycogen

FEW STUDIES HAVE EXAMINED the effects of hyperoxia on human skeletal muscle metabolism during the onset of exercise (7, 22, 23, 37) or during prolonged steady-state exercise (9). Previously, our laboratory examined the effects of breathing 100% O2 on the rate of substrate phosphorylation at the onset of cycling exercise at 65% and 90% peak O2 uptake (V̇O2peak) (7, 37). Both studies reported no decrease in the rate of substrate phosphorylation during the initial 1.5–2 min of exercise during hyperoxia and concluded that oxidative phosphorylation at the onset of normoxic exercise was not limited by O2 availability. However, it has been argued that hyperoxia at the 100% O2 level may not increase bulk O2 delivery to active skeletal muscle, because blood flow in human subjects has been reported to decrease due to hyperoxia-induced vasoconstriction (28, 32, 43), although this is controversial (21, 33).

Consequently, the initial purpose of this study was to determine whether a lower O2 concentration (60%, balance N2), with reduced potential for vasoconstriction, would increase oxidative phosphorylation and decrease substrate phosphorylation at the onset of 70% V̇O2peak cycle exercise compared with room air. Our approach was not to estimate muscle oxidative phosphorylation from O2 uptake measurements at the mouth but to directly measure substrate phosphorylation [phosphocreatine (PCr) degradation and lactate accumulation] in the muscle and assume a reciprocal relationship with oxidative phosphorylation. We hypothesized that 60% O2 would increase oxidative phosphorylation and decrease substrate phosphorylation at the onset of exercise compared with room air.

Numerous studies have reported a decrease in the respiratory exchange ratio (RER) during hyperoxia, suggesting that muscle metabolism is altered during aerobic exercise by decreasing the reliance on carbohydrate and promoting fat oxidation (for review see Ref. 42). However, it is widely noted that exercise measurements of O2 uptake (V̇O2) are technically difficult during hyperoxia and may overestimate V̇O2 and lead to an artificially low RER (29). The often-noted decrease in exercise blood lactate concentration during hyperoxia may also indicate less carbohydrate use in the muscle (1, 14, 22, 27). The only previous study to directly measure muscle lactate and glycogen contents during steady-state (>5 min) exercise reported decreased lactate accumulation during 30 min of exercise at 75% V̇O2peak at 21% inspired O2 compared with 60% but no change in net muscle glycogen use (9). Given the difficulty with indirect calorimetry measurements and the paucity of direct muscle measurements during steady-state exercise with hyperoxia, the second purpose of this study was to estimate carbohydrate use and oxidation during 15 min of cycling at 70% V̇O2peak while breathing gas with either 21% or 60% O2. We measured muscle glycogen utilization, coupled with pyruvate...
and lactate measurements, to estimate net carbohydrate use and measured the activity of pyruvate dehydrogenase (PDH) in its active form (PDHα), to estimate carbohydrate oxidation. It has been well established that measurements of PDHα activity during steady-state cycle and knee extensor exercise closely matched estimates of skeletal muscle carbohydrate oxidation in well-fed subjects (16, 31). We hypothesized that 60% O2 would not affect steady-state exercise PDHα activity and therefore that muscle carbohydrate oxidation would be unaltered.

**METHODS**

**Subjects.** Ten active male subjects volunteered to participate in this study. None was taking any medications or engaging in endurance training more than three times per week. Their mean (± SE) age, height, and \( V_\text{O}_2 \text{peak} \) were 20.3 ± 0.6 yr, 74.8 ± 2.3 kg, and 52.1 ± 1.6 ml·kg\(^{-1}\)·min\(^{-1}\) respectively. The experimental protocol and associated risks were explained both orally and in writing to all subjects, before written consent was obtained. The ethics committees associated with all study protocols. Before all visits, subjects abstained from intense physical activity and consumed a regular diet of 16% carbohydrate, 31% protein during the preceding day. Subjects visited the laboratory in the fed state (16, 31). We hypothesized that 60% O2 match estimates of skeletal muscle carbohydrate oxidation in previously (31). The remainder of the muscle sample was freeze-cycling and immediately frozen in liquid N2. Fewer than 30 s elapsed between cessation of exercise, obtaining of the muscle biopsy, and study.

**Preexperimental protocol.** Subjects initially performed a continuous incremental cycling test to exhaustion to determine pulmonary \( V_\text{O}_2 \text{peak} \) (Quinton Q-plex 1, Quiton Instruments, Seattle, WA) on a cycle ergometer (Lode Instrument, Groningen, The Netherlands). After the \( V_\text{O}_2 \text{peak} \) test, subjects visited the laboratory on three occasions: once for a practice ride and two visits for the experimental protocol. Before all visits, subjects abstained from intense physical activity and consumed a regular diet of ~50% carbohydrates, 30% fat, and 20% protein during the preceding day. Subjects visited the laboratory in the fed state (~2 h after a standardized meal) and repeated this preexperimental meal on all subsequent visits. Subjects underwent a full practice trial, which required them to breathe through a mouthpiece for 20 min at rest and during 15 min of cycling. The practice trial was designed to familiarize subjects with the protocol and breathing continually through the mouthpiece and to confirm the exercise power output of ~70% \( V_\text{O}_2 \text{peak} \). The mean (± SE) absolute power output for the trials was 189 ± 12 W.

**Experimental protocol.** The two experimental trials were conducted at least 1 wk apart. The trials were randomized, and the subjects were blinded to the inspired O2 concentration. During each experimental trial, subjects arrived at the laboratory, and a catheter was inserted into an antecubital vein for blood sampling while they rested quietly on a bed. A resting blood sample was taken, and saline was continuously infused to maintain a patent line. Three incisions were made over the vastus lateralis muscle of one leg under local anesthesia (2% lidocaine, no epinephrine) for muscle biopsy sampling. The subjects then breathed 21% or 60% O2, with balance N2, through a mouthpiece for 20 min at rest and 15 min of cycling. Inspired gases were mixed, analyzed, and stored in a 150-liter tissot spirometer. Blood samples were taken at −20 and −10 min, immediately before the start of exercise (0 min), and at 5, 10 and 15 min during the exercise protocol. Immediately before exercise, with subjects on the bed, a resting biopsy was taken and immediately frozen in liquid N2. Subjects then moved to the cycle ergometer and cycled at their predetermined power output. Additional muscle samples were taken after 1 and 15 min of cycling and immediately frozen in liquid N2. Fewer than 30 s elapsed between cessation of exercise, obtaining of the muscle biopsy, and recommencing of cycling. Muscles samples remained in liquid N2 until analysis. Expired pulmonary gases were collected from 6–8 and 12–14 min and analyzed for expired O2 and CO2 concentration during the 21% inspired O2 trial. Only ventilation measurements were taken during the 60% trials.

**Analyses.** A small piece of frozen wet muscle (~10–15 mg) was removed under liquid N2 for the determination of PDHα as described previously (31). The remainder of the muscle sample was freeze-dried, dissected free of all visible blood and connective tissue, and powdered for metabolite and glycogen analyses. An aliquot of freeze-dried muscle (~10–12 mg) was extracted with 0.5 M perchloric acid (HClO₄) containing 1 mM EDTA and neutralized with 2.2 M KHCO₃. The supernatant was used for the determination of creatine, PCR, ATP, lactate, and glucose-6-phosphate (G-6-P) by enzymatic spectrophotometric assays (3, 11) and for acetyl-CoA and acetylcarnitine with radiometric measures (5). Pyruvate and citrate were analyzed fluorometrically (26). Muscle glycogen content was determined from a second aliquot of freeze-dried muscle (~4–6 mg) from the resting (0 min) and 15-min biopsy samples. All muscle measurements were normalized to the highest total creatine measured among the six biopsies from each subject.

Venous whole blood was placed in a heparinized tube, and a portion was immediately deproteinized in a 1:5 ratio with 0.6% (wt/vol) perchloric acid. The supernatant was stored at −20°C and analyzed for glucose and lactate (3). A second portion of blood was immediately centrifuged, and 400 μl of plasma were added to 100 μl of NaCl and incubated at 56°C for 30 min. The plasma was stored at −20°C and analyzed for free fatty acids (FFA) with a colorimetric assay (Wako NEFA C test kit, Wako Chemicals, Richmond, VA).

**Calculus of ADP, AMP, and substrate phosphorylation.** Muscle free ADP (ADP̄) and AMP (AMP̄) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (6). Specifically, ADP̄ was calculated by using the measured ATP, creatine, and PCR̄ values; an estimated H⁺ concentration; and the creatine kinase constant of 1.66 × 10⁹. The H⁺ concentration was estimated from the measured lactate and pyruvate contents as described previously (35). AMP̄ was calculated from the estimated ADP̄ and measured ATP content using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate (P_i) was calculated by adding the estimated resting free phosphate of 10.8 mmol/kg dry wt (6) to the difference in PCR̄ content (Δ[PCR̄]) minus the accumulation of G-6-P between rest and selected exercise time points. Substrate level phosphorylation was calculated between rest and 1 min using the following equation:

\[
\text{ATP provision rate} = 1.5 \left( \Delta [\text{lactate} - \text{pyruvate}] \right) + \Delta [\text{PCR}] 
\]

where Δ is the difference between rest and 1-min values and brackets indicate concentration (39).

**Statistics.** All data are presented as the means ± SE. A two-way repeated-measures ANOVA (treatment × time) was used to determine significant differences between treatments. When a significant F-ratio was obtained, post hoc analyses were completed using a Student-Newman-Keuls test. A single-tailed paired t-test was used to determine significant differences between treatments during exercise in net glycogen utilization. Statistical significance was accepted at \( P < 0.05 \).

**RESULTS**

**Respiratory measures.** Because of the limitations of indirect calorimetry during hyperoxia, \( V_\text{O}_2 \) and CO2 production (\( V_\text{CO}_2 \)) were not assessed. However, the \( V_\text{O}_2 \), \( V_\text{CO}_2 \), and RER during normoxia were \( 2.80 ± 0.12 \) l/min, \( 2.76 ± 0.11 \) l/min, and \( 0.98 ± 0.01 \) at 7 min and were \( 2.84 ± 0.12 \) l/min, \( 2.74 ± 0.11 \) l/min, and \( 0.96 ± 0.01 \) at 13 min of cycling, respectively. Ventilation was higher (\( P < 0.05 \); trial effect) in the 21% trial at 7 and 13 min (79.3 ± 2.9 and 83.7 ± 3.2 l/min, respectively) compared with 60% hyperoxia (60% O2: 75.9 ± 4.6 and 79.4 ± 4.2 l/min).

**PDHα.** In the 21% O2 trial, PDHα increased significantly from a resting value of 0.49 ± 0.05 to 1.69 ± 0.20 mmol·kg wet wt \(^{-1}\)·min\(^{-1}\) after 1 min of exercise (Fig. 1). After 15 min of exercise, PDHα increased (\( P < 0.05 \)) further to 2.71 ± 0.31...
mmol·kg wet wt\(^{-1}\)·min\(^{-1}\). Hyperoxia had no effect on PDH\(_a\), with similar levels of PDH activation at rest and during exercise compared with 21% O\(_2\) (Fig. 1).

**Muscle metabolites.** PCR degradation and creatine accumulation were similar between trials (Table 1). The muscle content of ATP was unaffected by hyperoxia or exercise in all trials (Table 1). ADP\(_r\), AMP\(_r\), and P\(_{if}\) increased significantly throughout exercise with no difference between trials (Table 1).

There was no difference in the resting and 15-min muscle glycogen contents between trials (Table 2), but there was a decrease (P < 0.05) in the net glycogen utilization over 15 min of exercise in the 60% O\(_2\) trial vs. 21% O\(_2\) (Fig. 2). Muscle G-6-P at rest and during the exercise period was unaffected by level of inspired O\(_2\) or exercise (Table 2). Pyruvate contents were similar between both trials at rest and increased (P < 0.05) similarly during the exercise period (Table 2). Muscle lactate contents were unaffected by hyperoxia at rest and at 1 min of exercise, but they were ~30% lower (P < 0.05) at 15 min in the 60% O\(_2\) vs. 21% O\(_2\) trial (Fig. 3). This resulted in a reduced (P < 0.05) net muscle lactate accumulation from 1 to 15 min of exercise in the 60% O\(_2\) trial (8.6 ± 5.1 mmol/kg dry wt) compared with 21% O\(_2\) (22.8 ± 5.8 mmol/kg dry wt).

Acetyl-CoA, citrate, and acetylcarnitine contents were similar at rest and increased (P < 0.05) similarly between trials during the exercise period (Table 2).

The calculated substrate-level phosphorylation between 0 and 1 min was not different between trials (21%: 52.2 ± 11.1, 60%: 54.0 ± 9.5 mmol ATP/kg dry wt).

**Blood measures.** Blood glucose was unaffected by treatment or exercise throughout the 20-min resting period and the

Table 1. High-energy phosphate contents at rest and during 15 min of exercise at ~70% \(V_{O2}\) peak while breathing air with 21% or 60% \(O_2\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Time, min</th>
<th>0</th>
<th>1</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>55.4±3.7</td>
<td>72.2±5.4(^*)</td>
<td>93.1±5.5(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>51.9±3.4</td>
<td>70.2±6.4(^*)</td>
<td>89.2±6.0(^++)</td>
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<tr>
<td>PCR, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>77.9±2.5</td>
<td>57.1±5.2(^*)</td>
<td>84.8±5.3(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>78.4±2.6</td>
<td>58.2±6.0(^*)</td>
<td>42.4±4.9(^++)</td>
</tr>
<tr>
<td>ATP, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>26.1±0.7</td>
<td>25.5±1.4</td>
<td>23.6±0.9</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>25.0±1.3</td>
<td>22.7±0.8</td>
<td>24.5±1.0</td>
</tr>
<tr>
<td>ADP(_r), μmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>107.4±10.2</td>
<td>152.6±19.7(^*)</td>
<td>254.1±40.3(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>94.3±10.7</td>
<td>144.0±21.6(^*)</td>
<td>253.0±46.2(^++)</td>
</tr>
<tr>
<td>AMP(_r), μmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>0.45±0.08</td>
<td>0.98±0.24</td>
<td>3.30±0.36(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>0.37±0.08</td>
<td>1.03±0.26</td>
<td>2.28±0.76(^++)</td>
</tr>
<tr>
<td>P(_{if}), mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>10.8</td>
<td>24.4±3.3(^*)</td>
<td>46.6±6.2(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>10.8</td>
<td>31.4±3.4(^*)</td>
<td>45.0±3.7(^++)</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects. \(V_{O2}\) peak oxygen uptake; Cr, creatine; PCR, phosphocreatine; P\(_{if}\), free inorganic phosphate. Resting P\(_{if}\) of 10.8 mmol/kg dry wt assumed from Dudley et al. (6). *Significantly different from 0 min, P < 0.05. ++Significantly different from 1 min, P < 0.05.

Table 2. Muscle metabolite data at rest and during 15 min of exercise at ~70% \(V_{O2}\) peak while breathing air with 21% or 60% \(O_2\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
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<th>1</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>418.6±24.4</td>
<td>ND</td>
<td>225.9±16.1(^*)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>427.8±21.8</td>
<td>ND</td>
<td>298.3±24.4(^*)</td>
</tr>
<tr>
<td>G-6-P, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>1.08±0.23</td>
<td>1.93±0.36(^*)</td>
<td>2.62±0.29(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>1.21±0.20</td>
<td>1.66±0.28(^*)</td>
<td>2.96±0.32(^++)</td>
</tr>
<tr>
<td>Pyruvate, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>0.26±0.04</td>
<td>0.32±0.05</td>
<td>0.53±0.05++</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>0.23±0.02</td>
<td>0.37±0.03</td>
<td>0.48±0.07(^++)</td>
</tr>
<tr>
<td>Lactate, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>4.7±0.6</td>
<td>25.7±5.3(^*)</td>
<td>50.7±7.9(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>4.0±0.5</td>
<td>26.4±3.6(^*)</td>
<td>35.8±5.7(^*)</td>
</tr>
<tr>
<td>Acetyl-CoA, μmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>4.0±0.5</td>
<td>9.8±2.0</td>
<td>15.2±3.1(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>3.6±0.8</td>
<td>7.7±2.6</td>
<td>16.3±3.7(^++)</td>
</tr>
<tr>
<td>Citrate, mmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>0.36±0.08</td>
<td>0.32±0.04</td>
<td>0.52±0.05(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>0.28±0.03</td>
<td>0.35±0.03</td>
<td>0.54±0.05(^++)</td>
</tr>
<tr>
<td>Acetylcarnitine, μmol/kg</td>
<td>21% O(_2)</td>
<td>dry wt</td>
<td>4.5±0.8</td>
<td>6.0±0.8(^*)</td>
<td>16.2±0.8(^++)</td>
</tr>
<tr>
<td></td>
<td>60% O(_2)</td>
<td>dry wt</td>
<td>4.3±0.7</td>
<td>6.0±1.2(^*)</td>
<td>16.9±1.3(^++)</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects. G-6-P, glucose-6-phosphate; ND, not determined. *Significantly different from 0 min, P < 0.05. †Significantly different from 1 min, P < 0.05. ‡Significantly different from corresponding time point for 60% O\(_2\), P < 0.05.

Fig. 2. Net glycogen utilization from rest to 15 min of exercise at ~70% \(V_{O2}\) peak while breathing air with 21% or 60% O\(_2\).

Fig. 1. Pyruvate dehydrogenase activity of the active form (PDH\(_a\)) at rest and during 15 min of exercise at ~70% peak \(O_2\) uptake while breathing air with 21% or 60% O\(_2\). Values are means ± SE for 10 subjects. \(w_w\), Wet weight. *Significantly different from 0 min, P < 0.05. †Significantly different from 1 min, P < 0.05.
lactate concentration was also lower during 15 min of steady-state exercise in the hyperoxic trial.

**Effect of hyperoxia on substrate and oxidative phosphorylation at the onset of exercise.** At the onset of exercise, ATP production from oxidative phosphorylation cannot match the rate of ATP hydrolysis (2, 16, 36). This transient shortfall in oxidative energy supply is made up by substrate phosphorylation (PCr utilization and lactate accumulation). One mechanism that may contribute to this lag in oxidative phosphorylation during the onset of exercise is the finite time required to activate the processes of metabolism, or the so-called “metabolic inertia.” A second possibility is that some muscle fibers initially experience a suboptimal O2 supply in the electron transport chain, thereby limiting mitochondrial ATP production (10, 19, 40, 46).

Our unique approach in this study was not to estimate oxidative phosphorylation from measurements of pulmonary VO2, but to directly measure substrate phosphorylation in the muscle, and assume a reciprocal relationship with oxidative phosphorylation at the onset of exercise. A novel finding of the study was that muscle glycogen use was reduced by approximately 30% during hyperoxia. In agreement with our second hypothesis, there was no difference in PDH activity leading to a maintenance of carbohydrate oxidation at the normoxic rate, leaving less pyruvate for conversion to lactate. In line with these findings, the accumulation of muscle lactate was reduced by nearly 30% and blood glucose was elevated by 10.2 ± 0.33 mmol·L⁻¹ during the 15-min exercise period.
measured by 31P-magnetic resonance spectroscopy (12). This suggests that PCr kinetics at exercise onset was not limited by O2 driving pressure during submaximal exercise. Whereas we previously concluded that O2 was not limiting during the onset of exercise at this power output (7, 37), it has been argued that inspiration of 100% O2 may lead to vasoconstriction of skeletal muscle blood flow, such that bulk O2 transport may not be increased to the working skeletal muscle.

The classic work by Bannister and Cunningham (2a) suggested that the optimal hyperoxic level for exercise performance was 60–70% inspired O2 and that higher concentrations may be detrimental. Hyperoxia, at the 100% O2 level, has been shown to decrease blood flow to active skeletal muscles in dogs, leaving bulk O2 delivery unchanged (4, 15, 44, 47). Conversely, two studies using 100% inspired O2 reported no effect on blood flow and an increased O2 delivery during maximal knee extensor and cycle exercise (21, 33). In a related study using VO2 measurements at the mouth, MacDonald et al. (254) previously concluded that O2 was not limiting during the onset of exercise at 70% VO2 peak while breathing 21% O2. It is not readily apparent why the results of these two studies are different, because both relative cycling intensity and inspired O2 contents were the same. Perhaps one explanation could be that the present study utilized 10 subjects, which would have given greater statistical power when examining glycogen breakdown over the 6 subjects used in the Graham et al. study.

The mechanism responsible for the decrease in muscle glycogenolysis during hyperoxia is not clear in the present study because the known allosteric posttransformational regulators of glycogen phosphorylase, ADPf, AMPf, or Pif (16), were unaffected by hyperoxia. Glycogen phosphorylase can also be regulated hormonally by epinephrine. Whereas regulation via epinephrine is generally believed to exert a significant effect on muscle glycogenolysis during prolonged aerobic exercise, it is possible that a reduction in plasma epinephrine with hyperoxic breathing contributed to the reduced glycogenolysis over this short time period. Epinephrine was not measured in this study, but two previous studies demonstrated that increases in epinephrine during submaximal cycle exercise were significantly reduced while breathing 60 or 100% O2 compared with 21% O2 (13, 17). Additional studies need to measure the activity of glycogen phosphorylase in the “a” form as well as blood epinephrine concentrations to better elucidate this as a potential mechanism for the glycogen sparing effect of hyperoxia found in the present study.

Muscle and blood lactate accumulations. Differences in muscle and blood lactate accumulations during steady-state exercise while subjects breathed either hyperoxic or normoxic air could be due to differences in lactate production or differences in lactate removal or a combination of the two. As already noted, there was a 33% decrease in net glycogen breakdown in the 60% vs. 21% O2 trial. Because there was no difference between trials in PDHa, carbohydrate oxidation was maintained at the normoxic rate, leaving less pyruvate for conversion to lactate in the 60% O2 trial. This improved match of carbohydrate utilization vs. oxidation during hyperoxia resulted in an ~29% decrease in the net muscle lactate accumulation over 15 min of cycling compared with the normoxic trial. This “tighter” metabolic control between muscle glycogenolysis and carbohydrate oxidation (PDHa activity) is similar to that found following even short-term endurance exercise training, without any differences in muscle O2 consumption (30). However, unlike the previous training study, the present changes cannot be explained by decreases in ADPf, AMPf, or Pif.

The lactate findings in the present study are similar to previous reports of decreased blood (1, 14, 22, 27) and muscle (9) lactate accumulations with hyperoxia. It has been proposed that the decreased lactate with hyperoxia may be due to decreased lactate production, secondary to reduced glycogenolysis, glycolysis and pyruvate production, and/or increased lactate clearance (1, 14). The novel results of this study argue that decreased lactate production is the major determinant of the reduced muscle and blood lactate accumulation during hyperoxia.

During exercise, blood flow to the liver and other mesenteric organs decreases (24, 34). It has therefore been suggested that less of a decrease in splanchnic blood flow during hyperoxia
may increase lactate clearance by these tissues, compared with the normoxic situation (1, 34). Whereas this has been studied in rats with equivocal results, there is a clear lack of data examining splanchnic blood flow in exercising human subjects during hyperoxia. The only study we are aware of reported a nonsignificant trend of increased hepatosplanchnic blood flow during exercise while breathing 30% O₂ (24). Therefore, the strength of this mechanism for explaining the lower lactate levels remains to be elucidated. However, to clarify this issue, it will be necessary to estimate the production of muscle lactate in normoxic and hyperoxic environments by measuring muscle lactate accumulations coupled with measures of lactate release across the working muscles during steady-state exercise.

Summary. This study investigated the effects of hyperoxia (60% O₂) compared with room air on muscle carbohydrate metabolism at the onset of and during steady-state cycling. Direct measurements of substrate phosphorylation (PCr hydrolysis and muscle lactate accumulation) and activation of PDH during the initial minute of exercise were unaffected by hyperoxia. Therefore, we concluded that O₂ availability does not limit the rate of oxidative phosphorylation and activation of PDH or decrease the requirement for substrate phosphorylation during the onset of exercise at 70% VO₂peak while breathing room air. Hyperoxia decreased net glycogen utilization, but had no effect on carbohydrate oxidation, estimated from PDH activity. Consequently, the production of pyruvate more closely matched the oxidation of pyruvate during hyperoxia, which resulted in a decreased accumulation of muscle and blood lactate levels. Considering that there were no differences between trials in many of the known regulators of glycogen phosphorylase, the mechanism responsible for the decrease in muscle glycogenolysis during hyperoxia is not clear in the present study.

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