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Journal of Applied Physiology 98:250-256, 2005. First published Sep 17, 2004;
doi:10.1152/jappphysiol.00897.2004

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

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Submitted 18 August 2004; accepted in final form 14 September 2004

Stellingwerff, Trent, Lee Glazier, Matthew J. Watt, Paul J. LeBlanc, George J. F. Heigenhauser, and Lawrence L. Spriet. 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/jappphysiol.00897.2004.—This study compared the effects of inspiring 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 (PDH_a), 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%: 52.2 ± 11.1; 60%: 54.0 ± 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 (192.7 ± 25.3 vs. 138.6 ± 16.8 mmol glycosyl units/kg dry wt). Hyperoxia had no effect on PDH_a, because it was similar to the 21% O₂ trial at rest and during exercise (21%: 2.20 ± 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 (PDH_a) 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 ($\dot{V}O_{2\text{ peak}}$) (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% $\dot{V}O_{2\text{ peak}}$ 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 ($\dot{V}O_2$) are technically difficult during hyperoxia and may overestimate $\dot{V}O_2$ 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% $\dot{V}O_{2\text{ peak}}$ 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% $\dot{V}O_{2\text{ peak}}$ while breathing gas with either 21% or 60% O₂. We measured muscle glycogen utilization, coupled with pyruvate

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and lactate measurements, to estimate net carbohydrate use and measured the activity of pyruvate dehydrogenase (PDH) in its active form (PDH_a), to estimate carbohydrate oxidation. It has been well established that measurements of PDH_a activity during steady-state cycle and knee extensor exercise closely match estimates of skeletal muscle carbohydrate oxidation in well-fed subjects (16, 31). We hypothesized that 60% O₂ would not affect steady-state exercise PDH 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 (\pm SE) age, height, and $\dot{V}O_{2\text{ peak}}$ 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 of the University of Guelph and McMaster University approved the study.

Preexperimental protocol. Subjects initially performed a continuous incremental cycling test to exhaustion to determine pulmonary $\dot{V}O_{2\text{ peak}}$ (Quinton Q-plex 1, Quinton Instruments, Seattle, WA) on a cycle ergometer (Lode Instrument, Groningen, The Netherlands). After the $\dot{V}O_{2\text{ peak}}$ 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% $\dot{V}O_{2\text{ peak}}$. The mean (\pm 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 PDH_a 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 μ l of plasma were added to 100 μ l 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).

Calculation of ADP_f, AMP_f and substrate phosphorylation. Muscle free ADP (ADP_f) and AMP (AMP_f) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (6). Specifically, ADP_f was calculated by using the measured ATP, creatine, and PCr values; an estimated H⁺ concentration; and the creatine kinase constant of 1.66×10^9 . The H⁺ concentration was estimated from the measured lactate and pyruvate contents as described previously (35). AMP_f was calculated from the estimated ADP_f and measured ATP content using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate (P_{if}) was calculated by adding the estimated resting free phosphate of 10.8 mmol/kg dry wt (6) to the difference in PCr content (Δ [PCr]) 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:

$$\text{ATP provision rate} = 1.5 (\Delta[\text{lactate} = \text{pyruvate}]) + \Delta[\text{PCr}]$$

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

Statistics. All data are presented as the means \pm SE. A two-way repeated-measures ANOVA (treatment \times 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, $\dot{V}O_2$ and CO₂ production ($\dot{V}CO_2$) were not assessed. However, the $\dot{V}O_2$, $\dot{V}CO_2$, 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).

PDH_a. In the 21% O₂ trial, PDH_a 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, PDH_a increased (*P* < 0.05) further to 2.71 ± 0.31

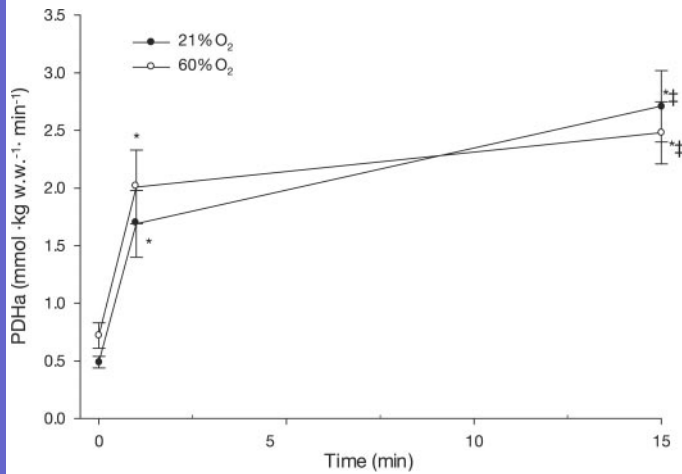


Fig. 1. Pyruvate dehydrogenase activity of the active form (PDH_a) at rest and during 15 min of exercise at ~70% peak O₂ uptake while breathing air with 21% or 60% O₂. Values are means ± SE for 10 subjects. ww, Wet weight. *Significantly different from 0 min, *P* < 0.05. ‡Significantly different from 1 min, *P* < 0.05.

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% O₂ (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_f, AMP_f, 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% O₂ trial vs. 21% O₂ (Fig. 2). Muscle G-6-P at rest and during the exercise period was unaffected by level of inspired O₂ 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

Table 1. High-energy phosphate contents at rest and during 15 min of exercise at ~70% $\dot{V}O_{2\ peak}$ while breathing air with 21% or 60% O₂

Parameter	Condition	Time, min		
		0	1	15
Cr, mmol/kg	21% O ₂	55.4 ± 3.7	72.2 ± 5.4*	93.1 ± 5.5*†
dry wt	60% O ₂	51.9 ± 3.4	70.2 ± 6.4*	89.2 ± 6.0*†
PCr, mmol/kg	21% O ₂	77.9 ± 2.5	57.1 ± 5.2*	38.4 ± 5.3*†
dry wt	60% O ₂	78.4 ± 2.6	58.2 ± 6.0*	42.4 ± 4.9*†
ATP, mmol/kg	21% O ₂	26.1 ± 0.7	25.5 ± 1.4	23.6 ± 0.9
dry wt	60% O ₂	25.0 ± 1.3	22.7 ± 0.8	24.5 ± 1.0
ADP _f , μmol/kg	21% O ₂	107.4 ± 10.2	152.6 ± 19.7*	254.1 ± 40.3*†
dry wt	60% O ₂	94.3 ± 10.7	144.0 ± 21.6*	253.0 ± 46.2*†
AMP _f , μmol/kg	21% O ₂	0.45 ± 0.08	0.98 ± 0.24	3.30 ± 0.96*†
dry wt	60% O ₂	0.37 ± 0.08	1.03 ± 0.26	2.28 ± 0.76*†
P _{if} , mmol/kg	21% O ₂	10.8	24.4 ± 3.3*	46.6 ± 6.2*†
dry wt	60% O ₂	10.8	31.4 ± 3.4*	45.0 ± 3.7*†

Values are means ± SE for 10 subjects. $\dot{V}O_{2\ peak}$, 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% $\dot{V}O_{2\ peak}$ while breathing air with 21% or 60% O₂

Parameter	Condition	Time, min		
		0	1	15
Glycogen, mmol/kg	21% O ₂	418.6 ± 24.4	ND	225.9 ± 16.1*
dry wt	60% O ₂	427.8 ± 21.8	ND	298.3 ± 24.4*
G-6-P, mmol/kg	21% O ₂	1.08 ± 0.23	1.93 ± 0.36*	2.62 ± 0.29*†
dry wt	60% O ₂	1.21 ± 0.20	1.66 ± 0.28*	2.96 ± 0.32*†
Pyruvate, mmol/kg	21% O ₂	0.26 ± 0.04	0.32 ± 0.05	0.53 ± 0.05*†
dry wt	60% O ₂	0.23 ± 0.02	0.37 ± 0.03	0.48 ± 0.07*†
Lactate, mmol/kg	21% O ₂	4.7 ± 0.6	25.7 ± 5.3*	50.7 ± 7.9*†‡
dry wt	60% O ₂	4.0 ± 0.5	26.4 ± 3.6*	35.8 ± 5.7*
Acetyl-CoA, μmol/kg dry wt	21% O ₂	4.0 ± 0.5	9.8 ± 2.0	15.2 ± 3.1*†
dry wt	60% O ₂	3.6 ± 0.8	7.7 ± 2.6	16.3 ± 3.7*†
Citrate, mmol/kg	21% O ₂	0.36 ± 0.08	0.32 ± 0.04	0.52 ± 0.05*†
dry wt	60% O ₂	0.28 ± 0.03	0.35 ± 0.03	0.54 ± 0.05*†
Acetylcarnitine, μmol/kg dry wt	21% O ₂	4.5 ± 0.8	6.0 ± 0.8*	16.2 ± 0.8*†
dry wt	60% O ₂	4.3 ± 0.7	6.0 ± 1.2*	16.9 ± 1.3*†

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% O₂, *P* < 0.05.

min of exercise, but they were ~30% lower (*P* < 0.05) at 15 min in the 60% O₂ vs. 21% O₂ 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% O₂ trial (8.6 ± 5.1 mmol/kg dry wt) compared with 21% O₂ (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

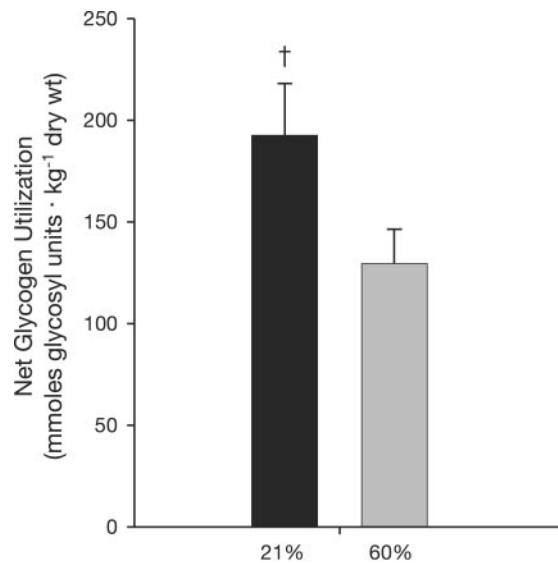


Fig. 2. Net glycogen utilization from rest to 15 min of exercise at ~70% peak O₂ uptake while breathing air with 21% or 60% O₂. Values are means ± SE for 10 subjects. †Significantly different from corresponding time point for 60% O₂, *P* < 0.05.

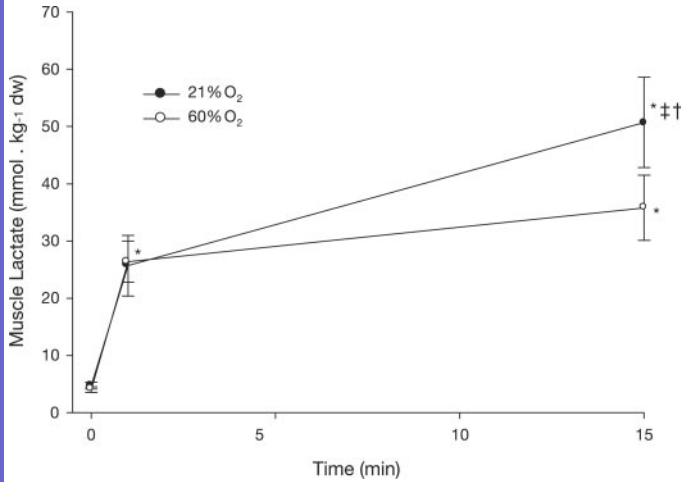


Fig. 3. Muscle lactate at rest and during 15 min of exercise at ~70% peak O₂ uptake while breathing air with 21% or 60% O₂. Values are means ± SE for 10 subjects. dw, Dry wt. *Significantly different from 0 min, *P* < 0.05. ‡Significantly different from 1 min, *P* < 0.05. †Significantly different from corresponding time point for 60% O₂, *P* < 0.05.

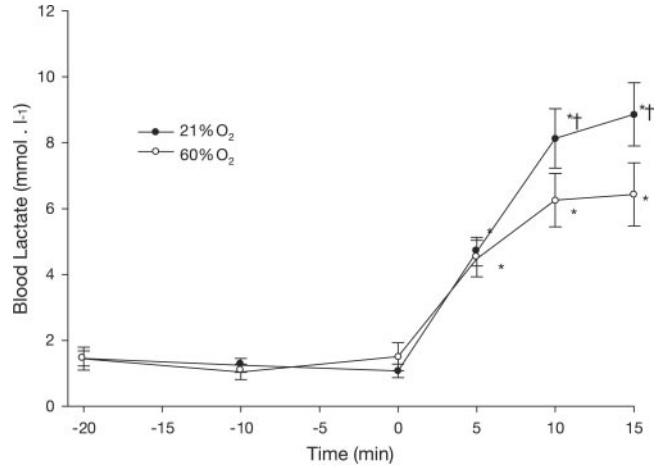


Fig. 4. Blood lactate at rest and during 15 min of exercise at ~70% peak O₂ uptake while breathing air with 21% or 60% O₂. Values are means ± SE for 10 subjects. *Significantly different from 0 min of same condition, *P* < 0.05. †Significantly different from corresponding time point for 60% O₂, *P* < 0.05.

15-min exercise period, whereas serum FFA decreased similarly between treatments during the 15-min exercise period (Table 3). Resting and 5-min exercise blood lactate concentrations were similar between trials (Fig. 4). However, at 10 and 15 min of exercise, blood lactate was significantly lower in the 60% vs. 21% O₂ trial (Fig. 4).

DISCUSSION

The initial purpose of this study was to investigate whether the decreased O₂ content of room air, compared with 60% inspired O₂, contributed to the delay in oxidative phosphorylation at the onset of 70% $\dot{V}O_{2\text{ peak}}$ cycle exercise. Contrary to our hypothesis we found no reduction in the level of substrate phosphorylation at the onset of exercise, suggesting that O₂ availability did not limit oxidative phosphorylation in the 21% O₂ trial. A second aim of this study was to test whether hyperoxia altered the reliance on carbohydrate metabolism during steady-state aerobic exercise, by simultaneously measuring muscle glycogen use and estimating carbohydrate oxidation from PDH_a measurements. A novel finding of the study was that muscle glycogen use was reduced by ~30% during hyperoxia. In agreement with our second hypothesis, there was no difference in PDH_a 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

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 $\dot{V}O_2$, but to directly measure substrate phosphorylation in the muscle, and assume a reciprocal relationship with oxidative phosphorylation at the onset of exercise. A novel aspect of the present work was to examine the hyperoxic level of 60% O₂. Our laboratory has previously demonstrated that breathing 100% inspired O₂ had no effect on substrate and oxidative phosphorylation at the onset of exercise at 65 and 90% $\dot{V}O_{2\text{ peak}}$ (7, 37), and the present study extended these conclusions to 60% inspired O₂. In support of these findings, a recent study also reported no difference in PCr hydrolysis at the onset of exercise while subjects breathed 10, 21, and 100% O₂, as

Table 3. Whole blood glucose and plasma free fatty acid concentrations at rest and during 15 min of exercise at ~70% $\dot{V}O_{2\text{ peak}}$ while breathing air with 21% or 60% O₂

Measure	Condition	Time, min					
		-20	-10	0	5	10	15
Glucose, mM	21% O ₂	4.25±0.15	4.20±0.26	4.38±0.15	4.09±0.17	3.75±0.20	3.97±0.24
	60% O ₂	4.21±0.19	4.40±0.16	4.51±0.15	4.22±0.13	4.21±0.16	4.10±0.25
Plasma FFA, mM	21% O ₂	0.26±0.06	0.24±0.05	0.26±0.05	0.16±0.03*	0.15±0.03*	0.16±0.04*
	60% O ₂	0.30±0.07	0.28±0.07	0.25±0.06	0.17±0.03*	0.16±0.03*	0.15±0.03*

Values are means ± SE for 10 subjects. FFA, free fatty acid. *Significantly different from 0 min, *P* < 0.05.



measured by ^{31}P -magnetic resonance spectroscopy (12). This suggests that PCr kinetics at exercise onset was not limited by O_2 driving pressure during submaximal exercise. Whereas we previously concluded that O_2 was not limiting during the onset of exercise at this power output (7, 37), it has been argued that inspiration of 100% O_2 may lead to vasoconstriction of skeletal muscle blood flow, such that bulk O_2 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 O_2 and that higher concentrations may be detrimental. Hyperoxia, at the 100% O_2 level, has been shown to decrease blood flow to active skeletal muscles in dogs, leaving bulk O_2 delivery unchanged (4, 15, 44, 47). Conversely, two studies using 100% inspired O_2 reported no effect on blood flow and an increased O_2 delivery during maximal knee extensor and cycle exercise (21, 33). In a related study using $\dot{V}\text{O}_2$ measurements at the mouth, MacDonald et al. (23a) reported that breathing 70% O_2 increased the $\dot{V}\text{O}_2$ kinetics ($\sim 80\%$ $\dot{V}\text{O}_{2\text{ peak}}$) but not below the ventilatory threshold ($\sim 50\%$ $\dot{V}\text{O}_{2\text{ peak}}$). Consequently, we examined the effects of 60% O_2 , where the potential vasoconstriction effects of hyperoxia would be reduced. Contrary to our hypothesis, substrate phosphorylation was unaffected while breathing 60% O_2 at the onset of exercise.

If the increase in O_2 provision at the onset of exercise while breathing 60% O_2 had increased oxidative phosphorylation and decreased substrate phosphorylation, we would have expected an increase in PDH_a at 1 min. However, PDH_a was unaffected by hyperoxia as were pyruvate and acetyl-CoA contents, and the ATP-to-ADP ratio, all known regulators of PDH activity (for reviews, see Refs. 20, 38). With these findings taken together, we conclude that O_2 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% $\dot{V}\text{O}_{2\text{ peak}}$ while breathing 21% O_2 .

Effect of hyperoxia on carbohydrate metabolism during steady-state exercise. Whereas previous studies have noted a decrease in the RER during hyperoxia (18, 25, 44, 47, 48), it is widely accepted that measurements of $\dot{V}\text{O}_2$ are technically difficult during hyperoxia (29, 41, 45). In the present study, we used a novel approach to assess the potential for hyperoxia to decrease carbohydrate oxidation during steady-state exercise by measuring PDH_a . It has been well established that measurements of PDH_a and tricarboxylic acid cycle flux during steady-state cycle and knee extensor exercise closely match estimates of skeletal muscle carbohydrate oxidation in well-fed subjects (8, 16, 31). In the present study we found no differences in PDH_a after 15 min of steady-state exercise at 70% $\dot{V}\text{O}_{2\text{ peak}}$, suggesting that carbohydrate oxidation was unaffected by hyperoxia. There was also no effect of hyperoxia on any of the measured regulators of PDH_a , including pyruvate content and the ATP-to-ADP ratio. These findings also suggest that fat oxidation was unaffected by hyperoxia during steady-state cycling at 70% $\dot{V}\text{O}_{2\text{ peak}}$.

Muscle glycogen. A novel finding in the current study was a $\sim 33\%$ reduction ($P < 0.05$) in glycogen utilization over the 15-min cycling period in the 60% O_2 trial vs. 21% O_2 . There are few data examining the effects of hyperoxia on glycogenolysis during steady-state exercise. To our knowledge only

Graham et al. (9) previously examined this and reported no effect of 60% vs. 21% O_2 on glycogen breakdown over 30 min of cycling at 70–75% $\dot{V}\text{O}_{2\text{ peak}}$. It is not readily apparent why the results of these two studies are different, because both relative cycling intensity and inspired O_2 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, ADP_f , AMP_f , or P_{if} (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% O_2 compared with 21% O_2 (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% O_2 trial. Because there was no difference between trials in PDH_a , carbohydrate oxidation was maintained at the normoxic rate, leaving less pyruvate for conversion to lactate in the 60% O_2 trial. This improved match of carbohydrate utilization vs. oxidation during hyperoxia resulted in an $\sim 29\%$ 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 (PDH_a activity) is similar to that found following even short-term endurance exercise training, without any differences in muscle O_2 consumption (30). However, unlike the previous training study, the present changes cannot be explained by decreases in ADP_f , AMP_f , or P_{if} .

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% $\dot{V}_{O_2\text{ peak}}$ 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.

ACKNOWLEDGMENTS

We sincerely thank Drs. Graham Jones and Kieran Killian of McMaster University Hospital, Hamilton, Ontario, for expert medical assistance.

GRANTS

This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada (L. L. Spriet) and the Canadian Institutes of Health Research (G. J. F. Heigenhauser).

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