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## Hyperoxia decreases muscle glycogenolysis, lactate production, and lactate efflux during steady-state exercise

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**Stellingwerff, Trent, Paul J. LeBlanc, Melanie G. Hollidge, George J. F. Heigenhauser, and Lawrence L. Spriet.** Hyperoxia decreases muscle glycogenolysis, lactate production, and lactate efflux during steady-state exercise. *Am J Physiol Endocrinol Metab* 290: E1180–E1190, 2006. First published January 10, 2006; doi:10.1152/ajpendo.00499.2005.—The aim of this study was to determine whether the decreased muscle and blood lactate during exercise with hyperoxia (60% inspired O<sub>2</sub>) vs. room air is due to decreased muscle glycogenolysis, leading to decreased pyruvate and lactate production and efflux. We measured pyruvate oxidation via PDH, muscle pyruvate and lactate accumulation, and lactate and pyruvate efflux to estimate total pyruvate and lactate production during exercise. We hypothesized that 60% O<sub>2</sub> would decrease muscle glycogenolysis, resulting in decreased pyruvate and lactate contents, leading to decreased muscle pyruvate and lactate release with no change in PDH activity. Seven active male subjects cycled for 40 min at 70%  $\dot{V}O_{2\text{ peak}}$  on two occasions when breathing 21 or 60% O<sub>2</sub>. Arterial and femoral venous blood samples and blood flow measurements were obtained throughout exercise, and muscle biopsies were taken at rest and after 10, 20, and 40 min of exercise. Hyperoxia had no effect on leg O<sub>2</sub> delivery, O<sub>2</sub> uptake, or RQ during exercise. Muscle glycogenolysis was reduced by 16% with hyperoxia ( $267 \pm 19$  vs.  $317 \pm 21$  mmol/kg dry wt), translating into a significant, 15% reduction in total pyruvate production over the 40-min exercise period. Decreased pyruvate production during hyperoxia had no effect on PDH activity (pyruvate oxidation) but significantly decreased lactate accumulation (60%:  $22.6 \pm 6.4$  vs. 21%:  $31.3 \pm 8.7$  mmol/kg dry wt), lactate efflux, and total lactate production over 40 min of cycling. Decreased glycogenolysis in hyperoxia was related to an ~44% lower epinephrine concentration and an attenuated accumulation of potent phosphorylase activators ADP<sub>f</sub> and AMP<sub>f</sub> during exercise. Greater phosphorylation potential during hyperoxia was related to a significantly diminished rate of PCr utilization. The tighter metabolic match between pyruvate production and oxidation resulted in a decrease in total lactate production and efflux over 40 min of exercise during hyperoxia.

carbohydrate oxidation; glycogen; pyruvate dehydrogenase activity; blood flow; arterial-venous measurements; oxidative and substrate phosphorylation

EVER SINCE THE CLASSIC WORK by Hill et al. (20) and Margaria et al. (34), it has been well known that breathing hyperoxic air (~60–100% O<sub>2</sub>) improves exercise performance and decreases steady-state exercise blood lactate concentrations compared with breathing room air (for review, see Ref. 54). Two studies

(14, 51) have also reported lower muscle lactate accumulation during exercise lasting longer than 15 min with hyperoxia. These measurements suggest that lactate production and efflux are reduced during exercise under hyperoxic conditions, possibly because of a greater O<sub>2</sub> availability and/or greater oxygen partial pressure than during room air breathing.

It has been proposed that the decreased blood lactate with hyperoxia may be due to decreased lactate production resulting from reduced glycogenolysis, glycolysis, and ultimately, a decreased pyruvate production and/or increased lactate clearance (1, 21). Indeed, we (51) recently examined the effect of hyperoxia on skeletal muscle carbohydrate metabolism and reported a ~33% reduction in glycogen utilization over a 15-min cycling period. We concluded that decreased lactate production via decreased glycogenolysis and pyruvate production with no change in mitochondrial pyruvate oxidation [estimated from pyruvate dehydrogenase (PDH) activity] was the major determinant of the reduced muscle and blood lactate during hyperoxia. However, to clarify these issues it is necessary to measure the production of muscle pyruvate and lactate in normoxic and hyperoxic conditions by measuring muscle pyruvate and lactate accumulations, coupled with measures of pyruvate and lactate release across working muscles during steady-state exercise. Few studies in humans have examined the effects of hyperoxia using arterial and venous (a-v) blood sampling, coupled with blood flow measurements, across the working muscles in humans. These studies found that during whole body cycling (28) or knee extensor exercise (35, 38) there was no effect of hyperoxia on lactate release at either submaximal or peak work rates.

Therefore, the primary aim of this study was to determine whether the decreased muscle lactate accumulation during exercise with hyperoxia is due to decreased muscle glycogenolysis and/or decreased lactate production. We also examined whether a decreased lactate efflux contributed to the lower blood lactate concentration reported during exercise when a hyperoxic gas was breathed. We accomplished this by measuring muscle glycogenolysis, leading to the quantification of the five fates of pyruvate: 1) pyruvate accumulation, 2) pyruvate oxidation [PDH activity (PDHa)], 3) reduction to muscle lactate, 4) lactate efflux, and 5) pyruvate efflux, to estimate total pyruvate and lactate production during 40 min of steady-state cycling at 70%  $\dot{V}O_{2\text{ peak}}$  when subjects breathed either 21 or 60% O<sub>2</sub>. We hypothesized that hyperoxia would decrease

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muscle glycogenolysis, resulting in decreased muscle pyruvate and lactate production and decreased muscle pyruvate and lactate release, with no change in pyruvate oxidation via PDH.

## METHODS

### Subjects

Seven active male subjects volunteered to participate in this study. None were taking medications and all engaged in recreational endurance training no more than five times per week. Their mean ( $\pm$ SE) age, height, weight, and  $\dot{V}O_{2\text{ peak}}$  were  $22.3 \pm 1.2$  yr,  $180 \pm 5$  cm,  $76.1 \pm 4.3$  kg, and  $52.8 \pm 3.0$  ml $\cdot$ kg $^{-1}\cdot$ 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 peak pulmonary oxygen uptake ( $\dot{V}O_{2\text{ peak}}$ , 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. Daily food records were conducted over a 48-h period, and subjects ate the same diet before all experimental trials. In the 24 h before all visits, subjects abstained from intense physical activity and caffeine consumption. Subjects visited the laboratory in the fed state (2–4 h after a standardized meal) for all visits. All subjects underwent a full practice trial, which required them to breathe through a mouthpiece for 20 min at rest and during 40 min of cycling. The practice trial familiarized the subjects with the experimental protocol and breathing through the mouthpiece and confirmed the  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$  power output. The mean ( $\pm$ SE) absolute power output for the trials was  $190 \pm 18$  W, and the relative power output was  $72.4 \pm 1.9\%$  of  $\dot{V}O_{2\text{ peak}}$ . Due to the limitations of indirect calorimetry during hyperoxia,  $\dot{V}O_2$  and  $\text{CO}_2$  production ( $\dot{V}CO_2$ ) were not assessed during the experimental trials. However, on average the  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , respiratory exchange ratio (RER), and ventilation during the 40-min normoxic practice ride were  $2.90 \pm 0.20$ ,  $2.71 \pm 0.16$ ,  $0.93 \pm 0.02$ , and  $80.8 \pm 5.8$  l/min, respectively.

### Experimental Protocol

The two experimental trials consisted of 40 min of cycling at  $70\%$   $\dot{V}O_{2\text{ peak}}$  when subjects breathed either 21 or  $60\%$  inspired  $O_2$  and were conducted at least 2 wk apart. The trials were randomized and the subjects blinded to the inspired  $O_2$  concentration. Before exercise, the radial artery was catheterized percutaneously with a Teflon catheter (20 gauge, 3.2 cm; Baxter, Irvine, CA) after local anesthesia with 0.5 ml of 2% lidocaine, without epinephrine, as previously described (6). The femoral vein was catheterized percutaneously (Thermodilution catheter, model no. 93-135-6F; Baxter) with the use of the Seldinger technique after administration of 3–4 ml of lidocaine (6). Catheters were maintained patent with nonheparinized isotonic saline. A resting (–20 min) arterial blood sample ( $\sim 9$  ml) was then taken. Leg blood flow was also determined at rest using the thermodilution method, as previously described (3). Specifically,  $\sim 10$  ml of nonheparinized saline were injected into the venous catheter, and leg blood flow was determined from the change in temperature as a function of time by use of a portable cardiac output monitor (Spacelab, Redmond, WA). At least three measurements were recorded at each time point and averaged. One leg was then prepared for muscle biopsy sampling; four incisions were made over the vastus lateralis muscle under local anaesthesia (2% lidocaine, no epinephrine).

The subjects then breathed 21 or  $60\%$   $O_2$ , balance  $N_2$ , through a mouthpiece for 20 min at rest in the seated position. Inspired gases were automatically mixed, analyzed for proper  $O_2$  concentration, and stored in a 150-L Tissot spirometer. Immediately before exercise, a resting (0 min) arterial blood sample was drawn, and a resting biopsy was taken, with subjects on the bed, and instantly frozen in liquid  $N_2$  for later analysis. Subjects then moved to the cycle ergometer and commenced cycling for 40 min at a power output to elicit  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$ . Another arterial blood sample was drawn 5 min into exercise, and then both arterial and femoral venous blood samples, combined with leg blood flow measurements, were taken at 10, 20, 30, and 40 min during exercise. Additional muscle samples were taken after 10, 20, and 40 min of cycling and immediately frozen in liquid  $N_2$ . Less than 40 s elapsed between cessation of exercise, the obtaining of the muscle biopsy, and recommencement of cycling.

### Muscle Analyses

A small piece of frozen wet muscle ( $\sim 10$ – $15$  mg) was removed under liquid  $N_2$  for the determination of PDH activity, as described previously (41). The remainder of the muscle sample was freeze-dried, dissected free of visible blood and connective tissue, and powdered for metabolite and glycogen analyses. An aliquot of freeze-dried muscle ( $\sim 10$ – $12$  mg) was extracted with 0.5 M perchloric acid ( $\text{HClO}_4$ ) containing 1 mM EDTA and neutralized with 2.2 M  $\text{KHCO}_3$ . The supernatant was used for the determination of creatine (Cr), phosphocreatine (PCr), ATP, and lactate by enzymatic spectrophotometric assays (5) and acetyl-CoA and acetylcarnitine with radiometric measurements (7). Pyruvate and citrate were analyzed fluorometrically (36). Muscle glycogen content was determined from a second aliquot of freeze-dried muscle ( $\sim 4$ – $6$  mg) from the resting (0 min) and 40-min biopsy samples. All muscle measurements were normalized to the highest total Cr content measured among the 8 biopsies from each subject.

### Blood Sampling and Analysis

Two separate arterial and venous blood samples were drawn at each time point into heparinized plastic syringes and placed on ice. One portion of the first sample was deproteinized in a 1-to-5 ratio with 0.6% PCA (wt/vol). This supernatant was stored at  $-80^\circ\text{C}$  and analyzed for glucose and lactate (5). A second portion of blood was immediately centrifuged, and 400  $\mu\text{l}$  of plasma were added to 100  $\mu\text{l}$  of NaCl and incubated at  $56^\circ\text{C}$  for 30 min to inactivate lipoprotein lipase activity. The plasma was stored at  $-80^\circ\text{C}$  and analyzed for free fatty acids (FFA) with a colorimetric assay (Wako NEFA C test kit; Wako Chemicals, Richmond, VA). A third portion of blood (1.5 ml) was added to 30  $\mu\text{l}$  of EGTA-GSH, mixed thoroughly, and centrifuged. The supernatant was stored at  $-80^\circ\text{C}$  and subsequently analyzed for plasma epinephrine by radioimmunoassay (Epinephrine RIA; Labor Diagnostika Nord, Nordhorn, Germany). The second arterial and venous sample drawn at each time point was analyzed for  $\text{PO}_2$ ,  $\text{PCO}_2$ , pH, and hematocrit via the GEM Premier 3000 blood gas analyzer (Instrumentation Laboratory, Richmond Hill, ON, Canada) and  $O_2$  saturation and hemoglobin (Hb) concentration (OSM3 Hemoximeter; Radiometer, Copenhagen, Denmark).

### Muscle Calculations

Free ADP ( $\text{ADP}_f$ ) and AMP ( $\text{AMP}_f$ ) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (10). Specifically,  $\text{ADP}_f$  was calculated using the measured ATP, Cr, and PCr values, an estimated  $\text{H}^+$  concentration (47), and the creatine kinase constant of  $1.66 \times 10^9$ .  $\text{AMP}_f$  was calculated from the estimated  $\text{ADP}_f$  and measured ATP content using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate ( $\text{P}_i$ ) was calculated by adding the estimated resting free phosphate of 10.8 mmol/kg dry wt (10) to the difference in PCr content ( $\Delta[\text{PCr}]$ ) minus



the accumulation of glucose 6-phosphate (G-6-P) between rest and selected exercise time points.

Blood O<sub>2</sub> and CO<sub>2</sub> Contents and Leg Respiratory Quotient Calculations

Blood O<sub>2</sub> contents (Ca<sub>O<sub>2</sub></sub>, Cv<sub>O<sub>2</sub></sub>) were calculated using measured Hb, Po<sub>2</sub>, and percentage of saturation:

Co<sub>2</sub> (ml/100 ml) = [(Hb × 1.34 × %saturation (So<sub>2</sub>)] + Pa<sub>O<sub>2</sub></sub> × 0.003

Blood CO<sub>2</sub> contents (Ca<sub>CO<sub>2</sub></sub>, Cv<sub>CO<sub>2</sub></sub>) were calculated using measured PCO<sub>2</sub>, PO<sub>2</sub>, pH, and Hb on both arterial and venous samples and were used via the following calculations by Douglas et al. (9):

Cco<sub>2</sub> (ml/100 ml) = plasma Cco<sub>2</sub> × [1 - (0.0289 × [Hb]) / ((3.352 - 0.456 × So<sub>2</sub>) × (8.142 - pH))]

where plasma CCO<sub>2</sub> was determined by the equations of Kelman et al. (27).

Leg respiratory quotient (RQ) was calculated from the ratio of venous-arterial CO<sub>2</sub> (v-a CO<sub>2</sub>) content difference and the arterial-venous O<sub>2</sub> (a-v O<sub>2</sub>) content difference:

leg RQ = leg CO<sub>2</sub> production (v-a CO<sub>2</sub> content difference) / leg O<sub>2</sub> uptake (a-v O<sub>2</sub> content difference)

Leg Uptake and Release of Metabolites

Leg uptake and release of blood metabolites (glucose, pyruvate and lactate) were calculated from their whole blood contents in arterial and venous blood and leg blood flow according to the Fick equation. Since there were differences in the hematocrit over time within a condition and between matched a-v samples, venous samples were corrected for fluid shifts that occurred during exercise. As calculated by the following equation, whole blood venous measurements were corrected for fluid shifts using the differences in Hb to a calculated percent change in blood volume (%ΔBV) (15)

%ΔBV = [(Hb<sub>arterial</sub>/Hb<sub>venous</sub>) - 1] × 100

This value was then multiplied by the measured venous value to yield a corrected value that was used in determining uptake/release for metabolites. Uptake and release of plasma FFA were determined as

above, but venous FFA values were corrected using changes in plasma water volumes (15).

Leg Fuel Utilization Calculations

The rate of pyruvate production from 0–10, 10–20, 20–40 and 0–40 min was calculated from the sum of five inputs: 1) muscle pyruvate accumulation, 2) pyruvate oxidation as estimated from PDH activity, 3) muscle lactate accumulation, 4) lactate efflux, and 5) pyruvate efflux. The rate of lactate production at each of the same time intervals was calculated as the sum of the rates of lactate accumulation and lactate efflux. Glycogenolysis was calculated two ways: 1) direct measurement of the pre- minus postexercise glycogen contents and 2) total pyruvate production minus the rate of glucose uptake plus the estimated rate of G-6-P accumulation. Muscle glucose accumulation was omitted from calculations, because it contributed to ~0.1% of total mmol of pyruvate produced over 40 min of exercise. Glycolysis was not calculated, because the rate of glucose uptake was similar between trials and amounted to only ~5% of the total rate of glycogenolysis. All values are calculated in three carbon units and reported in mmol·min<sup>-1</sup>·single leg<sup>-1</sup>. For all calculations, dry tissue was converted to wet tissue by a wet-to-dry ratio of 4.3, and we also assumed an active muscle mass of 5 kg/single leg (23, 40).

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 paired t-test was used to determine net glycogen utilization, pyruvate and lactate production, and total increases in ADP<sub>f</sub> and AMP<sub>f</sub> between trials. Statistical significance was accepted at P < 0.05.

RESULTS

Muscle metabolism

Muscle glycogenolysis. There was no difference in the resting and 40-min muscle glycogen contents between trials (Table 1). However, there was a 16% decrease (P < 0.05; paired t-test) in the net glycogen utilization over 40 min of cycling in the 60 vs. 21% O<sub>2</sub> trial. This difference translated into a decreased (P < 0.05) rate of glycogenolysis during hyperoxia

Table 1. Muscle metabolite data at rest and during 40 min of exercise at ~70% V̇O<sub>2 peak</sub> when breathing air with 21 or 60% O<sub>2</sub>

Table with 6 columns: Parameter, O2 Condition (%), and Time (min) with sub-columns for 0, 10, 20, and 40. Rows include Glycogen, Glucose, Pyruvate, Lactate, Citrate, Acetyl-CoA, and Acetylcarnitine.

Values are means ± SE, n = 7. V̇O<sub>2 peak</sub>, peak oxygen uptake. ND, not determined. \*Significantly different from 0 min (P < 0.05); †significantly different from previous time point (P < 0.05); ‡significant trial effect of 21% O<sub>2</sub> being different from 60% O<sub>2</sub> (P < 0.05).

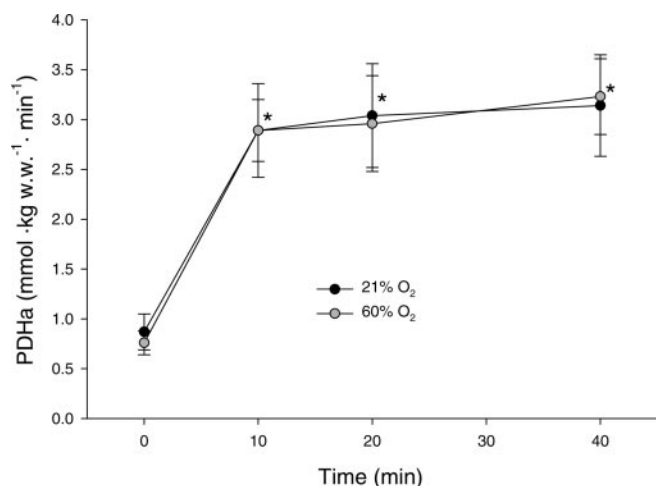


Fig. 1. Pyruvate dehydrogenase activity (PDHa) at rest and during 40 min of exercise at ~70%  $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60% O<sub>2</sub>. Values are means  $\pm$  SE,  $n = 7$ . \*Significantly different from 0 min ( $P < 0.05$ ).

compared with normoxia (60% O<sub>2</sub>:  $15.6 \pm 1.1$  vs. 21% O<sub>2</sub>:  $18.5 \pm 1.2$  mmol·min<sup>-1</sup>·single leg<sup>-1</sup>). The estimated glycogenolytic rates (total pyruvate production + G-6-P accumulation - glucose uptake) were very similar to the glycogen-derived glycogenolytic rates (60% O<sub>2</sub>:  $14.0 \pm 3.2$  vs. 21% O<sub>2</sub>:  $18.1 \pm 2.9$  mmol·min<sup>-1</sup>·single leg<sup>-1</sup>;  $P = 0.15$ ).

**Muscle glucose, pyruvate, and lactate.** Hyperoxia had no effect on glucose and pyruvate contents at rest or during exercise compared with normoxia (Table 1). Muscle lactate contents were consistently lower at rest and throughout the 40 min of cycling in the 60 vs. 21% O<sub>2</sub> trial ( $P < 0.05$ , trial effect; Table 1). This resulted in a reduced ( $P < 0.05$ ) net muscle lactate accumulation from rest to 40 min of exercise in the 60 vs. 21% O<sub>2</sub> trial (60%:  $22.6 \pm 6.4$  vs. 21%:  $31.3 \pm 8.7$  mmol/kg dry wt).

**PDHa.** PDHa increased significantly by 10 min of exercise and remained constant during the final 30 min of exercise, with no difference between trials (Fig. 1).

**Acetyl-CoA, citrate, and acetylcarnitine.** The level of inspired O<sub>2</sub> had no effect on either citrate and acetylcarnitine contents at rest nor during subsequent increases ( $P < 0.05$ )

during exercise in both (Table 1). In contrast, acetyl CoA contents were 23% higher ( $P < 0.05$ , trial effect) during the hyperoxic trial (Table 1).

**High-energy phosphates.** Muscle ATP contents were unaffected by exercise and hyperoxia (Table 2). PCr and Cr contents were similar between treatments at rest, but hyperoxia significantly ( $P < 0.05$ , trial effect) attenuated PCr degradation and Cr accumulation throughout exercise. Calculated ADP<sub>f</sub> and AMP<sub>f</sub> contents in the 60% O<sub>2</sub> trial at 20 and 40 min of exercise were significantly lower ( $P < 0.05$ ) than with 21% O<sub>2</sub> (Table 2). As well, the total increase in ADP<sub>f</sub> and AMP<sub>f</sub> from rest to 40 min was attenuated ( $P < 0.05$ , paired  $t$ -test) in the 60 vs. 21% O<sub>2</sub> trial (Table 2).

**Leg Blood Flow**

Resting (0 min) leg blood flow was similar between treatments after the 20-min equilibration period (Table 3). There were no differences between room air and hyperoxia for leg blood flow during exercise (Table 3).

**Blood Measures**

**Blood gas measurements.** Arterial PO<sub>2</sub>, PCO<sub>2</sub>, and percentage of O<sub>2</sub> saturation were significantly greater ( $P < 0.05$ ) in the 60% O<sub>2</sub> trial than with 21% O<sub>2</sub> at rest (0 min) and during the entire 40-min exercise period (Table 3). There was no effect of hyperoxia on arterial hematocrit or hemoglobin concentration, because both parameters increased significantly throughout the exercise period compared with rest (Table 3).

Hyperoxia significantly ( $P < 0.05$ , trial effect) elevated both CaO<sub>2</sub> and CvO<sub>2</sub> compared with room air (Table 3). Despite differences in arterial and venous O<sub>2</sub> contents, there was no effect of hyperoxia on the a-v O<sub>2</sub> difference (Table 3). Arterial CO<sub>2</sub> contents decreased during exercise in both normoxia and hyperoxia compared with rest, but there was significantly ( $P < 0.05$ ) greater decreases in the 21 vs. 60% O<sub>2</sub> trial (Table 3). Venous CO<sub>2</sub> contents were also lower ( $P < 0.05$ ) during normoxia during the 40 min of cycling (Table 3). However, hyperoxia had no effect on leg O<sub>2</sub> uptake or CO<sub>2</sub> production over the 40-min exercise period, and this produced no difference between trials in leg RQ (Table 3). There was also no difference between trials in connective leg O<sub>2</sub> delivery (CaO<sub>2</sub>  $\times$  leg blood flow; Table 3).

Table 2. High-energy phosphate contents at rest and during 40 min of exercise at ~70%  $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60% O<sub>2</sub>

Parameter	O <sub>2</sub> Condition, %	Time, min			
		0	10	20	40
Cr, mmol/kg dry wt	21‡	47.5 $\pm$ 2.0	96.7 $\pm$ 5.5*	96.7 $\pm$ 5.5*	101.0 $\pm$ 6.2*
	60	45.5 $\pm$ 1.5	91.3 $\pm$ 5.9*	88.8 $\pm$ 6.7*	90.3 $\pm$ 7.5*
PCr, mmol/kg dry wt	21‡	78.7 $\pm$ 2.1	31.1 $\pm$ 4.7*	29.8 $\pm$ 5.5*	28.9 $\pm$ 5.2*
	60	79.6 $\pm$ 2.5	34.6 $\pm$ 3.4*	35.9 $\pm$ 4.1*	38.7 $\pm$ 4.6*
ATP, mmol/kg dry wt	21	25.8 $\pm$ 0.8	23.8 $\pm$ 1.0	24.0 $\pm$ 0.8	23.1 $\pm$ 0.5
	60	24.8 $\pm$ 1.2	23.5 $\pm$ 1.2	25.0 $\pm$ 1.2	25.3 $\pm$ 1.6
ADP <sub>f</sub> , $\mu$ mol/kg dry wt	21‡	82.0 $\pm$ 5.3	281.6 $\pm$ 35.6*	360.2 $\pm$ 47.3*†	431.4 $\pm$ 23.3*†
	60	77.5 $\pm$ 4.9	265.4 $\pm$ 38.1*	275.8 $\pm$ 16.4*	304.8 $\pm$ 23.7*
AMP <sub>f</sub> , $\mu$ mol/kg dry wt	21‡	0.25 $\pm$ 0.03	3.45 $\pm$ 0.81*	6.92 $\pm$ 2.43*†	6.65 $\pm$ 1.55*†
	60	0.23 $\pm$ 0.02	3.12 $\pm$ 0.83*	4.09 $\pm$ 1.65*	3.44 $\pm$ 0.93*

Values are means  $\pm$  SE,  $n = 7$ . Cr, creatine; PCr, phosphocreatine; ADP<sub>f</sub>, free ADP; AMP<sub>f</sub>, free AMP. \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from corresponding time point for 60% O<sub>2</sub> ( $P < 0.05$ ); ‡significant trial effect of 21% O<sub>2</sub> being different from 60% O<sub>2</sub> ( $P < 0.05$ ).

**Table 3. Selected arterial and venous blood content parameters at rest and during 40 min of exercise at  $\sim 70\% \dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$** 

Parameter	$O_2$ Condition, %	Time, min				
		0	10	20	30	40
Arterial $PO_2$ , mmHg	21‡	111.6 ± 2.3†	102.1 ± 3.4*†	99.6 ± 3.4*†	101.1 ± 3.7*†	98.1 ± 4.5*†
	60	301.1 ± 3.1*	303.3 ± 4.0*	303.6 ± 4.3*	301.1 ± 5.8*	306.1 ± 4.8*
Arterial $PCO_2$ , mmHg	21‡	40.2 ± 0.8	36.5 ± 0.9*§†	34.7 ± 1.0*†	33.5 ± 1.0*†	33.8 ± 1.0*†
	60	40.6 ± 1.0	39.7 ± 1.5*§	36.9 ± 1.1*	38.24 ± 1.3*	37.7 ± 1.4*
Arterial % $O_2$ saturation	21‡	99.0 ± 0.1§†	97.3 ± 0.3*†	97.2 ± 0.3*†	97.2 ± 0.4*†	97.0 ± 0.4*†
	60	100.0 ± 0.1§	100.0 ± 0.1*	100.0 ± 0.1*	100.0 ± 0.1*	99.9 ± 0.1*
Arterial %hematocrit	21	44.0 ± 1.5	47.8 ± 1.5*	47.5 ± 1.5*	47.6 ± 1.6*	47.8 ± 1.5*
	60	44.0 ± 1.6	48.0 ± 1.8*	48.3 ± 1.9*	48.0 ± 1.8*	47.0 ± 1.5*
Arterial hemoglobin, g/l	21	137.4 ± 4.1§	151.4 ± 3.1*	150.3 ± 3.2*	150.1 ± 3.7*	149.0 ± 4.0*
	60	140.1 ± 5.7§	154.1 ± 6.3*	155.1 ± 6.5*	151.9 ± 6.1*	149.9 ± 5.9*
Arterial $O_2$ content, ml/dl	21‡	18.6 ± 0.6	20.1 ± 0.4	19.9 ± 0.4	19.9 ± 0.4	19.7 ± 0.5
	60	19.7 ± 0.9	21.6 ± 1.0	21.7 ± 1.0	21.2 ± 1.0	20.9 ± 0.9
Venous $O_2$ content, ml/dl	21‡	ND	4.6 ± 0.4	4.4 ± 0.5	4.3 ± 0.4	4.1 ± 0.4
	60	ND	5.5 ± 0.5	5.5 ± 0.4	5.4 ± 0.4	5.3 ± 0.4
a-v $O_2$ difference, ml/dl	21	ND	15.4 ± 0.4	15.5 ± 0.5	15.6 ± 0.6	15.5 ± 0.6
	60	ND	16.1 ± 0.7	16.2 ± 0.6	15.9 ± 0.7	15.7 ± 0.6
Arterial $CO_2$ content, ml/dl	21‡	53.9 ± 1.9	39.2 ± 2.1*†	39.4 ± 2.4*†	38.6 ± 2.3*†	39.0 ± 2.5*†
	60	54.7 ± 2.1	43.6 ± 2.3*§	44.3 ± 2.2*	44.7 ± 2.4*	45.1 ± 2.4*
Venous $CO_2$ content, ml/dl	21‡	ND	52.9 ± 1.6†	52.8 ± 2.0†	52.3 ± 1.8†	52.9 ± 1.8†
	60	ND	56.5 ± 1.9	58.4 ± 1.9	58.8 ± 2.0	59.3 ± 2.0
Leg $CO_2$ production, l/min	21	ND	1.42 ± 0.14	1.43 ± 0.17	1.41 ± 0.17	1.42 ± 0.16
	60	ND	1.33 ± 0.12	1.55 ± 0.15	1.46 ± 0.16	1.41 ± 0.11
Leg $O_2$ uptake, l/min	21	ND	1.34 ± 0.10	1.32 ± 0.13	1.32 ± 0.12	1.33 ± 0.12
	60	ND	1.26 ± 0.12	1.30 ± 0.12	1.28 ± 0.14	1.22 ± 0.12
Leg $O_2$ delivery, l/min	21	ND	1.74 ± 0.13	1.70 ± 0.15	1.69 ± 0.15	1.68 ± 0.14
	60	ND	1.69 ± 0.15	1.74 ± 0.18	1.72 ± 0.20	1.64 ± 0.17
Leg RQ	21	ND	1.05 ± 0.05	1.07 ± 0.06*	1.05 ± 0.05	1.06 ± 0.05
	60	ND	1.06 ± 0.03	1.19 ± 0.04*	1.14 ± 0.04	1.18 ± 0.05*
Leg blood flow, l/min	21	0.5 ± 0.1	8.7 ± 0.7	8.6 ± 0.8	8.5 ± 0.8	8.6 ± 0.8
	60	0.5 ± 0.1	7.9 ± 0.7	8.0 ± 0.7	8.1 ± 0.8	7.8 ± 0.8

Values are means ± SE,  $n = 7$ . a-v, arterial-venous; RQ, respiratory quotient. \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from 60%  $O_2$  ( $P < 0.05$ ); ‡significant trial effect of 21%  $O_2$  being different from 60%  $O_2$  ( $P < 0.05$ ); §significantly different from previous time point ( $P < 0.05$ ).

**Blood epinephrine.** There were no differences in epinephrine concentrations at rest (Fig. 2). During exercise, epinephrine concentrations increased in both trials but were attenuated throughout the exercise period in the 60%  $O_2$  trial, reaching significance by the 20-min mark (Fig. 2).

**Blood metabolites, concentrations, and leg fluxes.** Hyperoxia had no effect on arterial glucose concentrations and

glucose flux throughout 40 min of cycling (Table 4). Arterial pyruvate concentrations were similar at rest and increased to the same extent during exercise (Table 4). However, venous pyruvate was significantly ( $P < 0.05$ , trial effect) lower during exercise in hyperoxia than with 21%  $O_2$ . On average, this resulted in 27% less ( $P < 0.05$ , trial effect) pyruvate efflux in the hyperoxic trial (Table 4). There was no effect of hyperoxia on plasma FFA concentrations or FFA flux (Table 4).

At rest and after the 20-min breathing period there were no differences in arterial lactate between treatments (Fig. 3). Arterial lactate concentrations increased in both trials during exercise, but the increase was attenuated ( $P < 0.05$ ) in the hyperoxia trial. Venous lactate concentrations were also lower ( $P < 0.05$ , trial effect) during exercise in hyperoxia ( $4.1 \pm 0.5$  vs.  $5.6 \pm 0.8$  mmol/l, average during exercise). On average, lactate efflux was  $\sim 60\%$  greater at each individual time point during normoxia than with the 60%  $O_2$  trial ( $P = 0.11$ ; Fig. 4A). However, total lactate efflux over 40 min of exercise was significantly reduced during the hyperoxic trial (Fig. 4B).

#### Total Pyruvate and Lactate Production

During the first 10 min of exercise, the rate of pyruvate production was 20% decreased ( $P < 0.05$ ), which led to a 43% decreased ( $P < 0.05$ ) rate of lactate production in hyperoxia compared with normoxia (Figs. 4A and 5). There was a trend for a lower rate of pyruvate ( $P = 0.16$ ) production during the 10-to-20- and 20-to-40-min time periods, but this did not reach

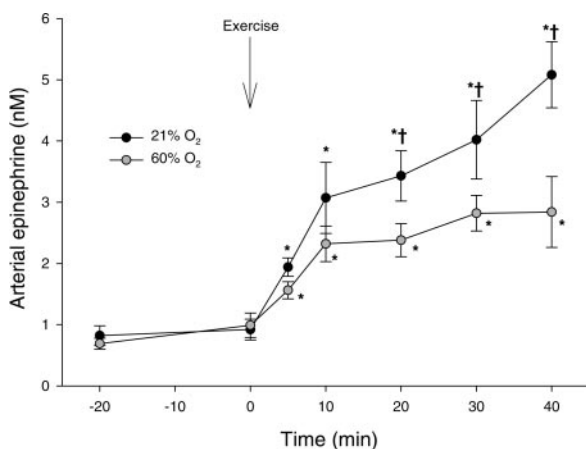


Fig. 2. Arterial epinephrine concentrations at rest and during 40 min of exercise at  $\sim 70\% \dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$ . Values are means ± SE,  $n = 7$ . \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from 60%  $O_2$  ( $P < 0.05$ ); ‡significantly different from 60%  $O_2$  ( $P < 0.05$ ).

Table 4. Concentrations of blood-borne substrates with selected flux data at rest and during 40 min of exercise at ~70%  $\dot{V}O_{2\text{ peak}}$  when breathing in air with 21 or 60%  $O_2$

Parameter	$O_2$ Condition, %	Time, min						
		-0	0	5	10	20	30	40
Arterial glucose, mmol/l	21	5.2±0.3	5.1±0.2	4.3±0.3	4.0±0.2*	3.9±0.4*	3.9±0.4*	4.3±0.4*
	60	4.7±0.4	5.0±0.2	4.8±0.2	4.5±0.3*	3.9±0.2*	4.2±0.2*	4.1±0.2*
Glucose flux, mmol/min	21	ND	ND	ND	4.27±1.47	-0.46±0.89*	2.15±1.55*†	1.89±1.28*
	60	ND	ND	ND	5.56±1.63	-1.36±1.47*	1.75±0.61*†	3.36±1.71*
Arterial pyruvate, $\mu\text{mol/l}$	21	24.2±3.4	24.1±3.4	32.0±7.3	44.6±7.7*	38.4±6.3*	36.6±4.5	31.4±3.4
	60	24.1±3.5	21.7±3.1	27.7±2.3	33.1±2.8*	35.1±3.1*	28.7±3.4	30.9±4.3
Venous pyruvate, $\mu\text{mol/l}$	21‡	ND	ND	ND	156.2±11.1	155.1±12.4	154.3±18.0	165.1±14.6
	60	ND	ND	ND	113.1±11.7	131.8±15.8	120.1±16.8	137.9±19.8
Pyruvate flux, mmol/min	21‡	ND	ND	ND	-0.96±0.10	-0.98±0.09	-0.95±0.10	-1.11±0.11*
	60	ND	ND	ND	-0.63±0.09	-0.76±0.11	-0.71±0.11	-0.83±0.17*
Arterial FFA, mmol/l	21	0.16±0.03	0.21±0.03	0.16±0.02	0.17±0.03	0.22±0.06	0.32±0.11*	0.36±0.10*
	60	0.24±0.05	0.25±0.06	0.16±0.03	0.17±0.03	0.19±0.03	0.29±0.05*	0.39±0.07*
FFA flux, mmol/min	21	ND	ND	ND	-0.05±0.10	0.13±0.07	0.08±0.05	0.08±0.07
	60	ND	ND	ND	-0.01±0.02	-0.03±0.03	0.04±0.05	-0.02±0.09

Values are means ± SE,  $n = 7$ . FFA, free fatty acids. \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from previous time point ( $P < 0.05$ ); ‡significant trial effect of 21%  $O_2$  being different from 60%  $O_2$  ( $P < 0.05$ ). For net flux data, positive numbers represent an uptake and negative numbers are release.

statistical significance. The attenuated lactate efflux, combined with decreased muscle lactate accumulation, resulted in significantly lower ( $P < 0.05$ ) amounts of accumulative lactate production from 0 to 10, 0 to 20, and 0 to 40 min in the hyperoxic treatment (Fig. 4B). When the entire 40-min exercise period was examined, there was a significant 15 and 56% decrease in total pyruvate and lactate production, respectively, in the 60 vs. 21%  $O_2$  trial (Figs. 4B and 5).

DISCUSSION

This study investigated the effects of breathing either 21 or 60% inspired  $O_2$  on skeletal muscle carbohydrate metabolism during 40 min of steady-state cycling at 70%  $\dot{V}O_{2\text{ peak}}$ . This study is the first to examine the effects of hyperoxia during whole body cycling using a-v blood sampling, leg blood flow measurements, and muscle measurements of glycogenolysis

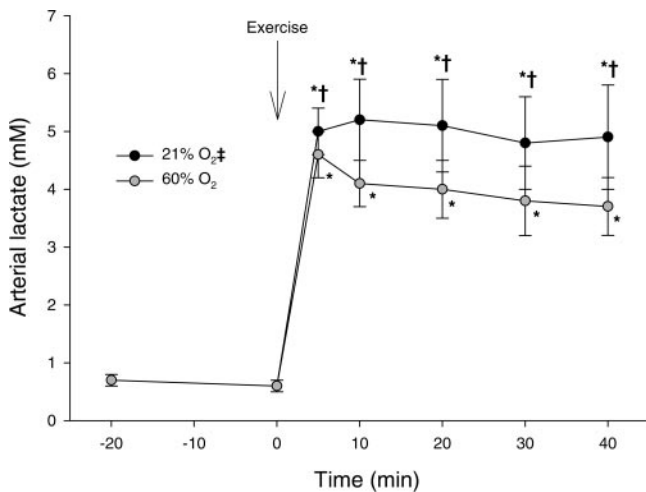


Fig. 3. Arterial lactate concentrations at rest and during 40 min of exercise at ~70%  $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$ . Values are means ± SE,  $n = 7$ . \*Significantly different from 0 min ( $P < 0.05$ ); †significantly different from 60%  $O_2$  ( $P < 0.05$ ); ‡significant trial effect of 21%  $O_2$  being different than 60%  $O_2$  ( $P < 0.05$ ).

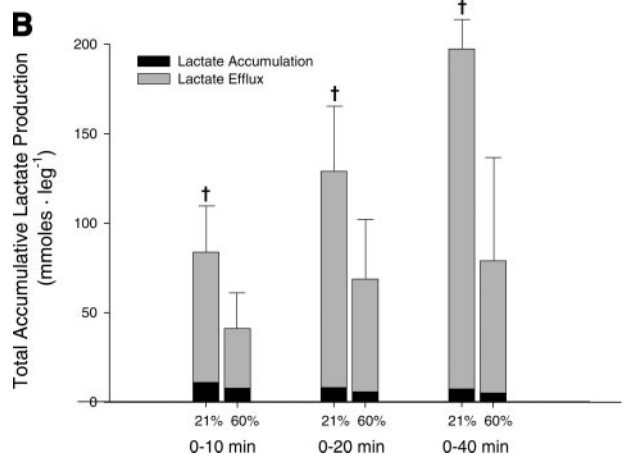
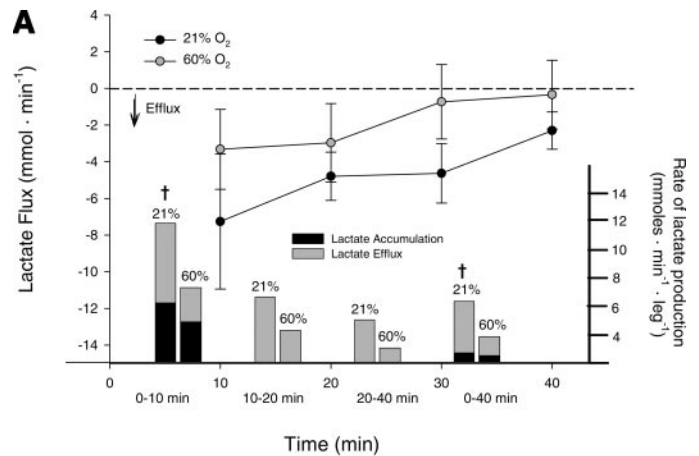


Fig. 4. A: rates of lactate production from 0 to 10, 10 to 20, 20 to 40, and 0 to 40 min of exercise and lactate efflux during exercise at ~70%  $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$ . Values are means ± SE,  $n = 7$ . †Significantly different from 60%  $O_2$  ( $P < 0.05$ ). B: total accumulative lactate production from 0 to 10, 0 to 20, and 0 to 40 min during exercise at ~70%  $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$ . Values are means ± SE,  $n = 7$ . †Significantly different from 60%  $O_2$  ( $P < 0.05$ ).

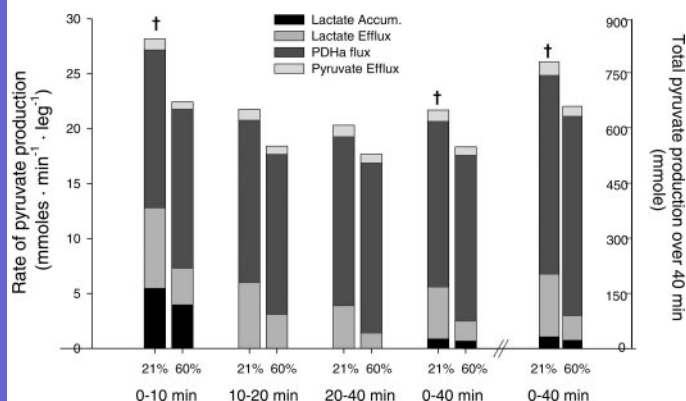


Fig. 5. Rates of pyruvate production from 0 to 10, 10 to 20, 20 to 40, and 0 to 40 min of exercise and total pyruvate production from 0 to 40 min exercise at  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$ . Values are means  $\pm$  SE,  $n = 7$ . †Significantly different from 60%  $O_2$  ( $P < 0.05$ ). Note that pyruvate accumulation was negligible.

and pyruvate and lactate production. Consistent with our hypothesis, we found a significant 16% reduction in glycogenolysis and a 15% decrease in pyruvate production over 40 min of exercise during hyperoxia compared with room air. During hyperoxia, in terms of the five metabolic fates of pyruvate, we found 1) no change in pyruvate accumulation, 2) no change in pyruvate oxidation via PDH, 3) less lactate accumulation, 4) less lactate efflux, and 5) diminished pyruvate efflux over 40 min of cycling (Fig. 6). Small, nonsignificant decreases in leg blood flow during hyperoxia offset the increased arterial  $O_2$  content, resulting into no effect of hyperoxia on leg  $O_2$  delivery. Hyperoxia also had no effect on leg  $O_2$  uptake,  $CO_2$  production, or RQ during exercise.

*Ca<sub>O<sub>2</sub></sub>*, Leg Blood Flow, O<sub>2</sub> Delivery, and Leg Fuel Utilization During Hyperoxia

We found a small,  $\sim 8\%$  decrease in blood flow during hyperoxia, which served to offset the  $\sim 7\%$  increase in arterial  $O_2$  content, and it resulted in no effect of hyperoxia on leg  $O_2$  delivery ( $Ca_{O_2} \times$  leg blood flow). It is well established that breathing hyperoxic air results in elevated  $Ca_{O_2}$  compared with room air (29, 38, 44, 55). However, it still remains controversial as to whether hyperoxia increases bulk  $O_2$  delivery to the active skeletal muscle. Some studies have reported decreased blood flow due to hyperoxia-induced vasoconstriction (38, 42, 55), which has resulted in no change in leg  $O_2$  delivery during both submaximal and maximal exercise. Conversely, other studies (29, 44) utilizing maximal exercise power outputs have found no effect of hyperoxia on leg blood flow, thus elevating leg  $O_2$  delivery. Because there was a significant, threefold increase in  $P_{O_2}$  during hyperoxia, it would imply a greater partial pressure to overcome diffusion limitations compared with normoxia. As well, as suggested by Pedersen et al. (38), a reduced leg blood flow during hyperoxia would also suggest a longer red cell transit time, which would also reduce the potential for diffusion limitations. Similar to previous findings (38), this increased  $P_{O_2}$ , resulting in increased  $Ca_{O_2}$ , had no effect on  $O_2$  uptake or  $CO_2$  production, which resulted into no difference between trials in leg RQ.

*Pyruvate Production Via Muscle Glycogenolysis*

The 16% decrease in glycogenolysis is the primary explanation behind the 15% reduction in pyruvate production during hyperoxia, because glucose uptake and accumulations were minimal and similar between trials (outlined in Fig. 6, *fate 1*). This finding of a decreased glycogen breakdown supports our previous findings of a significant 33% reduction in glycogen utilization during hyperoxia vs. normoxia over 15 min of cycling (51). The rate of glycogen breakdown is regulated by the rate-limiting enzyme glycogen phosphorylase (Phos), which degrades glycogen to glucose 1-phosphate. Phos activity is regulated by a two-stage process. The first stage, or transformation from the less active *b* form of Phos to the more active *a* form, is mediated by  $Ca^{2+}$  at exercise onset and hormonal control through epinephrine-stimulated pathways (8, 43). This transformation is considered to be a form of gross control, and far in excess of what is needed for actual flux through the enzyme, and represents the potential upper limit of flux. The second stage is posttransformational control, where the actual flux through the reaction is fine-tuned by substrate availability of  $P_i$  and direct allosteric regulation by  $AMP_f$  and  $ADP_f$ .

We believe that the decreased epinephrine concentration and attenuated accumulations of adenine nucleotides throughout the last 20 min of exercise during hyperoxia contributed to both the transformational and posttransformational regulation of Phos, resulting in the documented decreases in glycogenolysis and estimated flux through Phos. The decreased epinephrine response during submaximal cycling corroborates two previous findings (19, 25), which reported that epinephrine was significantly reduced when 60 or 100%  $O_2$  was breathed compared with 21%  $O_2$ . The attenuated accumulation of  $ADP_f$  and  $AMP_f$  during hyperoxia would also serve to decrease flux through Phos, because it has been shown that the accumulations of  $ADP_f$  and  $AMP_f$  are potent allosteric activators of Phos *a* in vitro (4, 31). Nevertheless, future studies should target Phos as

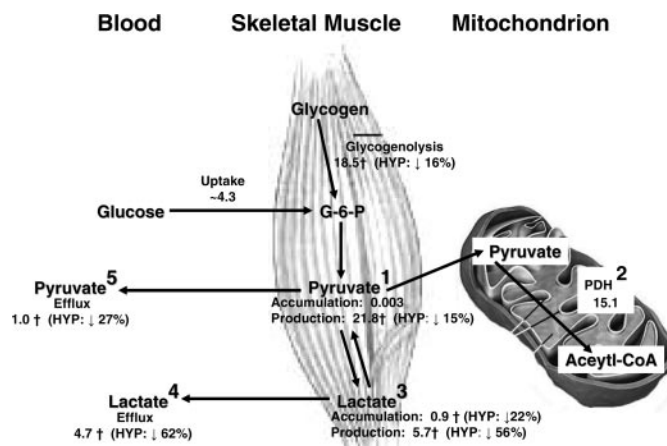


Fig. 6. Overview of skeletal muscle regulation during 40 min of exercise at  $\sim 70\%$   $\dot{V}O_{2\text{ peak}}$  when breathing air with 21 or 60%  $O_2$ . Values represent the normoxic rate in  $mmol \cdot min^{-1} \cdot leg^{-1}$  of glucose uptake, glycogenolysis, and the 5 primary fates of pyruvate: 1) pyruvate accumulation and production (*fate 1*), 2) oxidation via PDH (*fate 2*), 3) lactate accumulation and production (*fate 3*), 4) lactate efflux (*fate 4*), and 5) pyruvate efflux (*fate 5*). †Significantly different from 60%  $O_2$  ( $P < 0.05$ ); brackets indicate the hyperoxic % difference vs. normoxia.



a major regulatory site during hyperoxia by measuring the activity of Phos in the *a* form, coupled with estimates of glycogenolytic flux, to ultimately clarify this as the primary mechanism behind the reduced pyruvate production found during hyperoxia.

*Fates of Pyruvate*

The five major metabolic fates of pyruvate outlined in Fig. 6 include 1) pyruvate accumulation, 2) oxidation via PDH, 3) reduction to lactate, 4) lactate efflux, and 5) pyruvate efflux. Despite the substantial rates of pyruvate production, the rate of pyruvate accumulation was trivial and not different between trials due to the two primary fates of pyruvate, oxidation via PDH and reduction to lactate via the near-equilibrium enzyme lactate dehydrogenase (LDH).

*Pyruvate Oxidation Via PDH*

Similar to our previous study comparing 21 vs. 60% O<sub>2</sub> conditions (51), we found no effect of hyperoxia on PDHa at rest or during 40 min of steady state cycling at 70%  $\dot{V}O_{2\text{ peak}}$  (Fig. 6, *fate 2*).

PDH catalyzes the first irreversible step for carbohydrate (CHO)-derived pyruvate to enter the tricarboxylic cycle (TCA) within the mitochondria to ultimately be oxidized to acetyl-CoA (for reviews see Refs. 49 and 52). It is well established that the measured activity of PDH in the active form correlates highly with CHO flux through the TCA cycle and whole body CHO oxidation during steady-state exercise in well-fed subjects (12, 24, 41). It is also widely accepted that measurements of  $\dot{V}O_2$  and, therefore, RER, are technically difficult during hyperoxia (39, 53, 54). Previous studies (26, 56, 57, 59) have suggested an increase in fat oxidation during hyperoxia, as estimated through decreases in RER. Because there was no difference in PDH between trials, this suggests that CHO oxidation and, therefore, fat oxidation were unaltered by hyperoxia during steady-state cycling.

During rest and exercise conditions, PDH kinase (PDK) is stimulated by high ratios of ATP/ADP, which phosphorylates and inhibits PDH to its inactive *b* form. As well, pyruvate serves to inhibit PDK, thus leaving PDH in its more active *a* form. We found an attenuated accumulation of ADP<sub>f</sub> and AMP<sub>f</sub> and no difference in pyruvate contents throughout the exercise period under hyperoxic conditions compared with normoxia. It remains unknown as to why there were no changes in PDH between trials, because the differences in ADP<sub>f</sub> and AMP<sub>f</sub> would have predicted a diminished PDH activation during hyperoxia.

*Lactate Production and Efflux*

The lactate results of the present study are similar to previous reports of decreased blood (1, 21, 30, 37) and muscle (14, 51) lactate accumulations with hyperoxia (Fig. 6, *fates 3 and 4*). Previous studies in humans have found no effect of inspired O<sub>2</sub> concentration on lactate release during submaximal or peak work rate knee extension (35, 38) or during submaximal cycling between 20 and 92% maximal power output (28). In agreement with these previous findings, when examining lactate release at each individual time point, there was only a nonsignificant trend (*P* = 0.11) for reduced lactate efflux with 60% O<sub>2</sub> during the 40-min exercise period (Fig. 4A). However,

when examining total lactate efflux from 0 to 10, 0 to 20, and 0 to 40 min, there were significantly diminished amounts of lactate efflux throughout the hyperoxic trial (Fig. 4B). This decreased total lactate efflux, combined with decreased muscle lactate accumulation, resulted in ~56% less total lactate production in the hyperoxic treatment (Fig. 6).

Much controversy still exists as to what limits the rate of oxidative phosphorylation, and ultimately the accumulation of blood lactate, during steady-state exercise. The traditional viewpoint suggests that increased lactate formation with increasing exercise intensities is caused by an imbalance between O<sub>2</sub> supply and O<sub>2</sub> requirement, resulting in muscle hypoxia (for review, see Ref. 13). This suboptimal oxygen supply limits the production of ATP in the mitochondria of some fibers and results in increased substrate phosphorylation, glycolysis, and lactate production. An alternative view of muscle and blood lactate formation during steady-state exercise argues that it is primarily due to a mismatch between the rates of pyruvate production via glycogenolysis and pyruvate oxidation via the PDH enzyme complex (for review, see Ref. 50), and the present study seems to support this hypothesis.

Similar to our previous study (51), we found an improved match, or “tighter” metabolic control, during hyperoxia. Hyperoxia resulted in a decreased glycogen breakdown, which resulted in a decreased pyruvate production. Because there was no change in pyruvate oxidation (PDHa) between treatments, this decreased pyruvate production led to a reduced lactate accumulation and efflux, which resulted in decreased blood lactate concentrations, as found in previous studies utilizing hyperoxia during exercise (for review, see Ref. 54).

*Pyruvate Efflux*

The rate of leg pyruvate efflux in the present study was nearly fivefold lower than the rate of leg lactate efflux in the normoxic conditions (pyruvate: ~1 mmol/min vs. lactate efflux: ~4.75 mmol/min; Fig. 6, *fate 5*). During hyperoxia we found a significant, 27% decrease in the rate of pyruvate efflux vs. normoxia, which was due to a significant decrease in pyruvate production with no change in oxidation (PDHa). Our in vivo data of a greater lactate release vs. pyruvate release during normoxic exercise is supported by several studies in humans (2, 32). In contrast, a recent paper by Henderson et al. (18) reported similar rates of net release of pyruvate and lactate during steady-state cycling at 65%  $\dot{V}O_{2\text{ peak}}$  both pre- and posttraining in room air. The differences in pyruvate vs. lactate releases between the present study and Henderson et al. (18) are not readily apparent and are difficult to ascertain, because the former study does not report arterial or venous lactate concentrations or lactate efflux data but only reports lactate to pyruvate ratios. The most likely explanation was that the [<sup>3-<sup>13</sup>C</sup>]lactate tracer that was used to make some of their essential calculations has been proposed to overestimate lactate production and removal (46). Specifically, labeled lactate can easily equilibrate with pyruvate, and thus any metabolism of pyruvate can potentially saturate the TCA pool with label and result in an overestimation of lactate turnover, production, and oxidation. As well, whole blood can also allow interconversion of lactate to pyruvate and vice versa due to the presence of LDH associated with erythrocytes. Indeed, the isotopic equilibration and exchange of pyruvate and lactate in whole blood



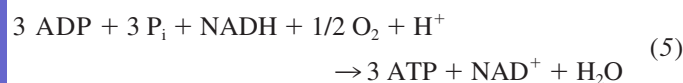
is very rapid in relation to the net production of these substrates (45). Therefore, blood itself can have an effect on the isotopic exchange between lactate and pyruvate and on subsequent calculations of lactate to pyruvate ratio kinetics (45).

In terms of maintaining skeletal muscle cytoplasmic redox state, it would be energetically unfavorable for a significant amount of pyruvate to escape the muscle directly instead of being reduced to lactate via cytoplasmic LDH. This reduction to lactate is vital during situations of high glycolytic flux, because the NADH that is produced by the glyceraldehyde-3-phosphate dehydrogenase reaction needs to be converted back to NAD<sup>+</sup> so that cytoplasmic redox state can be maintained, allowing glycolysis to proceed unimpeded. As well, the monocarboxylate transporter 4 (MCT4) has a much greater affinity to transport lactate out of the cell (*K<sub>m</sub>* is ~28) than pyruvate (*K<sub>m</sub>* is ~150) (33). Indeed, the MCT4 *K<sub>m</sub>* ratio of lactate to pyruvate is 1 to ~5.4, which is virtually equivalent to our finding of a nearly fivefold greater rate of lactate release vs. pyruvate release. Taken together, it would seem that the findings of Henderson et al. (18) are erroneous and at odds with the majority of published results.

*Increased Oxidative Phosphorylation Potential During Exercise With Hyperoxia*

Another novel finding of the present study is that we found a significantly diminished rate of PCr utilization throughout the hyperoxic exercise period compared with normoxia. In support of this, recent studies by Haseler et al. (17) and Hogan et al. (22) that utilized <sup>31</sup>P-magnetic resonance spectroscopy examined the effects of PCr hydrolysis when subjects breathed 10, 21, or 100% O<sub>2</sub> and also found an attenuated PCr utilization with increasing O<sub>2</sub> availability. Our laboratory has previously examined the effects of hyperoxia at exercise onset and found no effect of breathing either 100% (11, 48) or 60% O<sub>2</sub> (51) on calculated substrate phosphorylation, and therefore PCr utilization, during the initial minute of exercise. However, our previous studies (11, 48, 51) examined only exercise onset, and the attenuation of PCr hydrolysis with hyperoxia in the present study occurred primarily after 20 min of exercise. Indeed, there was 10, 17, and 25% less PCr degradation during hyperoxia vs. normoxia at the 10-, 20-, and 40-min points of exercise, respectively. In support of this, a more recent study (16) found that hyperoxia had no effect in altering exercise onset PCr kinetics (first 60 s of exercise) but reported a greater final percentage of PCr utilization, as inspired O<sub>2</sub> content decreased from 100 to 21% O<sub>2</sub> as exercise duration increased (>4 min).

Because we found an attenuated accumulation of ADP<sub>f</sub> and AMP<sub>f</sub>, coupled with decreased PCr utilization and lactate accumulation during hyperoxia, this indicates a decreased energy supply by substrate phosphorylation and a better match between ATP demand and oxidative ATP supply, suggesting that oxidative phosphorylation was increased. Mitochondrial oxidative phosphorylation via the electron transport chain (ETC) is represented by the following equation:



and is regulated by the ratios of [NAD<sup>+</sup>] to [NADH], [ATP] to [ADP] [P<sub>i</sub>], and the availability of O<sub>2</sub> (58). When one of these

two ratios or O<sub>2</sub> availability is altered, a compensatory change occurs in the other ratio to maintain the same driving force for oxidative phosphorylation (58). Despite the fact that we did not find a difference between treatments in leg O<sub>2</sub> delivery, there was significantly increased CaO<sub>2</sub> and PaO<sub>2</sub> during hyperoxia. Therefore, one mechanism for the potential increase in oxidative phosphorylation during hyperoxia could be due to an increase in mitochondrial O<sub>2</sub> supply, leading to the found decreases in ADP<sub>f</sub> and AMP<sub>f</sub>. However, other explanations exist, because a potentially increased fat provision leading to a decrease in the [NAD<sup>+</sup>]-to-[NADH] ratio or an alteration in acid-base status in the muscle [H<sup>+</sup>] could have also contributed to a potential increase in oxidative phosphorylation and, ultimately, the decreased muscle and blood lactate found with hyperoxia.

Although it is controversial and technically difficult to measure during hyperoxia, previous studies have suggested an increase in fat utilization during hyperoxia, as estimated through decreases in RER (26, 56, 57, 59), which would translate into increased NADH provision to the ETC. It should be noted that the decrease in lactate production over 40 min of exercise translates into a paltry decrease of ~190 ATP being provided by substrate phosphorylation during hyperoxia. Considering that 1 mmol of an 18-carbon fatty acid can provide 146 ATP via oxidative phosphorylation, a very minute increase in FFA uptake during hyperoxia, which would be very technically difficult to detect, could have potentially served to offset the decreased energy provision via substrate phosphorylation.

In summary, this is the first study to combine the measurements of glycogenolysis, pyruvate production, and the fates of pyruvate, which are pyruvate accumulation, oxidation (PDH activity), muscle lactate accumulation, and the efflux of lactate and pyruvate, when subjects breathed either 21 or 60% inspired O<sub>2</sub> during 40 min of steady-state cycling at 70%  $\dot{V}O_{2 \text{ peak}}$ . A small, nonsignificant decrease in leg blood flow during hyperoxia offset the increased arterial O<sub>2</sub> content, resulting in no effect on leg O<sub>2</sub> delivery. Despite this, muscle glycogenolysis was decreased by 16%, resulting in a closely matched 15% decrease in pyruvate production over 40 min of exercise during hyperoxia compared with room air. In terms of the metabolic fates of pyruvate, we found no change in pyruvate accumulation or oxidation via PDH, leaving less pyruvate for conversion to lactate in the hyperoxic condition. This tighter metabolic match of a decreased pyruvate production (via decreased glycogenolysis), with no change in pyruvate oxidation, resulted in a significant decrease in total lactate production and total accumulate lactate efflux over 40 min of cycling. Hyperoxia caused a potential increase in oxidative phosphorylation due to a significantly diminished rate of PCr utilization and attenuated accumulations of potent allosteric effectors ADP<sub>f</sub> and AMP<sub>f</sub> during exercise. These effects, coupled with a decreased epinephrine concentration, are the primary explanations for the decrease in glycogenolysis found during hyperoxia. Phos should be targeted as a major regulatory site to ultimately clarify it as the primary mechanism behind the reduced pyruvate production, leading to reduced lactate production and diminished pyruvate and lactate efflux found during exercise under hyperoxic conditions.



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