Thigh oxygen uptake at the onset of intense exercise is not affected by a reduction in oxygen delivery caused by hypoxia

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Christensen PM, Nordsborg NB, Nybo L, Mortensen SP, Sander M, Secher NH, Bangsbo J. Thigh oxygen uptake at the onset of intense exercise is not affected by a reduction in oxygen delivery caused by hypoxia. Am J Physiol Regul Integr Comp Physiol 303: R843–R849, 2012. First published August 29, 2012; doi:10.1152/ajpregu.00201.2012.—In response to hypoxic breathing most studies report slower pulmonary oxygen uptake (V\textsubscript{O\textsubscript{2}}) kinetics at the onset of exercise, but it is not known if this relates to an actual slowing of the V\textsubscript{O\textsubscript{2}} in the active muscles. The aim of the present study was to evaluate whether thigh V\textsubscript{O\textsubscript{2}} is slowed at the onset of intense exercise during acute exposure to hypoxia. Six healthy male subjects (25.8 ± 1.4 yr, 79.8 ± 4.0 kg, means ± SE) performed intense (100 ± 6 watts) two-legged knee-extensor exercise for 2 min in normoxia (NOR) and hypoxia [fractional inspired oxygen concentration (F\textsubscript{I\textsubscript{O\textsubscript{2}}} ) = 0.13; HYP]. Thigh V\textsubscript{O\textsubscript{2}} was measured by frequent arterial and venous blood sampling and blood flow measurements. In arterial blood, oxygen content was reduced (P < 0.05) from 191 ± 5 ml O\textsubscript{2}/l in NOR to 180 ± 5 ml O\textsubscript{2}/l in HYP, and oxygen pressure was reduced (P < 0.001) from 111 ± 4 mmHg in NOR to 63 ± 4 mmHg in HYP. Thigh blood flow was the same in NOR and HYP, and thigh oxygen delivery was consequently reduced (P < 0.05) in HYP, but femoral arterial-venous oxygen difference and thigh V\textsubscript{O\textsubscript{2}} were similar in NOR and HYP. In addition, muscle lactate release was the same in NOR and HYP, and muscle lactate accumulation during the first 25 s of exercise determined from muscle biopsy sampling was also similar (0.35 ± 0.07 and 0.36 ± 0.07 mmol·kg dry wt\textsuperscript{−1}·s\textsuperscript{−1} in NOR and HYP). Thus the increase in thigh V\textsubscript{O\textsubscript{2}} was not attenuated at the onset of intense knee-extensor exercise despite a reduction in oxygen delivery and pressure.

V\textsubscript{O\textsubscript{2}} kinetics; lactate release; O\textsubscript{2} pressure

AT THE ONSET OF EXERCISE the pulmonary and muscle oxygen uptake (V\textsubscript{O\textsubscript{2}}) increases markedly to meet the higher metabolic demand of the working muscles. It has been debated extensively whether inadequate O\textsubscript{2} delivery to the working muscles or a metabolic restraint related to enzyme activation and changes in high-energy phosphates is limiting the increase in V\textsubscript{O\textsubscript{2}} in the initial part of exercise (38).

Hypoxic breathing lowers arterial O\textsubscript{2} content and pressure (22, 42, 43). Slower pulmonary V\textsubscript{O\textsubscript{2}} kinetics has been reported in hypoxia when inhaling air with a fractional inspired oxygen concentration (F\textsubscript{I\textsubscript{O\textsubscript{2}}} ) of 0.12 (7, 8), 0.14 (20, 34), or 0.15 (46, 47) with the majority of the studies investigating only moderate intensity exercise. These findings support the concept that O\textsubscript{2} delivery is limiting the increase in V\textsubscript{O\textsubscript{2}} during exercise.

In contrast, \(\dot{V}_O\textsubscript{2}\) kinetics was not slowed in two studies using a F\textsubscript{I\textsubscript{O\textsubscript{2}}} of 0.15 and 0.12 (17), and 0.15 (8). Nevertheless, most studies suggest a slower \(\dot{V}_O\textsubscript{2}\) response at the onset of exercise in hypoxia, but it remains unclear whether the slower pulmonary response is caused by changes in the \(\dot{V}_O\textsubscript{2}\) of the active muscles. During low intensity exercise thigh \(\dot{V}_O\textsubscript{2}\) during a rest to work transition was reported to be unchanged by hypoxia (F\textsubscript{I\textsubscript{O\textsubscript{2}}} = 0.14) (29). However, thigh \(\dot{V}_O\textsubscript{2}\) at the onset of intense exercise under hypoxic conditions has not been determined.

Several studies measuring thigh \(\dot{V}_O\textsubscript{2}\) in normoxia suggest that O\textsubscript{2} delivery does not limit the rise in thigh \(\dot{V}_O\textsubscript{2}\) at the onset of moderate intensity exercise. In one study (15) using multiple measures of blood flow and blood sampling, it was found that leg blood flow and O\textsubscript{2} delivery was in excess of thigh \(\dot{V}_O\textsubscript{2}\) during moderate intensity cycling. Nyberg et al. (36) found an unchanged thigh \(\dot{V}_O\textsubscript{2}\) during moderate intensity one-legged knee-extensor exercise despite a 50% reduction in O\textsubscript{2} delivery in the initial 10 s of exercise and a ∼30% reduction in the remaining part of exercise. The difference in thigh O\textsubscript{2} delivery and thigh \(\dot{V}_O\textsubscript{2}\) in the control setting was highest in the initial phase (0–20 s) of exercise implying an overshoot in bulk blood flow and hence O\textsubscript{2} delivery to the muscles at the onset of exercise (36), which is also reported by others (3, 15, 23). Similarly, in a canine model no change in muscle \(\dot{V}_O\textsubscript{2}\) was seen when blood flow was elevated approximately threefold before stimulation of the muscle resembling moderate intensity exercise (13). In contrast, a reduction in O\textsubscript{2} delivery has been shown in that model to slow \(\dot{V}_O\textsubscript{2}\) kinetics at moderate intensity (12), which differs from the findings in humans when blood flow to the exercising muscles is reduced (36).

Thigh \(\dot{V}_O\textsubscript{2}\) in the initial phase of intense exercise has not been determined when O\textsubscript{2} delivery and the O\textsubscript{2} pressure gradient are manipulated. This is of interest since intense exercise involves recruitment of fast-twitch muscle fibers high in the motor unit hierarchy (25) that due to their lower capillary density and aerobic enzymatic capacity compared with slow-twitch fibers (9, 21) might be affected by changes in O\textsubscript{2} delivery and O\textsubscript{2} pressure as reported in animal models (19, 32). In support, Grassi et al. (14) reported faster \(\dot{V}_O\textsubscript{2}\) kinetics in the canine model when blood flow was elevated approximately fivefold before stimulations resembling intense exercise at 100% \(\dot{V}_O\textsubscript{2}\)max. In addition, the percentage of slow-twitch fibers appears to be correlated to fast \(\dot{V}_O\textsubscript{2}\) kinetics at heavy but not at moderate intensity exercise (40). At the muscular level \(\dot{V}_O\textsubscript{2}\) kinetics at high intensity are reported to be the same (24) or slower (23) than at moderate intensity exercise.
During moderate intensity knee-extensor exercise in hypoxia, thigh blood flow is increased to compensate for the reduction in arterial O₂ content (10, 22, 42, 43). Thus the slower pulmonary VO₂ kinetics in response to hypoxia during low intensity knee-extensor (7) and cycling (20, 34, 46, 47) exercise may have been caused by changes in O₂ pressure rather than O₂ delivery. During intense knee-extensor exercise maximal blood flow to the quadriceps muscle is similar in normoxia and hypoxia (6, 22, 43), and blood flow kinetics appear to be similar in hypoxia and normoxia (7, 29). Therefore, lowering the arterial O₂ content by breathing hypoxic air allows for the examination of the effect of a reduction in O₂ delivery and O₂ pressure on thigh VO₂ kinetics during intense exercise.

Thus the aim of the present study was to investigate whether a hypoxia-induced reduction in O₂ delivery and O₂ pressure causes slower VO₂ kinetics in the contracting muscles during the initial phase (2 min) of intense exercise.

**METHODS**

**Subjects**

Six healthy recreationally active male subjects at an average age of 25.8 ± 1.4 (means ± SE) yr, a body mass of 79.8 ± 4.0 kg, and a height of 180.2 ± 2.3 cm participated in the study. The subjects were informed of any risks and discomforts associated with the experiments before giving their written, informed consent to participate in accordance with Declaration of Helsinki. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg communities (H-A-2009-016).

**Study Design**

A randomized double-blinded study design was used in which the subjects performed two-legged knee-extensor exercise in a position with a 120° hip angle on two separate days in normoxia (NOR) and hypoxia (HYP). Fractional O₂ inspired (FiO₂) was 0.21 and 0.13 in NOR and HYP, respectively, with the latter condition corresponding to an altitude of 3,500 meters using the Altitude Trainer System (SMTEC, Nyon, Switzerland). In the 2 days before an experiment, the subjects were instructed to refrain from high intensity exercise and to continue their usual physical activity level during the period of the project.

To familiarize the subjects with the work mode, a series of three to five preliminary exercise tests were performed on separate days. An additional purpose was to establish an exercise intensity targeted at causing termination of exercise after 3–6 min, which would ensure a high work intensity resulting in recruitment of high-order motor units (fast-twitch fibers) and near-maximal blood flow. The subjects were told to maintain a kicking frequency of 60 per min, and exercise was terminated when the frequency was below 55 repetitions per minute (rpm). To ensure a stable kicking rhythm a screen displaying the kicking frequency was placed in front of the subjects and verbal instructions from the investigators were given whenever the frequency dropped or increased. The ergometer (a modified Monark 829E bike, Varberg, Sweden) used was electronically controlled, and power output was held constant during the entire work period regardless of small changes in the kicking frequency. All work bouts were preceded by 30 s of passive leg movement in which the load on the ergometer was applied and the flywheel of the ergometer was accelerated by an investigator to the desired kicking frequency of 60 rpm. After the familiarization trials, the subjects performed two main experiments separated by at least 3 wk, in randomized order.

**Main Experiments**

On the experiment days subjects consumed a light breakfast, and after ~60 min of rest, catheters were placed in the femoral artery and vein under local anesthesia. A thermistor was inserted in the venous catheter to measure thigh blood flow using the thermodilution technique (1). In addition, a muscle biopsy at rest was taken from vastus lateralis under local anesthesia using the Bergrönn needle (4). Each experiment day consisted of four exercise bouts separated by 30 min. On both experiment days the subject performed a 15-min control exercise bout at low intensity (30 watts) under normoxic conditions. Blood samples were collected and blood flow was measured after 5, 10, and 15 min. For the three time points as a whole, the average variation in blood flow and arterial-venous O₂ difference on the two experiment days was 15 ± 3% (range 3–25%) and 7 ± 2% (range 0–15%), respectively. The remaining three work periods were performed at a higher intensity (100 ± 6 watts). The first two periods lasted 25 s and the last exercise bout 2 min.

During the first 25-s work bout, arterial blood samples were drawn after 10 and 15 s of exercise. Venous blood samples were drawn after 3, 6, 9, 12, and 15 s of exercise using a rack of five stop-cocks enabling rapid blood sampling (3). In addition a muscle biopsy from vastus lateralis was obtained immediately after the exercise bout.

The second 25-s work bout was used to measure blood flow in the initial part of exercise, with saline infusions being made in the transition from passive to active exercise (from 5 s before exercise to 5 s of exercise) allowing a calculation of blood flow during the first 5 s of exercise and from 10 to 20 s of exercise.

During the exhaustive exercise bout, femoral venous blood was drawn at rest, 10 s before the start of exercise, and after 20, 40, 60, and 120 s. Arterial blood was drawn 5 s before the venous sampling. Venous and arterial blood sampling times were recorded and time at the capillary level was estimated based on the mean transit time at the various phases of exercise (3). Blood flow was measured after each venous blood sample with a time lag of ~6 s. Thirty seconds before each exercise bout, a cuff below the knee was inflated to avoid mixing of blood from the lower leg.

**Measurements and Analyses**

**Blood flow.** Thigh blood flow was determined using the thermodilution technique (1) as modified by Gonzalez-Alonso and colleagues (11). Venous blood temperature was measured during a constant infusion of ice-cold saline (~4°C). Thigh blood flow before and during exercise was not different in NOR and HYP. To reduce fluctuations in flow measurements performed on separate days, an average flow curve for NOR and HYP was constructed for each subject and used for calculations in accordance with previous studies (35). To validate this procedure an additional experiment was performed using the same ergometer and degree of hypoxia with seven healthy male subjects (age 27.1 ± 1.1 yr, weight 78.7 ± 3.0 kg, height 185.7 ± 1.5 cm). The subjects performed intense two-legged knee-extensor exercise (106 ± 6 watts) for 2 min on two separate days in NOR and HYP in randomized order, and thigh blood flow was measured with Doppler ultrasound (Logic E9, GE Healthcare) (33, 41) at rest, during passive exercise, and after 5, 10, 15, 20, 30, 45, 60, 90, and 120 s of exercise. There was no difference between NOR and HYP (Fig. 1). Blood flow measured with Doppler ultrasound was 3.4 ± 0.2 and 3.4 ± 0.2 ml/min in NOR and HYP after 20 s of exercise, increasing to 4.0 ± 0.3 and 4.1 ± 0.3 after 60 s, and 4.6 ± 0.2 and 4.9 ± 0.2 ml/min after 120 s in NOR and HYP, respectively.

**Blood analyses.** Arterial and venous samples were drawn in heparinized 2-ml syringes and placed on ice and analyzed within 60 min (ABL800 system, Radiometer, Copenhagen, Denmark) for hemoglobin (Hb) concentration (g/l), O₂ saturation (%), Po₂ (mmHg), PCO₂ (mmHg), lactate (mmol/l), and pH.

**Muscle analyses.** Muscle biopsies were frozen immediately in liquid nitrogen and stored at ~80°C until analyzed. The obtained
biopsies were freeze-dried and dissected free from fat, blood, and connective tissue and after freeze-drying analyzed for muscle lactate.

Calculations. O₂ content (ml/l) in arterial (a) and venous (v) blood was calculated as: O₂ content = Hb (g/l) × 1.34 ml O₂/g Hb × O₂ saturation (%) + PO₂ (mmHg) × 0.03 ml O₂-mmHg⁻¹-l⁻¹. Thigh O₂ delivery (ml/min) was calculated as: thigh O₂ delivery = thigh blood flow (l/min) × arterial O₂ content (ml/l). Thigh VO₂ (ml/min) was calculated using the Fick’s principle: VO₂ = thigh blood flow (l/min) × (arterial − venous O₂ content) (ml O₂/l). Lactate release (mmol/min) was calculated as: lactate release = thigh blood flow (l/min) × (venous − arterial lactate) (mmol/l). Net lactate accumulation (mmol·kg dry wt⁻¹·s⁻¹) for the first 25 s of exercise was calculated as: lactate accumulation = (muscle lactate 25 s − muscle lactate rest)/25 s assuming a negligible change in muscle lactate during the 30 s of passive exercise before the onset of exercise.

Statistics

Differences between HYP and NOR were examined using a two-way ANOVA for repeated measures with time and condition (HYP and NOR) as factors. If a significant main effect (P < 0.05) was observed, a Student-Newman-Keuls test was applied to identify at which time points NOR and HYP differed. Values are presented as means ± SE.

RESULTS

Thigh Blood Flow

Thigh blood flow was not different in HYP and NOR and thus averaged together (see METHODS). Immediately before exercise thigh blood flow was 1.69 ± 0.10 l/min increasing to 2.95 ± 0.20 and 4.13 ± 0.27 l/min after 16 and 60 s, respectively. After 2 min of exercise, thigh blood flow was 4.24 ± 0.35 l/min (Fig. 1).

Blood Gases

In HYP, arterial PO₂ was lower (P < 0.001) than in NOR throughout the exercise bout with average values being 63 ± 4 and 111 ± 4 mmHg, respectively (Fig. 2). At the onset of exercise there was an immediate drop in venous PO₂ in both HYP and NOR, with an overall effect (P < 0.05) for HYP showing marginally lower values (Fig. 2).

Thigh O₂ Delivery

Arterial O₂ content was lower (P < 0.05) in HYP compared with NOR with average values of 180 ± 5 and 191 ± 5 ml O₂/l, respectively (Fig. 3A). Leg O₂ delivery was reduced (P < 0.05) in HYP compared with NOR (Fig. 4A), with the difference being 21 ± 9, 29 ± 16, and 59 ± 13 ml O₂/min immediately before exercise, after 16 and 60 s of exercise, respectively, amounting to a 5–7% average reduction.

Thigh O₂ Uptake

Venous O₂ content was lower in five of six subjects in HYP with average values of 92 ± 7 and 101 ± 4 ml O₂/l in HYP and NOR, respectively, for all venous samples, but this did not reach statistical significance (P = 0.2). The venous O₂ content before exercise was 133 ± 11 and 145 ± 5 ml/l in HYP and NOR, respectively. After 16 s of exercise, it was 71 ± 9 and 79 ± 7 ml/l, for HYP and NOR, respectively, and 53 ± 7 and 61 ± 6 ml/l, respectively, after 60 s. After 2 min of exercise venous O₂ content was 53 ± 7 and 58 ± 4 ml/l in HYP and NOR (Fig. 3A).

Thigh O₂ extraction (arterial O₂ − venous O₂) was not different between the two conditions (Fig. 3B). Just before exercise the extraction was 46 ± 10 and 46 ± 8 ml/l in HYP and NOR, respectively, being 111 ± 7 and 113 ± 4 ml/l, respectively, after −16 s of exercise, and 125 ± 6 and 132 ± 5 ml/l, respectively, after −60 s and 132 ± 5 and 138 ± 5 ml/l, respectively, after 2 min of exercise.

Thigh VO₂ in HYP did not differ from NOR (Fig. 4B). Immediately before exercise it was 79 ± 18 and 79 ± 16 ml/min, being 320 ± 8 and 332 ± 18 ml/min, respectively,

![Fig. 1. Thigh blood flow during intense two-legged knee extensor exercise. Dashed line shows the average flow determined with the thermodilution technique in normoxia and hypoxia (AVG) used in the main experiment (100 ± 6 watts, n = 6), whereas the full lines shows blood flow in normoxia (NOR, filled symbols) and hypoxia (HYP, open symbols) measured with the Doppler ultrasound technique (106 ± 6 watts, n = 7). See METHODS for further details. Values are means ± SE.](image-url)
and the total net lactate release during the 2 min of exercise was not statistically different (14.7 ± 1.2 vs. 16.8 ± 1.7 mmol, respectively). Similarly, the net rate of muscle lactate accumulation (n = 4) for the first 25 s of exercise was not different reaching values of 0.36 ± 0.07 and 0.35 ± 0.07 mmol·kg dry wt⁻¹·s⁻¹ in HYP and NOR, respectively.

**DISCUSSION**

The main finding of the present study was that despite 5–7% lowering of O₂ delivery and a ~40% reduction of O₂ pressure caused by hypoxia (FIO₂ = 0.13), the increase in thigh VO₂ in the initial phase of intense two-legged knee-extensor exercise was not attenuated. Furthermore, muscle lactate production in hypoxia was not different from normoxia. These findings suggest that the increase in VO₂ during the initial phase of intense exercise is not limited by O₂ delivery or pressure in normoxia.

Arterial O₂ pressure was reduced by ~40% and O₂ delivery was lowered by 5–7% during the first 60 s of intense exercise by inhaling hypoxic air, without compromising the increase in thigh VO₂. Similarly, during knee-extensor exercise at a moderate intensity, thigh VO₂ was not affected by a similar degree of hypoxia as used in the present study (29) or a marked reduction (30–50%) of blood flow (36). Thus it appears that both during moderate and intense exercise the speed of which thigh VO₂ is raised is not lowered by a reduction in O₂ delivery and pressure. These findings suggest a limitation within the muscle fibers to the rise in thigh VO₂ in the initial phase of exercise.

Results related to anaerobic energy turnover are presented in Table 1. Both arterial and venous lactate levels were higher (P < 0.05) in HYP compared with NOR from 30 to 120 s of exercise. Just before exercise, the arterial and venous lactate concentration in HYP was 0.6 ± 0.0 and 0.8 ± 0.1 mmol/l, respectively, and 0.6 ± 0.0 and 0.7 ± 0.1 mmol/l in NOR. After 1 min of exercise, arterial and venous lactate in HYP was 2.8 ± 0.2 and 4.9 ± 0.4 mmol/l, respectively, and 1.8 ± 0.2 and 4.3 ± 0.3 mmol/l, respectively, in NOR. After 2 min arterial and venous values in HYP increased to 5.3 ± 0.3 and 8.0 ± 0.4 mmol/l, respectively, and 4.4 ± 0.5 and 7.2 ± 0.6 mmol/l, respectively, in NOR.

The lactate venous-arterial difference was not different between HYP and NOR. Values after 16 s were 0.6 ± 0.1 and 0.6 ± 0.1 mmol/l in HYP and NOR, respectively, and 2.1 ± 0.2 and 2.5 ± 0.2 mmol/l after 1 min and 2.7 ± 0.3 and 2.8 ± 0.7 mmol/l after 2 min. Similarly, no significant difference was observed in the net release of lactate between HYP and NOR,
Thigh V\textsubscript{O2} kinetics has been observed to be slower at high compared with moderate intensity knee-extensor exercise (23), and it has been proposed that the slower kinetics was related to a greater recruitment of fast-twitch fibers during the intense exercise, which is supported by findings of slower pulmonary V\textsubscript{O2} kinetics during high intensity cycling in subjects with a high proportion of fast-twitch fibers (40). Nevertheless, the finding in the present study suggests that even at a high workload with an expected large recruitment of fast-twitch fibers, thigh V\textsubscript{O2} is not sensitive to a reduction in O\textsubscript{2} pressure and O\textsubscript{2} delivery at the onset of exercise. This is in contrast to what has been observed in an animal model where hypoxia (F\textsubscript{IO2} = 0.1) caused larger increases in H\textsuperscript{+}, lactate, and AMP in muscles composed of a large proportion of fast-twitch muscle fibers (19), probably reflecting a reduced V\textsubscript{O2}. The difference in results may be related to the higher degree of hypoxia used in the animal study compared with the present study. In the present study it is not possible to distinguish between effects from the hypoxia-induced reduction in O\textsubscript{2} pressure (~40%) and O\textsubscript{2} delivery (~6%) on thigh V\textsubscript{O2} since both parameters were affected. Nevertheless it seems apparent that the diffusive driving pressure for oxygen from the capillaries to the myocytes has limited influence on V\textsubscript{O2} kinetics. Future studies may examine if muscle V\textsubscript{O2} during intense exercise in humans is affected by more marked reductions in O\textsubscript{2} delivery.

Muscle lactate accumulation during the first 25 s of exercise was similar in normoxia and hypoxia in the present study. In addition, the lactate efflux from the working muscles during the first 2 min of exercise was also similar, suggesting that glycolytic energy production was the same. The effect of hypoxia on muscle lactate release and accumulation in the initial phase of exercise does not appear to have been investigated previously. A few studies have compared the muscle lactate response during exercise in normoxia and hypoxia. Muscle lactate was higher after moderate intensity cycling for 4 min (148 watts, 53% V\textsubscript{O2}max in normoxia) at a F\textsubscript{IO2} of ~0.13 (26) and for 1 min (154 watts, ~55% V\textsubscript{O2}max in normoxia) at a F\textsubscript{IO2} of 0.11 (37). Untrained subjects cycled at moderate intensity (66% V\textsubscript{O2}max in normoxia) inhaling air with a F\textsubscript{IO2} of 0.14 and had higher muscle lactate after 1 and 3 min, with values of ~15 mmol/kg dry wt in normoxia and ~20 and 30 mmol/kg dry wt in hypoxia, but after 5 days of aerobic training the differences disappeared (16). In another study subjects cycled for 45 min at a low intensity (89 Watts) and muscle lactate was moderately elevated and higher in hypoxia (F\textsubscript{IO2} ~ 0.12) than in normoxia (23 vs. 11 mmol/kg dry wt), whereas lactate release was the same (5). Furthermore, lactate efflux was higher in hypoxia than in normoxia during incremental single-leg knee extensor exercise using 3–5 stages, each lasting 2–3 min at a F\textsubscript{IO2} of 0.12 (42) or after 7.5 min of steady-state two-legged knee-extensor exercise at 72 watts with a F\textsubscript{IO2} of 0.14 (2). The discrepancy between the results of these studies and the present study may be due to the higher intensity in the present study with the subjects having a high lactate production and efflux even in normoxia. The discrepancy could also be related to timing of the measurements. In the present study, lactate release was measured from the onset of exercise, and a muscle biopsy was taken already after 25 s of exercise. Nuclear magnetic resonance has been used to estimate changes in metabolites related to anaerobic energy turnover during exercise. In one study (18) submaximal exercise with the calf muscle in hypoxia (F\textsubscript{IO2} = 0.1) did not alter breakdown of creatine phosphate compared with a normoxic situation which may indicate an unchanged V\textsubscript{O2} given the close association between these two variables (44). Estimated intramuscular pH after 40 s of exercise however was reduced in hypoxia indicative of a higher anaerobic energy turnover. During intense knee-extensor exercise in moderate hypoxia (F\textsubscript{IO2} = 0.145) and normoxia in the prone position with fatigue established after ~6.5 and 8 min, respectively, changes in creatine phosphate, P\textsubscript{i}, and ADP were higher in hypoxia after 60 s, and that was also the case for P\textsubscript{i} after 120 s of exercise, indicating a higher anaerobic energy turnover in hypoxia (48). The apparent difference in the anaerobic response to hypoxia between this and the present study may be related to the exercise modality, since prone and supine exercise is associated with a low perfusion pressure, blood flow, and O\textsubscript{2} delivery compared with upright exercise in the present study. Indeed blood flow kinetics is slower during supine compared with upright exercise (28). Thus O\textsubscript{2} delivery may be close to a critical level already in normoxia in the prone position, and a threshold for oxygen delivery (O\textsubscript{2} demand in excess of O\textsubscript{2} delivery) (38) may be reached when combining the prone position with hypoxic breathing in the nuclear magnetic resonance study (48), but that was not evaluated since thigh or pulmonary V\textsubscript{O2} was not measured. Nevertheless, the findings in the present study of the same degree of muscle lactate accumulation and release in the initial phase of exercise in hypoxia and normoxia support that the aerobic metabolism was not compromised in the hypoxic condition.

An unchanged thigh V\textsubscript{O2} response during knee-extensor exercise despite a reduction in O\textsubscript{2} delivery in the present study is in agreement with what is reported using pulmonary measurements in untrained subjects inhaling air with a F\textsubscript{IO2} of 0.12 and 0.15 during moderate intensity cycling exercise (17) and during heavy intensity cycling at a F\textsubscript{IO2} at 0.15 (8). In contrast, pulmonary V\textsubscript{O2} kinetics was slower during cycling while inhaling air with a F\textsubscript{IO2} of 0.12 (8), 0.14 (20, 34), and 0.15 (46, 47) and also during knee-extensor exercise with a F\textsubscript{IO2} of 0.12 (7). Nevertheless, the discrepancy between the findings of Engelen et al. (8) and Delorey et al. (7) and the present study

| Table 1. Changes in lactate metabolism during intense two-legged knee extensor exercise for 2 min in hypoxia and normoxia |
|---------------------------------|----------|----------|
| Net muscle lactate accumulation (0–25 s), mmol·kg dry wt·s\textsuperscript{-1} | 0.36 ± 0.07 | 0.35 ± 0.07 |
| Net lactate release (0–25 s), mmol | 1.97 ± 0.25 | 1.96 ± 0.13 |
| Net lactate release (25–120 s), mmol | 12.75 ± 1.09 | 14.85 ± 1.79 |

Values are means ± SE. Intense two-legged knee extensor exercise was set at 100 ± 6 watts. HYP, hypoxia; NOR, normoxia.
may be explained by the lower inhalation O2 fraction (0.12) in the former two studies, and that a threshold for O2 delivery may have been reached (38). The slower pulmonary V\(\text{O}_2\) response may have been due to a reduction in the V\(\text{O}_2\) in the nonactive tissues since hypoxia augments the sympathetic nerve activity causing vasoconstriction in most tissues apart from the working muscles (31). This could be part of the explanation for the higher arterial lactate levels in the present study seen after 30 s of exercise in hypoxia despite no difference in lactate release from the thigh. Blood flow to the gut at rest in hypoxia (FI\(O_2\) \(\sim\) 0.10–0.12) is reduced by \(-\sim\) 15\% in humans (27), and the intestines are reported be become more reliant on anaerobic energy turnover during intense exercise at 5,000 m (FI\(O_2\) \(\sim\) 0.10) probably due a reduction in O2 delivery (30), but V\(\text{O}_2\) was not measured in these tissues. Calbet et al. (6) has estimated a decrease in O2 delivery of \(-\sim\) 0.3 and 0.4 l/min to tissue apart from the working muscles during one-legged knee-extensor exercise and cycling, respectively, in severe hypoxia (FI\(O_2\) = 0.105) at peak intensity during an incremental test. On the other hand, hypoxia increases both ventilation and cardiac output (8) which per se would increase whole body V\(\text{O}_2\) implying that the reduction in V\(\text{O}_2\) in tissues apart from the working muscles, which only constitutes \(-\sim\) 15\% of the pulmonary V\(\text{O}_2\) response (39) has to be quite pronounced to lower whole body V\(\text{O}_2\). It is clear that further studies are needed to investigate the effect of hypoxia on V\(\text{O}_2\) in tissues apart from the muscles during exercise.

The different thigh V\(\text{O}_2\) response to hypoxia using knee-extensor exercise and whole body exercise may also be explained by differences in the muscle mass recruited. During an incremental test, Calbet et al. (6) compared the responses to normoxia and severe hypoxia (FI\(O_2\) = 0.105) during cycling and one-legged knee-extensor exercise and found a reduction in O2 delivery to the quadriceps at peak effort of 47 and 26\%, respectively. The difference may be related to changes during exercise in arterial blood temperature and pH. At peak effort blood temperature was higher (0.5\°C) and blood pH lower (0.09) during cycling compared with isolated knee-extensor exercise. This in turn induces a more marked right shift in the O2 dissociation curve compared with exercise with a small muscle mass thereby reducing the affinity for O2 to bind to hemoglobin at the pulmonary level, thus reducing the O2 content in arterial blood to a higher extent during whole body exercise. The higher temperature and lower pH during cycling was estimated to account for 50\% of the difference in O2 delivery between exercise involving a small and large muscle mass. Blood flow to the quadriceps muscle, expressed as flow per unit of muscle mass, is likely to be lower when working at a high intensity with a large muscle mass as during cycling compared with knee-extensor exercise. With the use of peak femoral blood flow values at thigh V\(\text{O}_{2\max}\) from the study by Calbet et al. (6) of \(-\sim\) 9 and \(-\sim\) 6 l/min for each leg during cycling and knee-extensor exercise, respectively, and assuming that the blood flow feeds a muscle mass of \(-\sim\) 10 kg during cycling (49) and 2.5 kg in knee-extensor exercise (3), a mean perfusion of \(-\sim\) 1 and \(-\sim\) 2.5 l/min \text{kg}^{-1}\text{\,kg}^{-1} will be obtained at thigh V\(\text{O}_{2\max}\) for the former and latter condition (45). Thus at the onset of exercise the reduced perfusion of the active muscles during cycling may lead to an insufficient O2 supply, which may explain why hypoxia appears to have larger effects on V\(\text{O}_2\) kinetics during cycling than during exercise with a smaller muscle mass.

**Perspectives and Significance**

We show that thigh V\(\text{O}_2\) and anaerobic metabolism in the initial part of intense two-legged knee-extensor exercise were unaffected despite a \(-\sim\) 5\%–7\% reduction in O2 delivery and a \(-\sim\) 40\% reduction in arterial O2 pressure caused by inhaling hypoxic air, suggesting that in normoxia O2 delivery and, in particular the O2 pressure gradient, does not limit the increase in V\(\text{O}_2\) during intense exercise. However, to prove definitively that oxygen delivery is not limiting to the rise in oxygen uptake future studies are needed. It will be of interest during intense exercise to see if a larger reduction in oxygen delivery can slow the rise in oxygen uptake and likewise if a markedly elevated oxygen delivery before exercise will speed thigh oxygen uptake.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: P.M.C., N.B.N., L.N.N., S.P.M., M.S., N.H.S., and J.B. performed experiments; P.M.C. and S.P.M. analyzed data; P.M.C., N.B.N., N.H.S., and J.B. interpreted results of experiments; P.M.C., N.B.N., and J.B. prepared figures; P.M.C., N.B.N., L.N.N., S.P.M., M.S., N.H.S., and J.B. drafted manuscript; P.M.C., N.B.N., L.N.N., S.P.M., M.S., N.H.S., and J.B. edited and revised manuscript; P.M.C., N.B.N., L.N.N., S.P.M., M.S., N.H.S., and J.B. approved final version of manuscript; N.B.N. and J.B. conception and design of research.

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