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Limitations to oxygen transport and utilization during sprint exercise in humans: evidence for a functional reserve in muscle O_2 diffusing capacity

José A. L. Calbet[1,2], José Losa-Reyna[1,2], Rafael Torres-Peralta[1,2], Peter Rasmussen[3], Jesús Gustavo Ponce-González[1,2], A. William Sheel[4], Jaime de la Calle-Herrero[1], Amelia Guadalupe-Grau[1,2], David Morales-Alamo[1,2], Teresa Fuentes[1], Lorena Rodríguez-García[1], Christoph Siebenmann[3], Robert Boushel[4,5] and Carsten Lundby[3]

[1]*Department of Physical Education, University of Las Palmas de Gran Canaria, Campus Universitario de Tafira s/n, Las Palmas de Gran Canaria, Canary Islands, 35017, Spain*
[2]*Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, 35016 Las Palmas de Gran Canaria, Canary Islands, Spain*
[3]*Center for Integrative Human Physiology, Institute of Physiology, University of Zürich, Zürich, Switzerland*
[4]*School of Kinesiology, University of British Columbia, Vancouver, British Columbia, Canada*
[5]*Åstrand Laboratory, Swedish School of Sport and Health Sciences, Stockholm, Sweden*

Key points

- Severe acute hypoxia reduces sprint performance.
- Muscle $\dot{V}_{O_2}$ during sprint exercise in normoxia is not limited by O_2 delivery, O_2 offloading from haemoglobin or structure-dependent diffusion constraints in the skeletal muscle of young healthy men.
- A large functional reserve in muscle O_2 diffusing capacity exists and remains available at exhaustion during exercise in normoxia; this functional reserve is recruited during exercise in hypoxia.
- During whole-body incremental exercise to exhaustion in severe hypoxia, leg $\dot{V}_{O_2}$ is primarily dependent on convective O_2 delivery and less limited by diffusion constraints than previously thought.
- The kinetics of O_2 offloading from haemoglobin does not limit $\dot{V}_{O_2peak}$ in hypoxia.
- Our results indicate that the limitation to $\dot{V}_{O_2}$ during short sprints resides in mechanisms regulating mitochondrial respiration.

Abstract To determine the contribution of convective and diffusive limitations to $\dot{V}_{O_2peak}$ during exercise in humans, oxygen transport and haemodynamics were measured in 11 men (22 ± 2 years) during incremental (IE) and 30 s all-out cycling sprints (Wingate test, WgT), in normoxia (Nx, P_{IO_2}: 143 mmHg) and hypoxia (Hyp, P_{IO_2}: 73 mmHg). Carboxyhaemoglobin (COHb) was increased to 6–7% before both WgTs to left-shift the oxyhaemoglobin dissociation curve. Leg $\dot{V}_{O_2}$ was measured by the Fick method and leg blood flow (BF) with thermodilution, and muscle O_2 diffusing capacity (D_{MO_2}) was calculated. In the WgT mean power output, leg BF, leg O_2 delivery and leg $\dot{V}_{O_2}$ were 7, 5, 28 and 23% lower in Hyp than Nx ($P < 0.05$); however, peak WgT D_{MO_2} was higher in Hyp (51.5 ± 9.7) than Nx (20.5 ± 3.0 ml min^{-1} mmHg^{-1}, $P < 0.05$). Despite a similar P_{aO_2} (33.3 ± 2.4 and 34.1 ± 3.3 mmHg), mean capillary P_{O_2} (16.7 ± 1.2 and 17.1 ± 1.6 mmHg), and peak perfusion during IE and WgT in Hyp, D_{MO_2} and leg $\dot{V}_{O_2}$ were 12 and 14% higher, respectively, during WgT than IE in Hyp (both $P < 0.05$). D_{MO_2} was insensitive to COHb (COHb: 0.7 *vs.* 7%, in IE Hyp and WgT Hyp). At exhaustion, the *Y* equilibration index was well above 1.0 in both conditions, reflecting greater convective than diffusive limitation to the O_2 transfer in both Nx and Hyp. In conclusion, muscle $\dot{V}_{O_2}$ during sprint exercise is not limited by O_2 delivery, O_2 offloading from haemoglobin or structure-dependent diffusion constraints in

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the skeletal muscle. These findings reveal a remarkable functional reserve in muscle O_2 diffusing capacity.

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Corresponding author J. A. L. Calbet: Departamento de Educación Física, Campus Universitario de Tafira, 35017 Las Palmas de Gran Canaria, Canary Island, Spain. Email: lopezcalbet@gmail.com

Abbreviations a-vO_2diff, arteriovenous oxygen concentration difference; BF, blood flow; C_{aO_2}, arterial content of oxygen; CO, carbon monoxide; COHb, carboxyhaemoglobin; D_{LO_2}, lung O_2 diffusing capacity; D_{MO_2}, muscle O_2 diffusing capacity; D_{O_2}, O_2 diffusing capacity; ECG, electrocardiogram; F_{IO_2}, inspired oxygen fraction; FV, femoral vein; HR_{max}, maximal heart rate; HR_{peak}, peak heart rate during Wingate; Hyp, hypoxia; LBF, leg blood flow; Nx, normoxia; S_{O_2}, haemoglobin saturation with O_2; ODC, oxyhaemoglobin dissociation curve; P_{50}, partial oxygen pressure at 50% S_{O_2}; P_{aO_2}, arterial oxygen pressure; P_{CO_2}, carbon dioxide pressure; P_{O_2}, oxygen pressure; P_{O_2cap}, capillary O_2 pressure; P_{O_2mit}, mitochondrial O_2 pressure; P_{FVO_2}, femoral vein P_{O_2}; P_{IO_2}, inspiratory O_2 pressure; $\dot{V}_{CO_2}$, carbon dioxide production; $\dot{V}_{CO_2peak}$, peak carbon dioxide production; $\dot{V}_{Epeak}$, peak pulmonary ventilation; $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{O_2max}$, maximal oxygen consumption; $\dot{V}_{O_2peak}$, peak oxygen uptake; $W_{peak\text{-}i}$, instantaneous peak power output; $W_{mean\text{-}10}$, mean power output during the first 10 s of the sprint exercise; $W_{mean\text{-}30}$, mean power output during the whole sprint exercise; WgT, isokinetic 30 s Wingate test.

Introduction

Sprint exercise performance depends on the capacity to achieve high rates of ATP utilization, which must be matched by similar rates of ATP resynthesis. ATP is principally hydrolysed by the myosin-ATPase and the ion pumps, while it is resynthesized mostly by the oxygen-independent energy pathways (classically named anaerobic: phosphagens and glycolysis) and to a much lower extent by oxidative phosphorylation (Gaitanos *et al.* 1993; Bogdanis *et al.* 1998; Parolin *et al.* 1999). It remains unknown why the contribution of aerobic energy metabolism during sprint exercise is so low. Essentially there are three possible explanations: insufficient O_2 delivery to the mitochondria or reduced mitochondrial O_2 utilization, or both. Our main hypothesis is that during short sprints the aerobic energy contribution to exercise will not be limited by the supply of O_2 to the mitochondria, while during prolonged sprints (more than 10–15 s long) the aerobic energy yield will become O_2 delivery dependent. Testing this hypothesis requires the assessment of muscle O_2 delivery and muscle O_2 diffusing capacity at different time points during sprints performed in normoxia and hypoxia, in comparison with maximal values achievable during incremental exercise to exhaustion.

During all-out exercise lasting less than 60 s, skeletal muscles obtain most of the energy from phosphagens and glycolysis (Medbo & Tabata, 1993). During shorter sprints the contribution of phosphagens and glycolysis is even greater, and for sprint exercise durations lasting 10 s or less the contribution of aerobic metabolism is considered almost negligible (Gaitanos *et al.* 1993; Bogdanis *et al.* 1998; Parolin *et al.* 1999). In support hereof it has been argued that sprint performance is unaffected by moderate hypoxia in humans (Robach *et al.* 1997; Weyand *et al.* 1999), and it has also been demonstrated in elite cyclists that peak sprint power output is not reduced in severe hypoxia (Calbet *et al.* 2003*b*). However, previous studies attempting to determine the respective energy contributions to sprint exercise are based on extrapolations from vastus lateralis muscle biopsy samples, which are not sufficiently sensitive to assess small changes in, for example, pyruvate oxidation rates which may be affected by regional heterogeneities in muscle oxygen uptake ($\dot{V}_{O_2}$) (Heinonen *et al.* 2010). Moreover, lack of direct measurement of $\dot{V}_{O_2}$ during sprint exercise in normoxia (or hypoxia) precludes any definitive conclusion regarding the magnitude of the contribution of aerobic metabolism to muscle energy expenditure. In consequence, the extent to which sprint exercise performance in normoxia or severe acute hypoxia is O_2 delivery dependent remains unknown.

Previous studies using the knee extension ergometer found that during dynamic exercise at submaximal (Nyberg *et al.* 2010; Jones *et al.* 2012) and supramaximal (~120 % of peak thigh $\dot{V}_{O_2}$) intensities (Bangsbo *et al.* 2000), muscle $\dot{V}_{O_2}$ is not limited by O_2 delivery at the onset of exercise. Although sprint exercise was not tested in previous studies, it is well known that the quadriceps muscle is over-perfused relative to oxygen uptake during knee extension exercise (ml (250 g muscle mass)$^{-1}$) compared to lower perfusion rates achieved during whole-body exercise (ml (100 g muscle mass)$^{-1}$) (Knight *et al.* 1992; Calbet *et al.* 2004, 2009). Thus, the knee extension model may not be the most appropriate method to test whether O_2 delivery may limit VO_2 at the start of whole-body sprint exercise.

Oxygen delivery has a convective component, which determines the amount of O_2 arriving to the muscle capillaries, and a diffusive component that depends on the O_2 offloading in muscle capillaries and transfer of free O_2 from the capillaries across the sarcolemma to the mitochondria. Since blood lactate concentration barely

increases during the first 15 s of a sprint (Calbet *et al.* 2003*b*), a blunted right-shift of the oxyhaemoglobin dissociation curve (ODC) may limit the O_2 offloading in muscle capillaries, at least in the initial period of the sprint. However, based on mathematical modelling it has been suggested that changes in Hb affinity for O_2 (usually measured as the P_{O_2} eliciting a 50% haemoglobin saturation with O_2 (S_{O_2}) or P_{50}) do not appear to play a role as limiting factors of muscle $\dot{V}_{O_2}$ (Wagner, 1997). If this is the case, then a left-shift of the ODC elicited by a small amount of carbon monoxide (CO) should not have a negative impact on muscle $\dot{V}_{O_2}$ during sprint exercise, regardless of the inspired O_2 fraction (F_{IO_2}). Nevertheless, this has never been empirically tested in humans.

It has been suggested that the sarcolemma and other structural elements interposed between the erythrocytes and the mitochondria pose a resistance to O_2 diffusion during exercise (Roca *et al.* 1989; Richardson *et al.* 1995) and that due to the lower P_{O_2} gradient driving diffusion in hypoxia, this resistance plays a greater limiting role for peak oxygen uptake ($\dot{V}_{O_2peak}$) in hypoxia than in normoxia (Wagner, 1993). Assuming this model is correct, increasing HbCO should exacerbate this diffusion limitation due to the combined effect of a leftward shift of the ODC, a reduction in the functional haemoglobin concentration ([Hb]), and a lower level of acidification (lower Bohr effect) during sprint (McKenna *et al.* 1997) compared to during incremental exercise (van Hall *et al.* 2009). Accordingly, we hypothesized that leg $\dot{V}_{O_2peak}$ would be lower during sprint exercise in hypoxia when combined with CO than during incremental exercise to exhaustion performed at similar levels of hypoxia without CO.

Therefore the aims of this study were: (i) to assess to what extent leg $\dot{V}_{O_2}$ is limited by O_2 delivery at the onset of sprint exercise; (ii) to establish if blood O_2 affinity exerts a limiting role in leg $\dot{V}_{O_2}$ during sprint exercise in hypoxia, and (iii) to determine whether leg $\dot{V}_{O_2peak}$ is limited by diffusion during incremental exercise in hypoxia.

In this investigation healthy male subjects performed incremental cycle exercise to exhaustion in normoxia and severe acute hypoxia, followed by 30 s sprint exercise during which arterial and venous femoral gases where obtained concomitantly with measurements of cardiac output and leg blood flow with thermodilution. At the end of the last incremental exercise test in hypoxia, subjects inhaled a small amount of CO to elicit a leftward shift in their ODC during subsequent sprint exercise.

Methods

Subjects

Eleven healthy young men agreed to participate in these studies. Their mean ± SD age, height, weight and percentage of body fat were: 21.5 ± 2.0 years, 173.8 ± 8.0 cm, 72.3 ± 9.3 kg, and 16.1 ± 4.9%, respectively. Before volunteering, subjects received full oral and written information about the experiments and associated risks. Written consent was obtained from each subject. The study was performed in accordance with the *Declaration of Helsinki* and was approved by the Ethical Committee of the University of Las Palmas de Gran Canaria (CEIH-2010-01 and CEIH-2009-01).

General overview

This study was a part of larger project including several experiments designed to address the mechanisms limiting whole-body exercise performance in humans (Torres-Peralta *et al.* 2014; González-Henriquez *et al.* 2015; Losa-Reyna *et al.* 2015; Morales-Alamo *et al.* 2015). After a familiarization process including incremental and sprint exercise (Excalibur Sport 925900, Lode, Groningen, The Netherlands), the subjects participated in an invasive study including incremental exercise to exhaustion in normoxia (inspiratory O_2 pressure, $P_{IO_2} \approx 143$ mmHg) and severe acute normobaric hypoxia ($P_{IO_2} \approx 73$ mmHg) in random order with assessment of central and local haemodynamics combined with measurements of O_2 transport, as well as pulmonary and muscle gas exchange. A second set of experiments included two Wingate tests (a 30 s all-out sprint): one in normoxia and another in hypoxia ($P_{IO_2} \approx 73$ mmHg), performed on an isokinetic ergometer (Excalibur Sport 925900) with cadence fixed at 80 rpm, in random order. During the Wingate tests cardiac output and leg blood flow measurements were combined with arteriovenous differences to determine leg $\dot{V}_{O_2}$. The invasive experiments were complemented by a series of additional experiments performed on subsequent days, some of them included in our companion article.

Familiarization

On the first visit to the laboratory, anthropometric measurements and body composition analysis were performed. Thereafter, subjects reported to the laboratory on separate days to complete the following tests. First, their $\dot{V}_{O_2peak}$, maximal heart rate (HR_{max}) and maximal power output (W_{max}) in normoxia (Nx; $F_{IO_2} = 0.21$; barometric pressure 735–745 mmHg) and hypoxia (Hyp; $F_{IO_2} = 0.104$; barometric pressure 735–745 mmHg) were assessed with an incremental exercise test to exhaustion. One week later subjects were familiarized with the isokinetic 30 s Wingate test (80 rpm). Subjects were requested to refrain from ingesting caffeine and taurine-containing drinks and to not exercise 24 h before the experiments.

Body composition

Body composition was determined by dual-energy x-ray absorptiometry (DEXA) (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA, USA) as described elsewhere (Guadalupe-Grau *et al.* 2009). From the DEXA scans the leg muscle mass was calculated using the model of Wang *et al.* (1999).

Main experiments

On the experimental day, subjects reported to the laboratory at 07.00 h after an overnight fast from 22.00 h. After catheterization (see below) subjects were assigned to either an incremental exercise test to exhaustion in normoxia (30 W, 2 min) or hypoxia (20 W, 2 min; AltiTrainer200, SMTEC, Nyon, Switzerland), in random order and separated by 90 min rest. At exhaustion in hypoxia (Ex1), the subjects were rapidly switched to breath room air (normoxia) and requested to continue the exercise at the same load for 2 min, and then the load was increased by 20 W every 2 min until exhaustion (Ex2). This was followed by a lunch break (a sandwich and 200 ml of apple or pineapple juice) and a 120 min resting period. Thereafter, the incremental exercise in hypoxia was repeated. At exhaustion in hypoxia (Ex1) the subjects were requested to keep pedalling while a valve deviated the inspired breathing gas to a 30 litre anaesthesia bag filled with hypoxic gas ($F_{IO_2} \approx 13.3$, $P_{IO_2} \approx 91$ mmHg) and a small amount of CO (7 ml (kg body mass)$^{-1}$). The gas was breathed in an open circuit system in a well-ventilated room until the bag was almost emptied. The valve was then returned to the previous position such that the subjects continued the incremental test at this new level of hypoxia (F_{IO_2}: ∼13.3, P_{IO_2}: ∼91 mmHg). After 2 min at the load eliciting exhaustion, the intensity was increased by 20 W/2 min until a new exhaustion (Ex2). Again, subjects were requested to keep pedalling while they were switched to breath room air (normoxia). After 2 min, the load was increased by 20 W/2 min until exhaustion (Ex3). This was followed by another 2 h resting period. Thereafter, the subjects performed in random order two isokinetic Wingate tests, one in normoxia and the other in hypoxia ($P_{IO_2} \approx 73$ mmHg, AltiTrainer200) interspaced by a 90 min resting period. No warm-up was performed before the Wingate tests apart from 3 min of unloaded pedalling.

The first three incremental exercise tests to exhaustion were used to study the influence of different levels of oxygenation on the haemodynamic responses and fatigue mechanisms in hypoxia. Carbon monoxide was used to increase the arterial oxygen pressure (P_{aO_2}) while keeping the arterial content of oxygen (C_{aO_2}) at the level of severe hypoxia. The results from these experiments are presented and analysed in different articles (in preparation). The results obtained during the incremental exercise in normoxia and the incremental exercise tests in severe hypoxia up to Ex1 are reported here (the average of the two carried out), for comparison with the responses observed during the 30 s Wingate tests.

Catheterization and preparation for the experiments

Prior to the exercise tests, both femoral veins and one femoral artery were catheterized under local anaesthesia (2% lidocaine), as previously reported (Calbet *et al.* 2006). In the right femoral vein a 16G catheter (Arrow ES-04306, Teleflex, Morrisville, NC, USA) was inserted 3 cm below the inguinal ligament and advanced 12–13 cm distally. This catheter was used for the injection of ice-cold saline for the measurement of leg blood flow (LBF) using the thermodilution method (Andersen & Saltin, 1985). In the same femoral vein a thermodilution catheter (PV2014L16N, Pulsion Medical Systems AG, Munich, Germany) was inserted 2 cm below the inguinal ligament and advanced 12 cm cranially. This catheter was used to measure the temperature of the blood in the femoral vein. The same type of catheter was also inserted in the right femoral artery, advanced 12 cm cranially, and used to measure blood pressure and femoral artery blood temperature. A final 20G catheter was inserted in the contralateral femoral vein from 2 cm below the inguinal ligament and advanced 12 cm in the direction of the heart (Arrow ES-04150), and used for sampling femoral vein blood. All catheters were doubly sutured to the skin at the insertion point.

The right proximal femoral vein and the femoral artery catheters were connected to blood pressure transducers (Pulsion Medical Systems AG, Munich, Germany), positioned at the height of the parasternal fourth intercostal space. The two thermistors were connected to temperature processing boxes (Flemming Jessen Engineering, Copenhagen, Denmark) and blood pressure bridge amplifiers (ML-117, ADInstruments, Bella Vista, Australia).

For safety reasons, an electrocardiogram (ECG) was displayed on a monitor during catheterization and all the experimental procedures. The ECG, blood pressure and the temperature registered by the thermistors, as well as the infusate temperature, were collected using a 16 channel analog-to-digital data acquisition system (Power Lab ML880, ADInstruments), sampled at 200 Hz, and stored on a computer for subsequent analysis.

Blood sampling

During incremental exercise, simultaneous blood samples were obtained from the right femoral artery and left femoral vein 15 s before the end of each 2 min stage, and also as close as possible to exhaustion and blood gases immediately analysed. Prior to the sprint exercise a blood sample was obtained simultaneously from the

right femoral artery and the left femoral vein. Then, during the sprint blood was sampled every 5 s from the left femoral vein and every 10 s from the right femoral artery and used for determination of blood gases and haemoglobin concentrations. Samples were placed on ice and immediately analysed in the same order as they were taken. Blood gases were collected in Radiometer heparinized syringes and analysed with an ABL90 (Radiometer, Copenhagen, Denmark), calibrated according to the manufacturer's recommendations. Arterial and femoral vein blood gases and pH were corrected for blood temperature using the arterial and venous thermistors, respectively. Arterial P_{O_2} and pH were corrected using the equations of Severinghaus (1979), while P_{CO_2} was corrected using the equation $P_{CO_2tc} = P_{CO_2(37)} \times (10^{0.021} \times (T - 37))$ according to Siggaard-Andersen (1974), where P_{CO_2tc} is the temperature-corrected P_{CO_2}, $P_{CO_2(37)}$ is the P_{CO_2} measured at 37°C, and T is arterial blood temperature.

Power output and oxygen uptake

Power output during the sprints was reported as instantaneous peak power ($W_{peak\text{-}i}$), and mean power output during the first 10 s of the sprints ($W_{mean\text{-}10}$) and mean power output achieved during the full duration of the sprints ($W_{mean\text{-}30}$). Whole-body oxygen uptake was measured with a metabolic cart (V_{max} N29; Sensormedics, Yorba Linda, CA, USA), calibrated prior to each test according to the manufacturer's instructions, with high-grade calibration gases (Carburos Metálicos, Las Palmas de Gran Canaria, Spain). Respiratory variables were analysed breath-by-breath and averaged every 5 s during the Wingate test and every 20 s during the incremental exercise tests. The highest 20 s averaged $\dot{V}_{O_2}$ recorded in normoxia was taken as the $\dot{V}_{O_2max}$. The same criterion was applied to determine the $\dot{V}_{O_2peak}$ in hypoxia.

Leg blood flow and cardiac output

During incremental exercise and during the Wingate test, LBF was measured in the femoral vein using the constant infusion thermodilution technique (Andersen & Saltin, 1985). Briefly, iced saline solution was infused into the distal left femoral vein catheter at high and constant rates (140–160 ml min), using an automated pump (Harvard Apparatus, Millis, MA, USA). The temperature deflection in the femoral vein was used to determine the femoral vein blood flow, and that recorded in the femoral artery to measure cardiac output using the constant infusion transpulmonary thermodilution method (Calbet *et al.* 2015). Occasionally, the bolus thermodilution method was also used during incremental exercise to exhaustion. In this case, a 10–15 ml bolus of iced cold saline was injected into the distal left femoral vein catheter and the reading in the femoral vein thermistor used to compute the femoral vein blood flow and that in the femoral artery to calculate the cardiac output (Calbet & Boushel, 2015).

Calculations

Arterial and venous O_2 content (C_{aO_2} and C_{vO_2}) were computed from the saturation and [Hb] (i.e. $(1.34[Hb] \times S_{O_2}) + (0.03 \times P_{O_2})$). Arteriovenous $[O_2]$ difference (a-vO_2diff) was calculated from the difference in femoral arterial and femoral venous $[O_2]$. This difference was then divided by arterial concentration to give O_2 extraction. Oxygen delivery was computed as the product of blood flow and C_{aO_2}. Leg $\dot{V}_{O_2}$ was calculated as the product of LBF and the a-vO_2diff. The *in vivo* P_{50}, or P_{O_2} necessary to half-saturate the Hb not bound to CO, was calculated by the ABL90, accounting for the Bohr effect. The average O_2 muscle diffusing capacity (D_{MO_2}) and the mean capillary P_{O_2} of the leg muscles were determined as previously described (Roca *et al.* 1989; Calbet *et al.* 2005).

Statistics

Variables were normally distributed as shown by the Shapiro–Wilks test. Two-way repeated-measures ANOVA for oxygenation (two levels: normoxia *vs.* hypoxia) and time (6 levels: 0, 5, 10, 15, 20, 25 and 30 s) was used to analyse the responses observed during the sprints. Pairwise comparisons at specific time points were performed with Student's t test, and adjusted for multiple comparisons with the Holm–Bonferroni method. Comparisons between peak values obtained during the incremental exercise and Wingate tests were done using a paired t test. The relationship between variables was determined using linear regression analysis. Values are reported as the mean ± standard deviation (unless otherwise stated). $P < 0.05$ was considered significant. All statistical analysis was performed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Incremental exercise to exhaustion

The main responses to incremental exercise in normoxia and hypoxia are shown in Table 1. W_{max} and whole-body (or pulmonary) $\dot{V}_{O_2peak}$ were reduced in hypoxia by 42 and 34%, respectively; while peak heart rate during Wingate (HR_{peak}), $\dot{V}_{CO_2peak}$ and peak pulmonary ventilation ($\dot{V}_{Epeak}$) were 8, 29 and 33% lower in hypoxia than in

Table 1. Ergoespirometric responses and blood gases during incremental exercise and during isokinetic Wingate tests at 80 rpm performed in normoxia and acute hypoxia ($n = 9$)

	Incremental exercise at W_{max}		Wingate test (30 s all-out sprint)	
	Normoxia	Hypoxia	Normoxia	Hypoxia
F_{IO_2} (%)	20.80 ± 0.15	10.84 ± 0.21[a]	20.88 ± 0.06	10.75 ± 0.23[a]
P_{IO_2} (mmHg)	141.6 ± 1.2	74.0 ± 1.5[a]	142.9 ± 0.7	73.5 ± 1.7[a]
$W_{max}/W_{peak\text{-}i}$ (W)	286.7 ± 43.6	166.1 ± 37.2[a]	972.9 ± 300.2[b]	905.9 ± 377.4[b]
$\dot{V}_{O_2peak}$ (l min^{-1})	3.56 ± 0.34	2.37 ± 0.29[a]	2.75 ± 0.40[b]	2.19 ± 0.19[a]
$\dot{V}_{O_2peak}$ (ml kg^{-1} min^{-1})	50.7 ± 4.0	33.5 ± 2.6[a]	39.2 ± 5.9[b]	31.2 ± 3.0[a]
Leg $\dot{V}_{O_2peak}$ (ml min^{-1})	1.25 ± 0.22	0.76 ± 0.11[a]	1.12 ± 0.15[b]	0.87 ± 0.14[ab]
HR_{peak} (beats min^{-1})	192.9 ± 6.9	178.3 ± 7.4[a]	180.9 ± 5.5[b]	177.9 ± 7.7
$\dot{V}_{CO_2peak}$ (l min^{-1})	4.09 ± 0.43	2.92 ± 0.53[a]	3.29 ± 0.72[b]	3.27 ± 0.57
RER	1.15 ± 0.03	1.23 ± 0.11[a]	1.19 ± 0.16	1.48 ± 0.17[ab]
$\dot{V}_{Epeak}$ (l min^{-1})	135.6 ± 18.7	103.9 ± 21.0[a]	107.6 ± 34.9	113.1 ± 23.2[b]
P_{aO_2} (mmHg)	100.2 ± 7.3	33.3 ± 2.4[a]	101.8 ± 5.5	34.1 ± 3.3[a]
P_{FVO_2} (mmHg)	21.1 ± 2.0	10.6 ± 2.8[a]	16.2 ± 2.5[b]	9.9 ± 2.5[a]
P_{aCO_2} (mmHg)	34.2 ± 3.1	29.7 ± 2.0[a]	35.0 ± 3.1	31.3 ± 2.5[a]
P_{FVCO_2} (mmHg)	79.7 ± 7.2	53.5 ± 5.9[a]	82.3 ± 8.7	71.8 ± 6.7[ab]
A pH	7.225 ± 0.048	7.332 ± 0.084[a]	7.413 ± 0.025[b]	7.454 ± 0.029[ab]
FV pH	7.060 ± 0.058	7.206 ± 0.091[a]	7.179 ± 0.030[b]	7.220 ± 0.027[a]
A Hb (g l^{-1})	169.3 ± 4.7	163.2 ± 7.0[a]	153.7 ± 7.1[b]	154.9 ± 4.7[b]
S_{aO_2} (%)	96.1 ± 1.2	63.5 ± 6.1[a]	98.5 ± 0.4[b]	75.8 ± 6.2[ab]
S_{FVO_2} (%)	19.9 ± 5.3	11.8 ± 5.2[a]	22.4 ± 3.8	15.1 ± 3.9[a]
A O_2Hb (%)	94.9 ± 1.2	62.4 ± 5.9[a]	91.8 ± 1.0[b]	69.8 ± 5.7[ab]
FV O_2Hb (%)	18.3 ± 5.3	10.1 ± 5.2[a]	15.0 ± 4.0	6.9 ± 4.2[a]
A COHb (%)	0.5 ± 0.2	1.0 ± 0.4[a]	6.2 ± 0.9[b]	7.3 ± 2.1[b]
FV COHb (%)	0.7 ± 0.1	0.9 ± 0.5	6.6 ± 0.9[b]	7.3 ± 2.2[b]
A metHb (%)	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
FV metHb (%)	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
C_{aO_2} (ml l^{-1})	218.3 ± 7.7	137.4 ± 12.9[a]	192.1 ± 9.0[b]	146.0 ± 13.3[ab]
C_{FVO_2} (ml l^{-1})	42.4 ± 12.7	22.3 ± 11.7[a]	32.9 ± 9.9[b]	15.4 ± 9.4[a]
Leg fractional O_2 extraction (%)	80.6 ± 5.4	84.2 ± 7.0[a]	83.0 ± 4.5	89.6 ± 5.9[ac]
D_{LO_2} (ml min^{-1} mmHg^{-1})	64.2 ± 6.0	96.2 ± 10.7[a]	49.3 ± 7.1[b]	85.7 ± 5.4[ab]
D_{MO_2} (ml min^{-1} mmHg^{-1})	25.2 ± 5.2	46.0 ± 7.3[a]	20.5 ± 3.0[b]	51.5 ± 9.7[ab]

[a]$P < 0.05$ Normoxia *vs.* Hypoxia; [b]$P < 0.05$ compared to incremental exercise same level of oxygenation; [c]$P = 0.06$ compared to incremental exercise same oxygenation. A, arterial; FV, femoral vein; metHb, methaemoglobin D_{LO_2}, lung O_2 diffusing capacity.

normoxia. Arterial P_{O_2} was reduced to 33 ± 2 mmHg in hypoxia.

Sprint exercise power output and $\dot{V}_{O_2}$: first 10 s of the Wingate test

Power output, O_2 delivery and $\dot{V}_{O_2}$ are depicted in Fig. 1. Peak power output ($W_{peak\text{-}i}$) was not statistically different between normoxia and hypoxia (973 ± 300 and 906 ± 377 W, respectively, $P = 0.36$). However, during the first 10 s of the sprints, mean power output ($W_{mean\text{-}10}$) was 12% lower in hypoxia than normoxia (567 ± 90 and 499 ± 86 W, respectively, $P < 0.05$). The reduction in $W_{mean\text{-}10}$ was not due to lower $\dot{V}_{O_2}$, since the legs consumed a similar total amount of O_2 in normoxia and hypoxia (131 ± 34 and 129 ± 26 ml per leg, respectively, $P = 0.87$), which was confirmed by a similar pulmonary O_2 uptake (452 ± 145 and 478 ± 108 ml in normoxia and hypoxia, respectively, $P = 0.59$). This occurred despite a 22% lower leg O_2 delivery in hypoxia during the first 10 s of the sprints (1.50 ± 0.23 and 1.17 ± 0.17 l min^{-1}, $P < 0.05$), which was compensated by 14 units higher leg fractional O_2 extraction in hypoxia than normoxia (51.7 ± 9.2 and 65.9 ± 6.1%, respectively, $P < 0.05$).

Sprint exercise power output and $\dot{V}_{O_2}$: entire Wingate test

The mean power output during the entire sprints ($W_{mean\text{-}30}$) was reduced by 7%, from 494 ± 47 W in normoxia to 461 ± 52 W in hypoxia ($P < 0.05$). However, during the last 15 s of the sprints, mean power output was not different between conditions (434 ± 37 and 419 ± 49 W in normoxia and hypoxia, respectively,

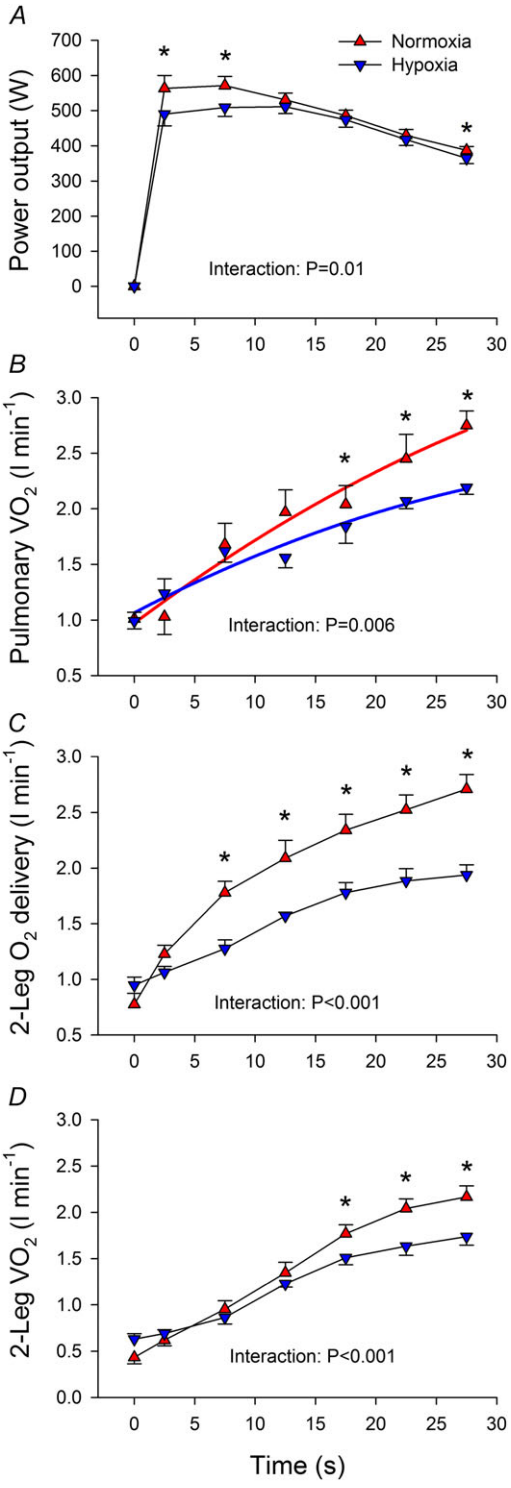

Figure 1. Power output, O_2 delivery and O_2 uptake during sprint exercise
Power output (*A*), pulmonary oxygen uptake ($\dot{V}_{O_2}$) (*B*), two-legged O_2 delivery (*C*), and two-legged $\dot{V}_{O_2}$ (*D*) during isokinetic Wingate tests at 80 rpm performed in normoxia ($P_{IO_2} \approx 143$ mmHg) and acute hypoxia ($P_{IO_2} \approx 73$ mmHg). *$P < 0.05$ normoxia (triangles pointing up) *vs.* hypoxia (triangles pointing down); $n = 10$ (one subject did not perform the Wingate test in hypoxia).

$P = 0.17$) (Fig. 1*A*). The rate of increase in pulmonary $\dot{V}_{O_2}$ was higher in normoxia than hypoxia ($F_{IO_2} \times$ time: $P < 0.05$). Consequently, the mean pulmonary $\dot{V}_{O_2}$ during the whole sprints was 12% lower in hypoxia than in normoxia ($P < 0.05$) (Fig. 1*B*).

During the last 15 s of the sprints, mean leg $\dot{V}_{O_2}$ was 19% lower in hypoxia (2.02 ± 0.29 and 1.63 ± 0.26 l min^{-1} in normoxia and hypoxia, respectively, $P < 0.05$; Fig. 1*D*), due to a 26% lower O_2 delivery (2.53 ± 0.40 and 1.87 ± 0.29 l min^{-1} in normoxia and hypoxia, respectively, $P < 0.05$; Fig. 1*C*), which was compensated only partly by higher fractional leg O_2 extraction (80.0 ± 3.5 and 87.2 ± 5.4% in normoxia and hypoxia, respectively, $P < 0.05$).

Peak oxygen delivery and utilization during the sprints

Peak oxygen delivery and utilization variables during incremental exercise to exhaustion and sprint exercise in normoxia and hypoxia are reported in Table 1. During the sprints, heart rate increased with the duration of the sprints to reach 180.9 ± 5.5 and 177.9 ± 7.7 beats min^{-1} in normoxia and hypoxia, respectively, $P = 0.09$). This represented 94 and 100% of the peak heart rate achieved during incremental exercise to exhaustion in normoxia and hypoxia, respectively. Peak cardiac output was 23.2 ± 2.5 and 21.0 ± 2.5 l min^{-1} in normoxia and hypoxia, respectively ($P < 0.05$). Peak systemic O_2 delivery was reduced by 32% in hypoxia (from 4.47 ± 0.59 to 3.06 ± 0.41 l min^{-1}, $P < 0.05$). However, $\dot{V}_{O_2peak}$ was reduced by only 20% (2.75 ± 0.40 and 2.19 ± 0.19 l min^{-1} in normoxia and hypoxia, respectively, $P < 0.05$), due to 10 units greater systemic O_2 extraction in hypoxia (62.7 ± 13.2 and 72.2 ± 6.4%, $P < 0.05$).

Peak leg blood flow during the sprints was 5% lower in hypoxia (7.04 ± 0.92 and 6.66 ± 0.95 l min^{-1}, $P < 0.05$), which combined with the 24% lower C_{aO_2} in hypoxia resulted in a 28% lower peak leg O_2 delivery in hypoxia (2.71 ± 0.39 and 1.94 ± 0.28 l min^{-1}, $P < 0.05$). Consequently, leg $\dot{V}_{O_2peak}$ was only 23% lower in hypoxia (2.24 ± 0.30 and 1.74 ± 0.28 l min^{-1}, $P < 0.05$) due to greater leg fractional O_2 extraction in hypoxia (83.0 ± 4.6 and 89.6 ± 5.9%, $P < 0.05$).

Muscle gas exchange during sprint exercise

The main variables determining muscle gas exchange are depicted in Fig. 2. Muscle O_2 diffusing capacity was progressively recruited throughout exercise in both conditions, but more markedly in hypoxia than in normoxia ($F_{IO_2} \times$ time: $P < 0.001$), to reach peak values of 20.5 ± 3.0 and 51.5 ± 9.7 ml min^{-1} mmHg^{-1} in normoxia and hypoxia, respectively ($P < 0.05$) (Fig. 2*B*).

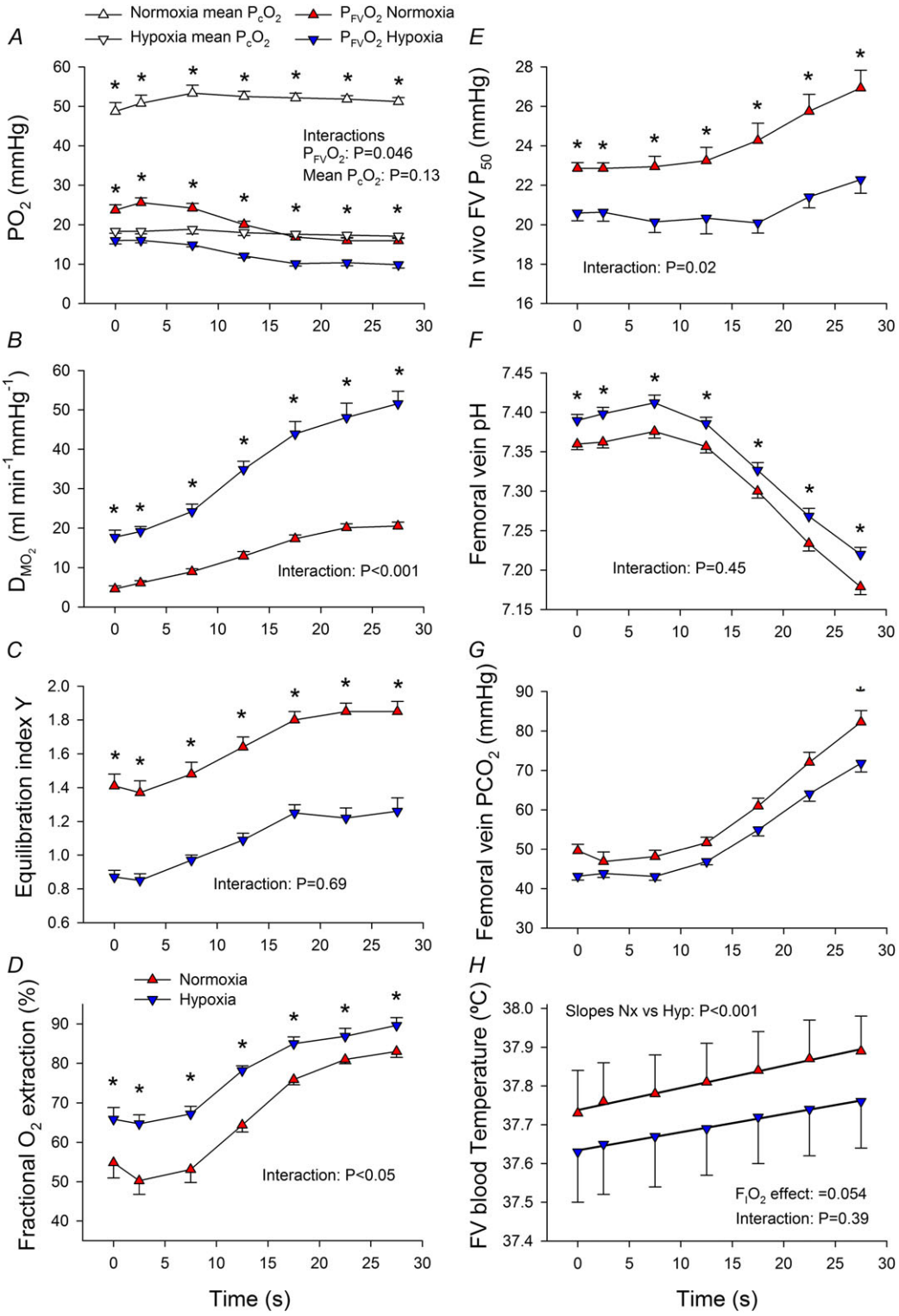

Figure 2. Muscle gas exchange during sprint exercise
Mean capillary oxygen pressure (P_{cO_2}) and femoral vein (FV) P_{O_2} (*A*), muscle O_2 diffusing capacity (*B*), equilibration index *Y* (dimensionless) (*C*), leg fractional O_2 extraction (*D*), *in vivo* femoral vein P_{50} (corrected for the Bohr effect) (*E*), femoral vein pH (*F*), femoral vein P_{CO_2} (*G*), and femoral vein blood temperature (*H*) during isokinetic Wingate tests at 80 rpm performed in normoxia ($P_{IO_2} \approx 143$ mmHg) and acute hypoxia ($P_{IO_2} \approx 73$ mmHg). $^*P < 0.05$ normoxia (triangles pointing up) *vs.* hypoxia (triangles pointing down); $n = 10$ (one subject did not perform the Wingate test in hypoxia).

During the Wingate test in normoxia, D_{MO_2} was 19% below the maximal value achieved during incremental exercise to exhaustion in normoxia. In contrast, during exercise in hypoxia D_{MO_2} was 12% higher during sprint than incremental exercise. In fact the highest value of D_{MO_2} was measured during the sprint exercise in hypoxia ($P < 0.05$, compared with all the other conditions).

The femoral vein P_{O_2} (P_{FVO_2}) was progressively reduced throughout the sprints in normoxia (from 23.9 ± 4.4 to 16.2 ± 2.5 mmHg) and hypoxia (from 16.0 ± 2.5 to 9.9 ± 2.5 mmHg, both $P < 0.05$) (Fig. 2*A*). The magnitude of the P_{FVO_2} decline was similar in both conditions. However, there was an F_{IO_2} × time interaction ($P < 0.05$), due to a 2 mmHg transient P_{FVO_2} increase above resting levels in the first 5 s of the sprints in normoxia ($P < 0.001$), which was not observed in hypoxia ($P = 0.96$). During the first 20 s of the sprints, muscle mean P_{cO_2} was increased by 3.7 mmHg in normoxia ($P < 0.05$), while in hypoxia it remained unchanged ($\Delta = -0.15$ mmHg, $P = 0.68$) (Fig. 2*A*). During the last 10 s of the sprints, muscle mean P_{cO_2} was maintained in normoxia (+3.1 mmHg above resting values, $P = 0.15$) but decreased in hypoxia (−1.1 mmHg, $P < 0.05$). This was a consequence of both the lower O_2 delivery in hypoxia (see above) and the greater fractional O_2 extraction in hypoxia (Fig. 2*D*). Fractional O_2 extraction was transiently reduced by 5 units ($P < 0.05$) at the onset of the sprints in normoxia (first 5 s) while it was maintained at resting levels over the same period in hypoxia (−1.1 units, $P = 0.59$). At exhaustion, fractional O_2 extraction was almost 7 units higher in hypoxia than normoxia ($P < 0.05$). This occurred despite a left-shift of the femoral vein ODC in hypoxia (Fig. 2*E*).

The femoral vein (FV) pH was 0.03 units higher in hypoxia than in normoxia, from rest to the end of the sprints. During the first 10 s, FV pH increased slightly ($P < 0.05$), but it declined sharply during the last 10–15 s of the sprints, with a similar pattern in both conditions (Fig. 2*F*). This was mirrored by the changes in P_{FVCO_2}, with a greater increase of P_{FVCO_2} in normoxia than hypoxia (F_{IO_2} × time: $P < 0.05$) (Fig. 2*G*). Femoral vein blood temperature increased linearly with the duration of the sprints ($r > 0.999$, $P < 0.05$), but the slope of the increase in blood temperature was 24% steeper in normoxia than hypoxia ($P < 0.05$) (Fig. 2*H*).

The beta parameter of the Piiper and Scheid model (Piiper & Scheid, 1981; Piiper & Haab, 1991), reflecting the slope of the blood O_2 equilibrium curve, was 4.3-fold higher in hypoxia than in normoxia. During the first 20 s beta was reduced linearly over time in hypoxia (0.05 units s^{-1}, $r = 0.996$), but not in normoxia. The *Y* equilibration index, indicating perfusion *vs.* diffusion limits, increased with the duration of the sprint with a similar pattern in both conditions. However, the *Y* equilibration index was 1.5-fold higher in normoxia than hypoxia. At exhaustion the *Y* equilibration index was well above 1.0 in both conditions, reflecting greater convective than diffusive limitation to the O_2 transfer in normoxia and hypoxia (1.85 ± 0.19 and 1.26 ± 0.23, respectively, $P < 0.05$). At exhaustion, the *Y* equilibration index measured in normoxia was correlated with that measured in hypoxia ($r = 0.74$, $P < 0.05$).

Discussion

This study demonstrates that severe acute hypoxia reduces sprint performance by a mechanism that is independent of O_2 delivery. In addition, we have shown that at the onset of all-out sprint exercise, leg $\dot{V}_{O_2}$ is not limited by O_2 delivery, the kinetics of O_2 offloading from haemoglobin, or diffusion constraints. Moreover, we have shown a large functional reserve of skeletal muscle D_{MO_2}, which is recruited during sprint exercise in hypoxia. The large reserve in D_{MO_2} implies that the structures crossed by O_2 in its transfer from the erythrocytes to the mitochondrial cytochrome *c* oxidase do not pose a restrictive resistance to the diffusion of O_2, at least in normoxia. This indicates that the main mechanism limiting $\dot{V}_{O_2}$ during sprint exercise in normoxia resides within the mitochondria. This is further supported by the fact that a lower P_{FVO_2} was consistently observed in hypoxia than in normoxia close to exhaustion. Finally, we have shown that during whole-body incremental exercise to exhaustion in hypoxia, the primary mechanism limiting skeletal muscle $\dot{V}_{O_2}$ is convective O_2 delivery, rather than an O_2 diffusion limitation secondary to a low O_2 pressure gradient driving diffusion in hypoxia. The latter interpretation is based on the greater leg $\dot{V}_{O_2peak}$ achieved during sprint exercise than during incremental exercise in severe hypoxia, despite a similar O_2 pressure gradient driving diffusion in both tests. Finally, by administering CO to left-shift the ODC, we have shown that the kinetic of O_2 offloading from the Hb does not limit $\dot{V}_{O_2peak}$ in hypoxia. Collectively our findings suggest that muscle $\dot{V}_{O_2}$ during whole-body incremental exercise and during prolonged sprint exercise is primarily limited by convective oxygen delivery. However, during short sprints the limitation to muscle $\dot{V}_{O_2}$ resides in mechanisms regulating mitochondrial respiration.

Severe acute hypoxia reduces sprint performance by mechanisms independent of O_2 delivery

During the first 10 s of sprint, mean power output was 12% lower in hypoxia relative to normoxia despite a similar muscular and pulmonary oxygen uptake and sufficient availability of muscle energy substrates. These findings suggest that central mechanisms limiting motor unit recruitment and/or activation are likely to be the main factors explaining the reduction in mean power

output during the first 10 s of sprint exercise in hypoxia. This observation also implies that in normoxia sprint performance, as expected, is not limited by oxygen delivery. This agrees with previous studies testing this hypothesis using one legged knee extension exercise although at intensities far below those reached during all-out sprint exercise (Bangsbo *et al.* 2000; Nyberg *et al.* 2010; Jones *et al.* 2012). In addition, our data demonstrate that for sprints performed in hypoxia and lasting longer than 15 s, leg $\dot{V}_{O_2}$ becomes O_2 delivery limited, although power output can be maintained at the normoxic level at the expense of increasing the glycolytic rate. Since to sustain a given power output, the rate of ATP resynthesis must match the rate of ATP utilization, a 19% lower leg $\dot{V}_{O_2}$ during the last 15 s of the sprints in hypoxia compared to normoxia implies that alternative sources of ATP resynthesis must have provided the required energy to maintain similar mean power output levels during the last 15 s of the sprints in hypoxia and normoxia. Given the small amount of skeletal muscle phosphagens remaining after 15 s of all-out exercise (Bogdanis *et al.* 1998; Parolin *et al.* 1999), the only feasible energy source that could compensate for the diminished aerobic ATP resynthesis is glycolysis. In support, a 50% higher glycolytic rate has been reported during sprint exercise in severe acute hypoxia (Morales-Alamo *et al.* 2012). This leaves two open questions: (i) why is glycolysis not more involved in ATP resynthesis during sprint exercise in normoxia when a substantial functional glycolytic reserve exists? and (ii) why is the effect of severe hypoxia on power output more pronounced during the first 10 s of the sprint, which is not limited by aerobic ATP resynthesis or by the availability of ATP from phosphocreatine and glycolysis? Could this be due to reduced recruitment or increased inhibition of high-threshold motor units in severe acute hypoxia? In support of an inhibition hypothesis, animal studies have shown that acute arterial hypoxaemia eliciting arterial and femoral vein P_{O_2} values similar to those observed during sprint exercise in the present investigation increases the baseline discharge frequency of group III and especially of group IV muscle afferents in resting cats (Hill *et al.* 1992; Lagier-Tessonnier *et al.* 1993) and rabbits (Arbogast *et al.* 2000). This is likely to occur independent of muscle metabolic changes, since the resting level of lactate and hydrogen ions, which together are known stimuli of small-diameter afferents, were unaffected at mean P_{aO_2} values of 21 mmHg in cats (Hill *et al.* 1992). Likewise, blood lactate concentration does not increase (Calbet *et al.* 2003*b*) and blood pH does not decrease during the first 10–15 s of all-out sprint exercise as demonstrated in the present investigation. A higher group III/IV muscle afferent firing rate may cause reflex inhibition of the α-motoneuron pool (for review see Amann & Kayser, 2009).

At the onset of all-out sprint exercise $\dot{V}_{O_2}$ is not limited by O_2 delivery

During the first seconds of sprint exercise in normoxia several variables indicate over-perfusion of the exercising muscles, i.e. ample O_2 delivery that is not fully utilized by the muscles. This contention is supported by the observed increase in femoral vein P_{O_2}, S_{O_2} and pH during the first 5–10 s of sprint combined with a reduction of the fractional O_2 extraction below the level observed just before the start of the sprint. In fact, a common observation when vasodilators are infused intra-arterially during exercise is an increase in femoral vein P_{O_2} and S_{O_2}, combined with a reduction of the fraction of O_2 extracted by the muscles (Rådegran & Calbet, 2001; Calbet *et al.* 2006). In addition, the small reduction of the equilibration index (Y) further suggests a transient lowering of the perfusion limitation at the start of the sprint in normoxia, i.e. over-perfusion. During the sprint in hypoxia, leg blood flow was similar to that observed during the sprint in normoxia, and therefore alternative compensatory mechanisms must have facilitated an increase of the fraction of O_2 extracted from the muscle capillaries.

The kinetics of O_2 offloading from oxyhaemoglobin does not limit muscle O_2 extraction regardless of P_{IO_2}

In the present experiments we diminished the functional haemoglobin concentration by administering a small amount of carbon monoxide. This resulted in carboxyhaemoglobin (COHb) levels similar to those found in smokers and reduced the O_2 carrying capacity of the haemoglobin by 6–7% during the sprints in normoxia and hypoxia. Despite a similar COHb% during the sprints performed in normoxia and hypoxia, the femoral vein blood P_{50} increased significantly less during exercise in hypoxia than normoxia (see Fig. 2*E*). Consequently, the *in vivo* P_{50} was ~5 mmHg lower at the end of the sprints in hypoxia than in normoxia. However, this had no negative impact on leg O_2 extraction or $\dot{V}_{O_2}$, which was higher than achieved during an incremental exercise to exhaustion in similar hypoxic conditions. Moreover, leg fractional O_2 extraction was higher and effluent blood desaturation greater during the sprints in hypoxia despite lower P_{50} in hypoxia than in normoxia.

The lower Hb P_{50} during the sprint performed in hypoxia was due to increased alkalization of blood, which began during the unloaded pedalling phase that preceded the sprints, combined with a lower P_{CO_2}, and lower femoral vein temperature during the sprints in hypoxia than in normoxia. The 0.1°C lower femoral vein temperature at the start of the sprints in hypoxia might have been caused

by the greater perfusion observed during the unloaded pedalling phase in hypoxia than normoxia, which may have elicited a slightly greater cooling effect (Krustrup *et al.* 2001). However, even when combined these three factors explain only 40% of the P_{50} difference between normoxia and hypoxia at the end of the sprints. The remaining 60% of the P_{50} difference can be accounted for by the effect of CO on the ODC, which shifts the ODC of the remaining haemoglobin (Hb not binding CO) to the left and also makes the shape of the ODC less sigmoidal and more hyperbolic (the Haldane effect). This effect is more pronounced at low saturation (Okada *et al.* 1976; Zwart *et al.* 1984) causing a lower FV P_{50} during the sprint in hypoxia. Nevertheless, without any Bohr effect, the FV P_{50} would have been approximately 5 and 7 mmHg lower in hypoxia and normoxia, respectively, at the end of the sprints.

Since the femoral vein P_{50} values in the presence of CO were leftward shifted even at peak exercise if compared to the values observed at rest without CO, our experiments indicate that O_2 offloading from Hb does not require a right-shift of the ODC. In other words, this study minimizes the role that can be attributed to acidification (the Bohr effect) as a mechanism facilitating O_2 offloading during exercise in general.

Muscle O_2 diffusing capacity does not limit $\dot{V}_{O_2}$ during sprint exercise in normoxia

The diffusion of O_2 from the muscle capillaries to the mitochondria is driven by the O_2 pressure gradient between the capillaries and the mitochondria, where the latter is close to 0 mmHg (~1.5-3 mmHg), particularly at near maximal exercise intensities (Severinghaus, 1994). In the case of O_2 diffusion limitation due to structural constraints (Richardson *et al.* 1995), as for example reduced capillary density (Hepple *et al.* 2000) or number of capillary-to-sarcolemmal contacts (Krogh, 1919*a*, *b*; Boushel *et al.* 2014*a*), a reduction of the pressure gradient driving O_2 diffusion should cause a reduction of $\dot{V}_{O_2}$. However, our experimental data demonstrate that muscle $\dot{V}_{O_2}$ was insensitive to severe hypoxia during the first 15 s of the sprints despite a reduction of the mean P_{O_2} gradient from 52 to 18 mmHg. This was possible since a functional reserve in D_{MO_2} exists that, like in the lungs, can be recruited when the P_{O_2} gradient driving diffusion is reduced, as occurred in hypoxia (see Fig. 2*A*). During the last 5 s of the sprints, the calculated D_{MO_2} was more than twofold higher in hypoxia than in normoxia (52 *vs.* 19 ml min^{-1} mm Hg^{-1}), and even higher than the 42 ml min^{-1} mmHg^{-1} reported during an incremental exercise to exhaustion in normoxia, in subjects of similar characteristics (Calbet *et al.* 2003*a*). Nevertheless, the fact that there is some O_2 left in the femoral vein and that P_{FVO_2} is ~10 mmHg suggests a diffusion limitation, although part of the O_2 remaining is certainly due to some venous admixture, with blood draining the bone marrow and the skin (Heinonen *et al.* 2013), tissues where O_2 extraction is expected to be lower than in the maximal exercising muscles. In addition, part of the P_{FVO_2} remaining at maximal exercise could be accounted for by some heterogeneity in the ratio perfusion/$\dot{V}_{O_2}$ between leg muscles (Heinonen *et al.* 2010; Cano *et al.* 2015).

Leg $\dot{V}_{O_2}$ during sprint exercise is more perfusion than diffusion limited, even in severe acute hypoxia

By applying the Piiper and Scheid model to quantify muscle O_2 transfer we have determined the relative roles of O_2 delivery (blood flow × C_{aO_2}) (perfusion) and diffusion in O_2 supply to exercising muscles. This model allows the calculation of the '*Y*' equilibration index (Piiper, 2000), which gives a quantitative assessment of the magnitude of perfusion-to-diffusion limitation ($Y > 3$ indicates perfusion limitation; $3 > Y > 0.1$, combined perfusion and diffusion limitation, $Y < 0.1$, diffusion limitation). Values above 1 indicate more perfusion than diffusion limitation and conversely values below 1 indicate more diffusion than perfusion limitation (Piiper & Scheid, 1981). In our experiments, *Y* always encompassed a range between 3 and 0.1, indicating that O_2 supply to the muscle is limited by both perfusion and diffusion, with the perfusion component of the limitation increasing throughout the duration of the sprint in both conditions. The fact that *Y* was always higher in normoxia than hypoxia indicates a greater diffusional component in hypoxia than in normoxia, as predicted by Wagner (1993) (Fig. 3). Moreover, *Y* values fall below 1 at the onset of the sprint in hypoxia, indicating that at the onset of the sprint in hypoxia the diffusion limitation predominates. This is caused by the lower O_2 delivery during the sprint in hypoxia than in normoxia. Despite this higher diffusion limitation in hypoxia, the flow of O_2 to the mitochondria was sufficient to match the normoxic $\dot{V}_{O_2}$ during the first 15 s of the sprint.

The limitation to leg $\dot{V}_{O_2}$ during sprint exercise resides within the muscle fibres

It has been suggested that the sarcolemma and other structural elements interposed between the erythrocytes and the mitochondria pose a resistance to O_2 diffusion during exercise (Krogh, 1919*a*, *b*; Wagner, 2000; Boushel *et al.* 2014*a*) and that this resistance plays a greater limiting role on $\dot{V}_{O_2peak}$ in hypoxia than in normoxia, due to the lower P_{O_2} gradient driving diffusion in hypoxia (Wagner, 1993). During the first 10 s of the normoxic sprint exercise, O_2 delivery was so high that O_2 fractional

extraction was even reduced, compared to that observed just before the start of the sprint. The fact that blood flow and leg vascular conductance (data not shown) increased similarly at the start of the sprint in normoxia and hypoxia suggests comparable levels of vasodilatation in both sprints. This implies that the recruitment of additional D_{MO_2} during hypoxia is not due to higher recruitment of muscle capillaries, i.e. vasodilatation. Therefore, in humans the limitation to muscle $\dot{V}_{O_2}$ at the start of the sprint is likely to reside within the muscle fibres and most likely in the mitochondria themselves, as previously shown in isolated canine muscle *in vivo* (Grassi *et al.* 1998) and isolated muscle fibres (Gandra *et al.* 2012) (for review see Clanton *et al.* 2013). Accumulation of muscle metabolites from anaerobic energy pathways (ADP, P_i, Cr/PCr, Ca^{2+}) is required to achieve high mitochondrial respiration rates (Boushel *et al.* 2014*b*), yet metabolite accumulation does not occur in substantial amount during the first 10 s of sprint exercise (Bogdanis *et al.* 1998). Thus, at the onset of exercise, a low [ADP] in the setting of high O_2 availability results in a low ADP/O_2 flux ratio due to ADP/ATP limitation. This pattern is explained by a lower proton flux into the matrix relative to proton leak. A higher accumulation of by-products of anaerobic metabolism may occur during hypoxic sprints (Morales-Alamo *et al.* 2012), but elevated [ADP] is known to increase mitochondrial P_{50} (Gnaiger, 2001), and therefore does not provide an obvious explanation for the higher D_{MO_2} in hypoxia that we observed. However, mitochondrial O_2 flux rate increases to a greater extent than that of P_{50} with increasing activation by ADP, and the P/O ratio is also increased in hypoxia in part due to a lower thermodynamic control of leak respiration. Despite very low reported mitochondrial P_{O_2} at maximal exercise in normoxia (~3.1 mmHg) and hypoxia (~2.1 mmHg) (Richardson *et al.* 1995), mitochondria respire at ~70–90% of maximum flux rate (V_{max}) (Gnaiger, 2001; Boushel *et al.* 2011) owing to excess capacity of mitochondria and high affinity of cytochrome *c* oxidase (COX). Yet, due to the oxygen dependence of mitochondrial respiration, the relative V_{max} of mitochondria is reduced in hypoxia, and accordingly, so is the P_{50} (Gnaiger, 2001). This effect allows for a greater O_2 extraction for a given P_{O_2} gradient in hypoxia ($\dot{V}_{O_2}$ mitochondria $= V_{max} \times P_{O_2}/(P_{O_2} + P_{50})$. An additional mechanism may relate to the O_2 affinity of COX. Of the two isoforms of COX, COXIV-2 has a higher O_2 affinity and is known to be upregulated in hypoxia (Fukuda *et al.* 2007; Desplanches *et al.* 2014). The isoform switch abolishes the allosteric ATP feedback inhibition of COX (Arnold, 2012) leading to increased [ATP] and in turn a higher ROS production as previously observed during sprint exercise in hypoxia (Morales-Alamo *et al.* 2012). Acute regulation of mitochondrial P_{50} by partial COX inhibition has been demonstrated (Larsen *et al.* 2011), and there is evidence for acute changes in COX O_2 affinity with acute exposure to hypoxia. In mouse cardiac and skeletal muscle ADP-stimulated respiration under fully oxygenated conditions is acutely blunted following brief periods of anoxia (Soltysinska *et al.* 2014), while the

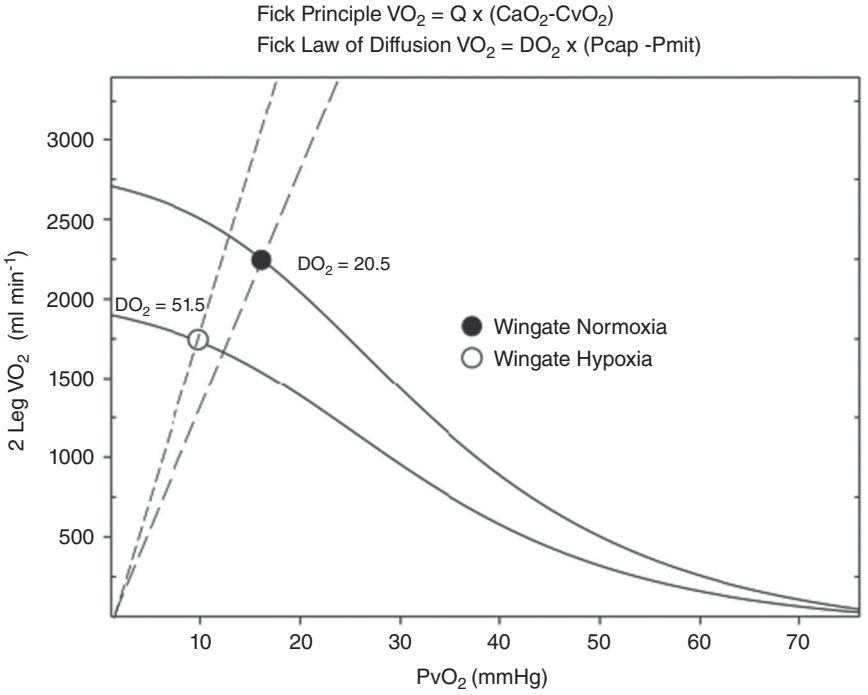

Figure 3. Wagner plot showing convection versus diffusion limitations during sprint exercise in normoxia ($P_{IO_2} \approx$ 143 mmHg) and hypoxia ($P_{IO_2} \approx$ 73 mmHg)
Two-leg $\dot{V}_{O_2}$ during the Wingate test in normoxia (filled symbols) and hypoxia (open symbols) represented by Fick's law of diffusion ($\dot{V}_{O_2} = D_{O_2} \times (P_{O_2cap} - P_{O_2mit})$) and the Fick principle ($\dot{V}_{O_2} = \dot{Q}(C_{aO_2} - C_{vO_2})$). The lines from the origin through the data points ($\dot{V}_{O_2}$) reflect Fick's Law of diffusion with their corresponding values for normoxia and hypoxia. The sigmoid curves represent $\dot{V}_{O_2}$ defined by the Fick principle. Despite lower O_2 delivery in hypoxia (vertical displacement in sigmoid curve), the steeper slope of the relationship between $\dot{V}_{O_2}$ and P_{vO_2} (Fick diffusion) shows the higher D_{MO_2} in hypoxia. $\dot{Q}$, two-legged blood flow; C_{aO_2}, arterial [O_2]; C_{vO_2}, venous [O_2]; D_{O_2}, O_2 diffusing capacity; P_{cap}, mean capillary P_{O_2}; P_{mito}, P_{O_2} at cytochrome *c* oxidase of mitochondria (assumed to approach zero at muscle $\dot{V}_{O_2max}$). $n = 9$.

opposite effect is observed in humans. Mitochondria isolated from quadriceps muscle acutely increase maximal O_2 flux capacity 20% above the pre-anoxia respiratory rate (F. J. Larsen and R. Boushel, unpublished observation). Thus, a COXIV-2 isoform switch with corresponding higher O_2 affinity is a plausible explanation for the high D_{MO_2} during sprint exercise in hypoxia.

Peak leg $\dot{V}_{O_2}$ is higher during sprint exercise than incremental exercise to exhaustion in severe acute hypoxia

Despite the short duration of the sprint, 14% higher values of peak Leg $\dot{V}_{O_2}$ were achieved during the sprint exercise in hypoxia compared to the incremental exercise in hypoxia. This was possible due to an almost similar peak leg blood flow and cardiac output during sprint and incremental exercise in severe hypoxia, combined with better pulmonary gas exchange during sprint exercise and a slightly greater leg O_2 extraction also during sprint exercise ($P = 0.06$). It should be highlighted that [Hb] was lower, in part due to blood sampling, but also due to lower exercise-induced haemoconcentration during the short sprints. Functional [Hb] was reduced due to a 7% COHb in blood, and the subjects could have been more tired when they performed the Wingate tests. Nevertheless, a more efficient pulmonary gas exchange, combined with left-shift of ODC resulted in a slightly higher C_{aO_2} during the sprint in hypoxia compared to the incremental exercise in hypoxia. The latter combined with an almost similar LBF permitted a greater leg O_2 delivery during sprint than incremental exercise to exhaustion in hypoxia and hence a greater leg $\dot{V}_{O_2}$, despite a similarly low P_{O_2} gradient (33.3 and 34.1 mmHg) for O_2 diffusion. Thus, leg $\dot{V}_{O_2}$ is also more perfusion than diffusion limited during incremental exercise to exhaustion in severe acute hypoxia. A higher $\dot{V}_{O_2}$ with a similar perfusion and P_{O_2} gradients is only possible if structural resistances in the pathway from the erythrocytes to the mitochondria play a minor role limiting O_2 trasnfer. In the latter case, at the same level of perfusion and P_{O_2} gradient, $\dot{V}_{O_2}$ should be increased just by increasing the C_{aO_2}, as actually observed during the hypoxic sprints in this investigation. Moreover, a recent study also suggests that lower [Hb] may be compensated by increased D_{MO_2} (Simonson *et al.*, 2015).

Limitations

We assumed that P_{O_2} at the level of cytochrome *c* oxidase is close to 0 mmHg in normoxia and hypoxia for the determination of D_{MO_2} at $\dot{V}_{O_2max}$ (Severinghaus, 1994) and during submaximal exercise (Richardson *et al.* 1995). This is a reasonable assumption (Cano *et al.* 2013) based on MbO_2 saturation determinations at submaximal and maximal exercise in normoxia and hypoxia ($F_{IO_2} = 0.12$) during knee extension exercise (Richardson *et al.* 1995). Intracellular P_{O_2} might have been slightly lower given the lower mean capillary P_{O_2} observed during whole-body incremental exercise compared to knee extension exercise (Calbet *et al.* 2009). However, potential small differences in intracellular (sarcoplasmic) P_{O_2} at $\dot{V}_{O_2max}$ would cause a small (~3–5%) underestimation of the actual D_{MO_2}, which is dominated by capillary P_{O_2} illustrated by the Fick diffusion equation ($\dot{V}_{O_2} = D_{O_2}(P_{O_2cap} - P_{O_2mit})$), where D_{O_2} is O_2 diffusing capacity, P_{O_2cap} is capillary O_2 pressure and P_{O_2mit} is mitochondrial O_2 pressure. It remains to be determined what the mechanisms allowing additional D_{MO_2} recruitment during exercise in severe acute hypoxia are. One possibility is through increased myoglobin-facilitated O_2 diffusion, as described in isolated canine muscle during exercise with severe anaemia (Honig & Gayeski, 1993). Another possibility could be related to changes in cytochrome *c* oxidase P_{50} (as already discussed). Limitations related to the procedures used to calculate D_{MO_2} have been described in the past (Roca *et al.* 1989).

In summary, we have demonstrated that muscle $\dot{V}_{O_2}$ during sprint exercise in normoxia is not limited by O_2 delivery, O_2 offloading from the haemoglobin or structure-dependent diffusion constraints in the skeletal muscle of young healthy men. Rather we have shown that a large functional reserve in muscle O_2 diffusing capacity exists and remains available at exhaustion during exercise in normoxia, and which is recruited during exercise in hypoxia. Consequently, even during whole-body incremental exercise to exhaustion in severe hypoxia, leg $\dot{V}_{O_2}$ is primarily dependent on O_2 delivery and less limited by diffusion constraints than previously thought.

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Additional information

Competing interests

None of the authors has any conflicts of interests.

Author contributions

Experiments were carried out at the Human Performance Laboratory of the University of Las Palmas de Gran Canaria. Conception and design of the experiments: J.A.C. Pre-testing and experimental preparation: J.L.R., R.T.P., J.P.G., A.G.G., J.C.H., D.M.A. and J.A.C. All co-authors participated in data collection during the main experiments. Data assembly and analysis: J.L.R., R.T.P., J.P.G., A.G.G., J.C.H., D.M.A., R.B., P.R., L.R. and J.A.C. The first version of the manuscript was written by J.A.C., which was critically reviewed by C.L. and R.B. All co-authors read, contributed comments to and approved the final version of the manuscript.

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