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# Limitations to oxygen transport and utilization during sprint exercise in humans: evidence for a functional reserve in muscle O<sub>2</sub> diffusing capacity

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## Key points

- Severe acute hypoxia reduces sprint performance.
- Muscle  $\dot{V}_{O_2}$  during sprint exercise in normoxia is not limited by O<sub>2</sub> delivery, O<sub>2</sub> offloading from haemoglobin or structure-dependent diffusion constraints in the skeletal muscle of young healthy men.
- A large functional reserve in muscle O<sub>2</sub> 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<sub>2</sub> delivery and less limited by diffusion constraints than previously thought.
- The kinetics of O<sub>2</sub> 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<sub>2</sub> diffusing capacity ( $D_{MO_2}$ ) was calculated. In the WgT mean power output, leg BF, leg O<sub>2</sub> 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<sup>-1</sup> mmHg<sup>-1</sup>,  $P < 0.05$ ). 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<sub>2</sub> transfer in both Nx and Hyp. In conclusion, muscle  $\dot{V}_{O_2}$  during sprint exercise is not limited by O<sub>2</sub> delivery, O<sub>2</sub> offloading from haemoglobin or structure-dependent diffusion constraints in

the skeletal muscle. These findings reveal a remarkable functional reserve in muscle O<sub>2</sub> diffusing capacity.

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**Abbreviations** a-vO<sub>2</sub>diff, arteriovenous oxygen concentration difference; BF, blood flow; C<sub>aO<sub>2</sub></sub>, arterial content of oxygen; CO, carbon monoxide; COHb, carboxyhaemoglobin; D<sub>LO<sub>2</sub></sub>, lung O<sub>2</sub> diffusing capacity; D<sub>MO<sub>2</sub></sub>, muscle O<sub>2</sub> diffusing capacity; D<sub>O<sub>2</sub></sub>, O<sub>2</sub> diffusing capacity; ECG, electrocardiogram; F<sub>IO<sub>2</sub></sub>, inspired oxygen fraction; FV, femoral vein; HR<sub>max</sub>, maximal heart rate; HR<sub>peak</sub>, peak heart rate during Wingate; Hyp, hypoxia; LBF, leg blood flow; Nx, normoxia; S<sub>O<sub>2</sub></sub>, haemoglobin saturation with O<sub>2</sub>; ODC, oxyhaemoglobin dissociation curve; P<sub>50</sub>, partial oxygen pressure at 50% S<sub>O<sub>2</sub></sub>; P<sub>aO<sub>2</sub></sub>, arterial oxygen pressure; P<sub>CO<sub>2</sub></sub>, carbon dioxide pressure; P<sub>O<sub>2</sub></sub>, oxygen pressure; P<sub>O<sub>2</sub>cap</sub>, capillary O<sub>2</sub> pressure; P<sub>O<sub>2</sub>mit</sub>, mitochondrial O<sub>2</sub> pressure; P<sub>FVO<sub>2</sub></sub>, femoral vein P<sub>O<sub>2</sub></sub>; P<sub>IO<sub>2</sub></sub>, inspiratory O<sub>2</sub> pressure;  $\dot{V}_{CO_2}$ , carbon dioxide production;  $\dot{V}_{CO_2\text{peak}}$ , peak carbon dioxide production;  $\dot{V}_{E\text{peak}}$ , peak pulmonary ventilation;  $\dot{V}_{O_2}$ , oxygen consumption;  $\dot{V}_{O_2\text{max}}$ , maximal oxygen consumption;  $\dot{V}_{O_2\text{peak}}$ , peak oxygen uptake; W<sub>peak-i</sub>, instantaneous peak power output; W<sub>mean-10</sub>, mean power output during the first 10 s of the sprint exercise; W<sub>mean-30</sub>, 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<sub>2</sub> delivery to the mitochondria or reduced mitochondrial O<sub>2</sub> 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<sub>2</sub> to the mitochondria, while during prolonged sprints (more than 10–15 s long) the aerobic energy yield will become O<sub>2</sub> delivery dependent. Testing this hypothesis requires the assessment of muscle O<sub>2</sub> delivery and muscle O<sub>2</sub> 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.* 2003b). 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<sub>2</sub> 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<sub>2</sub> 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)<sup>-1</sup>) compared to lower perfusion rates achieved during whole-body exercise (ml (100 g muscle mass)<sup>-1</sup>) (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<sub>2</sub> delivery may limit VO<sub>2</sub> at the start of whole-body sprint exercise.

Oxygen delivery has a convective component, which determines the amount of O<sub>2</sub> arriving to the muscle capillaries, and a diffusive component that depends on the O<sub>2</sub> offloading in muscle capillaries and transfer of free O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> (usually measured as the  $P_{O_2}$  eliciting a 50% haemoglobin saturation with O<sub>2</sub> ( $S_{O_2}$ ) 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> delivery at the onset of sprint exercise; (ii) to establish if blood O<sub>2</sub> 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 were 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  $\pm$  SD age, height, weight

and percentage of body fat were:  $21.5 \pm 2.0$  years,  $173.8 \pm 8.0$  cm,  $72.3 \pm 9.3$  kg, and  $16.1 \pm 4.9\%$ , 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<sub>2</sub> 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<sub>2</sub> 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 ( $F_{\text{IO}_2} \approx 13.3$ ,  $P_{\text{IO}_2} \approx 91$  mmHg) and a small amount of CO (7 ml (kg body mass)<sup>-1</sup>). 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 ( $F_{\text{IO}_2}$ :  $\sim 13.3$ ,  $P_{\text{IO}_2}$ :  $\sim 91$  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 ( $P_{\text{IO}_2} \approx 73$  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 ( $P_{\text{aO}_2}$ ) while keeping the arterial content of oxygen ( $C_{\text{aO}_2}$ ) 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 ( $W_{\text{peak-i}}$ ), and mean power output during the first 10 s of the sprints ( $W_{\text{mean-10}}$ ) and mean power output achieved during the full duration of the sprints ( $W_{\text{mean-30}}$ ). Whole-body oxygen uptake was measured with a metabolic cart ( $V_{\text{max}}$  N29; SensorMedics, Yorba Linda, CA, USA), calibrated prior to each test according to the manufacturer's instructions, with high-grade calibration gases (Carbueros 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  $\dot{V}_{O_2\text{max}}$ . The same criterion was applied to determine the  $\dot{V}_{O_2\text{peak}}$  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<sup>-1</sup>), 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.  $(1.34[\text{Hb}] \times S_{O_2}) + (0.03 \times P_{O_2})$ ). Arteriovenous  $[O_2]$  difference (a- $vO_2$ diff) 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- $vO_2$ diff. 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  $\pm$  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.  $W_{\text{max}}$  and whole-body (or pulmonary)  $\dot{V}_{O_2\text{peak}}$  were reduced in hypoxia by 42 and 34%, respectively; while peak heart rate during Wingate ( $HR_{\text{peak}}$ ),  $\dot{V}_{CO_2\text{peak}}$  and peak pulmonary ventilation ( $\dot{V}_{E\text{peak}}$ ) 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
$F_{I\text{O}_2}$ (%)	20.80 ± 0.15	10.84 ± 0.21 <sup>a</sup>	20.88 ± 0.06	10.75 ± 0.23 <sup>a</sup>
$P_{I\text{O}_2}$ (mmHg)	141.6 ± 1.2	74.0 ± 1.5 <sup>a</sup>	142.9 ± 0.7	73.5 ± 1.7 <sup>a</sup>
$W_{\max}/W_{\text{peak-i}}$ (W)	286.7 ± 43.6	166.1 ± 37.2 <sup>a</sup>	972.9 ± 300.2 <sup>b</sup>	905.9 ± 377.4 <sup>b</sup>
$\dot{V}_{\text{O}_2\text{peak}}$ (l min <sup>-1</sup> )	3.56 ± 0.34	2.37 ± 0.29 <sup>a</sup>	2.75 ± 0.40 <sup>b</sup>	2.19 ± 0.19 <sup>a</sup>
$\dot{V}_{\text{O}_2\text{peak}}$ (ml kg <sup>-1</sup> min <sup>-1</sup> )	50.7 ± 4.0	33.5 ± 2.6 <sup>a</sup>	39.2 ± 5.9 <sup>b</sup>	31.2 ± 3.0 <sup>a</sup>
Leg $\dot{V}_{\text{O}_2\text{peak}}$ (ml min <sup>-1</sup> )	1.25 ± 0.22	0.76 ± 0.11 <sup>a</sup>	1.12 ± 0.15 <sup>b</sup>	0.87 ± 0.14 <sup>ab</sup>
HR <sub>peak</sub> (beats min <sup>-1</sup> )	192.9 ± 6.9	178.3 ± 7.4 <sup>a</sup>	180.9 ± 5.5 <sup>b</sup>	177.9 ± 7.7
$\dot{V}_{\text{CO}_2\text{peak}}$ (l min <sup>-1</sup> )	4.09 ± 0.43	2.92 ± 0.53 <sup>a</sup>	3.29 ± 0.72 <sup>b</sup>	3.27 ± 0.57
RER	1.15 ± 0.03	1.23 ± 0.11 <sup>a</sup>	1.19 ± 0.16	1.48 ± 0.17 <sup>ab</sup>
$\dot{V}_{\text{Epeak}}$ (l min <sup>-1</sup> )	135.6 ± 18.7	103.9 ± 21.0 <sup>a</sup>	107.6 ± 34.9	113.1 ± 23.2 <sup>b</sup>
$P_{\text{aO}_2}$ (mmHg)	100.2 ± 7.3	33.3 ± 2.4 <sup>a</sup>	101.8 ± 5.5	34.1 ± 3.3 <sup>a</sup>
$P_{\text{FVO}_2}$ (mmHg)	21.1 ± 2.0	10.6 ± 2.8 <sup>a</sup>	16.2 ± 2.5 <sup>b</sup>	9.9 ± 2.5 <sup>a</sup>
$P_{\text{aCO}_2}$ (mmHg)	34.2 ± 3.1	29.7 ± 2.0 <sup>a</sup>	35.0 ± 3.1	31.3 ± 2.5 <sup>a</sup>
$P_{\text{FVCO}_2}$ (mmHg)	79.7 ± 7.2	53.5 ± 5.9 <sup>a</sup>	82.3 ± 8.7	71.8 ± 6.7 <sup>ab</sup>
A pH	7.225 ± 0.048	7.332 ± 0.084 <sup>a</sup>	7.413 ± 0.025 <sup>b</sup>	7.454 ± 0.029 <sup>ab</sup>
FV pH	7.060 ± 0.058	7.206 ± 0.091 <sup>a</sup>	7.179 ± 0.030 <sup>b</sup>	7.220 ± 0.027 <sup>a</sup>
A Hb (g l <sup>-1</sup> )	169.3 ± 4.7	163.2 ± 7.0 <sup>a</sup>	153.7 ± 7.1 <sup>b</sup>	154.9 ± 4.7 <sup>b</sup>
$S_{\text{aO}_2}$ (%)	96.1 ± 1.2	63.5 ± 6.1 <sup>a</sup>	98.5 ± 0.4 <sup>b</sup>	75.8 ± 6.2 <sup>ab</sup>
$S_{\text{FVO}_2}$ (%)	19.9 ± 5.3	11.8 ± 5.2 <sup>a</sup>	22.4 ± 3.8	15.1 ± 3.9 <sup>a</sup>
A O <sub>2</sub> Hb (%)	94.9 ± 1.2	62.4 ± 5.9 <sup>a</sup>	91.8 ± 1.0 <sup>b</sup>	69.8 ± 5.7 <sup>ab</sup>
FV O <sub>2</sub> Hb (%)	18.3 ± 5.3	10.1 ± 5.2 <sup>a</sup>	15.0 ± 4.0	6.9 ± 4.2 <sup>a</sup>
A COHb (%)	0.5 ± 0.2	1.0 ± 0.4 <sup>a</sup>	6.2 ± 0.9 <sup>b</sup>	7.3 ± 2.1 <sup>b</sup>
FV COHb (%)	0.7 ± 0.1	0.9 ± 0.5	6.6 ± 0.9 <sup>b</sup>	7.3 ± 2.2 <sup>b</sup>
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
$C_{\text{aO}_2}$ (ml l <sup>-1</sup> )	218.3 ± 7.7	137.4 ± 12.9 <sup>a</sup>	192.1 ± 9.0 <sup>b</sup>	146.0 ± 13.3 <sup>ab</sup>
$C_{\text{FVO}_2}$ (ml l <sup>-1</sup> )	42.4 ± 12.7	22.3 ± 11.7 <sup>a</sup>	32.9 ± 9.9 <sup>b</sup>	15.4 ± 9.4 <sup>a</sup>
Leg fractional O <sub>2</sub> extraction (%)	80.6 ± 5.4	84.2 ± 7.0 <sup>a</sup>	83.0 ± 4.5	89.6 ± 5.9 <sup>ac</sup>
$D_{\text{LO}_2}$ (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	64.2 ± 6.0	96.2 ± 10.7 <sup>a</sup>	49.3 ± 7.1 <sup>b</sup>	85.7 ± 5.4 <sup>ab</sup>
$D_{\text{MO}_2}$ (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	25.2 ± 5.2	46.0 ± 7.3 <sup>a</sup>	20.5 ± 3.0 <sup>b</sup>	51.5 ± 9.7 <sup>ab</sup>

<sup>a</sup> $P < 0.05$  Normoxia vs. Hypoxia; <sup>b</sup> $P < 0.05$  compared to incremental exercise same level of oxygenation; <sup>c</sup> $P = 0.06$  compared to incremental exercise same oxygenation. A, arterial; FV, femoral vein; metHb, methaemoglobin  $D_{\text{LO}_2}$ , lung O<sub>2</sub> diffusing capacity.

normoxia. Arterial  $P_{\text{O}_2}$  was reduced to  $33 \pm 2$  mmHg in hypoxia.

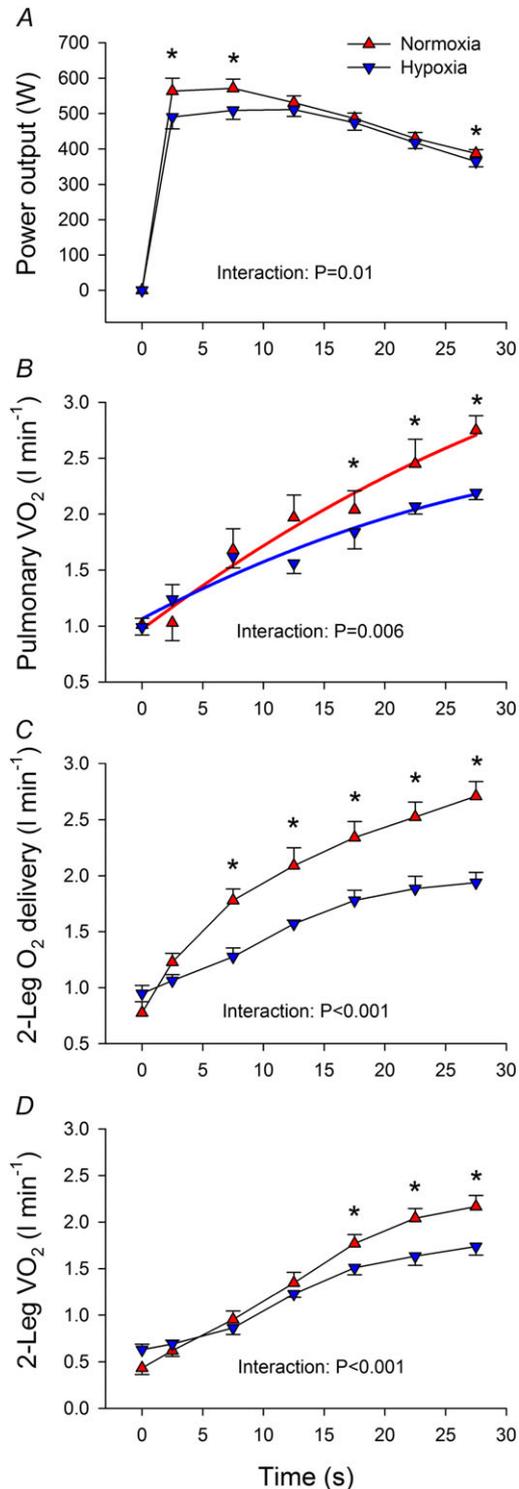
### Sprint exercise power output and $\dot{V}_{\text{O}_2}$ : first 10 s of the Wingate test

Power output, O<sub>2</sub> delivery and  $\dot{V}_{\text{O}_2}$  are depicted in Fig. 1. Peak power output ( $W_{\text{peak-i}}$ ) was not statistically different between normoxia and hypoxia ( $973 \pm 300$  and  $906 \pm 377$  W, respectively,  $P = 0.36$ ). However, during the first 10 s of the sprints, mean power output ( $W_{\text{mean-10}}$ ) was 12% lower in hypoxia than normoxia ( $567 \pm 90$  and  $499 \pm 86$  W, respectively,  $P < 0.05$ ). The reduction in  $W_{\text{mean-10}}$  was not due to lower  $\dot{V}_{\text{O}_2}$ , since the legs consumed a similar total amount of O<sub>2</sub> in normoxia and hypoxia ( $131 \pm 34$  and  $129 \pm 26$  ml per leg, respectively,  $P = 0.87$ ), which was confirmed by a similar pulmonary O<sub>2</sub> uptake

( $452 \pm 145$  and  $478 \pm 108$  ml in normoxia and hypoxia, respectively,  $P = 0.59$ ). This occurred despite a 22% lower leg O<sub>2</sub> delivery in hypoxia during the first 10 s of the sprints ( $1.50 \pm 0.23$  and  $1.17 \pm 0.17$  l min<sup>-1</sup>,  $P < 0.05$ ), which was compensated by 14 units higher leg fractional O<sub>2</sub> extraction in hypoxia than normoxia ( $51.7 \pm 9.2$  and  $65.9 \pm 6.1\%$ , respectively,  $P < 0.05$ ).

### Sprint exercise power output and $\dot{V}_{\text{O}_2}$ : entire Wingate test

The mean power output during the entire sprints ( $W_{\text{mean-30}}$ ) was reduced by 7%, from  $494 \pm 47$  W in normoxia to  $461 \pm 52$  W in hypoxia ( $P < 0.05$ ). However, during the last 15 s of the sprints, mean power output was not different between conditions ( $434 \pm 37$  and  $419 \pm 49$  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. 1A). The rate of increase in pulmonary  $\dot{V}O_2$  was higher in normoxia than hypoxia ( $F_{iO_2} \times \text{time}$ :  $P < 0.05$ ). Consequently, the mean pulmonary  $\dot{V}O_2$  during the whole sprints was 12% lower in hypoxia than in normoxia ( $P < 0.05$ ) (Fig. 1B).

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

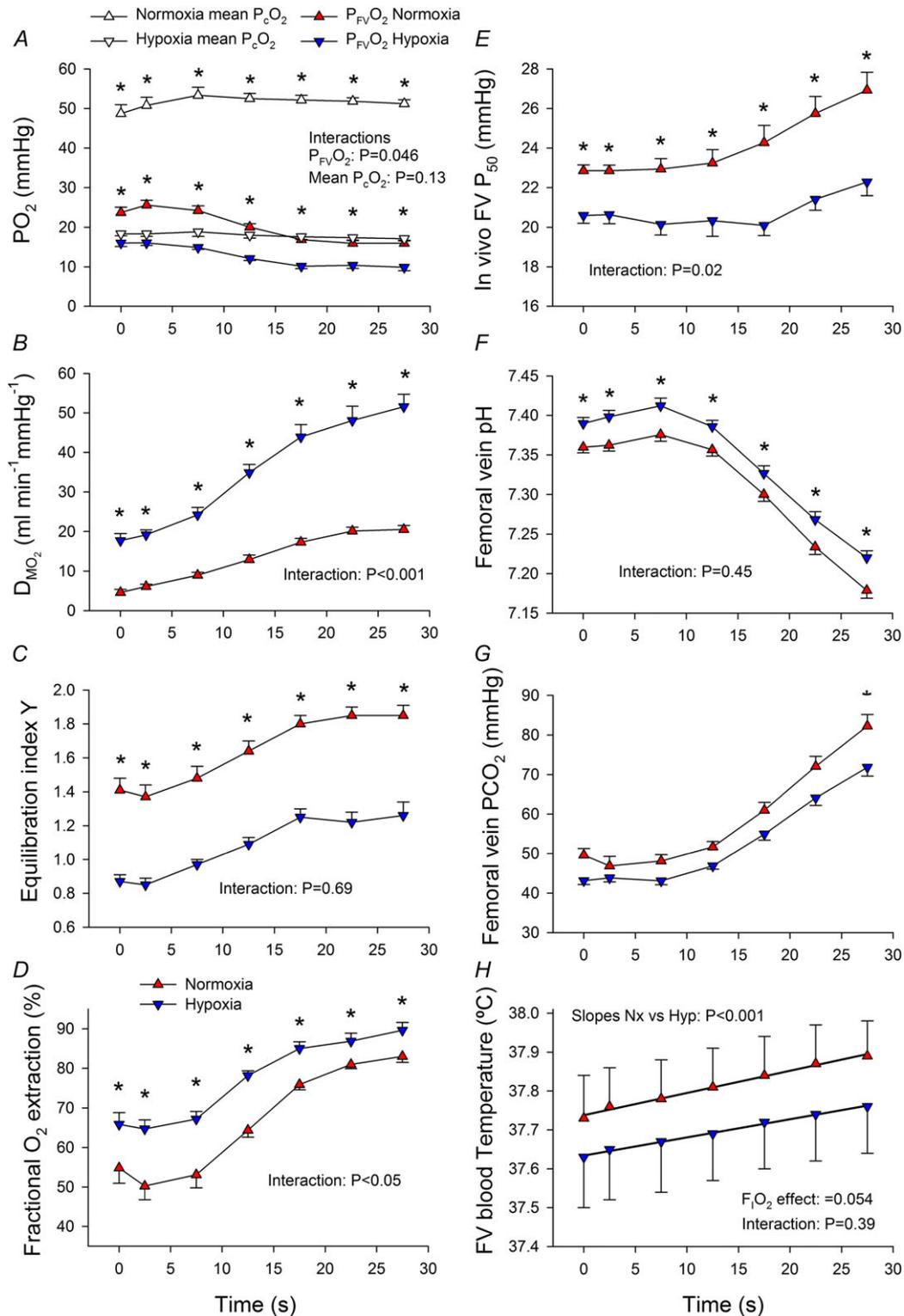
### Peak oxygen delivery and utilization during the sprints

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  $180.9 \pm 5.5$  and  $177.9 \pm 7.7$  beats min<sup>-1</sup> 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  $23.2 \pm 2.5$  and  $21.0 \pm 2.5$  l min<sup>-1</sup> in normoxia and hypoxia, respectively ( $P < 0.05$ ). Peak systemic  $O_2$  delivery was reduced by 32% in hypoxia (from  $4.47 \pm 0.59$  to  $3.06 \pm 0.41$  l min<sup>-1</sup>,  $P < 0.05$ ). However,  $\dot{V}O_{2\text{peak}}$  was reduced by only 20% ( $2.75 \pm 0.40$  and  $2.19 \pm 0.19$  l min<sup>-1</sup> in normoxia and hypoxia, respectively,  $P < 0.05$ ), due to 10 units greater systemic  $O_2$  extraction in hypoxia ( $62.7 \pm 13.2$  and  $72.2 \pm 6.4\%$ ,  $P < 0.05$ ).

Peak leg blood flow during the sprints was 5% lower in hypoxia ( $7.04 \pm 0.92$  and  $6.66 \pm 0.95$  l min<sup>-1</sup>,  $P < 0.05$ ), which combined with the 24% lower  $C_{aO_2}$  in hypoxia resulted in a 28% lower peak leg  $O_2$  delivery in hypoxia ( $2.71 \pm 0.39$  and  $1.94 \pm 0.28$  l min<sup>-1</sup>,  $P < 0.05$ ). Consequently, leg  $\dot{V}O_{2\text{peak}}$  was only 23% lower in hypoxia ( $2.24 \pm 0.30$  and  $1.74 \pm 0.28$  l min<sup>-1</sup>,  $P < 0.05$ ) due to greater leg fractional  $O_2$  extraction in hypoxia ( $83.0 \pm 4.6$  and  $89.6 \pm 5.9\%$ ,  $P < 0.05$ ).

### 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 ( $F_{iO_2} \times \text{time}$ :  $P < 0.001$ ), to reach peak values of  $20.5 \pm 3.0$  and  $51.5 \pm 9.7$  ml min<sup>-1</sup> mmHg<sup>-1</sup> in normoxia and hypoxia, respectively ( $P < 0.05$ ) (Fig. 2B).



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  $23.9 \pm 4.4$  to  $16.2 \pm 2.5$  mmHg) and hypoxia (from  $16.0 \pm 2.5$  to  $9.9 \pm 2.5$  mmHg, both  $P < 0.05$ ) (Fig. 2A). The magnitude of the  $P_{FVO_2}$  decline was similar in both conditions. However, there was an  $F_{IO_2} \times \text{time}$  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. 2A). 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. 2D). 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. 2E).

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. 2F). This was mirrored by the changes in  $P_{FVCO_2}$ , with a greater increase of  $P_{FVCO_2}$  in normoxia than hypoxia ( $F_{IO_2} \times \text{time}$ :  $P < 0.05$ ) (Fig. 2G). 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. 2H).

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 ( $1.85 \pm 0.19$  and  $1.26 \pm 0.23$ , 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-Tessonier *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.* 2003b) 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  $\alpha$ -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. 2E). Consequently, the *in vivo*  $P_{50}$  was  $\sim 5$  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<sub>2</sub> 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<sub>2</sub> offloading during exercise in general.

### Muscle O<sub>2</sub> diffusing capacity does not limit $\dot{V}_{O_2}$ during sprint exercise in normoxia

The diffusion of O<sub>2</sub> from the muscle capillaries to the mitochondria is driven by the O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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. 2A). 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<sup>-1</sup> mm Hg<sup>-1</sup>), and even higher than the 42 ml min<sup>-1</sup> mmHg<sup>-1</sup> 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<sub>2</sub> left in the femoral vein and

that  $P_{FVO_2}$  is ~10 mmHg suggests a diffusion limitation, although part of the O<sub>2</sub> remaining is certainly due to some venous admixture, with blood draining the bone marrow and the skin (Heinonen *et al.* 2013), tissues where O<sub>2</sub> 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

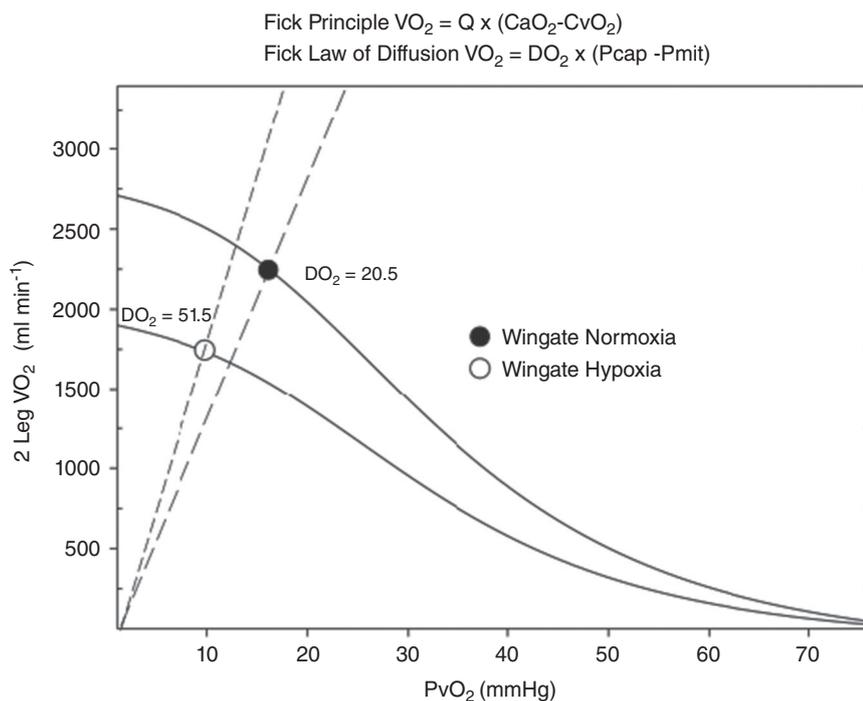
By applying the Piiper and Scheid model to quantify muscle O<sub>2</sub> transfer we have determined the relative roles of O<sub>2</sub> delivery (blood flow ×  $C_{aO_2}$ ) (perfusion) and diffusion in O<sub>2</sub> supply to exercising muscles. This model allows the calculation of the 'Y' equilibration 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<sub>2</sub> 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<sub>2</sub> delivery during the sprint in hypoxia than in normoxia. Despite this higher diffusion limitation in hypoxia, the flow of O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> delivery was so high that O<sub>2</sub> 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.* 2014b), 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 ( $\sim 3.1$  mmHg) and hypoxia ( $\sim 2.1$  mmHg) (Richardson *et al.* 1995), mitochondria respire at  $\sim 70$ – $90\%$  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 ( $\dot{V}O_2 = D_{O_2} \times (P_{O_2, cap} - P_{O_2, mit})$ ) and the Fick principle ( $\dot{V}O_2 = Q(C_{aO_2} - C_{vO_2})$ ). 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.  $Q$ , 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  $\dot{V}O_{2, max}$ ).  $n = 9$ .

opposite effect is observed in humans. Mitochondria isolated from quadriceps muscle acutely increase maximal O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> transfer. 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<sub>2</sub> 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 (sarcolemmal)  $P_{O_2}$  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<sub>2</sub> diffusing capacity,  $P_{O_2cap}$  is capillary O<sub>2</sub> pressure and  $P_{O_2mit}$  is mitochondrial O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> delivery, O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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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