On the mechanisms that limit oxygen uptake during exercise in acute and chronic hypoxia: role of muscle mass

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Peak aerobic power in humans ($\dot{V}_{O_2,peak}$) is markedly affected by inspired O_2 tension (F_{IO_2}). The question to be answered in this study is what factor plays a major role in the limitation of muscle peak $\dot{V}_{O_2}$ in hypoxia: arterial O_2 partial pressure (P_{a,O_2}) or O_2 content (C_{a,O_2})? Thus, cardiac output (dye dilution with Cardio-green), leg blood flow (thermodilution), intra-arterial blood pressure and femoral arterial-to-venous differences in blood gases were determined in nine lowlanders studied during incremental exercise using a large (two-legged cycle ergometer exercise: Bike) and a small (one-legged knee extension exercise: Knee) muscle mass in normoxia, acute hypoxia (AH) ($F_{IO_2} = 0.105$) and after 9 weeks of residence at 5260 m (CH). Reducing the size of the active muscle mass blunted by 62% the effect of hypoxia on $\dot{V}_{O_2,peak}$ in AH and abolished completely the effect of hypoxia on $\dot{V}_{O_2,peak}$ after altitude acclimatization. Acclimatization improved Bike peak exercise P_{a,O_2} from 34 ± 1 in AH to 45 ± 1 mmHg in CH ($P < 0.05$) and Knee P_{a,O_2} from 38 ± 1 to 55 ± 2 mmHg ($P < 0.05$). Peak cardiac output and leg blood flow were reduced in hypoxia only during Bike. Acute hypoxia resulted in reduction of systemic O_2 delivery (46 and 21%) and leg O_2 delivery (47 and 26%) during Bike and Knee, respectively, almost matching the corresponding reduction in $\dot{V}_{O_2,peak}$. Altitude acclimatization restored fully peak systemic and leg O_2 delivery in CH (2.69 ± 0.27 and 1.28 ± 0.11 l min^{-1}, respectively) to sea level values (2.65 ± 0.15 and 1.16 ± 0.11 l min^{-1}, respectively) during Knee, but not during Bike. During Knee in CH, leg oxygen delivery was similar to normoxia and, therefore, also $\dot{V}_{O_2,peak}$ in spite of a P_{a,O_2} of 55 mmHg. Reducing the size of the active muscle mass improves pulmonary gas exchange during hypoxic exercise, attenuates the Bohr effect on oxygen uploading at the lungs and preserves sea level convective O_2 transport to the active muscles. Thus, the altitude-acclimatized human has potentially a similar exercising capacity as at sea level when the exercise model allows for an adequate oxygen delivery (blood flow × C_{a,O_2}), with only a minor role of P_{a,O_2} *per se*, when P_{a,O_2} is more than 55 mmHg.

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It has been reported that during single leg knee extension exercise in normoxia, peak muscle blood flow and $\dot{V}_{O_2}$ may reach values between 2.5–4 l kg^{-1} min^{-1} and 0.35–0.60 l kg^{-1} min^{-1}, respectively (Andersen & Saltin, 1985; Richardson *et al.* 1995; Rådegran *et al.* 1999), implying that the human has the potential to reach $\dot{V}_{O_2,max}$ values just by activating maximally ∼10 kg of muscle mass. However, the amount of muscle mass that can be perfused maximally during exercise depends on the maximal cardiac output, which has a finite magnitude (Calbet *et al.* 2004*a*). Thus, during exercise with a large muscle mass, $\dot{V}_{O_2,max}$ is mainly limited by O_2 delivery, which in turn depends on blood flow and the oxygen content of the arterial blood (C_{a,O_2}). However, during exercise with a small muscle mass, $\dot{V}_{O_2,max}$ is not limited by cardiac output since, at fatigue, cardiac output has not reached maximal values (Roach *et al.* 1999).

During exercise with a large muscle mass in severe acute hypoxia and chronic hypoxia, cardiac output and leg blood flow are reduced (Calbet *et al.* 2003*a*,*b*; Lundby *et al.* 2006). The combination of a lower peak leg blood flow with a lower C_{a,O_2} explains the marked reduction in $\dot{V}_{O_2,max}$ observed during exercise in severe acute hypoxia (Calbet *et al.* 2003*a*). With altitude acclimatization,

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$\dot{V}_{O_2,max}$ improves marginally and remains reduced despite a marked recovery of maximal systemic oxygen delivery (Calbet *et al.* 2003*b*). This dissociation between maximal systemic O_2 delivery and $\dot{V}_{O_2,max}$ has been attributed to a 'peripheral factor' and it has been suggested that it could be an O_2 diffusion limitation or reduced mitochondrial oxidative capacity induced by altitude acclimatization (Cerretelli, 1976; Wagner, 2000). However, an insufficient mitochondrial oxidative capacity as the mechanism behind the persistent reduction of $\dot{V}_{O_2,max}$ in chronic hypoxia has been ruled out since a moderate level of hyperoxia is able to re-establish sea level $\dot{V}_{O_2,max}$ values (Calbet *et al.* 2003*b*; Lundby *et al.* 2006). Moreover, we have recently observed that maximal mitochondrial respiration remains unaltered after 8–10 days of residence at 4550 m (R. Boushel, E. Gnaiger, C. Wright-Paradis, P. Robach, J. A. Calbet & C. Lundby, unpublished observations) and neither citrate synthase nor 3-hydroxyacil-CoA-dehydrogenase activities are affected by prolonged exposure to high altitude (Mizuno *et al.* 2008).

The fact that exercise is interrupted before the maximal cardiac output and muscle blood flow has been reached also in chronic hypoxia (Pugh, 1964; Reeves *et al.* 1987; Calbet *et al.* 2002, 2003*a*,*b*; Lundby *et al.* 2008*a*), a condition in which C_{a,O_2} is similar to sea level, may suggest that P_{a,O_2} plays a more important role than C_{a,O_2}. This may be tested by studying the exercise response to incremental exercise to exhaustion with a small and a large muscle mass in acute and chronic hypoxia. If the main mechanism causing fatigue is a low P_{a,O_2}, then reducing the size of the muscle mass activated during exercise will have no, or only a minor, effect in terms of attenuating the effect of hypoxia on $\dot{V}_{O_2,peak}$ and, hence, exercise capacity. By reducing the active muscle mass to the quadriceps muscle of one leg we expected the peak leg blood flow to be maintained or elevated, since the sympathetic activation is low and the maximal (pumping) functional reserve of the heart not fully exploited (Savard *et al.* 1989; Strange *et al.* 1993). Moreover, if the main limitation to peak $\dot{V}_{O_2}$ during exercise in hypoxia resides in a limitation to O_2 diffusion in the lung and contracting muscles, then there will be no benefit from an elevation of blood flow (or the benefit will be less than expected from the increase in O_2 delivery) during exercise with a small muscle mass. In addition, the main mechanism limiting pulmonary gas exchange during exercise in hypoxia is a diffusion limitation (Torre-Bueno *et al.* 1985; Hammond *et al.* 1986). Due to lower peak cardiac output and higher pulmonary transit times during exercise with a small compared to a large muscle mass we also expect a better pulmonary gas exchange with the reduction of the active muscle mass (Calbet *et al.* 2008).

Thus, to test the hypothesis that a low P_{a,O_2} is not the primary cause of the reduced peak $\dot{V}_{O_2}$ during exercise at altitude, we studied the influence of the size of the active muscle mass on the cardiovascular and respiratory response to exercise in healthy humans exposed to acute and chronic hypoxia. Altitude-acclimatized humans can reach sea level maximal exercise C_{a,O_2} values. This makes it possible to clearly evaluate which factor plays the major role in limiting muscle peak $\dot{V}_{O_2}$: arterial oxygen tension or its content.

Methods

These experiments were carried out during and after the Copenhagen Muscle Research Centre 1998 Expedition to Mount Chacaltaya (Bolivia). They included submaximal and maximal exercise on the cycle ergometer (Bike), as well as on the one-leg knee extension ergometer (Knee), which we report here for the first time. The respiratory, haemodynamic and blood gas responses during submaximal and maximal exercise on the cycle ergometer were previously reported (Boushel *et al.* 2001; Calbet *et al.* 2002, 2003*a*,*b*, 2004*b*; Wagner *et al.* 2002). In these articles a detailed description of the subjects and methods applied can be found.

In the present article we integrate new analyses from the previously published responses on Bike with the current Knee data into a unified article to compare the respiratory, haemodynamic and blood gases responses to large and small muscle mass exercise. Since the focus of the present study is $\dot{V}_{O_2,max}$, we limit our comparisons to the maximal exercise points, except for O_2 extraction. The latter allowed for determining if O_2 extraction is reduced during exercise with a small muscle mass when O_2 delivery is not restricted by blood flow. In addition, new calculations on oxygen conductances and mean capillary P_{O_2} for all these conditions are included in the present article. During sea level experiments, valid data were obtained in all subjects; however, due to uncompleted data collection in two subjects, chronic hypoxia results are based on the data obtained from the remaining seven subjects (3 females and 4 males).

Subjects

Nine healthy, physically active, Danish lowlanders (5 males and 4 females) volunteered to participate in these studies. Their mean (± S.E.M.) age, height and weight were 24.3 ± 0.5 years, 176 ± 3 cm and 74 ± 4 kg, and their $\dot{V}_{O_2,max}$ averaged 59 ± 2 ml kg^{-1} min^{-1} (range: 49–62 ml kg^{-1} min^{-1}) and 53 ± 4 ml kg^{-1} min^{-1} (range: 47–63 ml kg^{-1} min^{-1}) in the males and females, respectively. All subjects had normal resting ECG, liver, kidney and thyroid functions, as well as fasting plasma glucose and electrolyte concentrations. All subjects were informed about the procedures and risks of the study before giving written informed consent to participate as approved by the Copenhagen and Fredriksberg

Ethical Committee. All experiments conformed with the Declaration of Helsinki.

Experimental preparation

Catheterization. Catheters were inserted percutaneously under local anaesthesia (2% lidocaine) in the femoral vein, an antecubital vein and the femoral artery, as described elsewhere (Calbet *et al.* 2003*a*). Briefly, an 18-gauge, 20-cm-long catheter (Hydrocath, Ohmeda, Swindon, UK) was inserted in the femoral vein of either the right or left leg. This catheter was introduced 2 cm below the inguinal ligament and advanced 7 cm distally. The femoral vein catheter was used for venous sampling and downstream cold saline injection. Subsequently, a thin polyethylene-coated thermistor (model 94-030-2.5F T.D. Probe, Edwards Edslab, Baxter, Irvine, CA, USA) was inserted 3 cm below the inguinal ligament and advanced proximally 10 cm into the same femoral vein. A second 18-gauge catheter was placed into the femoral artery 2 cm below the inguinal ligament and advanced 14 cm proximally. An additional catheter was placed in a vein in the left upper arm for the injection of the Cardio-green dye for measurement of cardiac output. After the catheters were placed, the subjects rested in a supine position for 30 min.

Experimental design

Chronic hypoxia tests (CH) were conducted at altitude after 9–10 weeks residence at 5260 m on Mount Chacaltaya, Bolivia. Acute hypoxia tests (AH) at sea level were carried out at least 6 months after the subjects returned to Denmark, after just a few minutes exposure to hypoxic gas.

Subjects first performed leg knee extension exercise, which was followed after a 60 min rest by exercise on the cycle ergometer in the upright position. The exercise tests were carried out at altitude and at sea level with two different fractions of inspired O_2 (F_{IO_2}). During the experiments at altitude, the subjects inspired room air (408 mmHg, $P_{IO_2} = 76$ mmHg) and air from a tank containing 55% O_2 in nitrogen ($P_{IO_2} = 200$ mmHg). Experiments at sea level were carried out at a barometric pressure of 750–760 mmHg with two different levels of F_{IO_2}: 0.210 (room air) and 0.105 (from a pre-mixed tank of O_2 in nitrogen). The latter resulted in a P_{IO_2} similar to that observed at altitude (i.e. 75 mmHg).

Exercise protocol

The exercise protocol is depicted in Fig. 1. One-legged knee extension exercise was performed on a specifically designed leg extension ergometer that confines muscle contractile activity to the quadriceps muscle in each leg extension while the return to the starting position (about 90 deg knee angle) was brought about passively by the crank and fixed gear of the ergometer, as described elsewhere (Andersen & Saltin, 1985). Leg extension exercise consisted of 10 min of continuous exercise at an intensity of 30 W (warm up) followed by a rest period of 3 min. Then an incremental leg knee extension test to exhaustion was performed starting from 30 W. The 30 W intensity was maintained for 2 min, then the load was increased by 10–15 W increments every 2 min until exhaustion. At the end of the leg extension exercise subjects were allowed to rest for 60 min. Thereafter, they performed

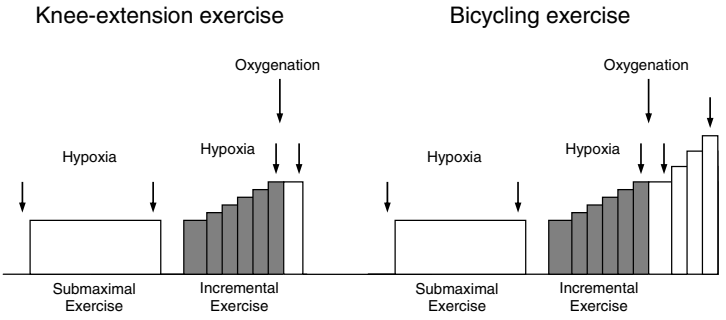

Figure 1. Experimental protocol
Vertical arrows indicate the time points at which measurements were performed. The same sequence was carried out in normoxia, acute hypoxia and chronic hypoxia. Leg extension exercise was performed first and followed after a 60 min rest by exercise on the cycle ergometer in upright position. The exercise tests were carried out at altitude and at sea level with two different fractions of inspired O_2 (F_{IO_2}). During the experiments at altitude, the subjects inspired room air (408 mmHg, $P_{IO_2} = 76$ mmHg) and air from a tank containing 55% O_2 in nitrogen ($P_{IO_2} = 200$ mmHg). Experiments at sea level were carried out at a barometric pressure of 750–760 mmHg with two different levels of F_{IO_2}: 0.210 (room air) and 0.105 (from a pre-mixed tank of O_2 in nitrogen). The latter resulted in a P_{IO_2} similar to that observed at altitude (i.e. 75 mmHg). In the acute and chronic hypoxic conditions and when the subjects were almost exhausted, the F_{IO_2} was increased to 0.21 and to 0.55 during acute hypoxia and chronic hypoxia, respectively, as indicated by 'oxygenation'.

submaximal exercise on the cycle ergometer (Monark 824E, Varberg, Sweden), which consisted of 10 min constant-intensity exercise at ∼120 W (102–141 W, at 80 r.p.m.). This was followed by a resting period of 10 min in CH and 20–30 min in AH and a maximal exercise test on the cycle ergometer. The latter was started at an initial intensity identical to that used in the submaximal test, which was maintained for 2 min. Exercise intensity was then increased by 20–40 W every minute until reaching the maximal exercise intensity ($\dot{W}_{max}$). Load increments were adjusted such that the exercise duration of the incremental exercise tests was approximately 6–7 min in all conditions.

During the exercise tests, subjects breathed through a 2-way valve inspiring room air (CH) or 10.5% O_2 (AH) for 5 min before the resting measurements were made (Fig. 1). At the end of exercise with hypoxia, subjects were vigorously encouraged to keep kicking or pedalling while they were switched to breathe hyperoxic air ($F_{IO_2} = 0.55$), giving a P_{IO_2} of about 200 mmHg in CH, or room air at sea level in the AH study. After 2 min at this P_{IO_2}, a further set of measurements was made, and finally the workload was increased again if possible by steps of 10–15 W (knee extension exercise) or 20–40 W (cycle ergometer exercise) every minute until exhaustion. Measurements were performed at ∼90% of $\dot{W}_{max}$ and repeated with each load increment until exhaustion. In this way, the same exercise protocols and O_2 exposure profiles were used in both acute and chronic hypoxia.

During the AH experiments, normoxic and hypoxic submaximal exercise bouts were administered in random order. When submaximal hypoxic exercise was performed first, the recovery periods were extended when needed, to allow venous blood lactate to reach similar values to those observed at rest. After the submaximal exercise, the normoxic maximal tests were carried out and then, after at least 1 h of rest in the semi-recumbent position breathing room air, the acute hypoxia tests were performed. Just at the end of the exercise with hypoxia, subjects were vigorously encouraged to keep kicking or pedalling while they were switched to breathing room air. After 2 min at the $\dot{W}_{max}$ attained in the hypoxic trial, the workload was increased again in steps of 20–40 W until exhaustion.

Measurements

Respiratory variables. Pulmonary $\dot{V}_{O_2}$, CO_2 production ($\dot{V}_{CO_2}$) and expired minute ventilation ($\dot{V}_E$) were measured with an on-line system (Medical Graphics CPX, Saint Paul, Minneapolis, MN, USA) while the subjects breathed through a low-resistance breathing valve and averaged every 15 s. Gases with known $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ concentration (micro-Scholander) were used for calibration of the gas analyser.

Blood flow. Femoral venous blood flow (i.e. leg blood flow) was measured in the femoral vein by constant-infusion thermodilution as described in detail elsewhere (Andersen & Saltin, 1985; Calbet, 2003; Calbet *et al.* 2003*a*). At peak effort, the measurements were made within 1 min of exhaustion. When possible, duplicate measurements of leg blood flow and femoral arteriovenous O_2 differences were made during the brief period of peak exercise. Heart rate (HR), arterial blood pressure, pulmonary $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and $\dot{V}_E$ were measured at the same time as leg blood flow and cardiac output.

Blood pressure and heart rate. Arterial blood pressure was monitored continuously by a disposable transducer (T100209A, Baxter, Unterschleissheim, Germany) placed at the level of the inguinal ligament as reported elsewhere (Calbet, 2003; Calbet *et al.* 2003*a*).

Cardiac output. Cardiac output was measured by indocyanine green (ICG, Akorn Inc., IL, USA) dye dilution (Dow, 1956; Boushel *et al.* 2001).

Blood analysis. Blood haemoglobin concentration ([Hb]) and O_2 saturation (S_{O_2}) were measured with a co-oximeter (OSM 3 Haemoximeter, Radiometer, Copenhagen, Denmark). P_{O_2}, P_{CO_2} and pH were determined with a blood gas analyser (ABL 5, Radiometer, Copenhagen, Denmark) and corrected for measured femoral vein blood temperature. Haematocrit was determined by microcentrifugation on triplicate samples. Arterial and venous O_2 content (C_{a,O_2} and C_{v,O_2}) were computed from the saturation and [Hb] (i.e. $(1.34[\text{Hb}] \times S_{O_2}) + (0.003 \times P_{O_2})$). Plasma noradrenaline and adrenaline concentrations were measured by HPLC with electrochemical detection (Hallman *et al.* 1978).

Calculations

Arteriovenous $[O_2]$ difference ($\Delta a - \bar{v}_{O_2}$) 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_{a,O_2}. Leg $\dot{V}_{O_2}$ was calculated as the product of leg blood flow and $\Delta a - \bar{v}_{O_2}$. Non-leg $\dot{V}_{O_2}$ was computed as the difference between pulmonary $\dot{V}_{O_2}$ and either one time leg $\dot{V}_{O_2}$ (knee extension exercise) or two times leg $\dot{V}_{O_2}$ (exercise on the cycle ergometer).

Statistical analysis

Differences in the measured variables among conditions and exercise levels were analysed with two-way ANOVA for

repeated measures, with condition and exercise intensity as within-subjects factors, followed by a LSD *post hoc* test for pair-wise comparisons. Significance was accepted at $P < 0.05$. Data are reported as means ± S.E.M.

Results

The size of the active muscle mass had a major impact on the tolerance to exercise in hypoxia. In acute hypoxia, reducing the size of the active muscle mass blunted the effect of hypoxia on $\dot{V}_{O_2,peak}$ by 62% and in chronic hypoxia the effect was completely abolished (Fig. 2). The main reasons accounting for these effects were a better pulmonary gas exchange and less of a shift to the right of the oxygen haemoglobin dissociation curve, which facilitated oxygen uploading in the lungs. The lack of impact of chronic hypoxia on whole-body and leg $\dot{V}_{O_2,peak}$ during knee extension is explained by the improvement of pulmonary gas exchange combined with the increase of blood haemoglobin concentration elicited by altitude acclimatization. These mechanisms allowed for a higher arterial oxygen content and oxygen delivery to the active muscles, and the other vascular beds. A detailed analysis of the impact of the size of the active muscle mass on the links of the oxygen transport cascade is reported below.

$\dot{V}_{O_2,peak}$ and pulmonary gas exchange

Compared to normoxia, acute hypoxia reduced whole-body $\dot{V}_{O_2,peak}$ by 47 and 18% (Fig. 2) and the maximal load achieved at the end of the incremental exercise to exhaustion by 31 and 8% during Bike and Knee, respectively. After altitude acclimatization, Bike whole-body and leg peak $\dot{V}_{O_2}$ were still reduced by 26 and 21%, respectively (Fig. 2). In contrast, altitude acclimatization allowed for a complete restoration of whole-body and leg peak $\dot{V}_{O_2}$ values during Knee (Fig. 2).

Pulmonary gas exchange was impaired during exercise in acute hypoxia but to a greater extent during Bike (Fig. 3). Acclimatization resulted in an improvement in pulmonary gas exchange which was more marked during Knee. The ventilatory response to exercise was exacerbated in hypoxia as reflected by the fact that the $\dot{V}_E/\dot{V}_{O_2}$ was higher during exercise in hypoxia than in normoxia. However, absolute ventilation values at exhaustion were lower in acute hypoxia than in normoxia when the exercise was performed on the cycle ergometer (Fig. 3). In contrast, exercise hyperventilation reached higher values during Knee in acute hypoxia compared to the normoxic condition (Fig. 3).

Compared to normoxia, P_{A,O_2} was reduced with acute hypoxia to the same extent in both models of exercise (53 and 54%, P = n.s.) (Fig. 3). Compared to acute hypoxia, peak exercise alveolar P_{A,O_2} was increased with acclimatization by 9 and 7% during Bike and Knee, respectively (Fig. 3). A similar response was observed for P_{a,CO_2} which was reduced by 23 and 20%, during Bike and Knee, respectively (Fig. 3). In the acclimatized subjects, P_{a,CO_2} was reduced to the same level, i.e. ∼20 mmHg during peak exercise regardless of muscle mass (Fig. 3).

At peak exercise in normoxia, P_{a,O_2} was lower during Bike than Knee (102 ± 3 and 110 ± 2 mmHg, respectively) (Fig. 3). Acute hypoxia resulted in a 66% lower P_{a,O_2} regardless of the exercise mode. However, P_{a,O_2} was 3.7 mmHg lower during Bike compared to Knee. Compared to acute hypoxia, acclimatization improved Bike peak exercise P_{a,O_2} by 34% (from 34 ± 1 to 45 ± 1 mmHg, in acute and chronic hypoxia, respectively) and Knee P_{a,O_2} by 44% (from 38 ± 1 to 55 ± 2 mmHg, in acute and chronic hypoxia, respectively), the improvement being significantly higher for Knee compared to Bike.

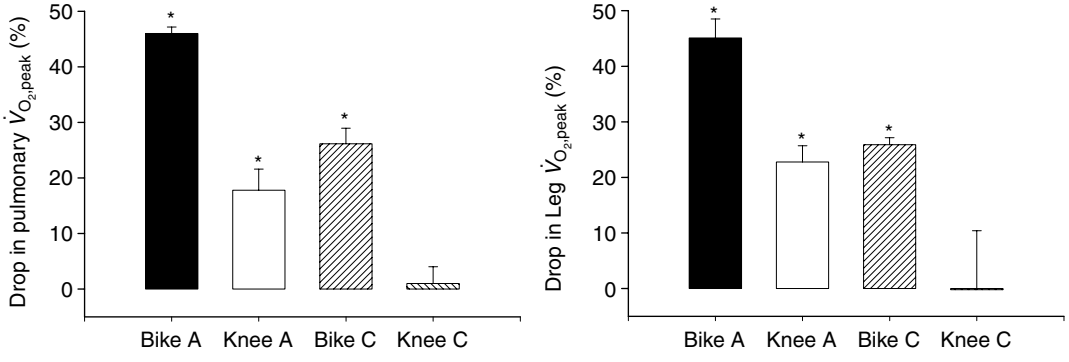

Figure 2. Effect of hypoxia on peak pulmonary and leg $\dot{V}_{O_2}$
Drop in pulmonary $\dot{V}_{O_2}$ during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in acute hypoxia (A) and after 9–10 weeks of residence at 5260 m (C). *Significantly different from normoxia ($P < 0.05$).

Consequently the $\Delta A - a_{O_2}$ was 8 mmHg higher during Bike exercise in AH compared to normoxia (23 ± 1 and 15 ± 1 mmHg, respectively) (Fig. 4). With acclimatization the maximal Bike exercise $\Delta A - a_{O_2}$ was reduced to 13 ± 1 mmHg. This effect of acclimatization on the $\Delta A - a_{O_2}$ was even more marked during leg extension exercise with a reduction of 13 mmHg (from 15 ± 1 mmHg in AH to just 2 ± 1 mmHg in CH).

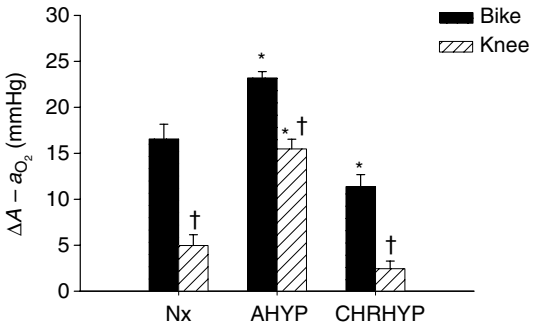

Figure 4. Pulmonary gas exchange
Alveolar–arterial P_{O_2} difference ($\Delta A - a_{O_2}$) during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

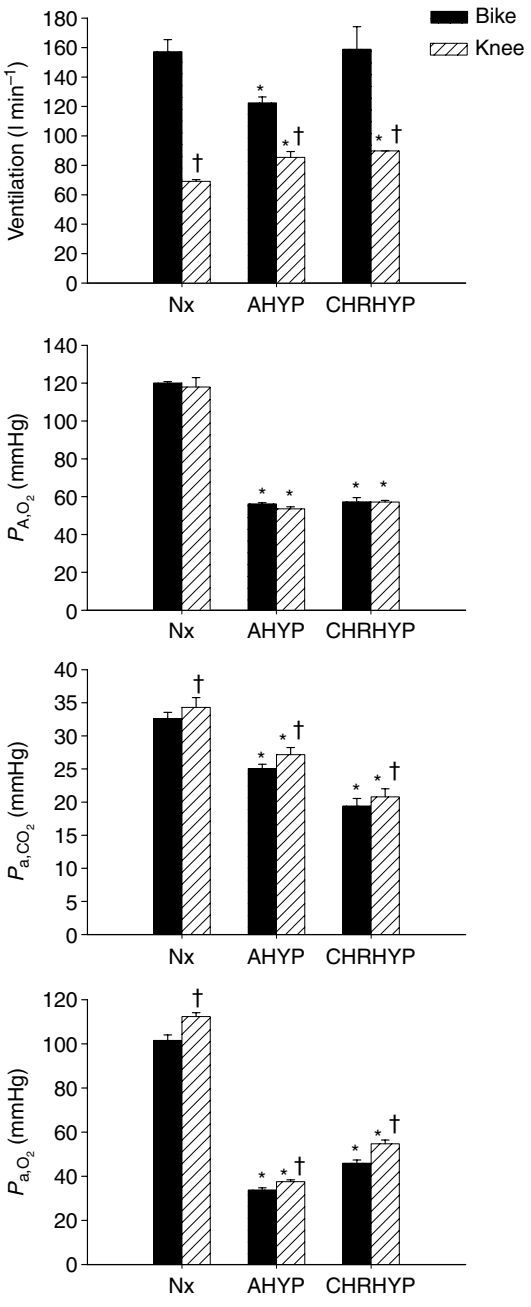

Figure 3. Pulmonary ventilation and blood gases
Pulmonary ventilation ($\dot{V}_E$), alveolar P_{O_2} (P_{A,O_2}), and arterial P_{CO_2} (P_{a,CO_2}) and P_{O_2} (P_{a,O_2}) during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

Despite the relatively small influence of exercising muscle mass on P_{a,O_2} during exercise in acute hypoxia, S_{a,O_2} was about 10 units higher during Knee (66.2 ± 2.7 and 76.5 ± 1.7%, respectively) (Fig. 5). About 40% of the

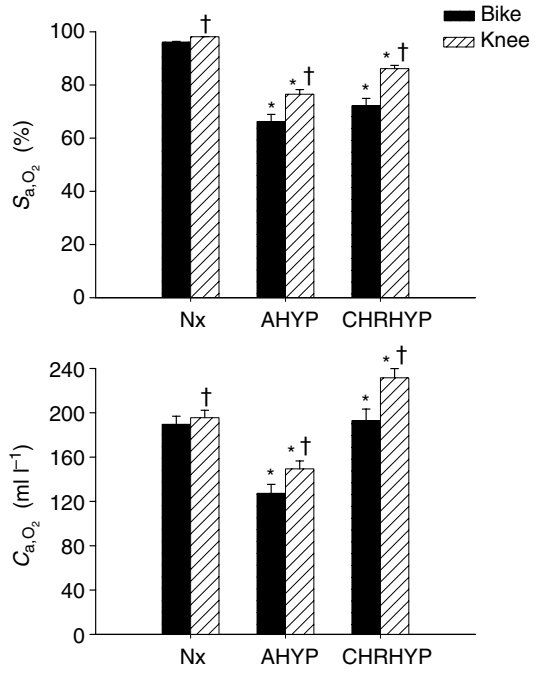

Figure 5. Oxygen content
Arterial O_2 saturation (S_{a,O_2}) and oxygen content (C_{a,O_2}) during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

difference in S_{a,O_2} could be accounted for by the higher arterial pH (+0.23°C) during Knee, and 10% by the higher blood temperature (+0.35°C) during Bike. The rest of the difference in S_{a,O_2} (45% of it) could be accounted for by the impact of the 3.7 mmHg lower P_{a,O_2} on S_{a,O_2} during Bike. Consequently at peak exercise in acute hypoxia, C_{a,O_2} was 17% higher during Knee than during Bike (Fig. 5). Compared to acute hypoxia, peak exercise S_{a,O_2} was improved dramatically with altitude acclimatization. This effect was more marked during Knee than during Bike (11 and 5 units more, respectively). In CH, S_{a,O_2} was also higher during Knee than during Bike (86.1 ± 1.2 and 72.2 ± 2.8%, respectively). Almost 16% of the difference in peak exercise S_{a,O_2} observed in CH between Bike and Knee could be accounted for by the higher arterial pH (+0.09) during Knee, and 8% by the higher blood temperature (+0.5°C) during Bike. The higher P_{a,O_2} during Knee explained 55% of the difference in S_{a,O_2}. At peak Knee in CH, the C_{a,O_2} was 53 and 18% higher than during exercise with the same model in AH and normoxia, respectively. Compared to Bike, C_{a,O_2} during Knee in CH was 20% higher.

Cardiac output and leg blood flow

During Bike, peak cardiac output and peak leg blood flow were reduced by 17 and 22% with acute hypoxia; however, acute hypoxia had no significant effect on peak cardiac output and leg blood flow during Knee (Fig. 6). During Knee and Bike in CH, peak cardiac output was similarly reduced by ∼14 and 15%, respectively. After altitude acclimatization, peak leg blood flow was reduced by 25% during Bike, but during peak Knee no significant differences were observed among the three conditions. At peak Bike effort in AH, heart rate, stroke volume and mean arterial pressure were reduced by 8, 9 and 5%, respectively. In contrast, AH had no significant effect on the heart rate, the stroke volume and the mean arterial blood pressure attained at the end of the Knee. Stroke volume at exhaustion in acute hypoxia was significantly higher during Bike than during Knee (118 ± 4 and 105 ± 7 ml, respectively).

In CH the heart rate response to Knee was 11 and 9% lower than in normoxia and AH, respectively. Neither F_{IO_2} nor altitude acclimatization influenced significantly the stroke volume at peak exercise during Knee. At exhaustion in CH, stroke volume was 46% higher during Bike than during Knee (130 ± 9 and 89 ± 6 ml, respectively).

Oxygen delivery and oxygen extraction

Acute hypoxia resulted in a reduction of systemic O_2 delivery (46 and 21%) and leg O_2 delivery (47 and 26%) during Bike and Knee, respectively, almost matching the corresponding reduction in $\dot{V}_{O_2peak}$ (Fig. 7). Altitude acclimatization almost restored peak systemic O_2 delivery during Bike, which remained 11% lower than during normoxic exercise under control conditions. In contrast, during Knee in CH, systemic O_2 delivery (2.69 ± 0.27 and 2.65 ± 0.15 l min^{-1}) and leg O_2 delivery (1.28 ± 0.11 and 1.16 ± 0.11 l min^{-1}) was similar to that observed in normoxia. Compared to AH, altitude acclimatization raised peak Knee systemic oxygen delivery by 28% and, hence, peak leg $\dot{V}_{O_2}$ attained a value similar to normoxia.

Regardless of F_{IO_2}, O_2 extraction was always higher during Bike (Fig. 8*A*). This was also true during submaximal exercise (Fig. 8*B*). Peak leg O_2 fractional extraction was increased from 85.5 ± 1.7% in normoxia

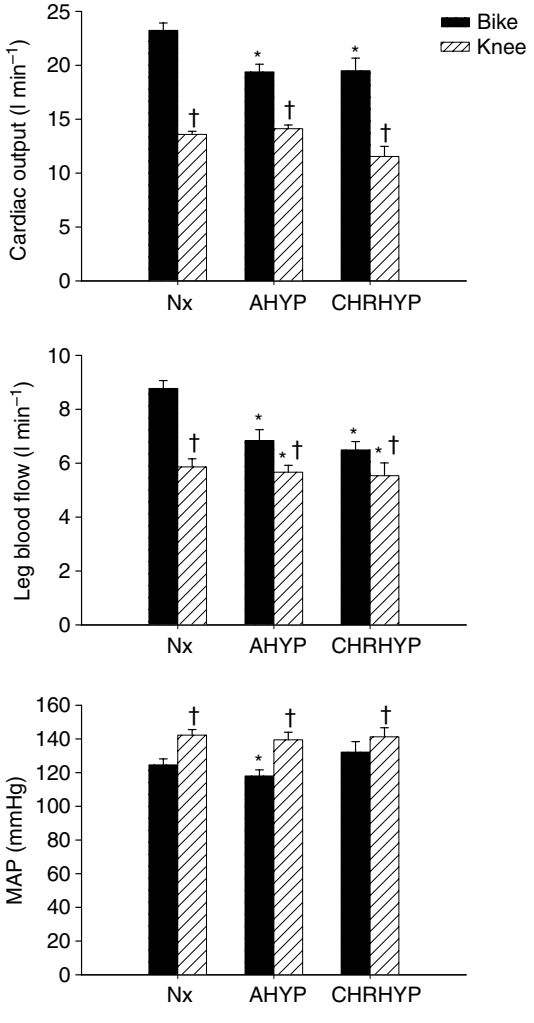

Figure 6. Cardiovascular variables
Cardiac output, leg blood flow and mean arterial blood pressure (MAP) during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

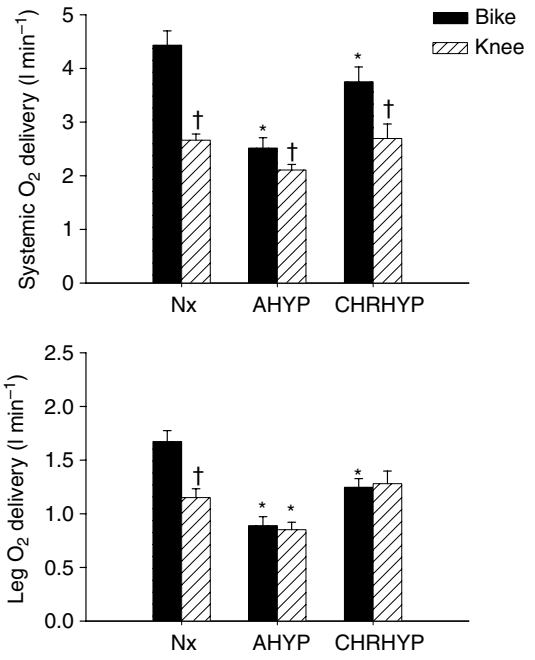

Figure 7. Oxygen delivery
Systemic and leg O_2 delivery during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

to 89.0 ± 2.3% during Bike in AH. A similar trend was observed during Knee (75.5 ± 2.2 to 78.4 ± 1.9%, in normoxia and AH, respectively, $P < 0.10$). After altitude acclimatization peak leg O_2 fractional extraction was higher during Bike than during Knee (87.7 ± 2.5 and 74.0 ± 2.3%, respectively). Compared to AH, peak leg O_2 extraction during Knee tended to be marginally lower after altitude acclimatization (79.7 ± 1.6 and 74.0 ± 2.3%, respectively).

Distribution of blood flow

In AH and in normoxia, the amount of blood flow directed to tissues apart from the active leg was quite similar (5.3–5.7 l min^{-1} (S.E.M.: 0.8 and 0.6) and 8.0–8.5 l min^{-1} (S.E.M.: 0.5) l min^{-1}) during Bike and Knee, being significantly higher during Knee than Bike. However, after altitude acclimatization, the amount of blood flow directed to tissues apart from the active leg was similar in Bike and Knee (6.6 ± 0.8 and 6.0 ± 0.7 l min^{-1}). The amount of flow directed to tissues apart from the active leg was significantly lower during Knee after altitude acclimatization compared to AH. This distribution of blood flow combined with the greater C_{a,O_2} during peak Knee made available almost twice as much O_2 delivery for the tissues apart from the active muscles during Knee than during Bike, when exercising in AH (Fig. 9). Moreover, during Knee in hypoxia, oxygen delivery to the tissues apart from the active muscles was similar, if not greater, than that observed at peak Bike in normoxia.

Plasma catecholamines

At exhaustion in normoxia, arterial noradrenaline and adrenaline concentrations were higher during Bike than during Knee (27 ± 4 and 5 ± 1 nmol l^{-1}, respectively). Acute hypoxia had no significant effect on the catecholamine responses to incremental exercise with the two models studied. After altitude acclimatization, the catecholaminergic response to Knee was not different from that observed in AH. The corresponding

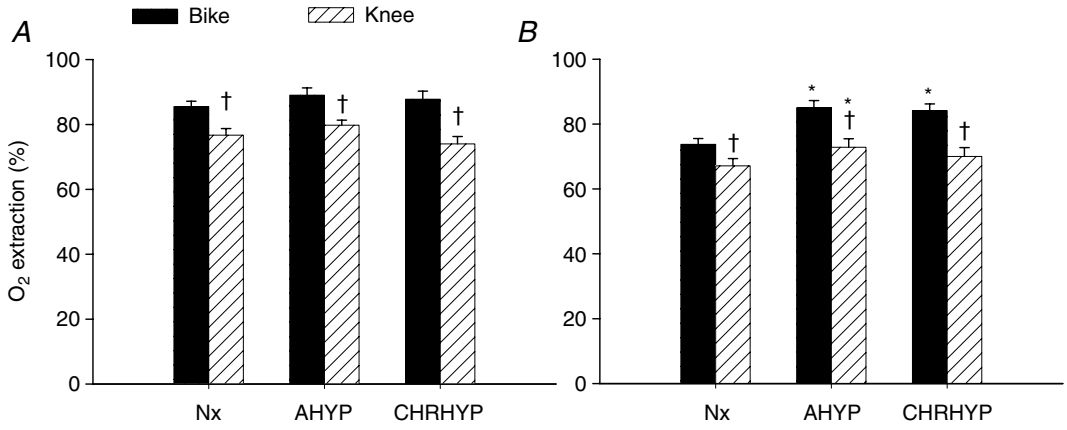

Figure 8. Oxygen extraction
Fractional O_2 extraction during maximal (*A*) and submaximal (*B*) exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

values for noradrenaline were 7 ± 2 and 5 ± 1 nmol l^{-1}, respectively, and for adrenaline 2 ± 1 and 2 ± 1 nmol l^{-1}, respectively. At exhaustion during Bike after altitude acclimatization, arterial noradrenaline was increased up to 52 ± 7 nmol l^{-1} and arterial adrenaline concentrations up to 11 ± 3 nmol l^{-1}, values that were greater than those observed during Knee.

Discussion

Summary of key findings

This study demonstrates the marked effect of the size of muscle mass activated during exercise on several links of the O_2 transport cascade in acute and chronic hypoxia (Fig. 10). During exhaustive exercise in acute and chronic hypoxia with the knee extensor muscles of one leg compared to ordinary bicycling:

(1) the pulmonary gas exchange is less perturbed,
(2) the O_2 dissociation curve of the haemoglobin is less shifted to the right,
(3) peak leg blood flow and peak cardiac output are the same as in normoxia.

With altitude acclimatization, pulmonary gas exchange is improved, but more during Knee than Bike, such that P_{a,O_2} remains above 55 mmHg at exhaustion during hypoxic Knee while it drops below this figure during Bike. The latter combined with the increase in blood haemoglobin concentration results in an unaltered peak $\dot{V}_{O_2}$ during hypoxic Knee while it remains still reduced by 26% during Bike.

In addition, during Knee in hypoxia the amount of flow available to perfuse other vascular beds apart from the active muscles is about 3 l min^{-1} higher than during Bike, reducing the likelihood of an insufficient O_2 delivery to the central nervous system, myocardium or respiratory muscles.

These experimental data lead to the main conclusion that the main mechanism accounting for the reduction of $\dot{V}_{O_2,peak}$ in both acute and chronic hypoxia is the reduction of O_2 delivery to the active muscles. In addition, our data demonstrate that even with a P_{a,O_2} value as low as 55 mmHg, the human skeletal muscle is able to achieve

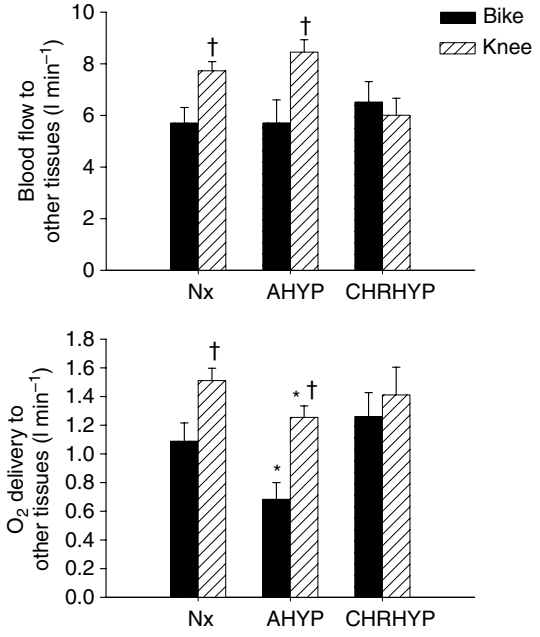

Figure 9. Distribution of blood flow and O_2 delivery
Blood flow directed to tissues apart from the exercising leg/s and oxygen delivery to the tissues apart from the active leg/s during peak exercise on cycle ergometer (Bike) and knee extension ergometer (Knee) in normoxia at sea level (Nx), acute hypoxia at sea level (AHYP) and after 9–10 weeks of residence at 5260 m above sea level (CHRHYP). *Significantly different from normoxia ($P < 0.05$). †Significantly different from exercise on the cycle ergometer (same F_{IO_2}) ($P < 0.05$).

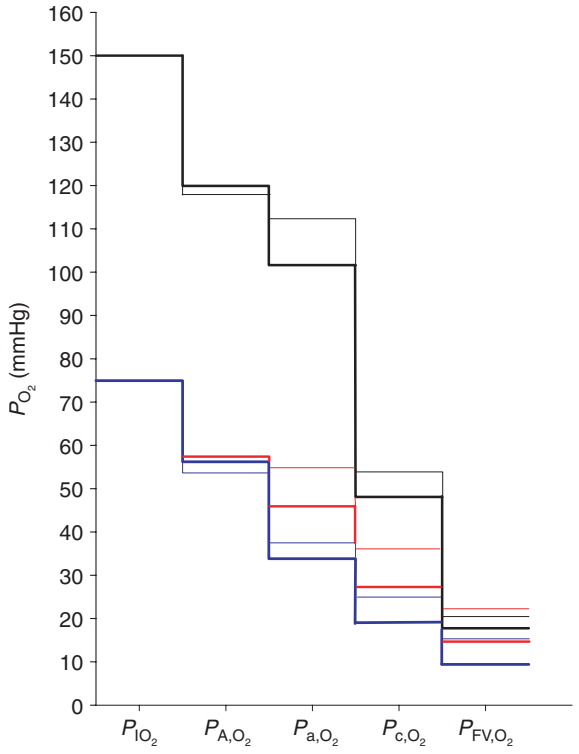

Figure 10. Oxygen cascade from the atmosphere to the femoral vein
The values of inspiratory O_2 pressure (P_{IO_2}), alveolar P_{O_2} (P_{A,O_2}), arterial P_{O_2} (P_{a,O_2}), estimated mean capillary P_{O_2} (P_{c,O_2}) and femoral vein P_{O_2} (P_{FV,O_2}) are represented during exercise on the cycle ergometer (thick lines) and knee extension exercise (thin lines), during normoxia (black lines), acute hypoxia (blue lines) and chronic hypoxia (red lines). Mean capillary P_{O_2} was calculated as previously described (Lundby *et al.* 2008*b*).

normoxic peak $\dot{V}_{O_2}$ values, if O_2 delivery is sufficient (Fig. 1). The latter argues against a major diffusional O_2 limitation during maximal exercise in human skeletal muscles, either in hypoxia or normoxia (Wagner, 1993; Calbet *et al.* 2002; Lundby *et al.* 2008*b*).

Cardiovascular responses

The combination of other studies (Stenberg *et al.* 1966; Koskolou *et al.* 1997*a*,*b*; Roach *et al.* 1999; Boushel *et al.* 2001; Lundby *et al.* 2008*a*) and the present investigation show that the reduction in maximal cardiac output during exercise in hypoxia is only observed when the exercise is performed with a large muscle mass and the level of hypoxia is higher than that observed at around 4000 m above sea level. Calbet *et al.* (2003*b*) hypothesized that the reduction of maximal cardiac output during exercise in severe hypoxia could be mediated by P_{a,O_2}, and probably by C_{a,O_2} and a S_{a,O_2} sensing mechanisms that regulate the output drive from cardiovascular nuclei in the central nervous system (Sun & Reis, 1994). An alternative explanation is that the cardioinhibitory effect of hypoxia could also have been mediated by activation of the peripheral chemoreceptors, which through the release of NO, may attenuate the activation of presympathetic vasomotor neurones at the rostral ventrolateral medulla during hypoxia (Zanzinger *et al.* 1998). The current study supports this theory since, although exercise with a small and a large muscle mass was performed with the same level of F_{IO_2} (0.105), the C_{a,O_2} was 17% and S_{a,O_2} 10 units higher during leg extension exercise, giving values of C_{a,O_2} and S_{a,O_2} similar to that observed during peak whole body exercise at ∼4000 m (Lundby *et al.* 2004) and, hence, peak cardiac output and leg blood flow were not reduced. Despite this marked difference in C_{a,O_2}, P_{a,O_2} differed only by 3.7 mmHg. This could indicate that P_{a,O_2} is not as strong a regulator as C_{a,O_2} (or brain O_2 delivery) to mediate these putative cardioinhibitory effects on the CNS in severe acute hypoxia. Accordingly, maximal cardiac output is not reduced in acute anaemia during two-legged knee extension exercise (Koskolou *et al.* 1997*b*) or bicycling (Ekblom *et al.* 1976). Neither the combination of acute isovolaemic anaemia (reduction of blood [Hb] by 20%) with acute hypoxia ($F_{IO_2} = 0.11$) (Roach *et al.* 1999), which decreased acutely systemic O_2 delivery at peak two-legged knee extension exercise by 38%, resulted in a lower peak cardiac output compared to that observed in normoxia (Roach *et al.* 1999). A particular feature of the two-legged knee extension exercise model is that at maximal exercise cardiac output values remain 4–5 l min^{-1} below those measured during maximal exercise on the cycle ergometer (Roach *et al.* 1999). In agreement, the difference between the actual maximal cardiac output, i.e. that attained during exercise on the cycle ergometer, and that measured during knee extension exercise in severe acute hypoxia was 9 l min^{-1} in the present investigation. Thus, during knee extension exercise in acute hypoxia with either one or two legs, O_2 delivery and $\dot{V}_{O_2,peak}$ are reduced in a similar proportion, while peak cardiac output is not increased to compensate for the reduction in C_{a,O_2}, despite ample functional reserve (4–9 l min^{-1}). Taken together, during 1- and 2-leg knee extension, cardiac output is maintained in acute hypoxia while during cycling exercise it is clearly reduced.

The present investigation shows that a major difference between small and large muscle mass exercise in acute hypoxia is that the amount of blood flow available to perfuse vascular beds apart form the active muscles is about 3 l min^{-1} greater during exercise with a small than a large muscle mass. This value is similar to that observed during combined acute anaemia and hypoxia in the two-legged extension ergometer (Roach *et al.* 1999). This implies that the amount of oxygen delivery to be shared by the tissues apart from the active muscle, which includes the myocardium, the respiratory muscles and the brain, is much lower during exercise with a large muscle mass. The present study also shows that the perfusion pressure is higher during exercise with a small compared to exercise with a large muscle mass. Therefore, a higher oxygen delivery combined with a higher perfusion pressure minimizes the risk of mismatch between O_2 delivery and demand in the myocardium, respiratory muscles and CNS during exercise with a small compared to a large muscle mass.

Our experimental data are compatible with a lower brain O_2 delivery during exercise in severe acute hypoxia with a large muscle mass, than during leg extension exercise (Amann & Calbet, 2008). A lower O_2 brain delivery could cause a cardioinhibitory reflex, establishing a lower ceiling for the elevation of maximal cardiac output and, hence, leg blood flow, leading ultimately to a reduction of O_2 delivery to the active muscle and consequently peak $\dot{V}_{O_2}$ and exercise performance.

Alternatively, low brain oxygenation in severe acute hypoxia could blunt central command (motor output to the active skeletal muscles) but such a possibility is unlikely since: (1) afferent feedback from the respiratory muscles is not able to reduce the central command (Savard *et al.* 1996); (2) maximal voluntary contraction declines to the same extent during incremental exercise in normoxia and acute hypoxia (Garner *et al.* 1990; Fulco *et al.* 1996); (3) the deficit of neural activation observed 2 min after the end of exhausting dynamic exercise with different C_{a,O_2} and F_{IO_2} values is similar (Amann *et al.* 2006); and (4) neither peak power output nor fatigue index during a Wingate test are affected by severe acute hypoxia (Calbet *et al.* 2003*c*).

Sensory input from the fatiguing muscles could also blunt motor output to the active skeletal muscles (Amann & Dempsey, 2008; Amann *et al.* 2008*a*,*b*). Due to

better muscle oxygenation during exercise with a small muscle mass, particularly after altitude acclimatization, this potential negative influence of sensory input on motor output is probably weaker when the size of the active muscle mass is small.

Respiratory responses

Pulmonary ventilation. In agreement with previous studies, exercise with a small muscle mass elicited a higher degree of relative hyperventilation than exercise with a large muscle mass (Asmussen & Nielsen, 1946). This effect may be explained by an enhancement of motor drive to the respiratory centres of the brain. If a given metabolic rate should be sustained by a lower muscle mass, then the effort is perceived as being harder and fatigue precipitates (Galbo *et al.* 1987). Both factors, fatigue and the perception of the exercise as harder, may increase the output drive from the motor cortex to the respiratory centres (Galbo *et al.* 1987; Piepoli *et al.* 1995).

With acclimatization, the ventilatory response to exercise was enhanced (Calbet *et al.* 2003*b*), but proportionally the same magnitude during both exercise models. Since the relatively higher exercise hyperventilation during exercise in hypoxia with a small muscle mass had a minor influence on P_{A,O_2} and P_{a,CO_2}, the differences in pulmonary ventilation could barely contribute to the higher C_{a,O_2} observed during exercise with a small compared to a large muscle mass either in acute or chronic hypoxia.

Pulmonary gas exchange. This study confirms our hypothesis and shows that exercise-induced impairment of pulmonary gas exchange (Hopkins *et al.* 1994, 2000) is attenuated when the exercise is performed with a small muscle mass. Although acute hypoxia exacerbates the exercise-induced impairment of pulmonary gas exchange (Hammond *et al.* 1986; Calbet *et al.* 2003*a*), we show that this effect is blunted when the exercise is performed with a small muscle mass. With acclimatization, the impairment of pulmonary gas exchange is reduced (Bebout *et al.* 1989; Calbet *et al.* 2003*b*) leading to an almost perfect (i.e an $\Delta A - a_{O_2}$ close to 0 mmHg) pulmonary gas exchange during peak leg extension exercise. Several factors can influence the pulmonary gas exchange, such as ventilation perfusion ($\dot{V}_A/\dot{Q}$, where $\dot{V}_A$ is alveolar ventilation and $\dot{Q}$ cardiac output) inequality, diffusion limitation, and to a lesser extend right-to-left shunts (Vogiatzis *et al.* 2008). Ventilation perfusion inequality increases with exercise intensity (Hammond *et al.* 1986; Hopkins *et al.* 1994) and duration (Hopkins *et al.* 1998), and has been shown to account for a large part of the $\Delta A - a_{O_2}$ during maximal exercise in normoxia (Hopkins *et al.* 1994) and hypoxia (Gale *et al.* 1985; Torre-Bueno *et al.* 1985). However, during exercise in hypoxia, diffusion limitation appears to be the most important mechanism accounting for the $\Delta A - a_{O_2}$ (Torre-Bueno *et al.* 1985; Hammond *et al.* 1986). The diffusion limitation is supposedly caused by too short a transit time of the blood through the pulmonary capillaries, which does not allow for a complete gas equilibration between the alveolar gas and the capillary blood (Calbet *et al.* 2008). This reduction in mean transit time has been attributed to high values of cardiac output based on the negative correlation that exists between pulmonary transit time and cardiac output (Hopkins *et al.* 1996). By combining the different experimental conditions included in our studies it becomes clear that cardiac output does not explain the observed difference in pulmonary gas exchange between the large and small muscle mass models (Calbet *et al.* 2008). For example, during submaximal exercise on the cycle ergometer in severe acute hypoxia (intensity ∼120 W) cardiac out was 16 l min^{-1} and the $\Delta A - a_{O_2}$ was 23 mmHg (Calbet *et al.* 2003*a*). However, during peak knee extension exercise in acute hypoxia the corresponding values were 14 l min^{-1} and 15 mmHg. Thus, despite an almost similar cardiac output and lower mean arterial pressure during exercise with a large muscle mass, the $\Delta A - a_{O_2}$ was 53% higher during exercise with a large muscle mass. Moreover, an increase in cardiac output from 14 to 16 l min^{-1} during submaximal exercise (∼120 W) with hypoxia in altitude-acclimatized humans induced by isovolaemic haemodilution (Calbet *et al.* 2002) was not accompanied by an impairment of pulmonary gas exchange, suggesting that for cardiac outputs up to 14–16 l min^{-1} pulmonary gas exchange is not limited by cardiac output (Calbet *et al.* 2008). In chronic hypoxia, peak leg extension exercise elicited a cardiac output of 12 l min^{-1} while the accompanying $\Delta A - a_{O_2}$ was only 2 mmHg. This condition can be compared with the values obtained during submaximal exercise on the cycle ergometer after acclimatization (∼120 W) which elicited a cardiac output of 14 l min^{-1} and a $\Delta A - a_{O_2}$ of 15 mmHg. The 13 mmHg difference in $\Delta A - a_{O_2}$ between knee extension and cycle ergometer exercise is too large to be explained by the small difference in cardiac output alone between these two conditions (Calbet *et al.* 2008).

Since the main two factors that explain the $\Delta A - a_{O_2}$ during exercise in acute hypoxia are diffusion limitation and $\dot{V}_A/\dot{Q}$ mismatch, the most likely is that the degree of $\dot{V}_A/\dot{Q}$ mismatch induced by exercise is attenuated during exercise with a small compared with a large muscle mass. However, this needs to be tested specifically.

Dissociation curve of the haemoglobin

This study shows that one of the main mechanisms by which exercise with a small muscle mass is associated with a markedly larger C_{a,O_2} than exercise with a large muscle mass is the less accentuated right-shift of the O_2

haemoglobin dissociation (ODC) curve during exercise with a small muscle mass. This difference in the position of ODC at the lungs accounts for 50% and ∼25% of the difference in C_{a,O_2} between small and large muscle mass exercise in acute and chronic hypoxia, respectively. Thus, mechanisms allowing an attenuation of exercise-induced hyperthermia and blood pH drop during exercise in hypoxia may have a rather favourable effect on exercise capacity, particularly when the exercise is performed in severe acute hypoxia, confirming our previous findings (Calbet *et al.* 2003*a*; Holmberg & Calbet, 2007).

Summary

The amount of active muscle mass engaged in the exercise has a major impact on the cardiovascular and respiratory response to exercise. The reductions in whole body and leg peak $\dot{V}_{O_2}$ are blunted by more than 50% during exercise in hypoxia with a small muscle mass, like leg extension exercise which recruits about 3 kg of muscle compared to exercise on the cycle ergometer which recruits more than 10 kg of muscle mass. Moreover, acclimatization abolishes the hypoxic effect in small muscle mass whereas whole body $\dot{V}_{O_2,max}$ and leg $\dot{V}_{O_2,max}$ are still remarkably reduced. The two main mechanisms that explain these findings are that during exercise with a small muscle mass pulmonary gas exchange is better preserved and the oxygen dissociation curve of the haemoglobin less shifted to the right as compared to exercise with a large muscle mass. These two mechanisms act together to enhance O_2 uploading in the lungs and, hence, C_{a,O_2}, permitting a relative larger O_2 delivery when exercise is performed with a small muscle mass. In addition our study shows that, in contrast with cycle ergometer exercise, knee extension exercise in severe acute hypoxia does not reduce peak leg blood flow and peak cardiac output. Consequently, the amount of flow available to perfuse the active muscles is similar in acute hypoxia and normoxia when the recruited muscle mass is small. The fact that C_{a,O_2} and perfusion pressure are much larger during peak knee extension exercise implies that the amount of O_2 delivery available to perfuse other vascular beds apart from the active muscles is markedly greater, reducing the likelihood of an imbalance between O_2 demand and delivery in critical regions such as the central nervous system, the myocardium or respiratory muscles. Thus, the altitude-acclimatized human has potentially a similar exercising capacity as at sea level when the exercise model allows for an adequate oxygen delivery (blood flow × C_{a,O_2}), with only a minor role of P_{a,O_2} *per se*, when P_{a,O_2} is more than 55 mmHg.

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