Maximal exercise and muscle oxygen extraction in acclimatizing lowlanders and high altitude natives

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The tight relation between arterial oxygen content and maximum oxygen uptake ($\dot{V}_{O_2max}$) within a given person at sea level is diminished with altitude acclimatization. An explanation often suggested for this mismatch is impairment of the muscle O_2 extraction capacity with chronic hypoxia, and is the focus of the present study. We have studied six lowlanders during maximal exercise at sea level (SL) and with acute (AH) exposure to 4100 m altitude, and again after 2 (W2) and 8 weeks (W8) of altitude sojourn, where also eight high altitude native (Nat) Aymaras were studied. Fractional arterial muscle O_2 extraction at maximal exercise was 90.0 ± 1.0% in the Danish lowlanders at sea level, and remained close to this value in all situations. In contrast to this, fractional arterial O_2 extraction was 83.2 ± 2.8% in the high altitude natives, and did not change with the induction of normoxia. The capillary oxygen conductance of the lower extremity, a measure of oxygen diffusing capacity, was decreased in the Danish lowlanders after 8 weeks of acclimatization, but was still higher than the value obtained from the high altitude natives. The values were (in ml min^{-1} mmHg^{-1}) 55.2 ± 3.7 (SL), 48.0 ± 1.7 (W2), 37.8 ± 0.4 (W8) and 27.7 ± 1.5 (Nat). However, when correcting oxygen conductance for the observed reduction in maximal leg blood flow with acclimatization the effect diminished. When calculating a hypothetical leg $\dot{V}_{O_2max}$ at altitude using either the leg blood flow or the O_2 conductance values obtained at sea level, the former values were almost completely restored to sea level values. This would suggest that the major determinant for $\dot{V}_{O_2max}$ not to increase with acclimatization is the observed reduction in maximal leg blood flow and O_2 conductance.

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When arterial oxygen content (C_{aO_2}) is increased by hyperoxia (Nielsen *et al.* 1998) or erythropoietin (rHuEPO) administration (Birkeland *et al.* 2000) maximal oxygen uptake ($\dot{V}_{O_2max}$) is increased. Conversely, when C_{aO_2} is reduced acutely by hypoxia, $\dot{V}_{O_2max}$ is reduced proportionally to the degree of hypoxia (Dill *et al.* 1966). Thus, there seems to be a tight relation between C_{aO_2} and $\dot{V}_{O_2max}$ within a given person (see Calbet *et al.* 2006 for a recent review). However, with acclimatization to high altitude C_{aO_2} is increased to sea level values while $\dot{V}_{O_2max}$ remains reduced (Calbet *et al.* 2003*b*; Lundby *et al.* 2004*a*). Red cell infusion at altitude has shown not to increase $\dot{V}_{O_2max}$ (Young *et al.* 1996). More recently, it has been shown that $\dot{V}_{O_2max}$ with acute hypoxic exposure does not increase despite increases in C_{aO_2} by novel erythropoiesis stimulating protein-induced erythropoiesis (Lundby & Damsgaard, 2006). Thus, in hypoxia $\dot{V}_{O_2max}$ may be limited by factors apart from C_{aO_2}, particularly when blood haemoglobin concentration is increased (Lundby & Damsgaard, 2006). A recent publication suggests that the failure to recover sea level $\dot{V}_{O_2max}$ after altitude acclimatization, despite an increase of C_{aO_2} to sea level values, is in part explained by two circulatory effects of altitude: (1) the persistent reduction of maximal cardiac output, and (2) the fact that during maximal exercise in chronic hypoxia a greater fraction of the available cardiac output is deviated to vascular beds away from the active skeletal muscles than during the same conditions before exposure to hypoxia (Calbet *et al.* 2003*b*). Another explanation often suggested is an impairment of muscle O_2 extraction capacity with chronic hypoxia (Wagner, 2000*b*). The present report focuses on O_2 extraction by the exercising muscles with altitude acclimatization in low- and highlanders.

The sigmoidal shape of the blood–O_2 equilibrium curve (OEC) facilitates blood oxygenation in the lung capillaries

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as virtually all haemoglobin is loaded with O_2 at a relative low arterial P_{O_2}. The ability of haemoglobin to bind O_2 is expressed as the P_{50} value, and represents the arterial P_{O_2} at which oxygen saturation is 50%. While the major determinant of the P_{50} is blood P_{O_2}, the binding of O_2 to haemoglobin can be greatly influenced by pH, P_{CO_2}, the concentration of 2,3-diphosphoglyceric acid (DPG), Mg^{2+}, ATP and Cl^-, temperature, and the amount of haemoglobin bound to CO (for review see Samaja *et al.* 2003). It is generally believed that a leftward shift in the OEC, i.e. a lowering of the P_{50} is advantageous for the loading of O_2 in the pulmonary capillaries, whereas a rightward shift is supposed to facilitate the unloading of O_2 at the tissue level (Bencowitz *et al.* 1982; Winslow, 1988). Accordingly, the increasing acidosis and hypercapnia in the capillary blood, combined with the increase in temperature, results in an *in vivo* rightward shift of OEC (increasing P_{50}), and thereby facilitates O_2 unloading to the exercising muscles (Stringer *et al.* 1994). Thus, in theory, if P_{50} is low, pulmonary O_2 uptake is enhanced whereas tissue O_2 unloading is impaired, and vice versa.

With acclimatization to 4000–5300 m of altitude, the blood–O_2 affinity of resting humans is decreased, as the standard P_{50} is increased by 2–5 mmHg (Wagner *et al.* 2002; Lundby *et al.* 2004*a*) probably due to increased blood content of DPG (Lenfant *et al.* 1971) and Mg^{2+}, ATP and Cl^- (Mairbaurl *et al.* 1993). Although an increase in standard P_{50} with altitude exposure may seem paradoxical, since the ability of haemoglobin to bind oxygen in the lung capillaries thereby decreases, the effect in this case on O_2 unloading to the exercising muscle may be beneficial. Favouring O_2 unloading to the muscle without concomitantly lowering capillary P_{O_2} could enhance O_2 delivery and potentially elevate $\dot{V}_{O_2max}$. In accordance with this concept, an increase in P_{50} from 33 to 53 mmHg in dogs lead to an increase of $\dot{V}_{O_2max}$ (Richardson *et al.* 1998). However, in a mathematical model of total body O_2 transport, $\dot{V}_{O_2max}$ proved to be insensitive to shifts in P_{50} (Wagner, 1997). Samaja (1988) postulated that changes in blood–O_2 affinity have different effects in various organs, depending on O_2 requirement and availability. For instance, in skeletal muscle, the high O_2 extraction at maximal exercise may diminish the advantage of a change in blood–O_2 affinity. But more important than the standard P_{50} is the '*in vivo*' P_{50}, which depends not only on the value of the standard P_{50}, but also on the local conditions for O_2 exchange within each tissue. Nevertheless, the potential advantage of an altitude-dependent shift in the standard P_{50} on tissue O_2 extraction remains unresolved since the *in vivo* P_{50} values within the skeletal muscles are unknown.

During a recent expedition to El Alto (altitude 4100 m) near La Paz, Bolivia, we explored the effects of altitude-acclimatization in lowlanders and in high altitude natives on HbO_2 affinity and O_2 extraction at the muscular level during maximal exercise. We aimed to test the specific hypotheses that (1) the arterial P_{50} ($P_{50(art)}$) increases with acclimatization, but (2) the *in vivo* P_{50} ($P_{50(muscle)}$) increases significantly less or not at all. In addition we tested whether the expected decrease in leg $\dot{V}_{O_2max}$ was caused, at least in part, by a decreased diffusing capacity for O_2 within skeletal muscle. To accomplish this, during maximal cycle ergometer exercise we determined the *in vivo* P_{50} in the arterial blood ($P_{50(art)}$) and in the femoral vein ($P_{50(muscle)}$), as well as leg O_2 conductance (a measure of oxygen diffusing capacity), fractional O_2 extraction, and whole body and leg $\dot{V}_{O_2max}$. To control for potential morphological changes during acclimatization, we also measured capillary density in muscle biopsies in all subjects and thigh muscle volume by magnetic resonance imaging (MRI) scanning before and after the altitude sojourn in the lowlanders.

Methods

Subjects

We studied six Danish male lowlanders with the following characteristics (mean (range)): age 26 years (22–31), height 187 cm (175–191) and weight 82 kg (75–91); and eight Bolivian male high altitude natives: 31 years (26–37), 163 cm (157–170) and 63 kg (52–70). The lowlanders were all physical education students participating regularly in a variety of club sports and outdoor recreational activities. Subjects were encouraged to remain active throughout the stay with activities such as cycling, soccer, basketball, hiking, and rock climbing. The high altitude natives were also physically active in combat sports and soccer clubs. All Bolivians were born in and lifetime residents of La Paz/El Alto, between 3700 and 4100 m above sea level, and all of Aymaran ancestry. All studies conformed to the standards set by the *Declaration of Helsinki*. Subjects received written and oral information in their native language and provided informed consent to the protocols. The protocol for the Danish subjects was approved by the Ethics Committee for Copenhagen and Frederiksberg (KF 11-050/01), and the protocol for the Danish and Bolivian subjects was approved by El Tribunal de Honor del Colegio Médico Departamental de La Paz, and the Ministerio de Previsión Social y Salud Pública de La Paz.

Acclimatization of the lowlanders

The Danish subjects performed six incremental bicycle exercise tests on a cycle ergometer. At sea level (Copenhagen, Denmark) the subjects were tested twice, with 3–4 weeks between the trials. In these tests the subjects were either breathing ambient air (sea level, SL) or a hypoxic gas mixture, 12.6% O_2 in N_2 (acute hypoxia,

AH). Approximately 1 month after the last test at sea level subjects travelled by plane to La Paz, Bolivia. Initially, they spent two nights in La Paz (~3700 m), and then moved to El Alto (4100 m; barometric pressure ~470 mmHg) for the remaining 8 weeks of the study. During weeks 3–7 of acclimatization, the subjects had short excursions from El Alto, but were not allowed to descend below 3700 m. The first testing period at altitude was between days 11 and 17 after arriving at La Paz. The incremental bicycle exercise test was carried out first while breathing ambient air (W2), followed approximately 2 h later by the second exercise test while breathing a high oxygen gas mixture eliciting sea level conditions, 38% O_2 in N_2 (W2N). The second test period at altitude was between days 52 and 60 after arriving at La Paz and was conducted exactly like the first tests (W8 and W8N).

Procedures and measurements

The procedures for the lowlanders at sea level and at altitude, and the high altitude natives were similar. The subjects had a light breakfast and reported to the laboratory at 08:00 h. Using local anaesthesia (lidocaine (lignocaine), 20 mg ml^{-1}), catheters were placed in a femoral artery (20G, 12 cm, Arrow) and vein (18G, Radiopack TFE, Cook, Bjaerverskov, Denmark) for blood sampling. The venous catheter was used to measure leg blood flow by the constant infusion thermodilution technique. In brief, a thermistor (model 94-030-2.5F T.D. probe, Edslab, Baxter, Irvine, CA, USA) was inserted to measure femoral blood temperature, while infusate temperature was determined with a flow-through chamber thermistor (model 93-505, Edslab, Baxter) connected to the venous catheter. The signal from both thermistors was conditioned and amplified by a custom-built interface (FBJ Industries, Denmark). During catheterization and the remainder of the study we continuously monitored the ECG (Bio amp, ADInstruments, UK) and arterial blood pressure. We used an analog–digital converter and data acquisition software (Powerlab/8SP and Chart 4, ADInstruments) to display and store the ECG, blood pressure and temperature data on a portable computer (Dell Inc.). After catheterization, the subjects remained supine for at least 30 min. Subjects were then seated on a bicycle ergometer (Monark 824E, Varberg, Sweden) and fitted with a mouthpiece and nose clip to enable measurements of ventilation, oxygen uptake ($\dot{V}_{O_2}$), and carbon dioxide production ($\dot{V}_{CO_2}$) from expired gas (oxygen analyser S-3 A/I, Ametek, USA; LB-2, Beckman, USA; VRDC/HC-1, ParVo Medics Inc., USA). Blood was sampled anaerobically in heparinized syringes and immediately analysed for haemoglobin (Hb) and oxygen saturation (S_{O_2}) (OSM3 haemoxymeter, Radiometer, Denmark), and blood pH, carbon dioxide (P_{CO_2}), and oxygen tensions (P_{O_2}) (ABL5, Radiometer, Denmark). Haematocrit (Hct) was determined by centrifuging designed capillary tubes. To address other scientific questions, additional blood samples and muscle biopsies from the vastus lateralis, using local anaesthesia (lidocaine, 20 mg ml^{-1}), were obtained both at rest, as well as after warm-up and exhaustive exercise. Metabolic, biopsy and leg blood flow measurements unrelated to the present study have been reported elsewhere (Lundby *et al.* 2003, 2004*a*,*b*).

Protocol

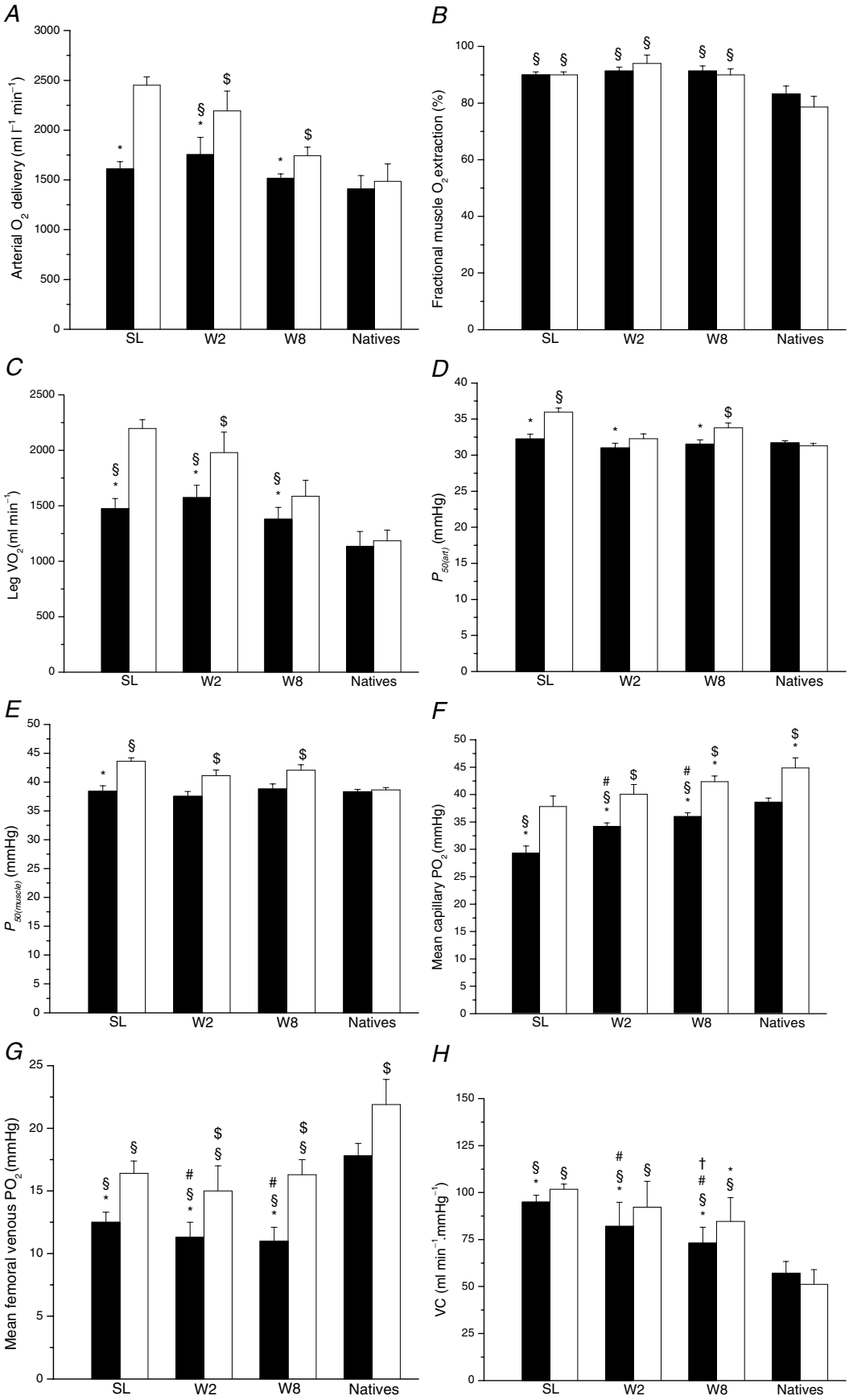
Resting measurements started 10 min after placement of the mouthpiece, while the subjects were seated, and at this time point the resting blood samples were obtained. Exercise started with a 15 min warm-up at 100 W for the lowlanders and 80 W for the high altitude natives. After warm-up the workload was increased by 40 W every 2.5 min until exhaustion for the lowlanders. The first increment for the high altitude natives after the warm-up was 20 W, followed by increments of 40 W until exhaustion. During the last minute of each workload, blood flow was measured followed by blood sampling and an additional blood flow measurement. At altitude, 2 h after the first incremental exercise, the protocol was repeated while breathing the oxygen-supplemented gas mixture (inspired O_2 fraction, $F_{IO_2} = 0.38$). All subjects were familiar with maximal exercise testing on cycle ergometers from participation in previous experiments, and were familiarized with the actual cycle ergometer used during pre-trials. All studies were performed with vigorous verbal encouragement as the subjects approached exhaustion.

Magnetic resonance imaging

Before and within one day of return to sea level after the high altitude sojourn the lowlanders were scanned in an MRI scanner in order to quantify the mass of m. quadriceps calculated from 28 individual images.

Calculations

The measured pH, oxygen tension (P_{O_2}), carbon dioxide (P_{CO_2}) and oxygen saturation (S_{aO_2}) were corrected for temperature according to Severinghaus (1979) using the blood temperature measured in the femoral vein. Plasma bicarbonate was calculated according to Siggaard-Andersen (1977) and blood base deficit was calculated based on the Van Slyke equation of Siggaard-Andersen (1977) and corrected for hypercarbia and oxygen desaturation according to Schlichtig (1997). The standard P_{50}, defined as the value of P_{O_2} that causes haemoglobin to be saturated by 50% when the O_2–Hb



equilibration curve is determined at 37°C, pH 7.40 and $P_{CO_2} = 40$ mmHg, was calculated from the whole set of arterial and venous gases obtained in each experiment. $P_{50(art)}$ and $P_{50(muscle)}$ values were calculated using the actual blood temperature, pH and P_{CO_2} using Kelman's (1966) equation. Muscle O_2 conductance and mean capillary P_{O_2} values were determined as previously described by Wagner (1992, 1993). The parameter β, which is the slope of the relationship between oxygen content and pressure (essentially the slope of the O_2 equilibrium curve with the haemoglobin), was calculated using the model proposed by Piiper (2000).

Statistical analysis

All data are expressed as mean ± s.D. For all data, the assumption of normal distribution was verified using the Shapiro-Wilk test, and the assumption of equal variances was verified using the F test. Differences between conditions in the Danish lowlanders were analysed with two-way ANOVA for repeated measures followed by Tukey's *post hoc* test. Student's t test for unpaired data was used to detect differences between high altitude natives and Danish lowlanders. Statistical significance was set at $P < 0.05$. The Bonferroni correction for significance level was used as appropriate.

Results

M. quadriceps volume data

M. quadriceps volume was not significantly changed in the Danish lowlanders during altitude exposure (2.21 ± 0.1 and 2.08 ± 0.1 l before and after 8 weeks at high altitude; $P = 0.12$). The m. quadriceps volume was not determined in the high altitude natives.

Resting data

The standard P_{50} of Hb (P_{50} at 37°C, pH 7.40, $P_{CO_2} = 40$ mmHg) was unchanged during acute hypoxia compared to sea level (sea level, 26.0 ± 0.3 mmHg; acute hypoxia, 26.2 ± 0.3 mmHg). As expected, P_{50} increased slightly (27.8 ± 0.4 and 27.9 ± 0.4 mmHg after 2 and 8 weeks, respectively, both $P < 0.05$ compared to sea level), and did not differ from the P_{50} of 28.3 ± 0.6 mmHg in the high altitude natives.

Data obtained at maximal exercise

Leg blood flow. Leg blood flow was 12.4 l min^{-1} in SL, and decreased to 11.0 l min^{-1} in AH. With chronic hypoxia leg blood flow remained reduced in W2 (9.6 ± 0.9 l min^{-1}) and decreased further compared to W2 to 8.3 l min^{-1} after 8 weeks of acclimatization (W8). In both W2 and W8, leg blood flow was not altered with the acute induction of normoxia. Leg blood flow was lower in the high altitude natives, and did not increase with acute normoxic exposure (Nat = 6.9 ± 0.7 and NatN = 6.5 ± 0.7 l min^{-1}, respectively).

Arterial O_2 delivery (Fig. 1*A*). At SL maximal arterial O_2 delivery was 2450 ± 83 ml min^{-1} and decreased to 1610 ± 72 ml min^{-1} in AH, and did not increase with acclimatization (1754 ± 172 and 1517 ± 43 ml min^{-1} in W2 and W8, respectively). With the acute induction of normoxia at altitude, arterial O_2 delivery was only increased significantly in W2N (3193 ± 199 ml min^{-1}). Arterial O_2 delivery was lower (1410 ± 134 ml min^{-1}) in the natives as compared to W2, and was not increased significantly with acute normoxia (1485 ± 174 ml min^{-1}).

Fractional arterial O_2 extraction across the leg (Fig. 1*B*). Fractional arterial O_2 extraction at maximal exercise was 90.0 ± 1.0% in the Danish lowlanders at sea level, and remained close to this value in all situations. In contrast to this, fractional O_2 extraction was 83.2 ± 2.8% in the high

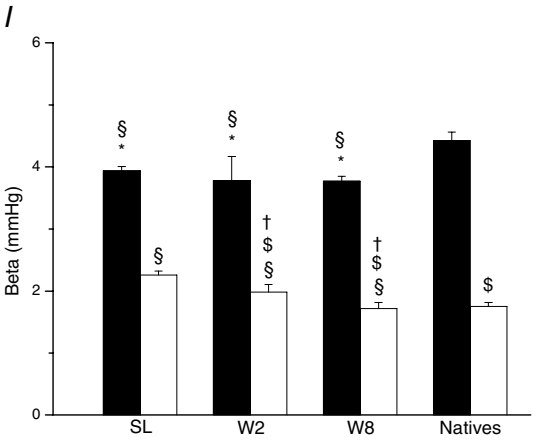


Figure 1
A, arterial O_2 delivery (ml min^{-1}); *B*, fractional muscle O_2 extraction (%); *C*, leg $\dot{V}_{O_2max}$ (ml min^{-1}); *D*, $P_{50(art)}$ (mmHg); *E*, $P_{50(muscle)}$ (mmHg); *F*, mean capillary P_{O_2} (mmHg); *G*, mean femoral venous P_{O_2} (mmHg); *H*, vascular conductance (VC; ml min mmHg^{-1}); *I*, β (mean slope of the O_2 dissociation curve; mmHg) at maximal exercise in lowlanders at sea level (SL), after 2 (W2) and 8 (W8) weeks of acclimatization to 4100 m, and in high altitude natives also studied at 4100 m. Filled bars, hypoxia; open bars, normoxia ($F_{IO_2} = 0.38$). *$P < 0.05$ to SL, §$P < 0.05$ to Nat, #$P < 0.05$ to AH, \$$P < 0.05$ to ambient condition, †$P < 0.05$ compared to previous time point within own condition.

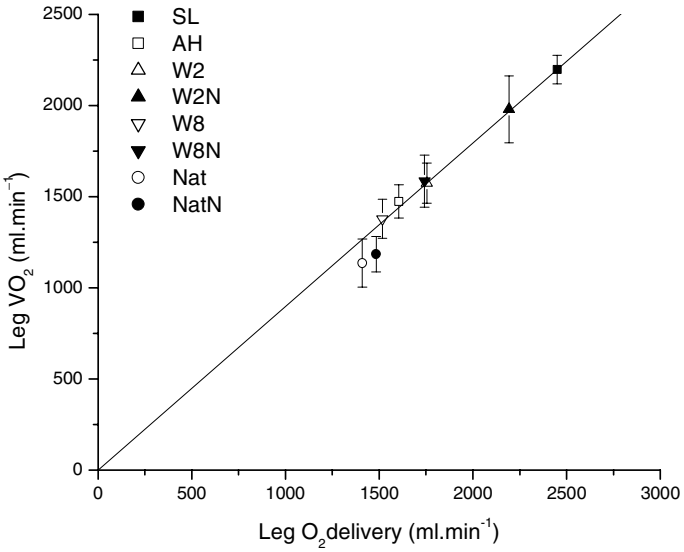


Figure 2
Relation between leg O_2 delivery (ml min^{-1}) and leg $\dot{V}_{O_2}$ (ml min^{-1}) at maximal exercise in lowlanders at sea level (squares; SL, sea level; AH, acute hypoxia), after 2 (triangles pointing up; W2, W2N) and 8 (triangles pointing down; W8, W8N) weeks of acclimatization to 4100 m, and in high altitude natives (circles; Nat, NatN) also studied at 4100 m. Open symbols, hypoxia; filled symbols, normoxia.

altitude natives, and did not change with the induction of normoxia.

***Leg* $\dot{V}_{O_2max}$ (Fig. 1*C*).** The induction of acute hypoxia reduced leg $\dot{V}_{O_2max}$ from 2198 ± 79 ml min^{-1} at sea level to 1474 ± 91 ml min^{-1} with hypoxia, and remained unchanged with acclimatization. With the induction of acute normoxia after 2 and 8 weeks of acclimatization leg $\dot{V}_{O_2max}$ was increased, whereas this was not the case in the high altitude natives (NatN). After 8 weeks of acclimatization, the acute induction of normoxia was insufficient to restore sea level leg $\dot{V}_{O_2max}$ values.

***$P_{50(art)}$*, *$P_{50(muscle)}$* and the mean slope of the O_2 dissociation curve (β) (Fig. 1*D*, *E* and *I*).** $P_{50(art)}$ was decreased from 36.0 ± 0.6 mmHg at sea level to 32.2 ± 0.7 mmHg in acute hypoxia, and did not decrease further with acclimatization ($W2 = 31.0 \pm 0.6$ mmHg; $W8 = 31.5 \pm 0.6$ mmHg). The values obtained in AH, W2 and W8 did not differ from the value obtained from the high altitude natives ($Nat = 31.7 \pm 0.3$ mmHg) (Fig. 1*D*). $P_{50(muscle)}$ decreased from 43.6 ± 0.6 mmHg at sea level to 38.8 ± 1.0 mmHg in acute hypoxia, and remained unchanged throughout the acclimatization period in the lowlanders ($W2 = 37.5 \pm 0.8$ mmHg; $W8 = 38.8 \pm 0.9$ mmHg), but was increased towards sea level values with acute induction of normoxia after 2 ($W2N = 41.1 \pm 1.0$ mmHg) and 8 ($W8N = 42.1 \pm 1.0$ mmHg) weeks of acclimatization. In the natives $P_{50(muscle)}$ did not change with the induction of normoxia ($Nat = 38.3 \pm 0.4$ mmHg; $NatN = 38.7 \pm 0.4$ mmHg). β was increased from 2.2 ± 0.1 at sea level to 4.0 ± 0.1 in acute hypoxia, and was not further changed with acclimatization, and was always lower than the 4.2 ± 0.1 in the natives. Acute normoxic exposure decreased β in all conditions.

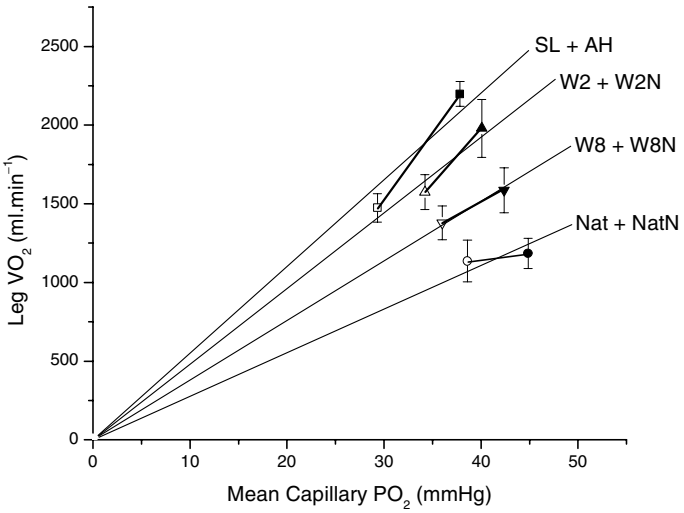


Figure 3
Oxygen conductance (ml min^{-1} mmHg^{-1}). Abbreviations as for Fig. 2.

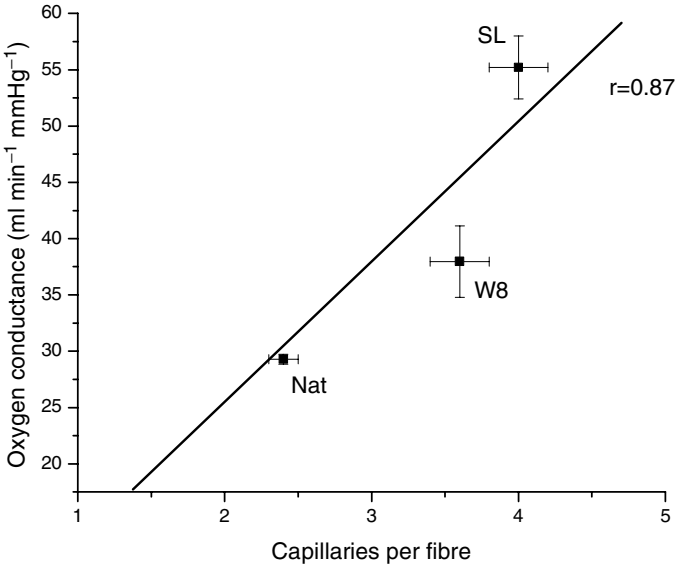


Figure 4
Relation between number of capillaries and oxygen conductance. Abbreviations as for Fig. 2.

Mean end capillary P_{O_2} and mean femoral venous P_{O_2} (Fig. 1*F* and *G*). Mean capillary P_{O_2} was 37.8 ± 1.9 mmHg at sea level and decreased to 29.3 ± 1.3 mmHg in acute hypoxia and increased to 34.2 ± 0.6 mmHg after 2 weeks of acclimatization. After 8 weeks exposure mean capillary P_{O_2} had not increased further compared to the 2 week point and was 36.0 ± 0.7 mmHg. Although mean capillary P_{O_2} had increased by 19% with acclimatization, the value obtained after 8 weeks was still lower than the 38.6 ± 0.8 mmHg observed in the high altitude natives. With normoxic breathing mean capillary P_{O_2} was increased in all conditions.

Oxygen conductance and capillarity (Fig. 4). Oxygen conductance was decreased in the Danish lowlanders after 8 weeks of acclimatization, but was still higher than the value obtained from the high altitude natives. The values were 55.2 ± 3.7, 48.0 ± 1.7, 37.8 ± 0.4 and 27.7 ± 1.5 ml min^{-1} mmHg^{-1} in SL, W2, W8 and Nat, respectively.

Discussion

The main findings are: (1) fractional oxygen extraction at maximal exercise is lower in high altitude natives than in lowlanders acclimatized to the same altitude where the natives live permanently; (2) $P_{50(art)}$ and $P_{50(muscle)}$ are decreased at maximal exercise with either acute or chronic hypoxia as compared to sea level; (3) with altitude acclimatization skeletal muscle capillary O_2 conductance, an estimation of muscle diffusing capacity, is lowered; however, this change can be attributed to reductions in

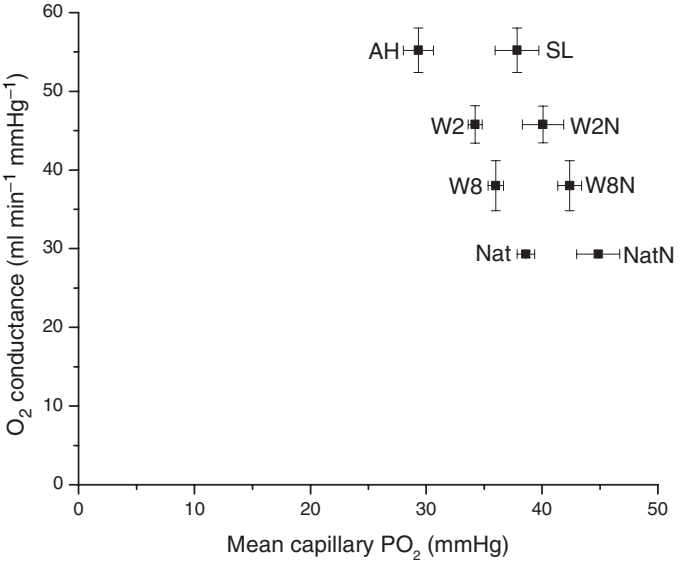


Figure 5
Relation between mean capillary P_{O_2} (mmHg) and O_2 conductance (ml min^{-1} mmHg^{-1}). Abbreviations as for Fig. 2.

Table 1. Arterial blood measurements under the different conditions and relative contribution of factors affecting $P_{50(art)}$

						Percentage contribution		
	pH	P_{aCO_2} (mmHg)	P_{aO_2} (mmHg)	T (°C)	P_{50} (mmHg)	pH	P_{CO_2}	T
SL	7.20 ± 0.01	33.8 ± 0.6	96.3 ± 1.9	39.3 ± 0.1	36.0 ± 0.6	58.4	6.3	40.7
AH	7.27 ± 0.02	29.2 ± 0.5	46.7 ± 1.0	38.4 ± 0.1	32.2 ± 0.7	52.6	3.9	39.5
W2	7.30 ± 0.01	23.4 ± 0.7	51.8 ± 1.1	38.5 ± 0.1	31.0 ± 0.6	58.6	12.8	27.0
W2N	7.28 ± 0.01	27.5 ± 0.8	118.1 ± 3.8	38.8 ± 0.1	32.3 ± 0.7	68.6	12.7	26.2
W8	7.28 ± 0.01	20.7 ± 1.0	55.8 ± 1.1	38.6 ± 0.1	31.5 ± 0.6	52.7	20.2	26.9
W8N	7.25 ± 0.01	21.5 ± 0.8	129.9 ± 4.1	39.0 ± 0.2	33.8 ± 0.6	41.4	11.8	24.5
Nat	7.29 ± 0.01	26.8 ± 0.8	58.3 ± 0.9	38.6 ± 0.1	31.7 ± 0.3	62.8	9.5	28.1
NatN	7.30 ± 0.01	27.7 ± 1.2	129.5 ± 1.7	38.7 ± 0.1	31.3 ± 0.3	71.5	14.7	29.4

Measurements were taken from lowlanders at sea level (SL), after 2 (W2) and 8 (W8) weeks of acclimatization to 4100 m, and in high altitude natives (Nat) also studied at 4100 m. Measurements were taken again after a period of acute normoxia in W2N, W8N and NatN. Values are mean ± S.D. The percentage contribution determines the relative contribution of pH, P_{aCO_2} and temperature (T) to the total changes in $P_{50(art)}$ for the given conditions as compared to SL. At SL the percentage contribution is derived from standard P_{50} values.

Table 2. Venous blood measurements under the different conditions and relative contribution of factors affecting $P_{50(muscle)}$

						Percentage contribution		
	pH	P_{vCO_2} (mmHg)	P_{vO_2} (mmHg)	T (°C)	P_{50} (mmHg)	pH	P_{CO_2}	T
SL	7.06 ± 0.01	80.9 ± 0.6	16.4 ± 1.0	39.3 ± 0.2	43.6 ± 0.6	67.3	11.0	30.3
AH	7.13 ± 0.02	62.5 ± 3.5	12.5 ± 0.8	38.4 ± 0.1	38.4 ± 0.1	43.7	5.2	32.1
W2	7.15 ± 0.02	58.1 ± 2.9	11.3 ± 1.2	38.3 + 0.1	37.5 ± 0.8	45.4	5.6	27.4
W2N	7.09 ± 0.02	75.0 ± 4.0	15.0 ± 2.0	38.8 ± 0.2	41.1 ± 1.0	40.0	0.0	40.0
W8	7.12 ± 0.02	60.4 ± 3.8	11.0 ± 1.1	38.6 ± 0.1	38.8 ± 0.9	46.0	14.6	31.9
W8N	7.07 ± 0.01	72.8 ± 3.1	16.3 ± 1.2	39.0 ± 0.2	42.1 ± 1.0	23.3	16.0	2.7
Nat	7.14 ± 0.01	64.3 ± 1.4	17.8 ± 1.0	38.6 ± 0.1	38.3 ± 0.4	55.7	10.0	28.9
NatN	7.16 ± 0.02	71.5 ± 2.7	21.9 ± 2.0	38.7 ± 0.1	38.7 ± 0.4	62.0	7.6	13.3

Values are mean ± S.D. The percentage contribution determines the relative contribution of pH, P_{vCO_2} and temperature (T) to the total changes in $P_{50(muscle)}$ for the given conditions as compared to SL. At SL the percentage contribution is derived from standard P_{50} values.

maximal leg blood flow; and (4) the reduction in leg blood flow and O_2 conductance are the main factors for $\dot{V}_{O_2max}$ not to increase with acclimatization to high altitude.

Several factors may account for the observed differences in muscular oxygen extraction at maximal exercise between high- and lowlanders, which depends on the interaction of the following: (1) kinetics of O_2 off-loading from haemoglobin, (2) capillary muscle O_2 conductance and the degree of mismatch between the metabolic demand and blood flow distribution, (3) muscle oxidative capacity, and (4) exercise intensity. These factors will be described first, followed by a discussion on why $\dot{V}_{O_2max}$ does not increase with acclimatization to high altitude with emphasis on blood flow regulation.

Kinetics of O_2 off-loading from haemoglobin

The standard P_{50} (37°C, pH 7.40, P_{CO_2} = 40 mmHg) is well known to increase with acclimatization, and also to be higher in high altitude natives as compared to lowlanders living at sea level (Lenfant *et al.* 1971; Mairbaurl *et al.* 1993; Wagner *et al.* 2002). This has been speculated to favour O_2 unloading to the tissues. However, the standard P_{50} does not represent the P_{50} at the site of O_2 unloading, and the present measurements of $P_{50(muscle)}$ (and $P_{50(art)}$) have previously not been performed in hypoxia. Quite surprising $P_{50(muscle)}$ and $P_{50(art)}$ values at maximal exercise were reduced by hypoxia (Danish lowlanders and natives showed a rather similar response). This decrease is in great part the consequence of a blunted reduction of arterial and venous pH at maximal exercise in acute and chronic hypoxia, i.e. the normal reduction of arterial and venous pH at maximal sea level exercise is attenuated in hypoxia. The remainder of the decrease in P_{50} values at maximal exercise in acute and chronic hypoxia may be accounted for by the changes in P_{CO_2} and blood temperature (Tables 1 and 2). This implies that for a given saturation the skeletal muscle capillary P_{O_2} will be lower in hypoxia, reducing the P_{O_2} gradient for O_2 diffusion. However, we did not observe any differences in $P_{50(muscle)}$ at maximal exercise between low- and highlanders, despite lower O_2 extraction in the highlanders, suggesting that other factors also play a role.

A leftward shift in the HbO_2 dissociation curve (decreased P_{50}) favours O_2 loading in the pulmonary capillaries, and this would benefit pulmonary oxygen uptake. This may be especially important during maximal exercise in hypoxia where arterial oxygen saturation decreases to levels approaching the steep part of the HbO_2 dissociation curve, as also reported in our subjects (AH = 73%, W2 = 77%, W8 = 79%, Nat = 83%) (Lundby *et al.* 2004*a*). In contrast, under normoxic conditions small changes in the position of the HbO_2 dissociation curve does not change saturation noticeably. Conversely, changes in $P_{50(muscle)}$ would have similar effects during normoxia and hypoxia, because the muscle capillary saturation during maximal exercise is on the steep part of the curve under both conditions.

O_2 conductance and capillary P_{O_2}

The capillary muscle O_2 conductance was calculated as leg $\dot{V}_{O_2}$ divided by mean capillary P_{O_2} (and not the capillary–mitochondrial oxygen tension gradient) thereby assuming that mitochondrial P_{O_2} is 0 mmHg during maximal exercise, a concept developed by Peter Wagner in the mid 1980s. We found that capillary muscle O_2 conductance decreased with acclimatization in the lowlanders, and even lower values for the high altitude natives. Since Krogh (1919), the radial distance from the capillaries to the most distant mitochondria (i.e. the diffusion distance) has been considered an important determination of O_2 diffusion, which can be estimated by determining capillary density. More recently, however, it was demonstrated that a substantial gradient exists between blood and intramyocyte P_{O_2} during maximal exercise, and therefore suggested that most of the limitation to O_2 flux is in the short diffusion path through plasma and capillary wall to the cytoplasm (Gayeski & Honig, 1986). This lead to the speculation that the number of capillary–myocyte contact points, estimated by the capillary to fibre ratio, rather than the diffusion distance (capillary density) determines O_2 flux. Subsequently, this concept was experimentally supported by Hepple *et al.* who reported that a 59% increase in capillary density had no effect on capillary muscle O_2 conductance (Hepple *et al.* 2000). In the present study, neither measurement of capillarization changed significantly during acclimatization of the lowlanders, and both capillary per fibre ratio and capillary density were lower in the high altitude natives compared to the Danish lowlanders (Lundby *et al.* 2004*b*). Therefore we cannot establish whether the number of contact points or the diffusion distance is more important compared to the other for muscle diffusing capacity. When oxygen conductance was calculated from the actual mean capillary P_{O_2} and blood gases, but with the blood flow attained at sea level, hardly any differences in oxygen conductance was observed between the different experimental conditions. Thus, from the present study, it would seem that the reduction in maximal leg blood flow is the main reason for oxygen conductance to decrease with acclimatization.

We are not the first to report decreases in O_2 conductance with exposure to high altitude. In Operation Everest II (OEII), at considerably higher altitudes than those used in the present study, Wagner (2001) estimated a 20% reduction at around 6000 m, whereas no changes were estimated at lower simulated altitudes. However, neither leg blood flow nor peak leg $\dot{V}_{O_2}$ was measured during OEII. Since peak leg blood flow is reduced with altitude acclimatization, as shown in the present study, and also in previous investigations (Calbet *et al.* 2003*b*), it seems likely that Wagner (2001) overestimated diffusion capacity (D_{O_2}) during OEII. In this study, subjects increased capillary density by reducing the muscle fibre diameter (MacDougall *et al.* 1991). Thus, despite a lower diffusion distance capillary O_2 conductance decreased in that study, which argues against the diffusion distance as an important factor. Part of the reduction reported by Wagner(2001) should be ascribed to the reduction of muscle mass experienced by their subjects (Green *et al.* 1989). Muscle mass recruited during exercise is a major determinant of the muscle capillary O_2 conductance (since capillary O_2 conductance = absolute leg $\dot{V}_{O_2max}$/mean capillary P_{O_2}; and the absolute leg $\dot{V}_{O_2max}$ depends, among other factors, on the absolute muscle mass of the leg). Due to their smaller body size, the high altitude natives also had a smaller muscle mass in the legs than the lowlanders, which could explain some of the difference observed in muscle diffusing capacity between acclimatized lowlanders and natives. However, since no significant differences in upper leg volume was found in the Danish lowlanders before and after 8 weeks of altitude exposure, we can rule out a change in muscle mass as a mechanism for the decreasing O_2 conductance with altitude acclimatization in our study. However, since we had only a limited number of subjects, a statistical type II error cannot be excluded, and the reduction in muscle mass of 6% should not be ignored.

The present capillary O_2 conductance of 55 ml min^{-1} $mmHg^{-1}$ at a $\dot{V}_{O_2max}$ of ~55 ml min^{-1} kg^{-1} at sea level is comparable to previous studies of our group. With maximal cycle ergometer exercise we found a capillary O_2 conductance of 42 ml min^{-1} $mmHg^{-1}$ in active subjects ($\dot{V}_{O_2max}$ ~ 55ml min^{-1} kg^{-1}) (Calbet *et al.* 2003*b*), and 55 ml min^{-1} $mmHg^{-1}$ in amateur cyclists with a $\dot{V}_{O_2max}$ of 60 ml min^{-1} kg^{-1} (Gonzalez-Alonso & Calbet, 2003). Since $\dot{V}_{O_2max}$ did not change in the Danish lowlanders with acclimatization (45 ml min^{-1} kg^{-1} in acute hypoxia and 47 ml min^{-1} kg^{-1} after 8 weeks exposure), and since there were no differences in $\dot{V}_{O_2max}$ between low- and highlanders (43 ml min^{-1} kg^{-1}), the changes in muscle

O_2 conductance in the present study do not appear to be linked to changes in aerobic performance, and as stated above, the decrease seems to be mediated by the lowering of maximal leg blood flow.

With acclimatization in the lowlanders, and after life-long exposure to high altitude in the highlanders, we observed an increase in mean femoral venous P_{O_2} at maximal exercise (Fig. 1*G*). This does not seem to be an effect of changes in $P_{50(muscle)}$ since we were not able to detect any difference in this parameter with acclimatization in low- and highlanders. This increase in femoral venous P_{O_2} may be the consequence of a reduction of muscle diffusing capacity in chronic hypoxia. Another explanation for this increase in maximal exercise mean femoral venous P_{O_2} could be a mechanism whereby the muscles stop using O_2 when there is still a substantial amount of O_2 left in the capillaries leaving the active muscles fibres. Such a mechanism could be, for example, a centrally induced fatigue (Kayser, 2003).

Muscle oxidative capacity

In vivo animal studies support the notion that reductions in oxidative capacity lead to concomitant reductions in O_2 extraction (McAllister *et al.* 1990; Robinson *et al.* 1994; Hepple *et al.* 2002). In the present study we did not quantify the activity of enzymes generally associated with oxidative capacity. However, even after 75 days of exposure to 5250 m altitude, we have previously not been able to detect any differences in the activity of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase in the vastus lateralis (B. Saltin, unpublished observation). Whether the correlation between oxidative capacity and O_2 extraction in animal studies can be directly linked to human beings is questionable since bed rest-induced reductions in mitochondrial volume and muscle oxidative enzyme activity are associated with high O_2 extraction at maximal exercise (Saltin *et al.* 1968). In human experiments differences in skeletal maximal muscle O_2 extraction capacity can hardly be ascribed to differences in muscle oxidative capacity, as recently summarized (Calbet *et al.* 2005).

Mean transit time, heterogeneity, blood flow distribution and exercise intensity

The observed differences in O_2 extraction could be related to differences in the mean transit time (MTT) of the erythrocyte when crossing the capillaries (Piiper, 2000), i.e. slow MTT = high extraction, and fast MTT = low extraction. The MTT of the erythrocytes crossing the muscle capillaries during maximal exercise is estimated from MTT = CBV/MBF, where CBV is the capillary blood volume and MBF the muscle blood flow. In turn, the capillary volume may be calculated from the capillary density (Kayar *et al.* 1994). Assuming an inner mean capillary diameter of 6.0 μm this corresponds to a MTT at maximal exercise of 766 ms at sea level and 1123 ms after 8 weeks of acclimatization in the lowlanders, and to 1173 ms in the high altitude natives. Therefore, MTT does not seem to be of importance for the differences found in O_2 extraction. When applying the calculations of the relative importance of perfusion and diffusion in O_2 transfer ($Y = D/Q\beta$, where D is O_2 diffusion capacity, Q is leg blood flow, β is the mean slope of the O_2 dissociation curve) as proposed by Piiper (2000) it is seen that Y decreases from 2.0 at sea level to 1.25 and 1.09 after 2 and 8 weeks of acclimatization. (Normal values for Y are between 0 and 3: with decreasing Y, diffusion limitation increases and perfusion limitation decreases, whereas $Y > 3$ indicates predominant perfusion limitation; $3 > Y > 0.1$ suggests combined perfusion and diffusion limitation, and $Y < 0.1$ indicates a prevailing diffusion limitation.) Hence, using this approach, the limitation in O_2 transfer due to oxygen diffusion seems to increase with acclimatization. However, when the O_2 diffusion capacity is calculated with the leg blood flows obtained at sea level, Y is increased to 1.78 and 1.82 after 2 and 8 weeks of acclimatization suggesting that altitude acclimatization does not alter the relative importance of perfusion and diffusion in limiting O_2 transfer.

Since our measurements were carried out at maximal exercise, any mismatch (perfused areas/areas where $\dot{V}_{O_2}$ is occurring) or shunting (apart from the active muscle fibres) in blood flow distribution should be negligible (Wagner, 2000*a*). Approximately 30% of the arterial O_2 is extracted in resting legs of humans, and this increases gradually to around 90% with maximal exercise, and even to 97% in some elite endurance athletes (Calbet *et al.* 2005). Based on levelling off in $\dot{V}_{O_2}$ at maximal exercise, high respiratory exchange ratios, and high arterial lactate and low pH values in all subjects, we are confident that the differences found in O_2 extraction are not caused by differences in exercise intensity.

Why does $\dot{V}_{O_2max}$ not increase with acclimatization to high altitude?

In the above sections we have demonstrated that, although muscle diffusing capacity is reduced, fractional muscle O_2 extraction is not impaired with altitude exposure, and that the explanation(s) for the reported mismatch between C_{aO_2} and $\dot{V}_{O_2max}$ with acclimatization must be found elsewhere. Recently we demonstrated that blood flow to the legs at maximal exercise was reduced from 6.6 l min^{-1} to 4.8 l min^{-1} after 8 weeks exposure to 5250 m altitude compared with acute exposure, and that it was reduced by 25% compared to sea level (Calbet *et al.* 2003*b*).

Table 3. Leg $\dot{V}_{O_2max}$ (ml l^{-1}) when calculated with leg blood flows or O_2 conductance obtained at sea level

	SL	AH	W2	W2N	W8	W8N
Leg $\dot{V}_{O_2max}$ calculated with sea level blood flow (ml l^{-1})	2198 ± 79	1618 ± 54*	2011 ± 45*	2478 ± 65*	2033 ± 87*	2365 ± 93*
Leg $\dot{V}_{O_2max}$ calculated with sea level O_2 conductance (ml l^{-1})	2198 ± 79	1640 ± 133	2038 ± 151	2385 ± 236	2117 ± 129	2497 ± 169

Values are mean ± S.D. *$P < 0.05$ compared with SL.

Table 4. Venous plasma concentrations of substances associated with venoconstriction or vasodilatation

	SL	AH	W2	W2N	W8	W8N	Nat	NatN
Noradrenaline (nmol l^{-1})	11.9 ± 4§	17.6 ± 3*	43.3 ± 9*§#	48.8 ± 3*§#	49.2 ± 10*§#	57.1 ± 7*§#	21.8 ± 6	18.3 ± 3
K^+ (mmol l^{-1})	5.9 ± 0.2	5.8 ± 0.3	5.2 ± 0.3*#	5.8 ± 0.2	5.7 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2
pH	7.06 ± 0.01§	7.13 ± 0.02*	7.15 ± 0.02*	7.09 ± 0.02$	7.12 ± 0.02*	7.07 ± 0.01$	7.14 ± 0.01	7.16 ± 0.02
Lactate (mmol l^{-1})	16.0 ± 0.3	15.1 ± 1.2	13.7 ± 1.9	12.0 ± 0.9	15.7 ± 0.7	14.5 ± 0.9	15.2 ± 1.3	14.1 ± 1.4

All values were obtained at maximal exercise and are given as mean ± S.D. *$P < 0.05$ to SL, §$P < 0.05$ to Nat, #$P < 0.05$ to AH, $$P < 0.05$ to ambient condition.

The decrease in leg blood flow with acclimatization was explained by a higher fraction of maximal cardiac output being deviated to tissues other than the exercising legs (4.8 and 6.6 l min^{-1} in acute and chronic hypoxia, respectively). In the present investigation we did not quantify cardiac output, but in Fig. 1*A* it is clearly seen that O_2 delivery to the exercising leg is similar in acute hypoxia and after 2 and 8 weeks of acclimatization. Therefore, in the present investigation there is a mismatch between C_{aO_2} and $\dot{V}_{O_2max}$, whereas the relationship between O_2 delivery and $\dot{V}_{O_2max}$ is unaltered with altitude exposure (Fig. 2). The reason for peak leg blood flow to be decreased with chronic high altitude exposure remains unknown, but could be related to the increased haematocrit, reduced vascular reactivity to vasodilatory signals and/or a hypoxia-mediated change in the output from the cardiovascular nuclei in the CNS to ensure better oxygenation in some tissues.

In Table 3 we have calculated arterial oxygen delivery and leg $\dot{V}_{O_2max}$ with acute hypoxic exposure, and after 2 and 8 weeks of acclimatization. To evaluate the effects of the reduced leg blood flow, we performed the calculations with the leg blood flows obtained at sea level. This numerical analysis suggests that arterial oxygen delivery and $\dot{V}_{O_2max}$ would have been nearly restored to sea level values without the drop in peak leg blood flow with altitude acclimatization. The small difference between sea level arterial oxygen delivery and $\dot{V}_{O_2max}$ and the same values after acclimatization can be entirely explained by arterial desaturation in the hypoxic conditions. This is also indicated by the fact that arterial oxygen delivery and $\dot{V}_{O_2max}$ in the hyperoxic altitude trials are higher than the values at sea level. This would suggest that the major determinant for $\dot{V}_{O_2max}$ not to increase with acclimatization is the observed reduction in maximal leg blood flow.

Possible mechanisms for a reduced vascular reactivity (increased vasoconstriction or decreased vasodilatation) during exercise at altitude and its role in maximum leg blood flow

In chronic hypoxia vasodilatation may be limited due to a higher vasoconstricting tone. Vasoconstriction within exercising muscles may potentially be accomplished by sympathetic activity or endocrine/paracrine effects of peptides such angiotensin II or endothelin-1. Microneurographic recordings of muscle sympathetic nerve activity have provided evidence of a 3-fold increase in burst frequency in resting acclimatized lowlanders in a previous field study (Hansen & Sander, 2003) as well as in the present study (M. Sander, personal observation). The present field study also provided evidence of increased muscle sympathetic nervous activity (MSNA) in resting Aymara, in whom burst incidence equalled the acclimatized Danes. Direct microneurographic recordings of sympathetic activity is not feasible during whole body dynamic exercise; however, plasma noradrenaline is markedly increased at high altitude both in resting and exercising lowlanders as well as in exercising high altitude natives, and the maximal levels of noradrenaline during maximal ergometer cycle exercise at high altitude far exceed levels reached under any circumstances at sea level (Table 4).

Exercise hyperaemia is triggered by powerful vasodilatory signals. The precise underlying mechanisms remain enigmatic, but local release of agents such as K^+,

nitric oxide, adenosine, ATP and Endothelium-derived hyperpolarization fact (EDHF) could all be involved (Clifford & Hellsten, 2004). In the present study a small decrease in venous $[K^+]$ was observed at maximal exercise after 2 weeks of acclimatization. However, since this did not occur after 8 weeks of acclimatization, and with a concomitant unchanged leg blood flow as compared to 2 weeks, it seems unlikely that changes in $[K^+]$ can explain the differences in flow. Interstitial accumulation of H^+ and lactate are also involved in the regulation of vascular tone. Thus, a lowering of pH can reduce the intracellular $[Ca^{2+}]$ and thereby cause relaxation of smooth muscle cells (Peng *et al.* 1998). The effects of chronic hypoxia on the local accumulation of vasodilators in exercising muscle are largely unknown. In the present study femoral venous pH values at maximal exercise were elevated at high altitude compared to sea level, whereas maximum femoral venous lactate levels were similar between high altitude and sea level. This combination could perhaps imply an interstitial environment less prone to vasodilatation. The interplay between increasing sympathetic activation and accruing vasodilator signals within heavily exercising skeletal muscle is complex. At sea level, there is evidence for a metabolic inhibition of sympathetic vasoconstriction in the exercising thigh and forearm (Dinenno *et al.* 2002; Wray *et al.* 2004). Mechanistic studies suggest that decreasing pH may be involved in this phenomenon (Tateishi & Faber, 1995). If so, the higher pH at high altitude may lead to less inhibition of sympathetic vasoconstriction. Acute lowering of muscle oxygen tension is accompanied by intensified metabolic inhibition of sympathetic actions (Hansen *et al.* 2000). Thus, the lower arterial and mean capillary oxygen tension in acute hypoxia might cause a more potent inhibition of sympathetic vasoconstriction. However, with acclimatization to altitude arterial P_{O_2} and mean capillary P_{O_2} increase, which may reduce the efficiency of this sympatholytic mechanism. In addition, hyperoxia at altitude does not restore sea level vascular conductance in the acclimatized human.

Our data from this and previous studies (Calbet *et al.* 2003*b*) show that leg vascular conductance at peak exercise is reduced in chronic hypoxia. This effect is probably a consequence of a shift in the balance between vasocontricting and vasodilating mechanisms in chronic hypoxia. However, further studies are needed to decipher which vasoactive systems are involved in this change.

In summary this study shows that with altitude acclimatization skeletal muscle capillary O_2 conductance is reduced in lowlanders to values a little higher or similar to those observed in altitude natives, and that this is the result of a reduced peak leg blood flow. When calculating a hypothetical leg $\dot{V}_{O_2max}$ at altitude using either the O_2 conductance or the leg blood flow values obtained at sea level, the former values were almost completely restored to sea level values. This suggests that the major determinants for $\dot{V}_{O_2max}$ not increasing with acclimatization are the observed reduction in maximal leg blood flow and O_2 conductance.

References

Bencowitz HZ, Wagner PD & West JB (1982). Effect of change in P50 on exercise tolerance at high altitude: a theoretical study. *J Appl Physiol* **53**, 1487–1495.

Birkeland KI, Stray-Gundersen J, Hemmersbach P, Hallen J, Haug E & Bahr R (2000). Effect of rhEPO administration on serum levels of sTfR and cycling performance. *Med Sci Sports Exerc* **32**, 1238–1243.

Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD & Saltin B (2003*b*). Why is VO2 max after altitude acclimatization still reduced despite normalization of arterial O_2 content? *Am J Physiol Regul Integr Comp Physiol* **284**, R304–R316.

Calbet JA, Holmberg HC, Rosdahl H, Van Hall G, Jensen-Urstad M & Saltin B (2005). Why do the arms extract less oxygen than the legs during exercise? *Am J Physiol Regul Integr Comp Physiol* **289**, R1448–1458.

Calbet JA, Lundby C, Koskolou M & Boushel R (2006). Importance of hemoglobin concentration to exercise: acute manipulations. *Resp Physiol Neurobiol*; DOI:10.1016/j.resp.2006.01.014.

Clifford PS & Hellsten Y (2004). Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol* **97**, 393–403.

Dill DB, Myhre LG, Philips EE & Brown DK (1966). Work capacity in acute exposure to altitude. *J Appl Physiol* **21**, 1168–1176.

Dinenno FA, Dietz NM & Joyner MJ (2002). Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. *Circulation* **106**, 1349–1354.

Gayeski TE & Honig CR (1986). O_2 gradients from sarcolemma to cell interior in red muscle at maximal VO_2. *Am J Physiol* **251**, H789–H799.

Gonzalez-Alonso J & Calbet JA (2003). Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation* **107**, 824–830.

Green HJ, Sutton JR, Cymerman A, Young PM & Houston CS (1989). Operation Everest II: adaptations in human skeletal muscle. *J Appl Physiol* **66**, 2454–2461.

Hansen J & Sander M (2003). Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J Physiol* **546**, 921–929.

Hansen J, Sander M, Hald CF, Victor RG & Thomas GD (2000). Metabolic modulation of sympathetic vasoconstriction in human skeletal muscle: role of tissue hypoxia. *J Physiol* **527**, 387–396.

Hepple RT, Hagen JL & Krause DJ (2002). Oxidative capacity interacts with oxygen delivery to determine maximal O_2 uptake in rat skeletal muscles in situ. *J Physiol* **541**, 1003–1012.

Hepple RT, Hogan MC, Stary C, Bebout DE, Mathieu-Costello O & Wagner PD (2000). Structural basis of muscle O_2 diffusing capacity: evidence from muscle function in situ. *J Appl Physiol* **88**, 560–566.

Kayar SR, Hoppeler H, Jones JH, Longworth K, Armstrong RB, Laughlin MH *et al*. (1994). Capillary blood transit time in muscles in relation to body size and aerobic capacity. *J Exp Biol* **194**, 69–81.

Kayser B (2003). Exercise starts and ends in the brain. *Eur J Appl Physiol* **90**, 411–419.

Kelman GR (1966). Digital computer subroutine for the conversion of oxygen tension into saturation. *J Appl Physiol* **21**, 1375–1376.

Krogh A (1919). The supply of oxygen to the tissue and the regulation of the capillary circulation. *J Physiol* **52**, 457.

Lenfant C, Torrance JD & Reynafarje C (1971). Shift of the O_2-Hb dissociation curve at altitude: mechanism and effect. *J Appl Physiol* **30**, 625–631.

Lundby C, Calbet JA, Van Hall G, Saltin B & Sander M (2004*a*). Pulmonary gas exchange at maximal exercise in Danish lowlanders during 8 wk of acclimatization to 4,100 m and in high-altitude Aymara natives. *Am J Physiol Regul Integr Comp Physiol* **287**, R1202–R1208.

Lundby C & Damsgaard R (2006). Exercise performance in hypoxia after novel erythropoiesis stimulating protein treatment. *Scand J Med Sci Sports* **16**, 35–40.

Lundby C, Pilegaard H, Andersen JL, Van Hall G, Sander M & Calbet JA (2004*b*). Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle. *J Exp Biol* **207**, 3865–3871.

Lundby C, Pilegaard H, Van Hall G, Sander M, Calbet J, Loft S & Møller P (2003). Oxidative DNA damage and repair in skeletal muscle of humans exposed to high-altitude hypoxia. *Toxicology* **192**, 229–236.

McAllister RM, Ogilvie RW & Terjung RL (1990). Impact of reduced cytochrome oxidase activity on peak oxygen consumption of muscle. *J Appl Physiol* **69**, 384–389.

MacDougall JD, Green HJ, Sutton JR, Coates G, Cymerman A, Young P & Houston CS (1991). Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude. *Acta Physiol Scand* **142**, 421–427.

Mairbaurl H, Oelz O & Bartsch P (1993). Interactions between Hb, Mg, DPG, ATP, and Cl determine the change in Hb-O2 affinity at high altitude. *J Appl Physiol* **74**, 40–48.

Nielsen HB, Madsen P, Svendsen LB, Roach RC & Secher NH (1998). The influence of PaO_2, pH and SaO_2 on maximal oxygen uptake. *Acta Physiol Scand* **164**, 89–87.

Peng HL, Ivarsen A, Nilsson H & Aalkjaer C (1998). On the cellular mechanism for the effects of acidosis on vascular tone. *Acta Physiol Scand* **164**, 517–525.

Piiper J (2000). Perfusion, diffusion and their heterogeneities limiting blood-tissue O_2 transfer in muscle. *Acta Physiol Scand* **168**, 603–607.

Richardson RS, Tagore K, Haseler LJ, Jordan M & Wagner PD (1998). Increased VO_2 max with right-shifted Hb-O_2 dissociation curve at a constant O_2 delivery in dog muscle in situ. *J Appl Physiol* **84**, 995–1002.

Robinson DM, Ogilvie RW, Tullson PC & Terjung RL (1994). Increased peak oxygen consumption of trained muscle requires increased electron flux capacity. *J Appl Physiol* **77**, 1941–1952.

Saltin B, Blomqvist G, Mitchell JH, Johnson RL Jr, Wildenthal K & Chapman CB (1968). Response to exercise after bed rest and after training. *Circulation* **38**, VII1–78.

Samaja M (1988). Prediction of the oxygenation of human organs at varying blood oxygen carrying properties. *Respir Physiol* **72**, 211–217.

Samaja M, Crespi T, Guazzi M & Vandegriff KD (2003). Oxygen transport in blood at high altitude: role of the hemoglobin-oxygen affinity and impact of the phenomena related to hemoglobin allosterism and red cell function. *Eur J Appl Physiol* **90**, 351–359.

Schlichtig R (1997). [Base excess] and [strong ion difference] during O_2-CO_2 exchange. *Adv Exp Med Biol* **411**, 97–102.

Severinghaus JW (1979). Simple, accurate equations for human blood. O_2 dissociation computations. *J Appl Physiol* **46**, 599–602.

Siggaard-Andersen O (1977). The van Slyke equation. *Scand J Clin Lab Invest Suppl* **37**, 15–20.

Stringer W, Wasserman K, Casaburi R, Porszasz J, Maehara K & French W (1994). Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *J Appl Physiol* **76**, 1462–1467.

Tateishi J & Faber JE (1995). Inhibition of arteriole alpha 2- but not alpha 1-adrenoreceptor constriction by acidosis and hypoxia in vitro. *Am J Physiol* **268**, H2068–H2076.

Wagner PD (1992). Gas exchange and peripheral diffusion limitation. *Med Sci Sports Exerc* **24**, 54–58.

Wagner PD (1993). Algebraic analysis of the determinants of VO_2max. *Respir Physiol* **93**, 221–237.

Wagner PD (1997). Insensitivity of VO_2max to hemoglobin-P50 as sea level and altitude. *Respir Physiol* **107**, 205–212.

Wagner PD (2000*a*). Diffusive resistance to O_2 transport in muscle. *Acta Physiol Scand* **168**, 609–614.

Wagner PD (2000*b*). Reduced maximal cardiac output at altitude – mechanisms and significance. *Respir Physiol* **120**, 1–11.

Wagner P (2001). Gas exchange. In *High Altitude. An Exploration of Human Adaptation*, vol. 161, ed. Hornbein T & Schoene R, pp. 199–234. Marcel Dekker, Inc., New York, Basel.

Wagner PD, Araoz M, Boushel R, Calbet JA, Jessen B, Radegran G, Spielvogel H, Sondegaard H, Wagner H & Saltin B (2002). Pulmonary gas exchange and acid-base state at 5,260 m in high-altitude Bolivians and acclimatized lowlanders. *J Appl Physiol* **92**, 1393–1400.

Winslow RM (1988). Optimal hematologic variables for oxygen transport, including P50, hemoglobin cooperativity, hematocrit, acid-base status, and cardiac function. *Biomater Artif Cells Artif Organs* **16**, 149–171.

Wray DW, Fadel PJ, Smith ML, Raven P & Sander M (2004). Inhibition of alpha-adrenergic vasoconstriction in exercising human thigh muscles. *J Physiol* **555**, 545–563.

Young AJ, Sawka MN, Muza SR, Boushel R, Lyons T, Rock PB, Freund BJ, Waters R, Cymerman A, Pandolf KB & Valeri CR (1996). Effects of erythrocyte infusion on VO_2max at high altitude. *J Appl Physiol* **81**, 252–259.