During hypoxic exercise some vasoconstriction is needed to match O\textsubscript{2} delivery with O\textsubscript{2} demand at the microvascular level

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To test the hypothesis that the increased sympathetic tonus elicited by chronic hypoxia is needed to match O\textsubscript{2} delivery with O\textsubscript{2} demand at the microvascular level eight male subjects were investigated at 4559 m altitude during maximal exercise with and without infusion of ATP (80 \(\mu\)g (kg body mass\(^{-1}\) min\(^{-1}\)) into the right femoral artery. Compared to sea level peak leg vascular conductance was reduced by 39% at altitude. However, the infusion of ATP at altitude did not alter femoral vein blood flow (7.6 ± 1.0 \textit{versus} 7.9 ± 1.01 \textit{min}^{-1}) and femoral arterial oxygen delivery (1.2 ± 0.2 \textit{versus} 1.3 ± 0.21 min\(^{-1}\); control and ATP, respectively). Despite the fact that with ATP mean arterial blood pressure decreased (106.9 ± 14.2 \textit{versus} 83.3 ± 16.0 mmHg, \(P < 0.05\)), peak cardiac output remained unchanged. Arterial oxygen extraction fraction was reduced from 85.9 ± 5.3 to 72.0 ± 10.2% (\(P < 0.05\)), and the corresponding venous O\textsubscript{2} content was increased from 25.5 ± 10.0 to 46.3 ± 18.5 ml\textsuperscript{-1} (control and ATP, respectively, \(P < 0.05\)). With ATP, leg arterial–venous O\textsubscript{2} difference was decreased (\(P < 0.05\)) from 139.3 ± 9.0 to 116.9 ± 8.4 \textit{min}^{-1} and leg \(V_{O2,\text{max}}\) was 20% lower compared to the control trial (1.1 ± 0.2 \textit{versus} 0.9 ± 0.1 \textit{min}^{-1}) (\(P = 0.069\)). In summary, at altitude, some degree of vasoconstriction is needed to match O\textsubscript{2} delivery with O\textsubscript{2} demand. Peak cardiac output at altitude is not limited by excessive mean arterial pressure. Exercising leg \(V_{O2,\text{peak}}\) is not limited by restricted vasodilatation in the altitude-acclimatized human.

(Resubmitted 2 October 2007; accepted 9 October 2007; first published online 11 October 2007)

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When arterial oxygen content (\(C_{aO2}\)) is increased by hypoxia \cite{Nielsen1998} or erythropoietin (rHuEPO) administration \cite{Ekblom1991} maximal oxygen uptake (\(V_{O2,\text{max}}\)) is increased. Conversely, when \(C_{aO2}\) is reduced acutely by hypoxia, \(V_{O2,\text{max}}\) is reduced in proportion to the degree of hypoxia \cite{Dill1966, Calbet2003a, Lundby2004}. Thus, there appears to be a tight relation between \(C_{aO2}\) and \(V_{O2,\text{max}}\) for a given individual. However, with acclimatization to altitudes above 4000 m, \(C_{aO2}\) is normalized to sea level values without concomitant normalization of \(V_{O2,\text{max}}\) \cite{Andersen1985, Mazzeo1994, Calbet2003b, Lundby2004, Schuler2007}. More recently, it has been shown that \(V_{O2,\text{max}}\) with acute hypoxic exposure does not increase despite increases in \(C_{aO2}\) with an erythropoietin analogue \cite{Lundby2006}, and similar results have been obtained at altitude with autologous erythrocyte infusion \cite{Young1996}. Thus, in hypoxia \(V_{O2,\text{max}}\) may be limited by factors apart from \(C_{aO2}\).

It has been suggested that the failure to recover sea level \(V_{O2,\text{max}}\) after altitude acclimatization despite an increase of \(C_{aO2}\) to sea level values is in part explained by hypoxia-associated decrements in maximal cardiac output and leg blood flow, resulting in an unchanged leg oxygen delivery with acclimatization \cite{Calbet2003b, Lundby2004}. The reason why peak blood flow is reduced in chronic hypoxia remains unknown. We have shown that leg vascular conductance at peak exercise in chronic hypoxia is reduced compared to normoxia or acute hypoxia \cite{Calbet2003b, Lundby2006}. The latter could be explained by increased vasoconstrictor
signals, reduced vasodilatory signals, increased vascular responsiveness to vasoconstrictors, or reduced sensitivity to vasodilators. In support of an increased vasoconstrictor tone as the main mechanism causing the reduction of peak exercise muscle vascular conductance with acclimatization, very high femoral artery noradrenaline concentrations have been reported at maximal exercise in acclimatized humans (Lundby et al. 2006). This increase in sympathetic tonus with chronic hypoxia may be needed to match O₂ delivery with O₂ demand at the microvascular level. On the other hand, excessive sympathetic activation could limit bulk O₂ delivery to the exercising muscles directly by reducing leg blood flow and indirectly by contributing to elevated systemic vascular resistance, which could result in lowered maximal cardiac output. Interestingly, in heart failure patients sympathetic overactivity contributes to the exercise intolerance that is a common hallmark of this condition, and \( \dot{V}_{\text{O}_2}\text{max} \) has been shown to be inversely related to resting muscle sympathetic activity, i.e. the greater the sympathetic tonus the more exercise capacity is limited (Notarius et al. 1999).

In the present investigation we tested the hypothesis that some degree of vasoconstriction in the active muscles is needed during exercise at altitude to match O₂ delivery with O₂ demand at the microvascular level, and thereby allowing a maximal degree of O₂ extraction. To test this hypothesis we investigated sea level dwellers after acclimatization to 4559 m during maximal exercise with and without infusion of the well-known vasodilator ATP, which is able to completely eliminate noradrenaline-induced vasoconstriction (Rosenmeier et al. 2004; Calbet et al. 2006).

Methods

Subjects

Eight healthy males, age 26 ± 1 years, height 181 ± 1 cm, and weight 81 ± 2 kg, volunteered to participate in the study. The subjects had a maximal oxygen uptake (\( \dot{V}_{\text{O}_2}\text{max} \)) of 4.4 ± 0.11 min⁻¹ or 54 ± 2 ml kg⁻¹ min⁻¹, assessed during an incremental test to exhaustion at sea level. All subjects were informed about possible risks and discomfort involved before giving their written consent to participate. This study was carried out according to the Declaration of Helsinki and was approved by the Scientific Ethics Committee of Copenhagen and Frederiksborg Municipalities, Denmark.

The experiments were conducted first at sea level in Copenhagen, and then 6 weeks later at altitude as described below. Sea level data have been published elsewhere (Calbet et al. 2006). When necessary for comparison purposes we report the mean value obtained during maximal exercise at sea level (\( n = 6 \)).

High-altitude procedure

The profile of exposure to hypoxia was as follows: the first day consisted of transport by cable car from 1300 m to 3200 m, followed by a 2 h climb to an altitude of 3611 m (Capanna Gnifetti), where the subjects stayed overnight (day 1). The next day, the subjects stayed inactive for 24 h at 3611 m (day 2), in order to minimize the risk of acute mountain sickness upon arrival at 4559 m. On the morning of the third day, after a 5 h climb, they reached the altitude of 4559 m (day 3) and stayed in the Margeritha Hut for the next 13 days.

Experimental preparation

On study days the subjects reported to the laboratory at 08.00 h, and the following catheters were positioned under local anaesthesia (2% lidocaine (lignocaine)). A 20 gauge catheter (Hydrocath, Ohmeda, Wiltshire, UK) was inserted percutaneously using the Seldinger technique into the right femoral artery, 2 cm below the inguinal ligament and advanced 5–10 cm in the proximal direction. This catheter was connected to a blood pressure transducer positioned at the height of the fourth intercostal space (T100209A, Baxter, Unterschleissheim, Germany) and was also used for arterial blood sampling. A similar catheter was inserted in the same femoral artery 5 cm below the inguinal ligament and advanced 5–10 cm in the proximal direction for intra-arterial infusion of ATP. In the right femoral vein, a venous catheter with side holes (Radiopack TFE, Cook, Bjaerverskov, Denmark) was inserted and advanced ~5 cm proximal to the inguinal ligament for the injection of iced physiological saline solution (Andersen & Saltin, 1985). A thin polyethylene-coated thermodilometer (model 94–030–2.5F T.D. Probe, Edwards Edwards Edslab, Baxter, Irvine, CA, USA) was inserted through this catheter for blood flow measurements by the constant infusion thermodilution technique (Andersen & Saltin, 1985). A flow-through chamber (model 93–505, Edslab) was connected to the entry of this catheter to measure infusate temperature during ice-cold saline infusion. In the same vein an additional 20 gauge catheter (Hydrocath, Ohmeda, Wiltshire, UK) was also inserted, 2–3 cm below the inguinal ligament, and advanced 7–10 cm in the distal direction, beyond the merger with the saphenous vein. This catheter was connected to another blood pressure transducer positioned at the height of the fourth intercostal space (T100209A, Baxter, Unterschleissheim, Germany) and used femoral vein pressure measurements and venous blood sampling. Finally, in four subjects an additional 20 gauge catheter (Hydrocath, Ohmeda, Wiltshire, UK) was also inserted in the left femoral vein, 2–3 cm below the inguinal ligament, and advanced 7–10 cm in the distal direction, beyond the merger with the saphenous vein. This catheter was used to sample femoral venous blood

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from the left leg. All sampling catheters were connected to a three-way stopcock and sutured to the skin to minimize the risk of movement during exercise. A three-lead electrocardiogram (ECG) was displayed on a monitor during catheterization and the rest of the experimental procedures (Dialogue 2000, Danica, Copenhagen, Denmark). The ECG, blood pressure and the temperatures registered by the thermistor, as well as the infuseus temperatures were recorded simultaneously with the data acquisition system (MacLab 8/s ADInstruments, Sydney, Australia).

Cardiac output was measured by bolus indocyanine green (ICG; Akorn, USA) dye-dilution as previously described (Boushel et al. 2001). Four to five milligrams of dye was injected rapidly into the femoral vein from a calibrated syringe followed by a 5–10 ml flush of isotonic saline. Blood from the femoral artery was withdrawn with a pump (Harvard, 2202A) at 20 ml min\(^{-1}\) through a linear photodensitometer (Waters CO-10, Rochester, MN, USA) for measurement of the arterial dye concentration. Dye curves were collected in a data acquisition system (PowerLab, ADInstruments). Withdrawn arterial blood was reinfused into the femoral vein in a closed loop system. Cardiac output was computed as the ratio of dye injected to the average arterial ICG concentration over the time interval of the curve and expressed per minute. Following each experiment an ICG calibration curve was derived for each subject by measuring the voltage deflection from three separate samples of blood with incremented concentrations of ICG added. Approximately 30 min after all catheters were in place the resting experiments started (reported elsewhere).

**Experimental protocol**

Two maximal exercise tests to exhaustion were performed separated by at least 1 h of resting. The tests were done under infusion of ATP (Sigma; dissolved in isotonic saline to 8 mg ml\(^{-1}\)) into the right femoral artery at a constant rate of 80 μg (kg body mass)\(^{-1}\) min\(^{-1}\) using a Harvard infusion pump (Harvard pump, Harvard Apparatus, Millis, MA, USA) or without infusion (control), in random order. Half of the subjects received ATP during the first exercise trial, and the other half in the second trial. Heart rate and blood pressures were averaged over 15 s concurrent with the blood flow measurements. During the incremental exercise test ˙\(V_O_2\) was averaged every 15 s. The exercise protocol was performed on a cycle-ergometer (Monark E834, Varberg, Sweden) and consisted of a 15 min warm-up period at 150 W, following which the workload was increased by 40 W every 1.5 min until exhaustion.

**Respiratory variables**

Pulmonary ˙\(V_O_2\), CO\(_2\) production (˙\(V_CO_2\)), and expired minute ventilation (˙\(V_E\)) were measured continuously using an automated metabolic cart (Quark b2, Cosmed Srl., Rome, Italy). Before each test ambient conditions were measured, then the gas analyser and the flowmeter were calibrated with high precision gases. During submaximal and maximal exercise the ˙\(V_O_2\) values were recorded as averages of 15 s. The highest 15 s measurement of ˙\(V_O_2\) was taken as representative of the ˙\(V_O_2\)max. The reason for using this short interval is that leg blood flow, leg ˙\(V_O_2\), and blood pressures were also assessed during a similar time interval.

**Blood samples and analytical procedures**

Blood was sampled anaerobically in heparinized syringes and immediately analysed for haemoglobin (Hb), oxygen saturation (OSM3, Radiometer, Copenhagen, Denmark), CO\(_2\) and O\(_2\) tension (ABL700, Radiometer). Blood gases were corrected for measured femoral vein blood temperature. Blood O\(_2\) content (C\(_{a_O_2}\) and femoral vein C\(_{v_O_2}\)) was computed from the saturation and [Hb], i.e. \((1.34[Hb] − S_{O_2}) + (0.003 − P_{O_2})\). Alveolar P\(_{O_2}\) was corrected according to Severinghaus.

**Statistical analysis**

The effect of ATP was examined using the Wilcoxon rank tests for related samples. To reduce the likelihood of a type II error, no corrections for multiple comparisons were performed (Perneger, 1998). The significance level was set at  \(P < 0.05\). Data are expressed as mean ± standard deviation (s.d.), unless otherwise stated.

**Results**

**Resting data**

With altitude acclimatization C\(_{a_O_2}\) was increased by 13% reaching values similar to those observed at sea level in normoxic conditions (197.3 ± 19.2 and 200.9 ± 11.0 ml l\(^{-1}\), respectively). This increase of C\(_{a_O_2}\) was accounted for by a 14% increase in arterial blood haemoglobin concentration (150.3 ± 10.2 and 174.2 ± 10.5 g ml\(^{-1}\), respectively). Mean arterial blood pressure was increased from 80.8 ± 7.7 at sea level to 103.9 ± 6.8 mmHg at altitude. Leg and systemic vascular conductance were 11.2 ± 3.9 and 70.8 ± 10.4 at sea level and 10.4 ± 3.8 and 71.6 ± 19.4 ml m\(^{-1}\) min\(^{-1}\) mmHg\(^{-1}\) at altitude.

\(\dot{V}_{O_2,max}\) at sea level, in acute hypoxia, at altitude, and at altitude while breathing a mild hyperoxic gas

When the subjects were exposed to acute hypoxia in the laboratory at sea level, ˙\(V_{O_2,max}\) was reduced by approximately 31% (from 4.3 ± 0.1 to 3.0 ± 0.5 l min\(^{-1}\),...
and, as expected, did not increase from this value with exposure to altitude (3.0 ± 0.6 l min⁻¹). At altitude, however, $V_{O_2,max}$ was restored to sea level values by breathing mild hyperoxia.

### Ventilation and pulmonary gas exchange at maximal exercise with and without ATP infusion at altitude

Pulmonary ventilation (135.9 ± 57.9 versus 129.6 ± 63.0 l min⁻¹), alveolar oxygen pressure (61.2 ± 3.1 versus 62.3 ± 1.6 mmHg), arterial oxygen saturation (69.8 ± 4.3 versus 72.9 ± 3.8%), and arterial oxygen content (164.3 ± 13.3 versus 169.3 ± 11.9 ml l⁻¹) did not differ between control and ATP infusion, respectively (Table 1).

### Cardiovascular response to maximal exercise at altitude with and without ATP, compared to sea level

At sea level maximal exercise heart rate, stroke volume, cardiac output and mean arterial pressure were: 182.4 ± 5.3 beats min⁻¹, 141.6 ± 14.0 ml min⁻¹, 25.7 ± 1.9 l min⁻¹ and 82.9 ± 6.7 mmHg, respectively.

Maximal heart rate was reduced at altitude, but there were no significant effects of ATP infusion on heart rate (159.4 ± 9 versus 161.8 ± 6 beats min⁻¹). Peak exercise stroke volume was similar at altitude (131.9 ± 14.4 ml) and at sea level, and was not affected by ATP infusion (132.1 ± 22.6 ml). Peak exercise cardiac output was reduced by 19% at altitude (20.9 ± 1.4 l min⁻¹) and remained unaffected by the ATP infusion (21.3 ± 3.0 l min⁻¹). In contrast, peak exercise mean blood pressure was increased at altitude to 106.9 ± 14.2 mmHg. The infusion of ATP reduced mean arterial pressure to a value that was comparable to that observed at sea level (83.3 ± 16.0 mmHg). Peak exercise leg blood flow at sea level was 9.3 ± 2.1 l min⁻¹, and was decreased to 7.6 ± 1.0 l min⁻¹ at altitude, remaining at this level with the infusion of ATP (7.9 ± 1.0 l min⁻¹). At sea level peak leg oxygen delivery was 1.9 ± 0.5 l min⁻¹ and was decreased to 1.2 ± 0.2 l at altitude, and was not altered with ATP infusion (1.3 ± 0.2 l min⁻¹) (Fig. 1).

Compared to sea level peak exercise leg vascular conductance was reduced at altitude (115.7 ± 35.0 and 70.7 ± 9.3 ml l⁻¹ min⁻¹ mmHg⁻¹, respectively). With ATP peak exercise vascular conductance was increased to 98.3 ± 24.4 ml l⁻¹ min⁻¹ mmHg⁻¹ ($P = 0.08$, compared to control altitude). When peak leg vascular conductance was adjusted by the respective peak power output, ATP resulted in a significantly greater peak leg vascular conductance. Similar effects were observed for systemic vascular conductance.

### Effects of ATP infusion on oxygen extraction, leg $V_{O_2}$, and pulmonary $V_{O_2}$

At sea level $O_2$ extraction was 85.0 ± 5.4% and remained unchanged with altitude exposure (85.9 ± 5.3%), but was decreased by 16% to 72.0 ± 10.2% with ATP infusion at altitude. Leg $V_{O_2}$ at sea level was 1.6 ± 0.2 l min⁻¹. In all altitude conditions leg $V_{O_2,max}$ was lower than at sea level, the values being 1.1 ± 0.2 versus 0.9 ± 0.1 l min⁻¹, respectively. The 20% difference in altitude leg $V_{O_2}$ between conditions was not statistically different ($P = 0.069$). Pulmonary $V_{O_2}$ was 3.0 ± 0.6 and 3.0 ± 0.5 l min⁻¹ in both conditions.

### Discussion

This study shows that in chronic hypoxia peak exercise leg vascular conductance is reduced and that peak exercise mean arterial pressure is increased. The fact that the infusion of ATP almost re-established sea level leg vascular conductance confirms that at altitude the exercise-induced vasodilatory response is restrained. This limitation of

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**Table 1. Blood oxygen parameters at peak exercise at altitude with and without ATP infusion**

<table>
<thead>
<tr>
<th></th>
<th>Control experiment</th>
<th>ATP infusion</th>
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<tr>
<td></td>
<td>Infused leg</td>
<td>Contralateral leg</td>
</tr>
<tr>
<td>$P_{aO_2}$ (mmHg)</td>
<td>19.1 ± 2.0</td>
<td>45.6 ± 2.2</td>
</tr>
<tr>
<td>$P_{aCO_2}$ (mmHg)</td>
<td>9.8 ± 4.0</td>
<td>69.8 ± 1.7</td>
</tr>
<tr>
<td>$S_{O_2}$ (%)</td>
<td>25.5 ± 10.0</td>
<td>30.9 ± 8.2</td>
</tr>
<tr>
<td>$S_{CO_2}$ (%)</td>
<td>85.9 ± 5.3</td>
<td>83.8 ± 2.7</td>
</tr>
<tr>
<td>$P_{aco_2}$ (ml l⁻¹)</td>
<td>51.7 ± 2.6</td>
<td>24.6 ± 1.1</td>
</tr>
<tr>
<td>$P_{vco_2}$ (ml l⁻¹)</td>
<td>51.4 ± 2.8</td>
<td>24.6 ± 1.1</td>
</tr>
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$P_{aO_2}$ (mmHg), $P_{aCO_2}$ (mmHg), $S_{O_2}$ (%), $S_{CO_2}$ (%), $C_{O_2}$ (ml l⁻¹), $C_{CO_2}$ (ml l⁻¹), fractional oxygen extraction (FOE; %), $P_{aco_2}$ (mmHg) and $P_{vco_2}$ (mmHg). Blood samples were drawn in the infused leg, and also in the contralateral leg in both experimental conditions. Values are mean ± s.d., *$P < 0.05$ compared to control situation.
the exercise-induced vasodilatation at altitude is probably mediated by the sympathetic nervous system which is overactive in chronic hypoxia (Calbet, 2003; Hansen & Sander, 2003). Sympathetic overactivity is generally presumed as a negative side-effect of chronic hypoxia (Heindl et al. 2001) and severe medical conditions such as chronic kidney failure (Converse et al. 1992) and chronic heart failure (Notarius et al. 1999). Moreover, in heart failure patients sympathetic activity measured under resting conditions is associated with lower $\dot{V}O_2\text{max}$ (Notarius et al. 1999). It has been suggested that sympathetic activation may limit exercise performance by restraining muscle blood flow in chronic heart failure patients (Notarius et al. 1999). Moreover in patients with idiopathic hyperhidrosis, which has been attributed to overactivity of the sympathetic fibres, sympathectomy increases peak forearm vascular conductance and exercise capacity during handgrip exercise (Kardos et al. 2000). However, although acute sympathetic inhibition with clonidine in patients with chronic heart failure results in greater vascular conductance and peak exercise blood flow in the exercising legs it does not affect exercise performance (Lang et al. 1997). In agreement with the present investigation, Lang et al. (1997) observed that in chronic heart failure patients sympathetic inhibition has no impact on maximal cardiac output or systemic $\dot{V}O_2\text{peak}$, while it increases venous $C_{aO_2}$, i.e. sympathetic inhibition was associated with a lower leg $O_2$ extraction.

In accordance with this, the present investigation indicates that some degree of sympathetic overactivity is actually necessary to maintain an adequate match between $O_2$ delivery and $O_2$ demand at the microvascular level in the exercising muscles. Counteracting the

Figure 1

Peak exercise cardiac output ($A$, l min$^{-1}$), leg blood flow ($B$, l min$^{-1}$), mean blood pressure ($C$, mmHg), leg vascular conductance ($D$, ml$^{-1}$ min$^{-1}$ mmHg$^{-1}$), leg $O_2$ delivery ($E$, l min$^{-1}$), fractional $O_2$ extraction ($F$, %), leg $\dot{V}O_2$ ($G$, l min$^{-1}$), and pulmonary $\dot{V}O_2$ ($H$, l min$^{-1}$) at sea level and at altitude with and without ATP infusion. Values are mean ± s.d., *$P < 0.05$ compared to control situation.
vasoconstricting signals with ATP results in such a reduction in O$_2$ extraction, that despite a concomitant increase of vascular conductance to almost sea level values, leg $V_{\text{O}_2}\text{peak}$ is reduced. This confirms similar results, but of lower magnitude, obtained with this experimental approach at sea level (Calbet et al. 2006).

ATP has long been recognized as a potent vasodilator, eliciting dilatation by activating P$_{2Y}$ purinergic receptors on vascular endothelial cells and thereby causing a release of vasodilators such as nitric oxide (NO), prostaglandins (PGs), and endothelium-derived hyperpolarizing factor (EDHF) (Ralevic & Burnstock, 1998). Recently it was shown that circulating plasma levels of ATP are increased in an exercise-intensity-dependent manner (Gonzalez-Alonso et al. 2002). The source of circulating ATP remains unknown, but may be released from endothelial cells, sympathetic nerve terminals, or red blood cells. The vasodilating effects of ATP infusions have been studied in humans during mild submaximal knee extensor exercise (20 W), and in this situation leg blood flow was augmented from 3.4 to 5.3 l min$^{-1}$ with a concomitant decrease in arterial O$_2$ extraction, keeping leg $V_{\text{O}_2}$ unchanged (Rosenmeier et al. 2004). We recently demonstrated in normoxia at sea level that it was possible to increase leg blood flow by infusing ATP in the femoral artery during ergometer cycling at near maximal exercise intensities (94% of $V_{\text{O}_2}\text{max}$). However, although blood flow was increased at this intensity from 8.5 to 10.2 l min$^{-1}$, blood flow at maximal exercise was not altered with ATP infusion (10.6 and 10.8 l min$^{-1}$, control and ATP, respectively), and subjects fatigued at the same exercise intensity (Calbet et al. 2006).

In contrast to what is observed at sea level, at altitude both maximal cardiac output and muscle blood flow are reduced, while exercise mean arterial pressure is elevated (Calbet et al. 2003b; Lundby et al. 2006), implying that the exercising muscles should be even more sensitive to ATP than observed at sea level. ATP infusion increased leg blood flow only marginally by 320 ml (non-significant, $P = 0.455$) to 7.9 l min$^{-1}$, and as a result arterial O$_2$ delivery was also altered minimally (92 ml min$^{-1}$, $P = 0.291$).

A key question to be answered with the present results is why it was not possible to restore the altitude-induced reduction in leg blood flow, and hence leg $V_{\text{O}_2}\text{max}$ back to sea level values with the infusion of ATP. In the present study, leg arterial O$_2$ extraction decreased markedly (16%, $P < 0.05$) and leg $V_{\text{O}_2}\text{max}$ was ultimately lowered by 91 ml min$^{-1}$ ($P = 0.069$). This finding indicates that despite a strong vasodilator stimulus locally in one limb, approximately 450 ml of the femoral blood flow was diverted to combined regions of less active muscle beds and other tissues such as skin and connective tissue which also possess P$_{2Y}$ receptors. Thus, ATP-induced diversion of blood to less (or non-) active muscle or towards other tissues where extraction is already maximal, caused a mismatch between blood flow distribution and muscle oxygen demand, altering an apparently optimal pattern of flow distribution to the active musculature. This, combined with the lower perfusion pressure in the limb, probably lowered the effective perfusion pressure to the most active muscle vascular beds. In control exercise conditions sympatholysis in contracting muscle with increasing exercise intensity despite greater sympathetic drive may be greatest in the most activated muscle regions serving to precisely match blood flow increases to the most activated muscle fibres maximising extraction (Strandell & Shepherd, 1967). Meanwhile sympathetic vasoconstrictor tone is preserved in less active tissue regions providing an optimal balance of vasodilatation and perfusion pressure for the muscle fibres with the greatest oxygen demand. In addition, the feed-forward dilatation induced by ATP probably interrupts the precisely coordinated ascending vasodilatory signals from arteriolar networks emerging from muscle with the greatest metabolic demand governing total flow into muscle (Segal, 2005). Based on previous studies and the present one, it is clear that the infusion of ATP causes leg vasodilatation when infused intra-arterially at exercise intensities ranging from rest to maximal exercise. Despite this vasodilatory response, peak leg blood flow is only increased up to very high submaximal exercise intensities, and it fails to increase leg blood flow at maximal exercise.

The intra-arterial infusion of ATP may have failed to increase peak leg blood flow during exercise in chronic hypoxia due to several reasons. Cardiac output may be regulated ‘upstream’ of the working muscles, limiting further blood supply to contracting muscle despite powerful vasodilator signals. In comparison to sea level, chronic hypoxia reduced peak cardiac output to the same extent as that reported previously (Stenberg et al. 1966; Calbet et al. 2003b, 2006). Interventions aiming at reducing afterload (isovolaemic haemodilution), increasing preload (hypervolaemic haemodilution), or restoring sea level heart rate by parasympathetic blockade have all failed to elevate cardiac output in chronic hypoxia (Boushel et al. 2001; Calbet et al. 2002, 2004b). In this study we attempted to elevate limb blood flow by inducing a strong vasodilator stimulus locally, which we expected would reduce cardiac afterload and thus also increase cardiac output, which would then be directed to the vasodilated leg. The intra-arterial infusion of ATP resulted in a reduction of afterload but without the expected compensatory increase of cardiac output to maintain arterial pressure, through the activation of the baroreflex (Strandell & Shepherd, 1967). This finding concurs with our previous results (Calbet et al. 2002); however, in this study we applied a novel experimental approach to accomplish this effect which does not interfere with $C_{\text{O}_2}$. ATP infusion was associated with a drop in mean blood pressure from approximately 107 to 83 mmHg at maximal exercise. This is in contrast to the response at
sea level, where no differences in mean blood pressure were found with ATP infusions at maximal exercise (Calbet et al. 2006). The different response may have been induced by a stronger local dilator effect of ATP in chronic hypoxia, which is expected in the presence of a higher vasoconstricting tonus. The reason why this decrease in blood pressure was not compensated for by a baroreflex-mediated increase in cardiac output could be explained by reduced baroreflex sensitivity in hypoxia (Severe et al. 2001). Nonetheless, it is remarkable that $V_{O_2,max}$ (and leg blood flow) remains unaffected by such a large drop in pressure, approaching even resting levels. This would suggest that it is not so critical that blood pressure is increased during whole body exercise to drive blood flow as it has been suggested in the past.

Cardiac output may be restricted by medullary or CNS control. At sea level, exercise with a large muscle group is generally assumed to be limited by the cardio-respiratory oxygen transport capacity (Andersen & Saltin, 1985; Calbet et al. 2004a). However, as early as 1924, A. V. Hill suggested that the limitation to maximal exercise may occur within the CNS (Hill et al. 1924), and with exercise at altitude, using a large muscle mass, it has been repeatedly suggested that exercise capacity may be limited by the CNS (for review see Kayser, 2003). The earlier reports favouring this hypothesis found their support in reduced signs of metabolic fatigue within the exercising muscles, i.e. low lactate levels at termination of the exercise. However, recent experimental data have contradicted these early reports, and muscle lactate, ATP, glycogen and creatine phosphate levels at the point of exhaustion at altitude, may change to similar values as achieved at sea level (van Hall et al. 2006). Therefore, potential limiting signals (except $P_{O_2}$) from the muscles to the CNS must be equal in both environmental situations. Although muscular signalling to the CNS may be unaffected at altitude, negative feedback signals from the heart and/or respiratory muscles may be increased. In agreement, it was recently demonstrated that the central neural drive (central command) during hypoxic exercise (5 km time trial) is decreased, and it was further speculated that the purpose therein may be to prevent peripheral muscle fatigue from exceeding a critical value (Amann et al. 2006). Although this could be the case during a time trial type of exercise, it is unlikely to account for the responses during standard incremental exercise tests (Calbet, 2006). In fact, epidural anaesthesia does not delay fatigue during maximal incremental exercise in hypoxia (Kjaer et al. 1999). Taken together, evidence is emerging that the major mediator of reduced peak cardiac output in hypoxia resides in brainstem or CNS which therefore limits leg blood flow and ultimately $V_{O_2,max}$. It was recently suggested that CNS control of cardiac output may become even more pronounced the more severe the hypoxic stimulus (Amann et al. 2007).

In agreement with our findings, α-adrenergic blockade did not affect systemic $V_{O_2,max}$ in chronic hypoxia (Mazzeo et al. 2003). However, the latter study was not designed to address any compensatory effects of autonomic control with manipulations of leg vascular conductance, and haemodynamic effects were not limited to the exercising vascular beds. Taken together, these data suggest that the observed decrease in leg blood flow with acclimatization to high altitude is independent of increased sympathetic nervous activity.

In summary, this study shows that peak exercise leg vascular conductance is reduced at altitude, and that it can almost be restored to sea level values by intra-arterial infusion of ATP. We have also shown that peak leg blood flow is not limited by excessive vasoconstricting signals during exercise in chronic hypoxia, and that some degree of vasoconstriction is needed in the lower extremities during maximal exercise to preserve an optimal distribution of blood flow. Although at altitude, peak exercise mean arterial pressure is increased and cardiac output reduced, decreasing mean arterial blood pressure by peripheral vasodilatation does not influence peak cardiac output implying that peak cardiac output is not limited by excessive afterload during exercise at altitude, in the human adapted to chronic hypoxia.

References


