AltitudeOmics: Effects of 16 days acclimatization to hypobaric hypoxia on muscle oxygen extraction during incremental exercise

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Abstract

Introduction: Acute altitude exposure lowers arterial oxygen content (CaO_2) and cardiac output ($\dot{Q}c$) at peak exercise, whilst O_2 extraction from blood to working muscles remains similar. Acclimatization normalizes CaO_2 but not peak $\dot{Q}c$ nor peak oxygen consumption ($\dot{V}O_2p$). To what extent acclimatization impacts muscle O_2 extraction remains unresolved.

Methods: Twenty-one sea-level residents performed an incremental cycling exercise to exhaustion near sea level (SL), in acute (ALT1) and chronic (ALT16) hypoxia (5,260 m). Arterial blood gases, gas exchange at the mouth and oxy- (O_2Hb) and deoxyhaemoglobin (HHb) of the vastus lateralis were recorded to assess arterial O_2 content (CaO_2), $\dot{Q}c$, and $\dot{V}O_2$. The HHb-$\dot{V}O_2$ slope was taken as a surrogate for muscle O_2 extraction.

Results: During moderate-intensity exercise, HHb-$\dot{V}O_2$ slope increased to a comparable extent at ALT1 (2.13 ± 0.94) and ALT16 (2.03 ± 0.88) compared to SL (1.27 ± 0.12), indicating increased O_2 extraction. However, the HHb/CaO_2 ratio increased from SL to ALT1 and then tended to go back to SL values at ALT16. During high-intensity exercise, HHb-$\dot{V}O_2$ slope reached a break point beyond which it decreased at SL and ALT1, but not at ALT16.

Discussion/Conclusion: Increased muscle O_2 extraction during submaximal exercise was associated with decreased CaO_2 in acute hypoxia. The significantly greater muscle O_2 extraction during maximal exercise in chronic hypoxia is suggestive of an O_2 reserve.

New and Noteworthy

Near infrared spectroscopy (NIRS) allows for the continuous measurement of oxy- and deoxyhaemoglobin concentrations (O2Hb and HHb, respectively), providing estimates of local muscle tissue oxygenation across the whole range of exercise intensities and exposures to hypoxia. During incremental exercise HHb and $\dot{V}O_2$ both increase linearly and the slope of the relationship between HHb and $\dot{V}O_2$ can be interpreted as an indirect index of local muscle O_2 extraction. Beyond 70% $\dot{V}O_2p$, HHb increase is blunted but $\dot{V}O_2$ keeps increasing which is attributed to limitations in muscle O_2 extraction. The aim of this work was to characterize the HHb - $\dot{V}O_2$ relationship during incremental cycling exercise to exhaustion at sea level (SL), in acute (ALT1) and during chronic (ALT16) exposure to hypoxia at 5,260 m. NIRS measurements being scarce in chronic hypoxia, the present study would bring an important new piece of information on continuously, albeit indirectly, measured muscle O_2 extraction during graded exercise to exhaustion.

The demonstrated presence of a muscle O_2 extraction reserve during chronic exposure is coherent with previous studies on the same set of participants and others indicating both limited muscle oxidative capacity and decrease in motor drive during chronic exposure to 5,260 m.

Introduction

The increase in oxygen consumption by the activated muscles during incremental cycling exercise is met by a proportional increase in vascular mass oxygen transport [$\dot{Q}aO_2$ = cardiac output ($\dot{Q}c$) x arterial oxygen content (CaO_2)]. Acute exposure to an altitude of 5,260 m decreases peak oxygen consumption [($\dot{V}O_2p$) (1–3)]. Two thirds of this decrease can be attributed to a reduction in CaO_2 (4–6), and the remainder to reductions in $\dot{Q}c$ and muscle blood flow (6–8). These decreases in CaO_2 and blood flow are accompanied by somewhat increased levels of lower limb O_2 extraction as measured from femoral arterio-venous differences (9, 10). With acclimatization to altitude Hb concentration and SaO_2 increase, normalizing CaO_2 at rest, and almost normalizing it at peak exercise. Peak $\dot{Q}c$ remains blunted, limiting mass oxygen transport, whilst the arterio-venous O2 difference returns towards normoxic values with no change in $\dot{V}O_2p$ as a result (2, 9, 11).

The reports of lower limb oxygen extraction levels at peak exercise in acute and chronic hypoxia, as compared to normoxia, are based on femoral arterio-venous O_2 differences, which represent global lower limb oxygen use, including non-contracting tissue (12). It is inferred that the levels of extraction in the effluent venous blood from the activated muscles must be approaching maximal levels, but this has not been measured directly.

Previous studies using invasive techniques showed that O_2 extraction increased at a given absolute submaximal exercise intensity in both acute and chronic hypoxia as compared with normoxia. Leg blood flow was similar after acclimatization to chronic hypoxia compared with normoxia, indicative of the normalization of CaO_2 (10, 13). These studies also showed that at maximal exercise leg blood flow was reduced in chronic hypoxia. Mild hyperoxic gas was then administered upon exhaustion in chronic hypoxia, which resulted in an increased O_2 extraction thereby suggesting that O_2 extraction was not limited after acclimatization (13). Similar results have been reported using advanced analytical techniques from biopsies in the AltitudeOmics research program (14).

Near infrared spectroscopy (NIRS) allows for the continuous measurement of oxy- and deoxyhaemoglobin concentrations (O_2Hb and HHb, respectively), providing estimates of local muscle tissue oxygenation across the whole range of exercise intensities and exposures to hypoxia (15–17). The NIRS signal comprises both haemoglobin (Hb) and myoglobin (Mb) (18, 19), but the majority (≈ 70%) of the signal is derived from venous Hb of the capillary bed (16, 20) and HHb is thought to reflect local O_2 extraction with less contamination from skin blood flow (15, 21–23).

At sea level, HHb concentrations result from the (im)balance between O_2 delivery and $\dot{V}O_2$ in the tissue under the NIRS probe. Increased muscle oxygenation would indicate a greater increase in O_2 delivery than $\dot{V}O_2$, whereas a decrease in muscle oxygenation would indicate a greater increase in $\dot{V}O_2$ than O_2 delivery. The relationship between O_2 delivery and $\dot{V}O_2$ is arterial-venous difference, which is therefore reflected by HHb (21). The mechanisms that could explain differences in O_2 extraction in chronic hypoxia compared to sea level could be for example an increased O_2 carrying capacity from the increased haemoglobin mass which would implore study of the HHb/CaO_2 relationship. Another example would be a lower leg blood flow after acclimatization, which could extend mean transit time and facilitate O_2 extraction, which would implore study of the HHb/($\dot{Q}aO_2$) relationship.

During incremental exercise HHb and $\dot{V}O_2$ both increase linearly and the slope of the relationship between HHb and $\dot{V}O_2$ can be interpreted as an indirect index of local muscle O_2 extraction as a function of work rate (24). Beyond 70% $\dot{V}O_2p$, HHb increase is blunted but $\dot{V}O_2$ keeps increasing which is attributed to limitations in muscle O_2 extraction (25–27).

The aim of this work was to characterize the HHb - $\dot{V}O_2$ relationship during incremental cycling exercise to exhaustion near sea level (SL), in acute (ALT1) and during chronic (ALT16) exposure to hypoxia at 5,260 m. The hypotheses were: first, muscle O_2 extraction approaches a maximum in normoxia and acute hypoxia, as reflected by blunting of the HHb - $\dot{V}O_2$ slope. Second, muscle O_2 extraction does not approach a maximum in chronic hypoxia, as reflected by no blunting of the HHb - $\dot{V}O_2$ slope, due to normalization of CaO_2 and associated increase in muscle O_2 supply. NIRS measurements being scarce in chronic hypoxia, the present study would bring an important new piece of information on continuously, albeit indirectly, measured muscle O_2 extraction during graded exercise to exhaustion.

Methods

Participants Recruitment and Screening

This study was conducted as part of the AltitudeOmics project. Twenty-one young, healthy, sea-level residents [age: 20.8 years, range: 19–23 years], were recruited near sea level in the region of Eugene, Oregon, USA (130 m). Physical examinations and the U.S. Army Physical Fitness Test (APFT, push-ups, sit-ups, and a 3.2-km run) (28) were performed to characterize health and fitness status. Exclusion criteria included: being born at >1,500 m; having travelled to altitudes >1,000 m in the past three months (including air travel); using prescription medications; smoking; being pregnant or lactating; having a history of serious head injury (loss of consciousness); self or familial history of migraine; known hematologic or cardiovascular abnormality (e.g., sickle cell trait, cardiac arrhythmia); pulmonary function or diffusion capacity for carbon monoxide <90% of predicted; or failure to meet the minimal age/gender standards for the APFT (28). With the exception of CaO_2, watts at maximal exercise and V̇O2p, there is no overlap in the data presented (29).

Ethical Approval

The study was approved by the institutional review boards of the University of Colorado and the University of Oregon and by the Human Research Protection Office of the US Department of Defense and was performed according to the Declaration of Helsinki. The participants were informed about procedures of this study, and written consent was given prior to participation.

Experimental Design

Familiarization with the experimental procedures included an incremental exercise test up to exhaustion ($\dot{V}O_2p$ test) to assess the aerobic fitness of the participants and ensure that the inclusion criteria were met. After familiarization, the participants underwent experimental trials near sea level (SL) (130 m; barometric pressure ~749 mmHg) and two times at high altitude (5,260 m, Mt. Chacaltaya, Bolivia; barometric pressures ~409 - 410 mmHg, on the 1st and 16th day at high altitude, ALT1 and ALT16, respectively). For each participant, all ALT measurements were carried out around the same time of day to minimize any confounding effects of their circadian rhythm. During ascent (from 1,525 to 5,260 m) to Mt. Chacaltaya, the participants breathed supplemental oxygen (2 L/min, nasal cannula, or mask). Administration of O_2 was ceased 2 hours before the ALT1 measurements started. This ensured both standardized acute exposure at ALT1 and minimized influence of acute mountain sickness (AMS) during ALT1 exercise. On days 2 to 4 of altitude exposure, the participants lived La Paz, Bolivia (3,800 m; average barometric pressure ~487 mmHg) to continue acclimatizing at a lower altitude. On Day 4 the participants visited 5,260 m for four to six hours. On Day 5, they returned to 5,260 m, where they lived for an additional 13 days. No symptoms of AMS were observed at ALT16 due to acclimatization.

Experimental Protocol

Before entering the exercise room, the participants laid down in a room dedicated to the insertion of an arterial catheter (20–22 gauge) into a radial artery. After ~30 min of instrumentation, the participants underwent a resting protocol described elsewhere (29). The participants then moved on to the exercise protocol described hereafter. Participants were seated on an electrically braked cycle ergometer (Velotron Elite, Racermate, Seattle, WA, USA). The protocol begun with a 3-min resting baseline in pedalling position on the ergometer, with the right pedal down supporting the relaxed leg. The participants then completed four 3-min stages at 70, 100, 130 and 160 watts, followed by 15 watts/min increments until they could no longer maintain pedalling > 50 rpm despite strong verbal encouragement. No specific pedalling frequency was required of the participants. This protocol was developed to accommodate other research questions (30). Maximal power output

(watts) was calculated as: work rate of last stage completed + [(work rate increment) × (time into final stage/duration of stage in seconds)] (31).

Measurements

Arterial blood gas

Blood samples (2 ml) were taken during the resting baseline, at the end of each of the four three-min stages and immediately before the cessation of exercise (BL, 70W, 100W, 130W, 160W and MAX). All samples were analysed immediately for arterial PO_2 (PaO_2), (Rapidlab 248; Siemens Healthcare Diagnostics, Munich, Germany), haemoglobin concentration ([Hb]), and O_2 saturation (SaO_2) (Radiometer OSM3; Radiometer Medical ApS, Copenhagen, Denmark). CaO_2 (mlO_2/dl) was calculated as: CaO_2 = 1.39 mlO_2/g Hb × [Hb] g/dl × (SaO_2/100) + (PaO_2 mmHg × 0.003 mlO_2/ml/mmHg). Core body temperature was telemetrically recorded from an ingested pill (CorTemp; HQInc, Palmetto, FL) to correct the blood gas values to measured body temperature. Additionally, pulsed arterial O_2 saturation (SpO_2) was measured at the forehead by pulse oximetry (Nellcor N-200, Mansfield, MA, USA).

Cardiac output

Continuous blood pressure was measured using the arterial catheter connected to a pressure transducer put at heart level. Beat-to-beat cardiac output ($\dot{Q}c$) was estimated with pulse contour analysis of the blood pressure trace (32). The combination of arterial blood gas and cardiac output allowed us to compute oxygen delivery ($\dot{Q}aO_2$) by multiplying $\dot{Q}c$ by CaO_2.

Metabolic variables

Throughout the exercise protocol, the participants breathed through a mouthpiece connected to a pitot tube, which in turn was attached to a two-way, non-rebreathing valve (Hans-Rudolph 2700, Hans-Rudolph, Shawnee, KS, USA). Ventilation was measured using a turbine spirometer (Universal Ventilation Meter; Vacu·Med, Ventura, CA, USA) and corrected to BTPS. Expired fractions of O_2 and CO_2 were measured using a pair of fast-responding gas analysers (O_2Cap Oxygen analyser; Oxigraf, Mountain View, CA, USA) sampling from a port in the mouthpiece and 3-L mixing chamber simultaneously. The turbine was calibrated using a 3-liter syringe (Hans-Rudolph 5530) and the gas analysers were calibrated using gas mixtures of known concentrations of O_2 prior to each testing session. $\dot{V}O_2$ was calculated from ventilation and gas concentrations using the Haldane transformation. $\dot{V}O_2p$ was defined as the maximum 30 sec moving average of $\dot{V}O_2$.

NIRS measurements

Muscle HHb was obtained with a spatially resolved, continuous wave NIRS apparatus (Oxymon MKIII, Artinis, Zetten, The Netherlands). The probe was directly attached to the skin using double-faced transparent tape and wrapped with elastic non-transparent bandage to prevent any stray light contamination and to limit any effects of differences in air temperature on skin blood flow between measurements. It was placed on the right vastus lateralis 15 cm proximal and 5 cm lateral to the superior border of the patella, using a source-detector spacing of 3.8 cm and differential path length factor (DPF) of 4.0 (33). On the first experimental session, the location of the probe was marked on the skin with indelible ink to ensure identical replacement during the following experiments. HHb is expressed as absolute changes from resting baseline of each trial. This procedure is classically used in NIRS signal analysis when no absolute value of HHb is available. Thus, HHb values reflect changes from 0 at rest to maximal exercise, thus making between-condition comparisons possible. Sample rate was set at 50Hz. Adipose tissue thickness at the site of the NIRS probe was measured using a skinfold calliper.

Data Acquisition

All analogue data were sampled and recorded at 200 Hz on a personal computer for off-line analysis (Powerlab 16/30; ADInstruments, Dunedin, New Zealand).

Characterisation of muscle O_2 extraction

Emitted light from the NIRS probe diffuses through arterial, capillary, and venous blood. However, ~70% of HHb originates from the venous side of the capillary bed (16, 22), that is immediately after O_2 has been offloaded from haemoglobin to the muscle. In this study, the slope of HHb to $\dot{V}O_2$ was used to estimate muscle O_2 extraction as a function of work rate (24).

First, HHb and $\dot{V}O_2$ signals were filtered using a third order Butterworth filter with a cut-off frequency at 1 Hz, operated in both the forward and the reverse directions to ensure for a zero-phase distortion. Then, data from the start of exercise to $\dot{V}O_2p$ was divided in 20 windows of equal duration, and the averaged values for HHb and $\dot{V}O_2$ were calculated for each window. At each time point, the averaged HHb and $\dot{V}O_2$ responses were then normalised so that 70 W equals 0%, whilst $\dot{V}O_2p$ represents 100% (Figure 1). The relationship between the normalized HHb and $\dot{V}O_2$ was fitted to two linear segments, separated by a breakpoint using a piecewise equation. The first segment went from the start of exercise to the breakpoint, and the second segment continues from the breakpoint to exhaustion as previously described (24).

$y = s1 \cdot x + b1$ for $x \leq$ break point

$y = s2 \cdot x + b2$ for $x >$ break point

where s represents the slope of the relationship and b the y-intercept. Those two parameters were estimated by a least-square linear regression (Matlab R2019b, MathWorks, Natick, MA). For each exercise test, several possibilities were fitted, i.e., from 3 points in the first relationship and the rest in the second to all points except 3 in the first relationship and 3 in the second. For each of the relationship, the errors were computed as follows:

$\text{error1} = \sum_{n=1}^{n=x} |\mathrm{HHb}(x) - s1 \cdot \dot{V}O_2(x) + b1|$ for $x \leq$ break point

$\text{error2} = \sum_{n=x}^{n=20} |\mathrm{HHb}(x) - s2 \cdot \dot{V}O_2(x) + b2|$ for $x >$ break point

The best fit was obtained by minimizing the error of the two equations using the least square method (Figure 2).

Finally, results from ALT1 and ALT16 were expressed in percentage of the SL $\dot{V}O_2p$ (Figure 3).

Exercise intensities below the break point were considered moderate whereas exercise intensities above were considered high.

NIRS - CaO_2 relationship

In chronic hypoxia a significant increase in haemoglobin blood concentration ([Hb]) is expected and has been previously reported in the AltitudeOmics population (34). NIRS signals are likely affected by changes in [Hb]. Normalizing HHb by CaO_2 takes into account both the changes in [Hb] after acclimatization and the changes in oxygen content during hypoxic exercise. Therefore, the HHb/CaO_2 ratio was used to allow comparison between SL, ALT1 and ALT16.

Data analysis and statistics

All data are reported as mean ± SD. The slopes of the relationships, the x and y coordinates of breakpoint and the maximal power output at SL, ALT1 and ALT16 were compared using a one-way

ANOVA for hypoxic effect (Matlab R2019b, MathWorks, Natick, MA, USA) with an α level of 0.05. Slopes beyond the breakpoint were tested against a zero using Student's t-test. Other parameters at SL, ALT1 and ALT16 at rest and at maximal exercise were compared using a two-way ANOVA for hypoxic and exercise effects (Matlab R2019b, MathWorks, Natick, MA, USA) with an α level of 0.05. For significant interactions between hypoxia and exercise, pairwise comparisons were performed using Tuckey's HSD post-hoc test.

Results

Environmental conditions are summarized in Table 1, which is a reproduction from a previous publication (29). As expected, at rest and during incremental exercise, CaO_2 significantly decreased from SL to ALT1, due to a decrease in PaO_2 and SaO_2. There was a significantly increased CaO_2 from ALT1 to ALT16, largely due to an increase in Hb concentration as previously described in the set of participants of the present study (34), and an increase in SaO_2, but CaO_2 remained significantly lower at rest and during exercise at ALT16 compared to SL (Table 2).

Maximal power output, $\dot{V}O_2p$, $\dot{Q}c$ and CaO_2 data are summarised in Table 2, and results were as expected, showing reduction in hypoxic conditions. $\dot{Q}c$ at maximal exercise significantly decreased by 15±5% in ALT1 compared to SL and remained stable at ALT16 compared to ALT1, similarly $\dot{V}O_2p$ significantly decreased by 29±11% in ALT1 compared to SL and remained stable at ALT16 compared to ALT1. $\dot{Q}aO_2$ (the product of $\dot{Q}c$ and CaO_2) significantly decreased in ALT1 compared to SL from 100 W to MAX, whereas no significant differences were found between SL and ALT16. $\dot{Q}aO_2$ significantly increased from ALT1 to ALT16 from 100 W to MAX. NIRS data expressed in delta µM from resting baseline are summarised in Table 3. A significant increase in HHb during ALT1 exercise, but not MAX, compared to SL was observed, whereas there was no significant difference between SL and ALT16.

Muscle O_2 extraction during moderate exercise intensities significantly increased to a comparable extent at ALT1 and ALT16 compared to SL, as indicated by the slope of the HHb - $\dot{V}O_2$ relationship before the breakpoint (Table 4 and illustrated in Figure 3). Beyond the breakpoint, muscle O_2 extraction became blunted, at SL and ALT1, as indicated by the s2 slopes being significantly lower than before the break point, whereas no such blunting in muscle O_2 extraction was found at ALT16, the slope after the breakpoint being significantly greater than at SL and ALT1 (Table 4 and illustrated in Figure 3). Additionally, at SL, the slope beyond the breakpoint was not statistically different from zero, whereas at ALT1 and ALT16 it was different from zero. Yet, at ALT1 the slope beyond the breakpoint was not statistically different from SL (Table 4).

As expected, the breakpoint was found around 70% $\dot{V}O_2p$ at SL. In values relative to SL, this position was shifted to the left in both ALT1 and ALT16 compared to SL, with no difference between ALT1 and ALT16 (Table 4 and illustrated in Figure 3). The y-intercept of the breakpoint was also reduced to a comparable extent in ALT1 and ALT16 compared to SL. In values relative to each condition, the position of the breakpoint was not significantly different between SL and ALT1 (68.8 ± 5.5% and 69.5 ± 15.3%, respectively, see supplementary material) and was significantly right shifted in ALT16 (i.e., it happened later relative to $\dot{V}O_2p$ in each condition, 73.5 ± 6.7%). The y-intercept of the breakpoint was significantly reduced to a comparable extent in ALT1 and ALT16 (83.3 ± 18.2% and 84.4 ± 13.4%, respectively) compared to SL (96.2 ± 16.7%) in values relative to each condition.

The transition between 3-minute to 1-minute stages occurred at a different time than the break point of the HHB-$\dot{V}O_2$ relationship. At sea level, only 4 participants had the stage transition occurring between 65 and 75% of their $\dot{V}O_{2p}$ (so around their HHb-$\dot{V}O_2$ breakpoint) whilst 9 participants showed a stage transition below 65% $\dot{V}O_{2p}$ and 8 other participants had it above 75% $\dot{V}O_{2p}$ (both occurring at a different time than their HHb-$\dot{V}O_2$ breakpoint). At ALT1 and ALT16 the HHb-$\dot{V}O_2$ breakpoint systematically occurred before the stage transition (ALT1: 26±12 vs. 93±9 %; ALT16 30±11 vs. 94±9% $\dot{V}O_{2p}$, respectively, all $p < 0.05$).

The HHb/CaO_2 ratio significantly increased during submaximal exercise (up to 160 W) from SL to ALT1 and then tended to go back to SL values at ALT16 (Figure 4, panel C). These changes follow those of HHb and CaO_2 (Figure 4, Panel A and B, respectively), indicating that the recovery of both parameters seem comparable in magnitude and are likely interdependent. Ultimately the $HHb/\dot{Q}aO_2$ follows the same trend (Figure 4, panel D).

299 Skinfold thickness at the site of the NIRS probes did not significantly change from SL to ALT1
300 (16.2 ± 7.9 and 15.4 ± 7.3 mm respectively), whereas it had significantly decreased to 13.9 ± 7.0 mm
301 at ALT16.

302 Supplementary figures can be seen at https://doi.org/10.6084/m9.figshare.23653812.

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Discussion

The main finding of this study was that muscle O_2 extraction, as estimated with NIRS, was elevated in ALT1 and ALT16 compared to SL during exercise intensities below the breakpoint. During exercise intensities beyond the breakpoint, muscle O_2 extraction was blunted at SL and ALT1, but not at ALT16 (Table 4 and illustrated in Figure 3). The present work gives insight into muscle oxygenation dynamics during incremental exercise in acute and chronic hypoxia using an indirect yet physiologically meaningful continuous measure of O_2 extraction in the working muscle.

Low and moderate exercise intensities

During low to moderate exercise intensities there was a greater HHb change for a given change in $\dot{V}O_2$ in both ALT1 and ALT16 compared to SL indicated by the increased slopes before the breakpoint in Table 4. This finding indicates that during exercise in hypoxia, there may be increased O_2 extraction and decreased O_2 convective transport relative to metabolic demand in the working muscles (24). This increase in muscle O_2 extraction during hypoxic exercise is in accordance with previous reports of convective O_2 supply to the working limbs, whereas muscle O_2 conductance would seem unchanged (9, 35, 36). A previous invasive study showed that fractional O_2 extraction increased at the same absolute submaximal exercise intensity in acute and chronic hypoxia compared to normoxia (10). This increased muscle O_2 extraction could, at least in part, serve as a compensatory mechanism to counteract the effects of reduced O_2 supply during exercise in hypoxia, as supported by the decrease in $\dot{Q}aO_2$ observed in acute hypoxia. However, there was no difference in submaximal muscle O_2 extraction between ALT1 and ALT16 despite improvements in CaO_2 and $\dot{Q}aO_2$ after two weeks of acclimatization (9) (Table 2). Since $\dot{Q}aO_2$ increased from ALT1 to ALT16, the lack of improvement in muscle O_2 extraction at ALT16 is unlikely due to reduced O_2 supply. Moreover, no differences were observed between SL and ALT16, even though CaO_2 during exercise at ALT16, remained significantly lower than at SL. These results are in line with the CMRC Chacaltaya 1998 findings, when supplemental O_2 was given at exhaustion, allowing the participants to reach higher exercise intensities, whilst O_2 extraction was reduced. The latter indicated that in acclimatized lowlander, muscle O_2 extraction does not seem limited by O_2 delivery, at least during whole-body exercise.

High exercise intensities

When muscle O_2 extraction becomes limiting during high intensity exercise, as indicated by a blunting of the HHb-$\dot{V}O_2$ slope, any increase in $\dot{V}O_2$ beyond the breakpoint must be due to increases in muscle blood flow (24). Thus, beyond the breakpoint, only an increase in blood flow would increase O_2 supply and mitochondrial oxidative activity. In the present study, beyond the breakpoint there was a blunting of the HHb-$\dot{V}O_2$ slopes at SL and ALT1 (slopes not different and SL slope not different from zero), whereas no such blunting of the slope was observed at ALT16 (greater slope, Table 4), meaning that O_2 extraction slowed down at SL and ALT1 beyond the breakpoint but did less so at ALT16. This finding alludes to the presence of a muscle O_2 extraction reserve at high exercise intensities following acclimatization to high altitude (i.e., some O_2 is remaining in the effluent blood of the capillaries under the NIRS probe). Since $\dot{V}O2p$ remained similar between ATL1 and ALT16 whilst $\dot{Q}aO_2$ at ALT16 had returned towards SL values, there are two possibilities: i) muscle O_2 extraction reserve was not fully utilized at ALT16; or ii) the mechanisms limiting $\dot{V}O2p$ differ between ALT1 and ALT16. In support of these hypotheses, there is direct evidence that muscle oxidative capacity may have been limited at ALT16 in the present set of participants. Metabolomic, proteomic profiling, mitochondrial respirometry and blood gas analyses were integrated to comprehensively define the physiological responses of skeletal muscle energy metabolism. Results indicate that the mitochondrial respiratory capacity of skeletal muscle is preserved at ALT16 (14), suggesting an enhancement of muscle bioenergetics in chronic physiological hypoxia (37, 38). Mitochondria play a central role in the adaptive responses by supporting greater resting muscle phosphorylation

potential and enhancing the efficiency of fatty acid oxidation. At first sight this seemed contradictory with the findings of the present study, but this greater phosphorylation potential directs glucose toward pentose phosphate and one-carbon metabolism pathways that support cytosolic redox balance and purine nucleotide homeostasis (14). Muscle accumulation of free amino acids from protein catabolism appears to be a primary driver of this response by coordinating cytosolic and mitochondrial pathways to rid the cell of ammonia (14). This generates an anaplerotic imbalance that may be initially adaptive but could ultimately limit muscle oxidative capacity in vivo independent of oxygen availability (14).

In addition to muscle adaptations, previous work showed reduced muscle fatigue during exercise above ~4,500m, indicating early onset of motor drive inhibition (5, 6, 39–42). In chronic hypoxia this is partially corrected, as demonstrated by Goodall et al. (43) who reported reduced indices of supraspinal fatigue following acclimatization to high altitude (ALT16) in the present set of participants. It thus seems that increased O_2 supply in chronic hypoxia increased corticospinal excitability. "Thus, increased O_2 supply in chronic hypoxia appears to have increased corticospinal excitability. Yet, the increased excitability did not result in sufficient muscle activation requiring the use of all the available O_2, leaving an O_2 extraction reserve at the cessation of maximum exercise."In other conditions, such as one-leg exercise, this functional reserve could be used (44).

HHb to CaO_2 ratio

The fact that the HHb/CaO_2 ratio increases during exercise at ALT1 and tends to go back toward SL values at ALT16 strengthens the hypothesis that at ALT1, when O_2 supply was the most affected, one compensatory mechanism was to increase O_2 extraction during submaximal exercise; whereas at ALT16, when there was some recovery of O_2 supply, O_2 extraction partially returned towards SL values. In support, previous work demonstrated muscle adaptation to chronic hypoxia exposure, independent of other adaptations, which resulted in better contractility during and after repeated contractions (45).

Limitations

We used the relationship between HHb and $\dot{V}O_2$ as a surrogate measure of muscle O_2 extraction during exercise in humans. It is a non-invasive indirect measurement performed by another research group and with findings comparable with the present normoxic values (24).

The relationship between muscle O_2 extraction and $\dot{V}O_2p$ has been investigated for nearly a century (46) and remains matter of debate (47) since multiple factors along the O_2 transport system interact and contribute to limit $\dot{V}O_2p$. The present study is not intended to revisit this debate but rather brings a new important piece of information using an investigational technique scarcely used during altitude acclimatization. As previously suggested, oxygen diffusion at the muscle level contributes to limiting $\dot{V}O_2p$ at SL and ALT1, but likely does less so in ALT16, as indicated by the absence of a blunting of the HHb - $\dot{V}O_2$ relationship, whilst accompanied by normalization of $\dot{Q}aO_2$ values.

Normalisation of $\dot{Q}aO_2$ during high altitude acclimatization coincides with increases in [Hb], decreases in both subcutaneous fat thickness and skin blood flow (from decreased ambient temperature, Table 1). Angiogenesis could be another confounding factor but no angiogenesis was reported after height weeks of acclimatization at 4,100 m (48). HHb changes relative to the baseline in each condition were used, which minimizes the effects mentioned above. In addition the present results are consistent with previous catheter studies under similar experimental conditions, which also suggested that O_2 extraction was not full in chronic hypoxia (13) and with previous studies on the present set of participants (34, 43). Yet, future studies with more invasive methods are required to validate the information provided by NIRS data.

The AltitudeOmics project had several goals (29), and the present exercise protocol, which included stages of 3 minutes followed by stages of 1 minute, was a compromise to meet the needs for all. This special exercise design may have influenced the HHb-$\dot{V}O_2$ relationship due to their respective kinetics for the 3-minute and 1-minute stages. Yet, as stated in the result section, the stage transition (from 3-minute to 1-minute) occurred at a different time than the HHb-$\dot{V}O_2$ breakpoint in all conditions, likely underlying the fact that the HHb-$\dot{V}O_2$ breakpoints relies on a physiological mechanism rather than the exercise design. In addition, the values we found at sea level match previously reported values by another research group using another exercise design (24), suggesting a limited effect of the exercise design on the HHb-$\dot{V}O_2$ relationship.

Men and women were pooled in the present study which may be a confounding factor as the HHb-$\dot{V}O_2$ relationship may differ between sex.

Conclusions

The present study demonstrated increased muscle O_2 extraction during submaximal exercise during acute exposure to 5,260m, which may be attributable to decreased $\dot{Q}aO_2$. The presence of a muscle O_2 extraction reserve during chronic exposure is coherent with previous studies on the same set of participants (14, 43) and others (40, 49) indicating both limited muscle oxidative capacity and decrease in motor drive during chronic exposure to 5,260 m. Therefore, for the flow conditions and level of capillarization, the O_2 extraction reached is the maximal achievable, yet there is O_2 remaining in the effluent blood on the venous side. Future studies should include local blood flow measurements in parallel to muscle NIRS to relate the changes in O_2 extraction to expected changes in local blood flow and O_2 supply in acute and chronic hypoxia.

Authors' contribution

Conceived and designed the experiments: ATL, AWS and RCR. Performed the experiments: NB, OE, JLF, JEE and AWS. Analysed and interpreted data: NB and BK. Wrote the first version of the manuscript and prepared the figures: NB. Revised the manuscript: AWS, JLF, BK, RCR, ATL. All authors approved final version of the manuscript.

Acknowledgments

This paper is part of a series titled "AltitudeOmics" that together represent a group of studies that explore the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations have invested enormous amounts of time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity, and tenacity of our research participants. AltitudeOmics principal investigators were C.G. Julian, A.T. Lovering, A.W. Subudhi, and R.C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, participants management and data collection, supporting industry partners, and people and organizations in Bolivia that made AltitudeOmics possible is available in the first paper in this series (29). The authors are extremely grateful to J. Kern, J.E. Elliot, S.S. Laurie, and K.M. Beasley for their invaluable assistance in the blood gas data collection for this study.

Supplementary figure

10.6084/m9.figshare.23653812

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Figure Legends

Figure 1: Typical raw signals of deoxyhaemoglobin (HHb) top panel and oxygen consumption ($\dot{V}O_2$) bottom panel from rest to exhaustion during the incremental protocol in participant #1 in acute hypoxia (ALT1).

Figure 2: Example of fitted data using the double-linear method by Spencer et al. (24). Same data as in Figure 1 i.e., participant #1 in acute hypoxia (ALT1).

Figure 3: Mean fit for all participants using the double-linear method by Spencer et al. (24). Plain line: sea level (SL), dashed line: acute hypoxia (ALT1), dotted line: chronic hypoxia (ALT16). The breakpoints are located by the vertical and horizontal thin dotted lines in each condition. Sea Level $\dot{V}O_2p$ (%). SL: n = 21; ALT1: n = 16, ALT16: n = 21.

Figure 4: Panel A: deoxyhaemoglobin (HHb), Panel B: arterial oxygen content (CaO_2), Panel C: oxygen delivery ($\dot{Q}aO_2$), and Panel D: HHb over $\dot{Q}aO_2$ ratio as a function of $\dot{V}O_2$, at sea level (SL, plain line), in acute hypoxia (ALT1, dashed line), and chronic hypoxia (ALT16, dotted line). All values are normalized with respect to SL maximal exercise. All values mean ± SD. SL: n = 21; ALT1: n = 16, ALT16: n = 21.

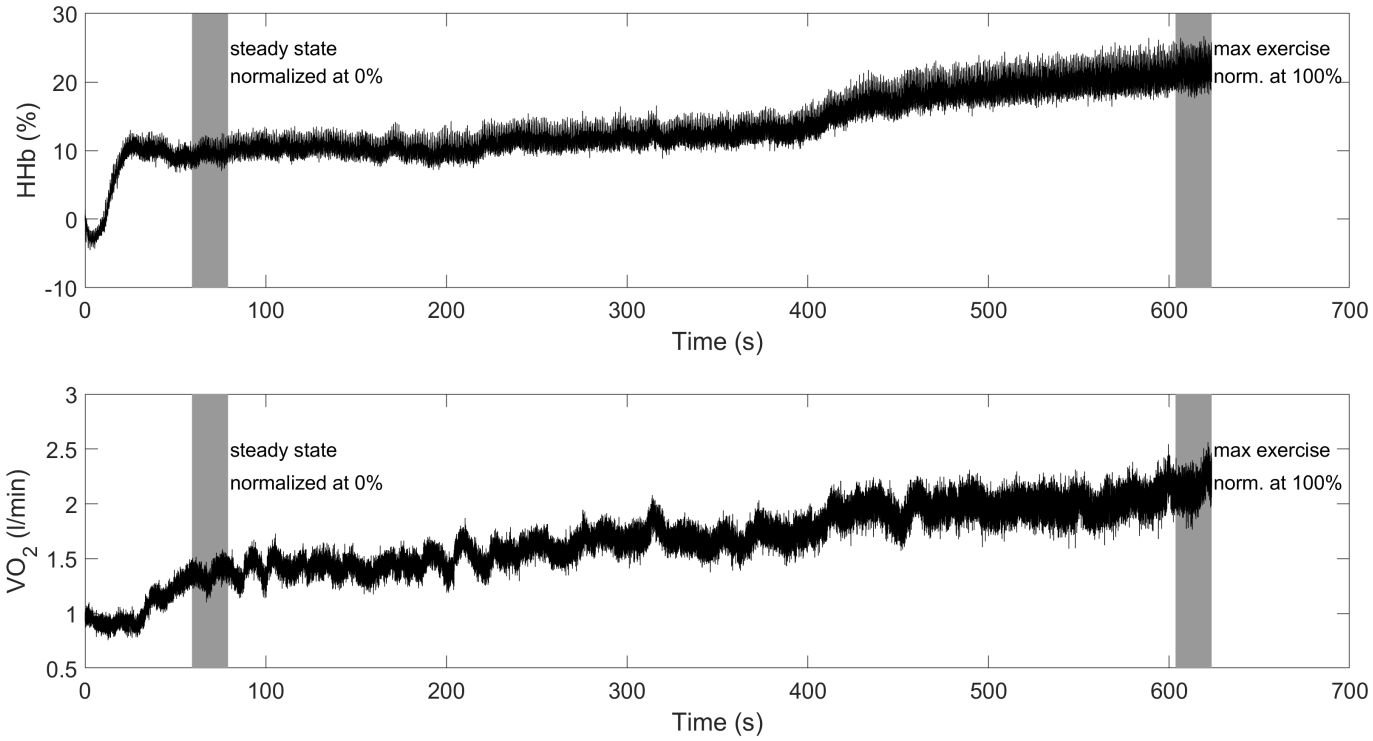


Figure 2

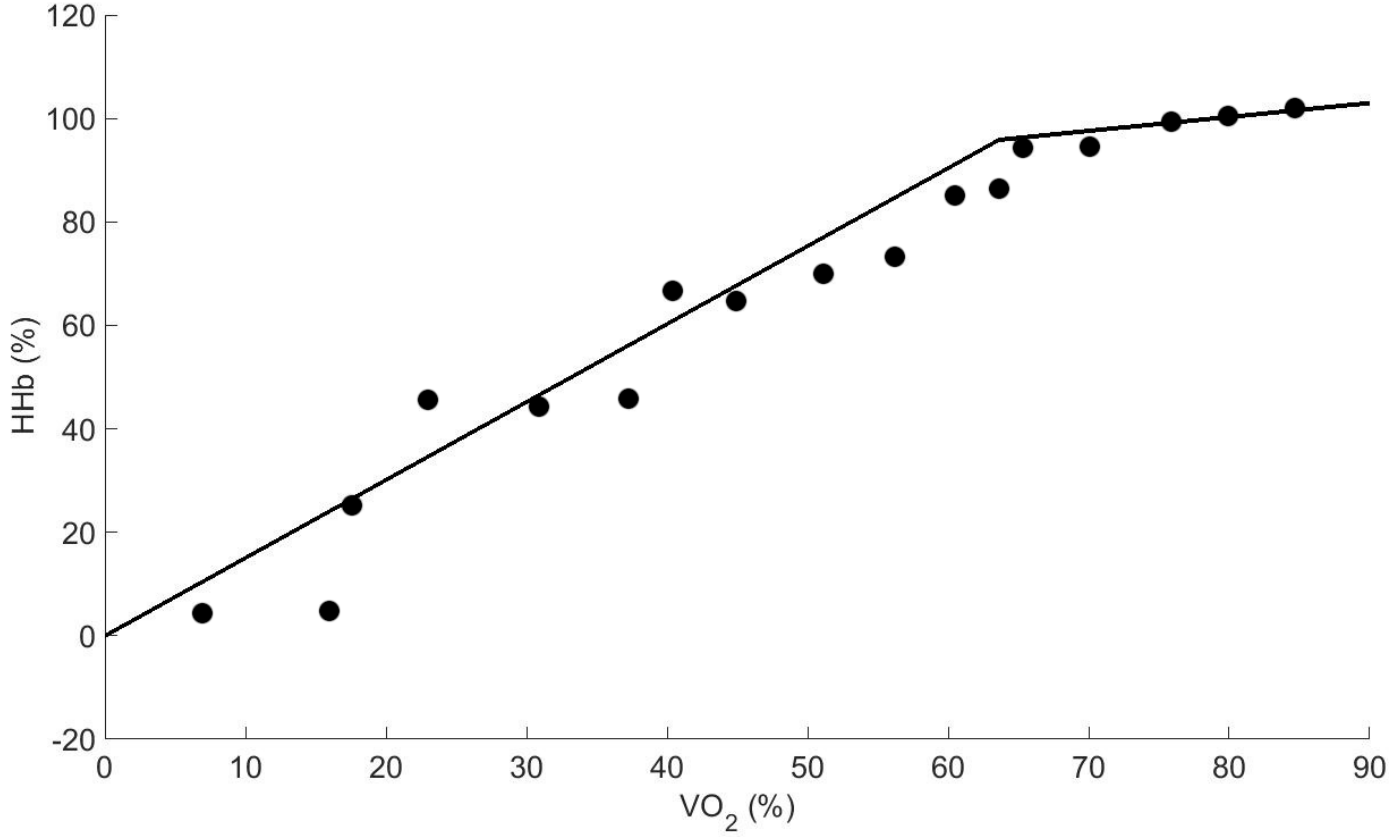


Figure 3

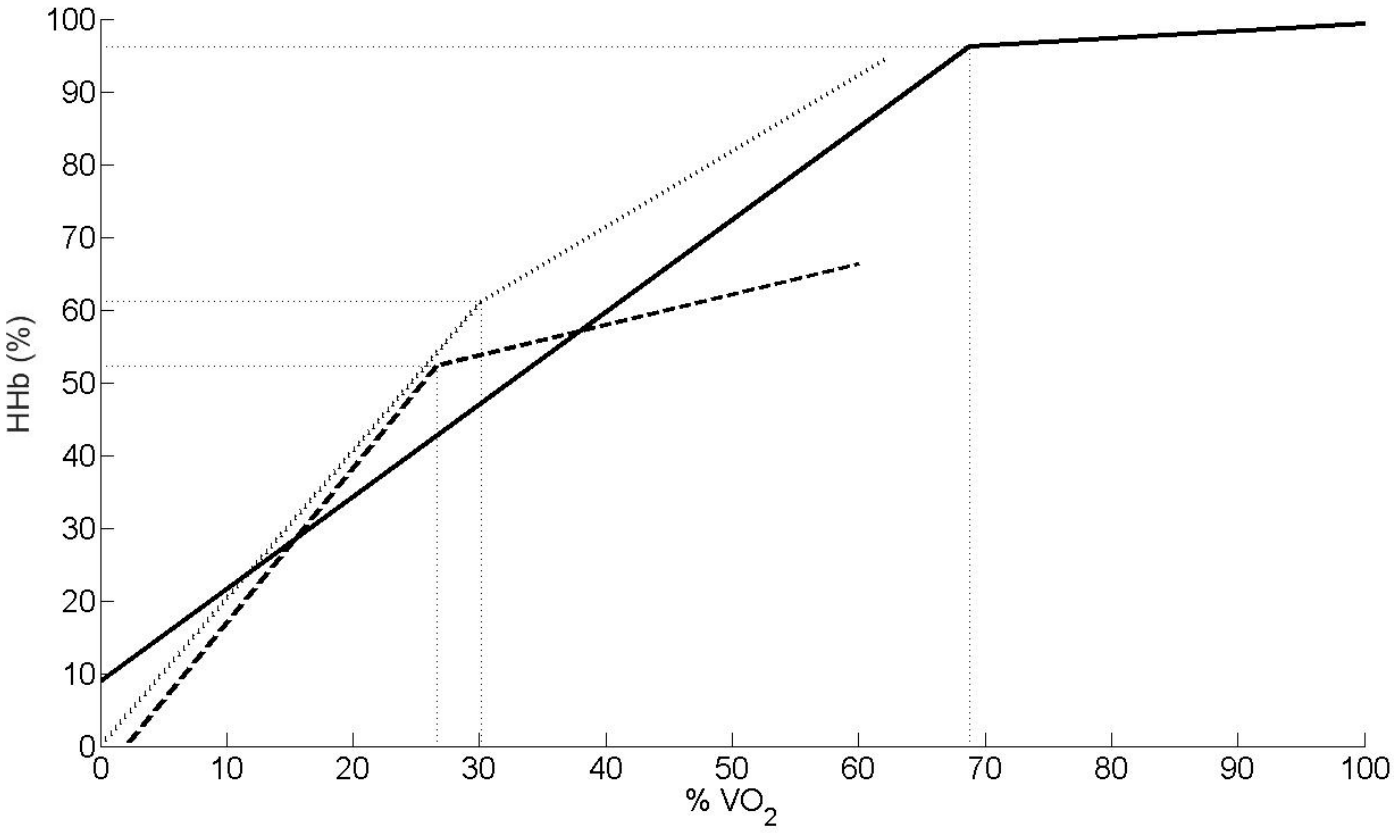


FIGURE 4

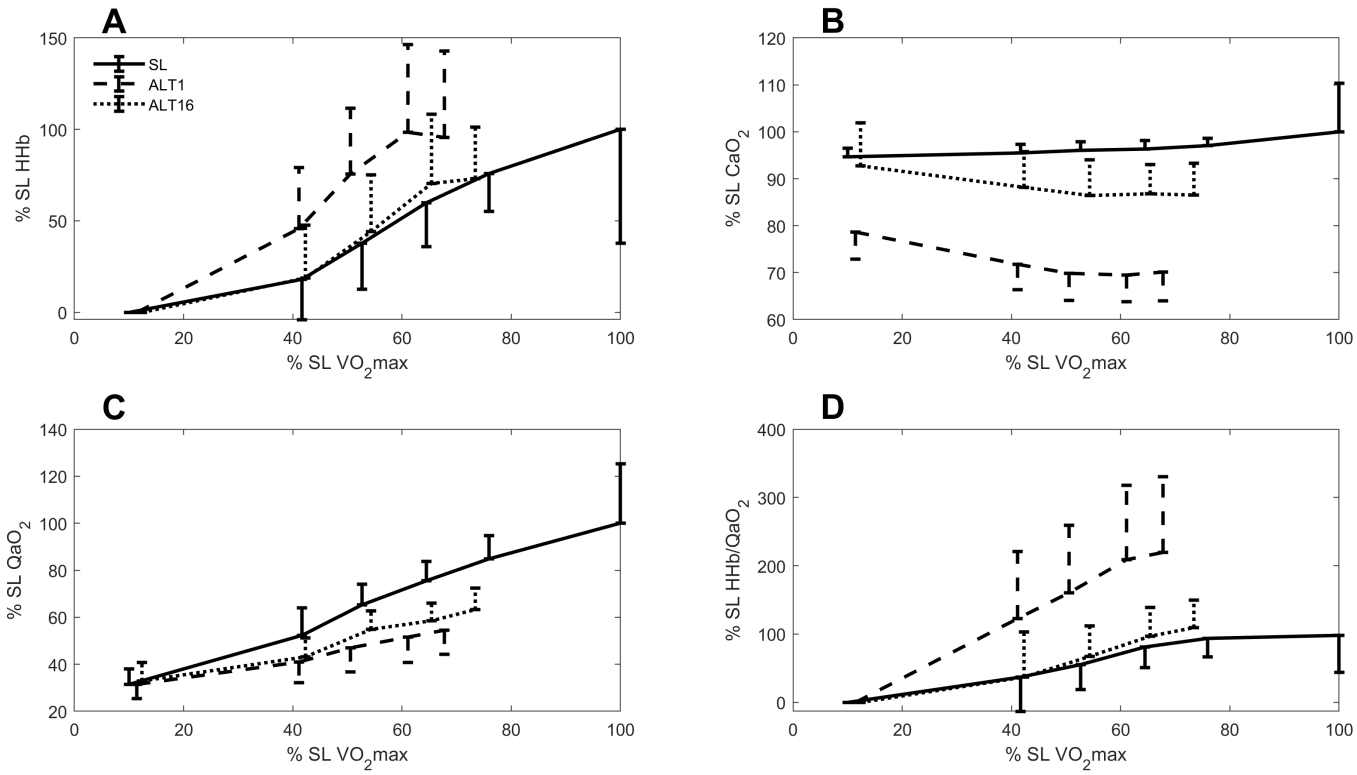


Table 1

	SL	ALT1	ALT16
Barometric pressure (mm Hg)	749.4 ± 2.4	409.1 ± 0.8 *	410.4 ± 1.0 *
Temperature (C)	22.8 ± 0.4	12.6 ± 2.2 *	19.1 ± 2.4 *†
Humidity (%)	39.2 ± 7.5	19.8 ± 7.5 *	14.2 ± 7.7 *†

All values are mean ± SD. * different from normoxia ($p < 0.05$), † different from ATL1 ($p < 0.05$).

Table 2

	SL	ALT1	ALT16
$\dot{V}O_2$ (mlO_2/min/kg)			
Rest	4.6 ± 0.9	5.0 ± 1.5	5.8 ± 1.5
70W	18.9 ± 2.5	18.0 ± 2.2	19.4 ± 2.7
100W	23.8 ± 2.9	22.0 ± 2.8	25.0 ± 3.6
130W	29.0 ± 4.1	26.1 ± 2.8	30.3 ± 3.5
160W	34.5 ± 5.1	29.5 ± 3.2	35.1 ± 4.1
Max	48.1 ± 8.8	30.7 ± 5.6 *	36.2 ± 6.2 *
$\dot{V}O_2$ (mlO_2/min)			
Rest	317 ± 74	361 ± 124	386 ± 99
70W	1270 ± 186	1277 ± 130	1281 ± 155
100W	1601 ± 108	1567 ± 187	1648 ± 174
130W	1951 ± 127	1917 ± 185	2051 ± 165
160W	2325 ± 182	2305 ± 222	2459 ± 279
Max	3345 ± 848	2239 ± 630 *	2427 ± 564 *
Power output (W)			
Max	258 ± 68	167 ± 43 *	173 ± 48 *
[Hb] (g/l)			
Rest	141 ± 14	143 ± 13	161 ± 21 *†
70W	143 ± 14	145 ± 13	164 ± 20 *†
100W	144 ± 14	146 ± 13	166 ± 20 *†
130W	145 ± 13	151 ± 13	172 ± 19 *†
160W	147 ± 13	156 ± 12	180 ± 14 *†
Max	154 ± 15	151 ± 14	173 ± 20 *†
PaO_2 (mmHg)			
Rest	102.2 ± 6.9	39.5 ± 4.2 *	47.7 ± 5.0 *†
70W	95.4 ± 4.9	32.9 ± 3.4 *	42.4 ± 3.4 *†
100W	97.0 ± 4.8	32.5 ± 2.9 *	41.2 ± 3.3 *†
130W	96.3 ± 5.8	33.0 ± 3.2 *	40.9 ± 3.3 *†
160W	95.6 ± 7.3	33.3 ± 3.0 *	41.4 ± 4.4 *†
Max	97.2 ± 8.3	34.3 ± 2.7 *	41.7 ± 3.9 *†
SpO_2 (%)			
Rest	96.1 ± 0.6	78.2 ± 4.9 *	83.0 ± 5.1 *†
70W	95.5 ± 0.5	70.1 ± 4.6 *	77.6 ± 4.3 *†
100W	95.4 ± 0.5	67.5 ± 4.5 *	75.0 ± 4.4 *†
130W	95.1 ± 0.7	66.3 ± 5.3 *	74.4 ± 3.8 *†
160W	94.8 ± 1.1	66.8 ± 5.3 *	73.7 ± 4.2 *†
Max	93.8 ± 1.6	65.9 ± 5.8 *	71.8 ± 5.0 *†
CaO_2 (mlO_2/dl)			
Rest	19.3 ± 1.8	15.7 ± 1.5 *	18.8 ± 3.2 †
70W	19.3 ± 1.9	14.3 ± 1.7 *	17.9 ± 2.8 *†
100W	19.4 ± 1.8	13.9 ± 1.6 *	17.6 ± 2.8 *†
130W	19.5 ± 1.8	14.0 ± 1.8 *	18.0 ± 2.4 *†
160W	19.6 ± 1.8	14.6 ± 1.6 *	18.5 ± 1.9 *†
Max	20.4 ± 2.0	13.9 ± 1.7*	17.4 ± 2.2*†
$PaCO_2$ (mmHg)			
Rest	36.2 ± 4.1	27.0 ± 3.6 *	20.0 ± 3.4 *†
70W	38.3 ± 2.6	26.7 ± 2.5 *	20.5 ± 2.4 *†
100W	38.8 ± 3.2	25.3 ± 2.8 *	20.2 ± 2.2 *†
130W	38.1 ± 3.7	23.3 ± 3.3 *	18.9 ± 2.2 *†
160W	37.2 ± 4.3	22.4 ± 2.7 *	16.6 ± 3.6 *†
Max	31.7 ± 4.8	21.3 ± 3.0 *	16.1 ± 2.6 *†
$\dot{Q}c$ (l/min)			
Rest	6.4 ± 1.5	7.6 ± 1.3 *	7.2 ± 1.6 *
70W	10.7 ± 3.4	11.3 ± 2.2	10.0 ± 2.0
100W	13.3 ± 2.3	13.3 ± 1.7	12.8 ± 1.4
130W	15.2 ± 2.8	15.0 ± 2.1	14.5 ± 1.4
160W	16.7 ± 2.9	16.3 ± 2.4	15.2 ± 2.2
Max	19.6 ± 3.9	16.5 ± 3.1 *	16.0 ± 2.2 *

All values are mean ± SD. $\dot{V}O_2$: oxygen consumption, [Hb]: blood haemoglobin concentration, SpO_2: pulsed arterial oxygen saturation, CaO_2: arterial oxygen content, $PaCO_2$: arterial pressure of carbon dioxide, HR: Heart Rate, $\dot{Q}c$: cardiac output. * different from SL ($p < 0.05$), † different from ATL1 ($p < 0.05$).

Table 3

	SL			ALT1			ALT16		
	O_2Hb	HHb	THb	O_2Hb	HHb	THb	O_2Hb	HHb	THb
Rest	0	0	0	0	0	0	0	0	0
70W	-5.3 ± 4.1	2.6 ± 2.9	-2.6 ± 3.9	-6.2 ± 3.5	5.4 ± 3.8 *	-0.7 ± 3.7	-4.0 ± 3.4	3.1 ± 4.2	-0.9 ± 4.0
100W	-6.0 ± 4.5	5.0 ± 3.8	-1.0 ± 4.5	-8.1 ± 4.1	8.6 ± 5.0 *	0.5 ± 3.9	-4.9 ± 3.7	6.2 ± 4.8	1.3 ± 4.8
130W	-6.3 ± 6.4	8.1 ± 5.1	1.8 ± 9.3	-10.7 ± 5.3	11.7 ± 7.1 *	1.0 ± 4.6 *	-5.9 ± 4.2	10.2 ± 6.1	4.3 ± 5.5
160W	-8.2 ± 6.6	10.6 ± 6.0	2.4 ± 9.3	-12.9 ± 6.5	15.1 ± 9.0 *	2.2 ± 6.0	-6.9 ± 5.2	14.2 ± 8.0	7.3 ± 6.3
Max	-12.3 ± 8.3	15.4 ± 9.6	3.1 ± 11.1	-11.4 ± 6.0	13.3 ± 10.0	1.9 ± 6.4	-7.6 ± 4.7	13.5 ± 10.4	6.0 ± 8.4

All values are mean ± SD. O_2Hb: NIRS oxyhaemoglobin, HHb : NIRS deoxyhaemoglobin, THb : NIRS total Haemoglobin. * different from SL ($p < 0.05$), † different from ATL1 ($p < 0.05$).

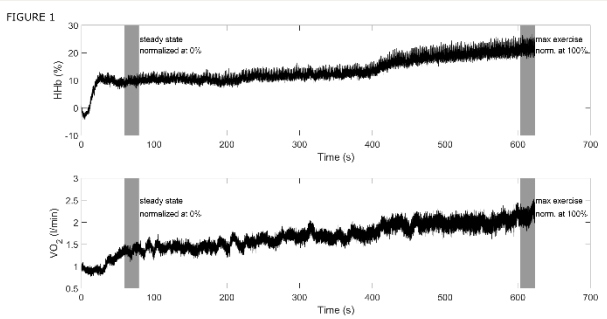
Table 4

	SL	ALT1	ALT16
s1	1.27 ± 0.12	2.13 ± 0.94 *	2.03 ± 0.88 *
s2	0.18 ± 0.44	0.42 ± 0.50	1.04 ± 0.58 *†
x (% $\dot{V}O_2$)	68.8 ± 5.5	26.6 ± 12.1 *	30.1 ± 11.5 *
y (% HHb)	96.5 ± 16.4	52.3 ± 21.7 *	61.1 ± 20.1 *

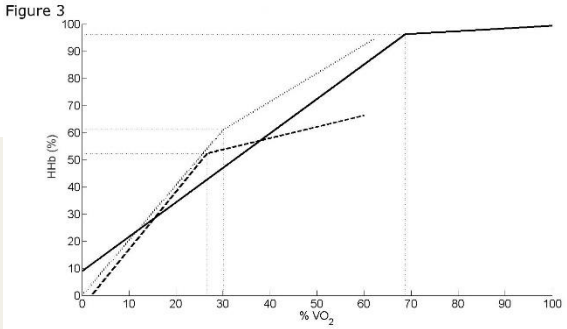
All values are mean ± SD. x: x coordinate of the breaking point, y: y coordinate of the breaking point, s1 slope before the breaking point, s2 slope beyond the breaking point. * different from normoxia ($p < 0.05$), † different from ATL1 ($p < 0.05$).

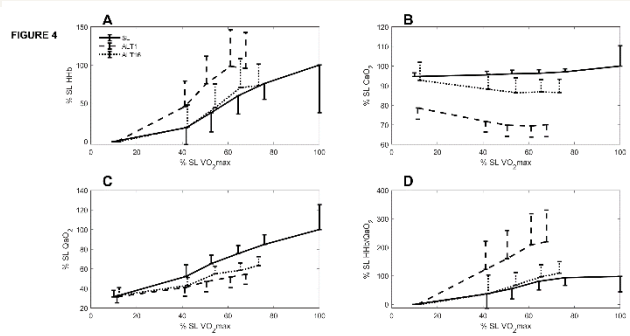
Effects of 16 days acclimatization to hypobaric hypoxia on muscle oxygen extraction during incremental exercise

METHODS



OUTCOME muscle O_2 extraction, as estimated with NIRS, was elevated in ALT1 and ALT16 compared to SL during exercise intensities below the breakpoint. During exercise intensities beyond the breakpoint, muscle O_2 extraction was blunted at SL and ALT1, but not at ALT16





CONCLUSION Increased muscle O_2 extraction during submaximal exercise was associated with decreased CaO_2 in acute hypoxia. The significantly greater muscle O_2 extraction during maximal exercise in chronic hypoxia is suggestive of an O_2 reserve.

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