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Improved $\dot{V}O_2$ uptake kinetics and shift in muscle fiber type in high-altitude trekkers

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Doria C, Toniolo L, Verratti V, Cancellara P, Pietrangelo T, Marconi V, Paoli A, Pogliaghi S, Fanò G, Reggiani C, Capelli C. Improved $\dot{V}O_2$ uptake kinetics and shift in muscle fiber type in high-altitude trekkers. *J Appl Physiol* 111: 1597–1605, 2011. First published August 25, 2011; doi:10.1152/japplphysiol.01439.2010.— The study investigated the effect of prolonged hypoxia on central [i.e., cardiovascular oxygen delivery ($\dot{Q}_aO_2$)] and peripheral (i.e., O_2 utilization) determinants of oxidative metabolism response during exercise in humans. To this aim, seven male mountaineers were examined before and immediately after the Himalayan Expedition Interamnia 8000–Manaslu 2008, lasting 43 days, among which, 23 days were above 5,000 m. The subjects showed a decrease in body weight ($P < 0.05$) and of power output during a Wingate Anaerobic test ($P < 0.05$) and an increase of thigh cross-sectional area ($P < 0.05$). Absolute maximal O_2 uptake ($\dot{V}O_{2max}$) did not change. The mean response time of $\dot{V}O_2$ kinetics at the onset of step submaximal cycling exercise was reduced significantly from 53.8 s ± 10.9 to 39.8 s ± 10.9 ($P < 0.05$), whereas that of $\dot{Q}_aO_2$ was not. Analysis of single fibers dissected from vastus lateralis biopsies revealed that the expression of slow isoforms of both heavy and light myosin subunits increased, whereas that of fast isoforms decreased. Unloaded shortening velocity of fibers was decreased significantly. In summary, independent findings converge in indicating that adaptation to chronic hypoxia brings about a fast-to-slow transition of muscle fibers, resulting in a faster activation of the mitochondrial oxidative metabolism. These results indicate that a prolonged and active sojourn in hypoxia may induce muscular ultrastructural and functional changes similar to those observed after aerobic training.

hypobaric hypoxia; oxygen consumption kinetics; single fiber mechanics

ADAPTATION TO LONG-TERM HYPOXIA entails several modifications, occurring at each step of the aerobic pathway, which affect oxygen (O_2) transport and utilization. For instance, at high altitude, the ability of the cardiovascular system to carry O_2 from the lungs to the muscles is improved by the increase of red blood cell mass brought about by the activation of the hypoxia-inducible factor–erythropoietin signaling pathways (12). It has been shown, however, that the positive effects of such improvement may be counteracted or blunted by the concomitant impairment in several muscular functions (11, 21).

Indeed, skeletal muscles change their structural and functional properties during exposure to hypobaric hypoxia, lasting >4–8 wk. At moderate altitude [4,000–5,000 m above sea level (asl)], the peripheral adaptations occurring at muscular level seem to mimic those observed after endurance training (44), and the addition of physical exercise to the hypoxic environment can be exploited to further improve aerobic performance (43). At altitudes above 6,000 m, however, a decrease of the activity of the oxidative enzymes and an increase of the activity of those implied in glycolysis have been described (22). In addition, muscle fiber atrophy occurs, mitochondrial mass is reduced (17, 28), and the muscles undergo biochemical and structural modifications reminiscent of those observed in ageing (11). Such alterations can give a substantial contribution to the reduction of the aerobic performance that has been observed during and after a prolonged stay at high altitude (10).

Muscular O_2 utilization rate during moderate intensity exercise (i.e., exercise performed below the intensity corresponding to the anaerobic threshold) is mainly controlled by peripheral cellular mechanisms rather than by bulk O_2 delivery to the muscles (7, 18). Therefore, the study of the dynamic response of oxidative metabolism at the onset of exercise may provide insightful information about the adaptation of the skeletal muscles occurring during hypobaric hypoxia.

The effects of prolonged sojourn at high altitude on pulmonary O_2 uptake ($\dot{V}O_2$) kinetics and O_2 deficit ($DefO_2$; i.e., the difference between the volume of O_2, which would have been consumed if a steady-state had been attained immediately at the beginning of the exercise, minus the O_2 volume taken up during the exercise) have been investigated only once (6). The quoted study documented a larger $DefO_2$ and a slower $\dot{V}O_2$ kinetics after a 6-wk permanence at 5,200 m asl.

In summary, hypoxia may produce positive and negative effects on skeletal muscles and on O_2 transport in blood—the trade-off between these contrasting effects still being unclear. To clarify the issue, it is necessary to investigate simultaneously the metabolic and respiratory responses to exercise and the functional and structural muscular changes in subjects exposed to a hypoxic environment for a long period.

On this ground, we sought to investigate the effect of a prolonged stay in high altitude on the dynamic response of the oxidative metabolism to the sudden imposition of exercise and to infer about the relative role of central (i.e., O_2 delivery) or peripheral (i.e., O_2 utilization) adaptations.

METHODS

Subjects

Seven sedentary, amateur male climbers (age 39.4 ± 14.9; height 1.72 m ± 0.09) of the mountaineer club "Interamnia 8000" participating in the expedition aimed to climb Mount Manaslu in the Himalaya range and volunteered to serve as subjects after being informed about the nature of the study. None of them suffered from

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cardiovascular or respiratory problems or metabolic or skeletal muscle diseases.

Experimental Design

The study included functional assessments, blood sampling, and muscular biopsies. All procedures were performed with the approval of the Ethics Committee of the Universities of Chieti-Pescara (Chieti, Italy) and Verona (Italy) and of CeRiSM (Rovereto, Italy) and were conducted in accordance with the 1964 Declaration of Helsinki. All individuals provided written, informed consent before participating in the study.

All assessments included in the experimental protocol were carried out before and after (2–5 days upon return to Kathmandu) the expedition at the laboratory of CeRiSM (Rovereto, Italy; 204 m asl) and at the Exercise Physiology Laboratory, Department of Neurosciences and Imaging, University of Chieti-Pescara (110 m asl), in strictly controlled environmental conditions (22–25°C, 55–65% relative humidity).

During the experimental sessions, anthropometric parameters, maximal voluntary contraction (MVC) of the leg extensors, maximal anaerobic power and plasma lactate concentration ($[La]_b$) in a Wingate Anaerobic test (WAnT) and $\dot{V}O_2$ during submaximal and maximal ergometric tests were measured, and blood and muscle samples were collected.

Expedition Profile

The expedition lasted 43 days (from September 8th to October 20th). After the flight from Rome to Kathmandu (1,300 m), the seven mountaineers reached Manaslu base camp at 5,000 m after 13 days of an acclimatization trek. All of the climbers spent 23 days at high altitude, and during this period, they moved to *camp 1* (5,900 m) and to *camp 2* (6,400 m) without O_2 supplementation. Due to adverse weather conditions, the summit was not reached. The climbers returned to Kathmandu with a trek of 7 days and flew back to Italy on October 20th. The total distance covered walking was ~450 km.

During the expedition, efforts were made to provide enough food to cover both the energy and the nutritional requirements of participants; yet, subjects were not following a specific or individualized dietary regimen.

Anthropometric Evaluation, Muscle Cross-Sectional Area, Muscle Power, and Strength

Body mass index, body fat, and quadriceps area. Body mass and stature were measured on the first testing day of each testing session before the incremental test (analogical scale 761 and portable stadiometer 214, Seca, Hamburg, Germany). Concurrently, subcutaneous skinfold thickness was measured sequentially, using a pincer-type caliber at six sites (biceps brachii, subscapular, iliac, thigh, and triceps surae; skinfold caliber, Holtain, Crymych, UK) for percent body fat and lean body mass calculations (15). Thigh circumference was measured by inextensible anthropometric tape at one-third of the trocanter-patella distance above the patella. Based on the thigh circumference and skinfold thickness, the muscle cross-sectional area (CSA) was calculated (1).

Isometric strength measurement. Bilateral isometric strength of the knee extensor muscles was measured during MVCs using a leg extension machine [Panatta Sport, Apiro (MC), Italy] equipped with a load cell [Globus Italia, Codognè (TV), Italy], which was calibrated before each experiment. Subjects were seated with the trunk thigh angle at 90° and the knee joint angle also at 90°. Subjects performed maximal voluntary isometric contractions of the knee extensors three times. Isometric contractions lasted for 5 s and were separated by 2-min rest intervals. The highest value of torque attained was taken as the MVC strength.

WAnT. WAnT was used to assess the maximal power (MP) on a mechanically braked cycle ergometer (Ergomedic 894E, Monark Exercise AB, Varberg, Sweden). The mechanical resistance (in kg), set equal to 7.5% of body mass, was applied after an unloaded acceleration phase, which lasted ~3 s and was preceded by a standardized warm-up of 5 min. The subjects were instructed to pedal as fast as possible, and they were verbally encouraged to maintain the highest possible pedaling rate throughout the test (30 s). After the test, subjects recovered sitting on the saddle of the cycle ergometer for 10 min. During the test, the following parameters were evaluated: *1*) MP, i.e., the highest mechanical power observed as a mean of 5-s epochs; *2*) average power (AP), corresponding to the mean power output maintained during the 30-s test (14). Before and every 2 min until the 10th min of recovery, the blood $[La]_b$ (mM) was determined using a portable blood lactate analyzer (Lactate Pro LT-1710, Arkray, Kyoto, Japan). Peak $[La]_b$ was selected as the highest value of $[La]_b$ measured in the recovery phase after the WAnT.

Maximal $\dot{V}O_2$ and Response to Submaximal Exercise

Maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$), ventilatory threshold (VT), and the response to submaximal exercise were evaluated in 2 subsequent days, both before and after the expedition. On the 1st day, the subjects performed a maximal incremental ramp test consisting of 3 min at rest and 5 min of priming exercise at 50 W, followed by a continuous increase in the workload by 20 W/min until exhaustion. The accepted criteria for maximal effort were: respiratory exchange ratio > 1.1, and heart rate $> 90\%$ of the predicted maximum based on age. On the next day, each subject performed two square-wave transitions. After 3 min at rest, the cardiac output ($\dot{Q}$) was measured by means of an inert gas rebreathing procedure. Then, after 3 min of exercise at 30 W, the workload was increased immediately to 100 W and for 7 min (i.e., a 70-W step transition was applied). The procedure was repeated after 10 min of recovery. $[La]_b$ was measured from capillary blood samples at rest, at the end of the priming phase, at the 3rd min of exercise at 100 W, and at the 1st, 3rd, and 5th min of recovery. Hemoglobin concentration ([Hb]; g 100 ml^{-1}) was assessed from arterialized capillary blood samples taken from the ear lobe at rest and at the end of the exercise.

All tests were performed on an electromechanically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands), operated by a personal computer, connected to a metabolic cart. The system allowed instantaneous step changes to predefined workloads. Breath-by-breath (B-by-B) $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) were measured continuously at the mouth (Quark b^2, Cosmed, Rome, Italy). Analyzers and respiratory flow transducer were calibrated following the manufacturer's instructions before each experimental run.

The left arm of the subjects was bent and suspended at the level of the xiphoid with a sling, while an arterial pressure profile was recorded continuously at a fingertip by using a noninvasive photoplethysmographic method (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands). Pressure values were corrected for the height difference between the heart and the fingertip.

$\dot{Q}$ at rest was measured by means of an inert gas rebreathing method (41) (Innocor, Innovision, Odense, Denmark).

$[La]_b$ was assessed by means of an electroenzymatic method (Biosen C_line, EKF Diagnostic, Barleben, Germany) on 10 μl blood samples from an earlobe; [Hb] was measured by a photometric technique (HemoCue, Ängelholm, Sweden) on 10 μl blood samples obtained from an earlobe; and percent saturation of O_2 in hemoglobin (S_aO_2) was measured by infrared oximetry (Siemens MICRO$_2$, Danvers, MA).

To determine VT, the $\dot{V}O_2$ and $\dot{V}CO_2$ B-by-B values measured during the incremental test were smoothed preliminarily by applying a three-sample moving average. The VT was then estimated individually, according to Beaver et al. (2). $\dot{V}O_{2max}$ was calculated as the average of the VO_2 values measured in the last 30 s before the end of the exercise.

Stroke volume was determined on a beat-by-beat basis by means of the Modelflow method (38), applied offline to the pulse-pressure profiles obtained from Portapres by using the BeatScope software package. As the aortic compliance used by Modelflow may substantially differ from that of a given individual, Modelflow data need to be corrected with an independent measure of $\dot{Q}$. To this aim, an individual correction factor was calculated as the ratio of inert gas rebreathing $\dot{Q}$ to the corresponding Modelflow data (38). Beat-by beat cardiovascular O_2 delivery ($\dot{Q}_aO_2$) was finally calculated as

$$\dot{Q}_aO_2 = \dot{Q} \cdot S_aO_2 \cdot [Hb] \cdot \alpha \quad (1)$$

where constant α is the physiological O_2 binding coefficient of hemoglobin (1.34 ml g^{-1}).

For the analysis of square-wave exercises, only the first 6 min of each repetition were considered. B-by-B $\dot{V}O_2$ values were interpolated to 1-s intervals (26), time aligned with the onset of exercise transition, and treated by subtracting the $\dot{V}O_2$ steady-state ($\dot{V}O_{2ss}$) value at 30 W. Then, the data from the two repetitions were combined in 5-s averages to obtain a single data file for each subject and condition. DefO_2 was calculated as the difference between the O_2, which would have been consumed if $\dot{V}O_{2ss}$ had been attained immediately at the beginning of the exercise and the O_2 volume actually taken up during the exercise. The first quantity was calculated by multiplying $\dot{V}O_{2ss}$ in ml O_2 s^{-1} by the duration of the exercise set equal to 360 s. The O_2 volume consumed during exercise was calculated by summing progressively the $\dot{V}O_2$ values expressed in ml O_2 s^{-1} from the onset of exercise to 360 s. Mean response time (MRT) of $\dot{V}O_2$ kinetics was finally calculated from the ratio of DefO_2 to the corresponding O_2 uptake above that of the free wheeling (8).

Also beat-by-beat $\dot{Q}_aO_2$ values of the two experimental series were interpolated to 1-s intervals and aligned with the onset of exercise. They were then treated by subtracting from them the $\dot{Q}_aO_2$ steady-state value at 30 W. Finally, the two series of data were overlapped and averaged (5 s) to obtain a single data file for each subject and condition. The kinetics of the net increment of $\dot{Q}_aO_2(t)$ at the onset of the exercise was described by means of a biexponential model (25)

$$\dot{Q}_aO_2(t) = \left[A_1\left(1 - e^{(t-TD_1)/\tau_1}\right)\right] + \left[A_2\left(1 - e^{(t-TD_2)/\tau_2}\right)\right] \quad (2)$$

where A_1 and A_2, τ_1 and τ_2, and TD_1 and TD_2 are the amplitudes, the time constants, and the time delays of the first and second component, respectively.

The parameters of the model were estimated by means of a weighted nonlinear least-squares procedure (9), implemented in LabVIEW 7.0 (National Instruments, Austin, TX). Initial guesses of the parameters of the model were entered after visual inspection of the data. Finally, the MRT of the $\dot{Q}_aO_2$ response to step exercise was calculated as

$$MRT = \left[A_1/(A_1 + A_2)\right](TD_1 + \tau_1) + \left[A_2/(A_1 + A_2)\right](TD_2 + \tau_2) \quad (3)$$

Muscle Biopsies

Muscle biopsies were taken using a semiautomatic needle (Precisa 13 gauge, Hospital Service, Rome, Italy) from the vastus lateralis muscle at one-third of the distance between the patella upper margin and the trochanter major after local anesthesia with Lidocaine (0.5%, w/v). In each subject, several samples were collected from the same needle insertion. Each sample (approximate weight 4 mg, corresponding to a cylinder of 0.7-mm diameter and 8-mm length) was immersed in skinning solution mixed with glycerol (50% v/v) and stored at −20° until used (30).

Single Muscle Fiber Mechanics

Muscle samples, collected during biopsy and stored at −20°C, as stated above, were transferred on the day of the experiment in a petri dish filled with ice-cold skinning solution and separated in small bundles. Single fiber segments were dissected manually from bundles under a stereomicroscope and then bathed for 1 h in skinning solution containing 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO) to ensure complete membrane solubilization. Light aluminium clips were applied at both ends of the segments.

Skinning, relaxing, preactivating, and activating solutions used for mechanical experiments with single fibers, were prepared as described previously (4), with the addition of protease inhibitors (E64 10 μM and leupeptine 40 μM).

Fiber segments were mounted in the experimental setup in a drop of relaxing solution between the force transducer (AME 801, Sensor-One Technologies, Sausalito, CA) and the electromagnetic puller (Scientific Instruments, Heidelberg, Germany), equipped with a displacement transducer (40). The signals from the force and displacement transducers were fed into a personal computer and stored in the hard disk after analog-to-digital conversion (CED 1401 plus interface, Cambridge Electronic Design, Cambridge, UK). For data storage, the recall and analysis software Spike2 (Cambridge Electronic Design) was used. All details of the setup are reported elsewhere (40).

Fiber segments were mounted in relaxing solution and after measuring length, diameters, and sarcomere length, were stretched by ~20%. They were then transferred into the preactivating solution for at least 2 min and finally, maximally activated by immersion in the activating solution (pCa 4.6). During maximal activation, isometric tension (P_0) was measured, and unloaded shortening velocity (V_0) was determined according to the slack test procedure (16). At the end of the experiment, the segment was removed from the setup and immersed in sample buffer for gel electrophoresis after removing the aluminium clips.

Gel Electrophoresis

At the end of the mechanical experiment, each fiber was classified on the basis of myosin heavy chain (MyHC) isoform separation with SDS-PAGE. To this aim, the composition in MyHC isoforms of homogenates of single muscle fibers was determined on 8% polyacrylamide slab gels after denaturation in SDS (SDS-PAGE) (37). Slabs, 18-cm wide, 16-cm high, and 1-mm thick, were used. Electrophoresis was run for 26 h at 70 V for 1.5 h and at 230 V for the remaining time. Gels were silver stained. Three bands were separated in the region of 200 kD, corresponding (in order of migration from the slowest to the fastest) to MyHC-1, MyHC-2A, and MyHC-2X. In addition, myosin light chain (MyLC) isoforms were separated on 12% polyacrylamide slab gels. A sample containing ~4 μg proteins was loaded on the gel and the electrophoresis run at 32 mA until the migration front reached the end of separating gels, which were stained with Coomassie blue. Two MyLC bands were separated in the region of 20 kD, corresponding to the fast (MyLC-2f) and slow (MyLC-2s) isoforms of regulatory MyLC. The bands were identified by Western blot using antibody against MyLC-2 (FL-172, Santa Cruz Biotechnology, Santa Cruz, CA). The relative proportions of MyHC and MyLC isoforms were determined by the measurement of the brightness area product (B.A.P.; i.e., the product of the area of the band by the average brightness subtracted local background after black-white inversion) after scanning the gels with the accuracy of 600 days postinfection.

Finally, a confirmatory analysis was performed by applying the same methods on residual muscle fiber bundles from the individual bioptic samples.

TOM20 is considered a well-established mitochondrial marker (13, 33). To determine the mitochondrial protein, TOM20 proteins from four subjects were separated by SDS-PAGE with the same protocol used for MyLC (see above in *Gel electrophoresis*) and electrotransferred onto nitrocellulose membranes. Antibodies against TOM20 (FL-145, Santa Cruz Biotechnology) were used, and immunodetection was performed with the chemiluminescence kit (ECL kit, Amersham

Biosciences, Uppsala, Sweden). Quantification was based on B.A.P. analysis, as stated above (see above in *Gel electrophoresis*) for myosin isoform densitometry. Care was taken that equal amounts of homogenate were loaded in all lanes, as checked by actin densitometry. Each determination of myosin isoforms or TOM20 was done in triplicate.

Statistical Analysis

Regarding functional and blood samples, data are shown as means with SDs, and pairwise comparisons were carried out by using Student's *t*-test for paired observations. The level of significance was set at $P < 0.05$.

Concerning the structural and functional parameters derived from muscle biopsies, average values of fiber CSA and mechanical characteristics were calculated as weighted averages (i.e., individual average value of each class of fiber was weighted by the corresponding numerousness within the subject). Cohen's d effect size was also determined (39).

Based on variances in in vivo functional parameters (e.g., the measure of $DefO_2$ in our laboratory is characterized by within-subject variability of 10%), using a power of 0.8 and α-level of 0.05, sample-size analysis for paired *t*-test (SigmaPlot version 11.0, Systat Software, Chicago, IL) indicated that the minimum number of subjects required to detect a significant difference (i.e., a 20% variation) was four. When the mechanical characteristics of isolated fibers are considered (i.e., a within-subject variability of 30%), the required sample size to detect a significant change (i.e., a 50% variation), using a power of 0.8 and α-level of 0.05, was five.

RESULTS

Variations of Anthropometric Parameters and Muscle Performance In Vivo

The anthropometric parameters of the climbers before and after the stay at altitude are shown in Table 1. Body weight ($P < 0.05$; effect size 0.23) and percent body fat ($P < 0.001$; effect size 0.72) were decreased significantly, whereas total lean body mass was unchanged. On the contrary, the CSA of thigh muscles was increased significantly ($P < 0.001$; effect size 0.75) after altitude exposure.

As shown in Table 2, MP, AP, and $[La]_b$ were significantly lower after the stay at altitude (effect size was 0.55, 0.67, and 1 for MP, AP, and $[La]_b$, respectively).

MVC did not significantly change before and after expedition. Actually, two subjects showed an increase (by +2.6% and +18.9%, respectively), whereas five subjects showed a decrease (range: 2.6–21.4%).

$\dot{V}O_{2max}$, $DefO_2$, and O_2 Delivery Response

The absolute values of $\dot{V}O_{2max}$, determined before and after expedition, were not significantly different (pre: 3.04 l·min^{-1} ± 0.56; post: 3.06 l·min^{-1} ± 0.51). On the contrary, $\dot{V}O_{2max}$ normalized for body mass was significantly higher after the expedition ($P < 0.05$; effect size 0.32; Table 2). $\dot{V}O_2$ at VT corresponded to the 59% ± 10 and to 58% ± 8 of $\dot{V}O_{2max}$ before and after exposure to hypobaric hypoxia, and it did not change significantly (pre: 22.4 ml·kg^{-1}·min^{-1} ± 5.9; post: 23.6 ml·kg^{-1}·min^{-1} ± 5.00).

Table 1. *Average ± SD values of body weight, percentage of body fat, and cross-sectional area of the thigh before and after the expedition*

Subject	Mean ± SD	
	pre	post
BW (kg)	79.3 ± 15.3	76.0* ± 12.2
BF (%)	26.3 ± 4.5	23.2† ± 4.1
CSA (cm^2)	158 ± 20	172† ± 17

BW, body weight; BF, body fat; CSA, cross-sectional area; pre, before expedition; post, after expedition. *$P < 0.05$; †$P < 0.01$.

Table 2. *Average ± SD values of maximal oxygen consumption and of maximal power output, average power output, and peak blood lactate concentration measured during the Wingate Anaerobic test before and after the expedition*

Subject	Mean ± SD	
	pre	post
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	38.8 ± 6.5	40.8* ± 6.2
MP (W·kg^{-1})	7.3 ± 1.1	6.7* ± 1.1
AP (W·kg^{-1})	5.9 ± 0.7	5.4* ± 0.8
$[La]_b$ (mM)	10.9 ± 2.3	8.6* ± 2.3

$\dot{V}O_{2max}$, maximal oxygen consumption; MP, maximal power output; AP, average power output; $[La]_b$, blood lactate concentration. *$P < 0.05$.

$\dot{V}O_{2ss}$ at 100 W amounted to 1.78 l·min^{-1} ± 0.21 and to 1.70 ± 0.14 in the control condition and upon the return from altitude, respectively, and corresponded to a net increment of O_2 uptake during the step transition from 30 to 100 W of 1.09 l·min^{-1} ± 0.10 and of 0.99 l·min^{-1} ± 0.11 (NS).

In Fig. 1, the $\dot{V}O_2$ and $\dot{Q}_aO_2$ kinetics of a typical subject, as measured after the exposure to hypobaric hypoxia, are shown. Average $DefO_2$ in the control condition amounted to 979.8 ml ± 221.8, and it was reduced significantly upon the return: 666.8 ml ± 205.2 ($P < 0.05$; effect size: 1.46). The two values corresponded to a MRT of 53.8 s ± 10.9 and of 39.8 s ± 8.8 ($P < 0.05$; effect size: 1.41).

The peak increment of $[La]_b$ amounted, on average, to 1.5 mM ± 0.7 and to 1.2 mM ± 0.6 before and after the expedition, respectively (NS).

[Hb] was 14.0 g 100 ml^{-1} ± 1.6 and 14.1 g 100 ml^{-1} ± 1.4 (NS) before and after exposure to hypoxia. S_aO_2 was not significantly different in the two occasions and amounted, on average, to 99%. Therefore, arterial concentration of O_2 was 19.8 ml 100 ml^{-1} ± 1.2 and 19.2 ml 100 ml^{-1} ± 2.0 (NS) before and after expedition, respectively.

During submaximal exercise, $\dot{Q}$ was equal to 12.9 l·min^{-1} ± 1.6 and to 12.7 l·min^{-1} ± 2.3 (NS) before and after exposure to hypoxia (NS); these two values corresponded to a $\dot{Q}_aO_2$ during exercise at steady-state of 2.3 l·min^{-1} ± 0.5 and of 2.2 l·min^{-1} ± 0.4 (NS). MRTs of the $\dot{Q}_aO_2$ kinetics turned out to be almost identical before (25.4 s ± 14.1) and after (26.4 s ± 7.1) expedition (NS).

Single Muscle Fibers, Myosin Isoforms, and Mitochondrial Content

Bioptic samples from two subjects had to be discarded for poor quality. Approximately 200 fibers from five subjects could be dissected and mechanically characterized in both pre- and postconditions.

The percent distribution of fibers based on MyHC isoform separation with SDS-PAGE is shown in Fig. 2*A*. Slow fibers increased from 34.7% to 52.2% ($P < 0.05$; effect size 1.43)

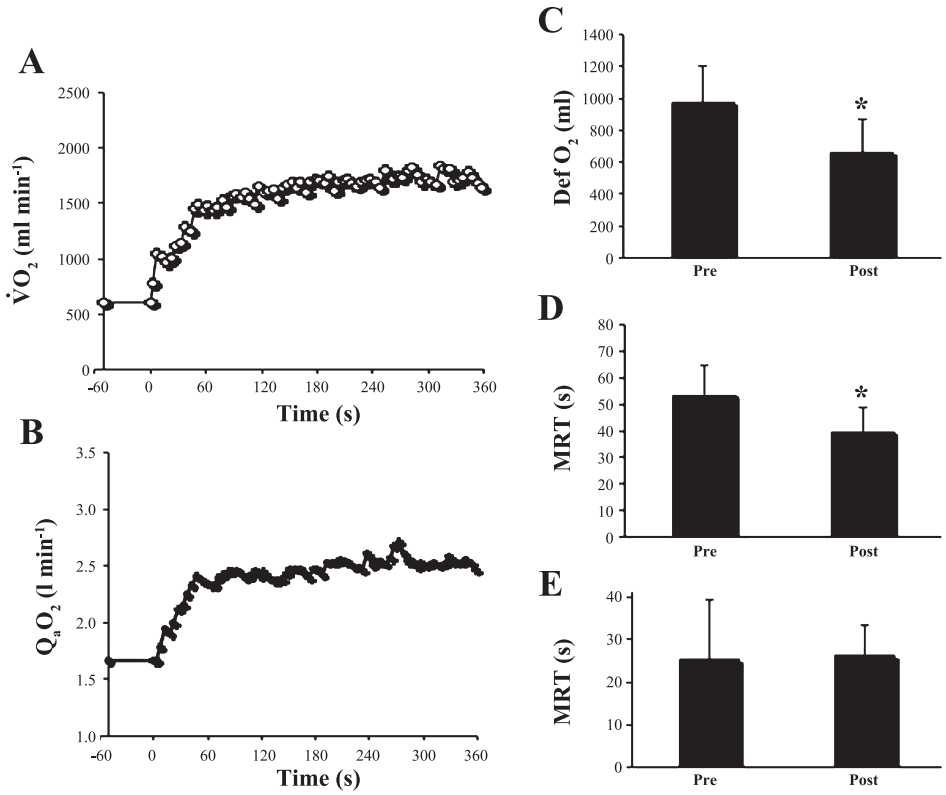

Fig. 1. Oxygen (O_2) uptake ($\dot{V}O_2$; *A*) and O_2 delivery ($\dot{Q}_aO_2$; *B*) kinetics at the onset of aerobic exercise of a typical subject assessed after exposure to hypobaric hypoxia. The average values of O_2 deficit ($DefO_2$), calculated before and after the expedition, are shown in *C*, with the corresponding mean response time (MRT; *D*) calculated from $DefO_2$ and $\dot{V}O_2$ at steady-state. $\dot{V}O_2$ on-kinetics turned out to be faster and $DefO_2$ smaller after exposure to hypobaric hypoxia, whereas MRT of the $\dot{Q}_aO_2$ on-kinetics turned out not to be significantly different after long-term hypoxia (*E*). Pre, before expedition; Post, after expedition. $*P < 0.05$.

and fast, intermediate 2A fibers decreased from 31.9% to 19.6% ($P < 0.05$; effect size 0.67), a finding compatible with the transition in fiber type.

The above results obtained in singles fibers were confirmed based on the analysis of the bundles remaining after single fiber dissection. In Fig. 2*B*, a significant and coherent transition in isoform distribution in MyHC-1 (from 40.5% to 55.5%; $P < 0.05$; effect size 6.8) and MyHC-2A (from 41.2% to 32.8%; $P < 0.05$; effect size 1.22) isoforms is shown. In addition, MyLC (or regulatory MyLC) isoform distribution was af-

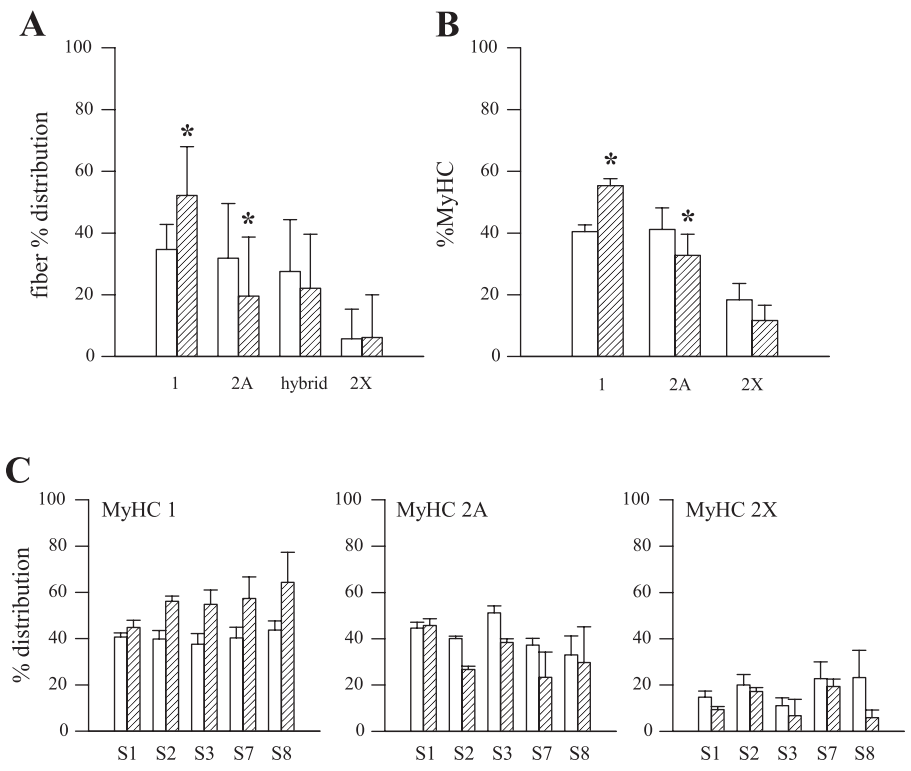

Fig. 2. *A* and *B*: percent distribution of fiber type and of myosin heavy chains isoforms (MyHC) before (open bars) and after (hatched bars) the expedition. $*P < 0.05$. *C*: individual percent distribution of MyHC isoforms MyHC-1, MyHC-2A, and MyHC-2X, observed in the 5 evaluated subjects (S1–S3, S7, and S8), before (open bars) and after (hatched bars) the expedition.

fected, since a significant increase in slow isoforms (MyLC-2s; from 48.2% to 57.4%; $P < 0.05$; effect size 0.66) and a significant decrease in the fast ones (MyLC-2f; from 51.8% to 42.7%; $P < 0.05$; effect size 0.60) occurred. Interestingly, when individual subjects were considered (Fig. 2*C*), the fast-to-slow transition is detectable in all subjects, although to a different extent.

In Fig. 3, the mean values of fiber CSA and of fiber mechanical parameters are reported. Fiber CSA (Fig. 3*B*) was greater after the stay at altitude than before (plus 17% on the average), without reaching statistical significance. Accordingly, active force (F_0), developed during maximal activation induced by calcium (Fig. 3*A*), was greater after the stay at altitude than before, but the difference did not attain the level of significance (plus 17% on the average). Thus P_0, i.e., F_0 normalized to fiber CSA, remained virtually unchanged (Fig. 3*C*). Surprisingly, unloaded V_0 was decreased significantly (1.27 L/s^{-1} to 0.39 L/s^{-1}; $P < 0.01$; effect size 3.5). Specifically, V_0 was decreased significantly both in slow fibers, expressing MyHC-1 (0.52 L/s^{-1} to 0.19 L/s^{-1}; $P < 0.05$; effect size 2.47), and in fast 2A fibers (1.38 L/s^{-1} to 0.78 L/s^{-1}; $P < 0.1$; effect size 1.15; Fig. 3*D*). The decrease in V_0 also entailed a proportional drop ($P < 0.01$; −64%) of the mechanical MP developed by the muscles, which was calculated as 10% of the product of $P_0 \times V_0$.

Finally, the residual fragments of the biopsy samples were sufficient to analyze a mitochondrial marker protein (TOM20) before and after the expedition in four subjects. The densitometric determination showed a trend to increase in the mitochondrial protein abundance with greater values in three subjects and no change in one. Moreover, the decrease of $DefO_2$ was highly correlated with the percent increase in mitochondrial protein TOM20 [$DefO_2$ (ml) = 61 + 10 increase TOM20 (%); $r^2 = 0.97$; $n = 4$].

DISCUSSION

The balance between detrimental and positive effects of high altitude on the regulation and functions of aerobic metabolism in humans is still an open issue. Whereas there is wide agreement that moderate hypoxia (i.e., altitude below 4,000–5,000 m) can positively affect aerobic power and exercise endurance [for a recent review, see (43)], on the contrary, exposure to severe hypoxia (i.e., above 6,000 m of altitude) has detrimental effects (22). The results obtained in the present study showed that a prolonged sojourn (>3 wk) above 5,000 m was not only well tolerated but also had, per se, a positive impact on the ability to use O_2 for energy production. Importantly, such conclusion comes from independent but fully consistent lines of evidence, namely, the analysis of $\dot{V}O_2$ kinetics and the study of structural and functional characteristics of the skeletal muscle.

In particular, the dynamic response of O_2 uptake at the muscular level was faster after exposure to long-term hypobaric hypoxia, despite the unchanged kinetics of bulk O_2 transport. At the same time, a switch from fast to slow fibers in the muscles of the lower limbs was shown, together with a trend to increased mitochondrial abundance. Therefore, a prolonged stay in a hypoxic condition above 5,000 m does not necessarily imply deterioration, but can even induce beneficial adaptations of muscle oxidative metabolism.

Some aspects of the correlation between the changes in noninvasive functional indexes and the molecular and functional adaptations of muscle fibers occurring at high altitude will be discussed in the following paragraphs.

$\dot{V}O_2$ Kinetics and Muscular Adaptations

The functional adaptations undergone by the oxidative energy pathway in exercising humans were quantified by calculating $DefO_2$ at the onset of a step exercise transition.

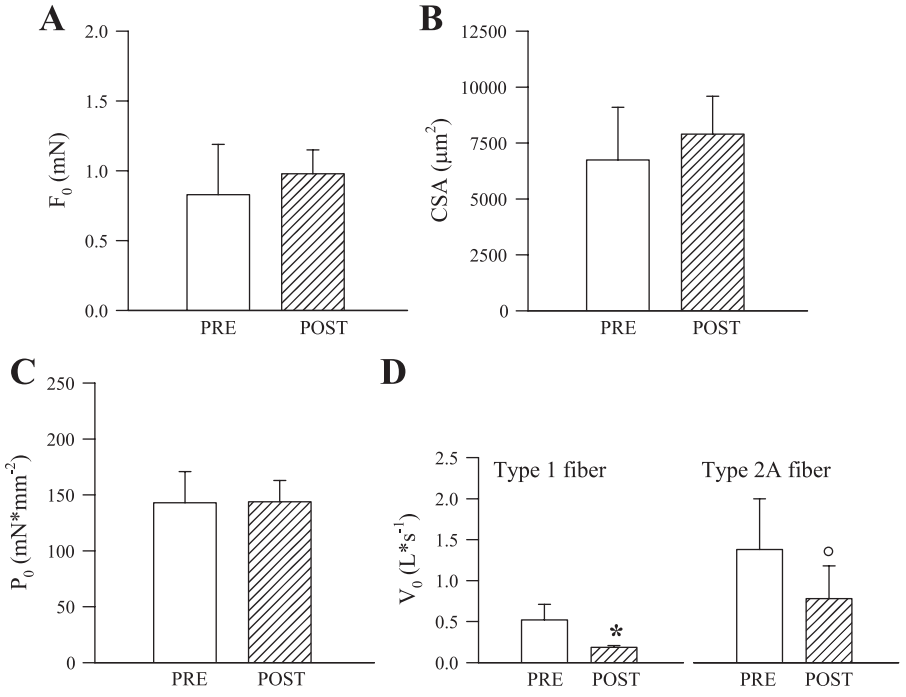

Fig. 3. Muscle fiber (weighted averages of ~200 fibers) characteristics before (open bars) and after (hatched bars) the expedition. *A*: force developed by single fibers during maximal calcium activation (F_0); *B*: cross-sectional area (CSA) of single fibers; *C*: tension (P_0), i.e., F_0 normalized over CSA; *D*: maximal shortening velocity (V_0) of type 1 and type 2A muscle fibers. $*P < 0.05$; $°P < 0.1$.

MRT—or effective time constant τ' (27)—was calculated from the ratio of Def_{O_2} to the corresponding net $\dot{V}O_{2ss}$. This approach allows circumventing the problems originated from fitting with a nonlinear exponential model B-by-B time series characterized by an inherently low sampling frequency and a low signal-to-noise ratio (as is the case when a low number of repetitions of the same exercise are available). This approach provides MRT values that are identical to those obtained by fitting the same response by a simple exponential function without time delay (8, 45).

Pulmonary $\dot{V}O_2$ kinetics is considered to be a proxy of the muscular O_2 dynamic response. Moreover, it is broadly accepted that at least during moderate exercise, $\dot{V}O_2$ kinetics is limited by the intrinsic inertia of the muscular oxidative system rather than by the local delivery of O_2 to the muscles (18, 19). As such, the modification of the O_2 dynamic response during exercise is a valuable tool to understand the effects of experimental interventions and/or environmental adaptations on the regulation of the oxidative metabolism in vivo.

For instance, it is known that even short periods of aerobic training are sufficient to induce a significant acceleration of $\dot{V}O_2$ kinetics (29). This response seems to anticipate both the increase of the maximal aerobic power induced by training and of mitochondrial protein content and mitochondrial enzyme activities (29). A theoretical model has been proposed recently (24) to explain the biochemical regulation and the training-induced adaptation of the $\dot{V}O_2$ kinetics in skeletal muscles. According to this model, the increase in mitochondria content is one of the factors inducing the acceleration of muscular $\dot{V}O_2$ kinetics and therefore, also of pulmonary $\dot{V}O_2$ response to exercise. The high correlation between the percent increase of mitochondrial TOM20 and the decrease in Def_{O_2} observed in a subgroup of subjects (four examined) is consistent with the above hypothesis.

Whereas the dynamic response of O_2 uptake at the muscular level improved after exposure to long-term hypobaric hypoxia, $\dot{Q}_aO_2$ kinetics did not change. These results seem to indicate and confirm that at least during moderate-intensity step transitions, pulmonary $\dot{V}O_2$ and muscular O_2 kinetics are not influenced directly by the dynamics of bulk O_2 delivery, but they are dictated by the intrinsic slowness of oxidative metabolism (18, 31).

We know that high-altitude hypoxia induces an increase of [Hb] and therefore, of blood O_2-carrying capacity secondary to enhanced emopoiesis. In the present study, however, no changes were detected in blood O_2-carrying capacity during the experiments performed after exposure to hypoxia. This may be due to a rapid return of [Hb] to sea level values after the end of the hypoxic stimulus, as it has been described in previous works (34). This fast recovery is mainly due to the high rate of disappearance of young and middle-aged red blood cells formed in response to the hypoxic stimulus (35).

Previous results showed that $\dot{V}O_2$ kinetics, upon the return to sea level after 6 wk at 5,200 m asl, was significantly slower than before the stay at altitude, implying that a larger Def_{O_2} was contracted at the onset of exercise (6). This finding was attributed to a greater use of the inner O_2 stores ascribed to the increase in [Hb] found after exposure to hypobaric hypoxia. In our subjects, however, [Hb] was unchanged compared with the value prevailing in the control condition. As a consequence, the inner O_2 stores of the body were unmodified, and they could not affect $\dot{V}O_2$ kinetics by any means.

The adaptation of hind-limb skeletal muscles was investigated on biopsy samples taken from vastus lateralis before and after the expedition. At variance with previous studies (20) that detected no changes in fiber-type distribution after prolonged exposure to moderate chronic hypoxia, we documented an increased proportion of slow fibers. Recent data (36) may help us reconcile the apparent discrepancy of our results with previous studies regarding fiber-type distribution changes (20). Namely, the coupling of physical exercise with hypoxia has been demonstrated to cause a larger increase in subsarcolemmal mitochondrial density of the trained muscles compared with training in a normoxic condition. The specific biochemical and structural adaptations are elicited by differential regulation of gene transcription occurring with exercise in the two different oxygenation conditions (36). Our subjects, during the approach to (and the descent from) the base camp and during their sojourn at high altitude, performed a higher amount of physical activity (i.e., an average of 30 km/day during the 14 days of trekking and an average of 1 h/day of intense physical activity during the stay at the base camp) compared with the pre-expedition lifestyle. Therefore, it cannot be excluded that physical exercise is responsible for the muscular adaptations observed in this study.

Slow fibers are characterized by a high reliance on oxidative metabolism and by a high capillary-to-fiber ratio (5). An increase in slow fiber proportion would imply an enhanced oxidative metabolism and an improved local microcirculation, which are in complete agreement with the acceleration of $\dot{V}O_2$ on-kinetics discussed above.

Anthropometrics and Muscle Performance

CSA of the lower limb significantly increased after the expedition. This finding is consistent with the tendency of the increase of fiber CSA. MVC was unchanged in full accordance with the results obtained at the single fiber level.

Further confirmation of a shift toward the slow oxidative fiber type comes from the results of the WAnT. Upon return to sea level, we observed a decline in MP and AP (approximately -10%) and in peak $[La]_b$ (approximately -20%). The drop in mechanical power is consistent with the observed shift from type 2 to type 1 muscle fibers (characterized by an intrinsically lower mechanical power) and reflects the decreased capacity of leg muscles to perform extremely powerful movements that require mainly anaerobic metabolic sources (3). It is worth observing that single fiber experiments revealed not only an increased abundance of slow fibers but also a decrease in their maximum V_0. The above findings are intriguingly similar to changes observed in slow fibers during aging (32), which are attributed to a post-translational modification caused by oxidative glycosylation of myosin (32). The contribution of the most abundant fiber types to the decrease in muscle power (power is the product of velocity by force) is fully understandable.

A reduced blood $[La]_b$ was observed at the end of the WAnT, upon return to sea level, compared with pre-expedition tests. A number of physiological phenomena may concur to fully explain such decrease: *1*) the above-mentioned reduction of power output; *2*) an increased reliance on aerobic metabolism for the production of ATP during supramaximal exercise;

3) the possible contribution of the still controversial phenomenon called "lactate paradox" (23, 42). All of the explanations are in full agreement with the independent results obtained from the analysis of the structural–functional muscle adaptations and from the $\dot{V}O_2$ kinetics.

We can therefore conclude that a prolonged stay in a hypoxic condition above 5,000 m does not necessarily imply deterioration but can even induce an improvement of the muscle oxidative metabolism. This is the main message coming from the present results. From the comparison with previous studies, it may be speculated, however, that there may be an altitude threshold, above which, the negative effects are predominant, and no compensation is possible.

In addition, these results confirm that at least during moderate intensity exercise, pulmonary $\dot{V}O_2$ and muscular O_2 kinetics are not influenced directly by the dynamics of bulk O_2 delivery, but they are dictated by the intrinsic slowness of the oxidative metabolism to adapt to changes in energy demand.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: V. V., S. P., G. F., C. R., and C. C. conception and design of research; C. D., L. T., V. V., P. C., T. P., V. M., S. P., and C. C. performed experiments; C. D., L. T., P. C., T. P., V. M., A. P., S. P., and C. R. analyzed data; A. P., S. P., G. F., C. R., and C. C. interpreted results of experiments; L. T. and S. P. prepared figures; C. D., T. P., S. P., G. F., C. R., and C. C. drafted manuscript; C. D., L. T., T. P., S. P., G. F., C. R., and C. C. edited and revised manuscript; C. C. approved final version of manuscript.

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