Molecular and cellular adaptations of skeletal muscles in seven mountaineers during the ascent of Manaslu

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Background (1)

- Skeletal muscles are composed of muscle fibres with distinct molecular and functional properties (fibre type)

- Skeletal muscle fibres can adapt to new conditions or functional demands by changing their type and/or their size

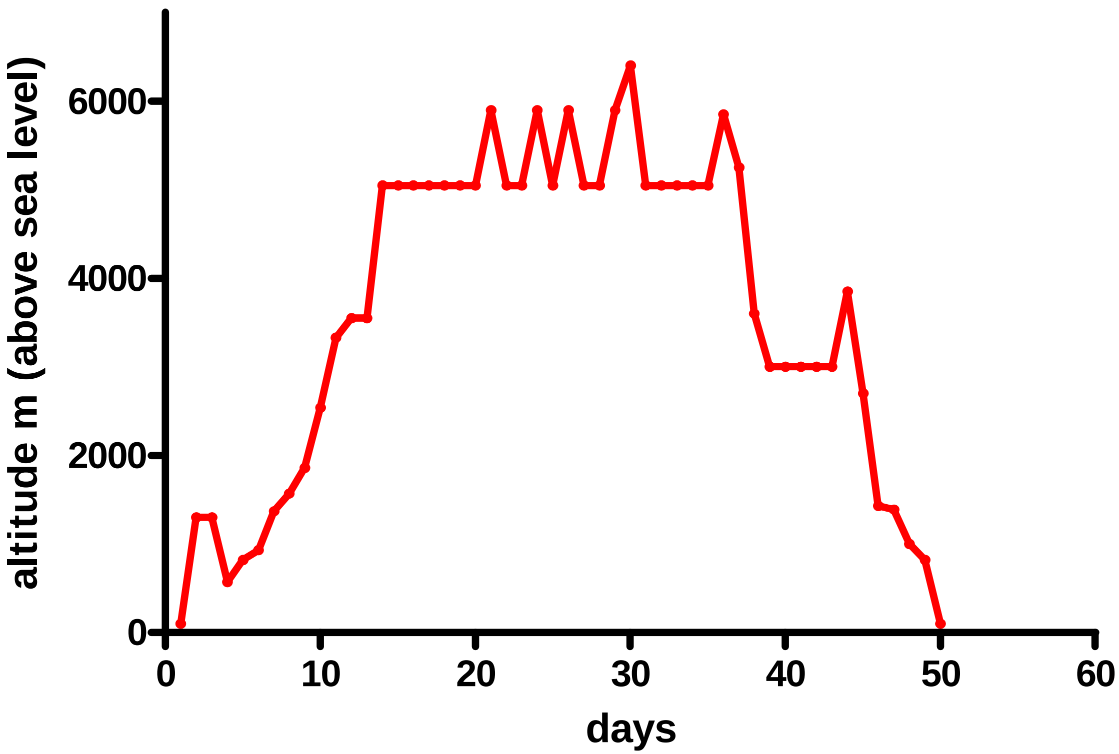
Background (2)

- “Muscle structural changes during typical mountaineering expeditions to the Himalayas were assessed on muscle biopsies. A significant reduction in muscle fiber size (-20%) and a loss of muscle oxidative capacity (-25%) were observed. The capillary network was not affected by catabolism. It is concluded that the oxygen supply to muscle mitochondria after high altitude exposure is thus improved.” (Hoppeler H, Howald H, Cerretelli P. - 1990)

- Similar results (reductions in muscle fiber size and slow-to fast transformation) in rats (Bigard et al 2000, Ishihara-Itoh 1992-2000)

Aim

- To analyse single muscle fibres before and after a long (5 week) stay above 5000 m
- To assess changes in fibre type, fibre size and fibre contractile performance

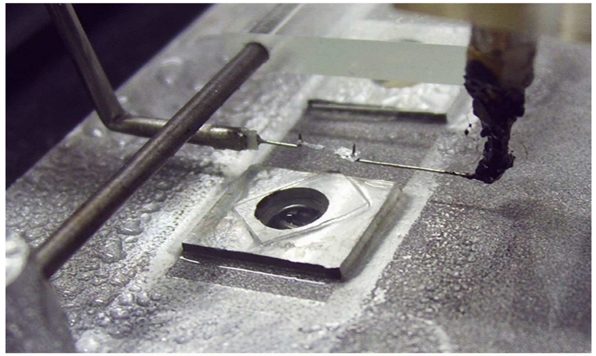


altitude m (above sea level)
6000
4000
2000
0
0
10
20
30
40
50
60
days

Methods

- Biopsy sampling before and after expedition
- Muscle fibre manual dissection
- Individual fibre characterization
  - Size or cross sectional area
  - Type (myosin isoforms as markers)
  - Contractile performance (maximal isometric tension, maximum shortening velocity)

Methods



Muscle fibre dissection

↓

Muscle fibre mounting in the set-up → Cross sectional area (FIBRE SIZE)

→ Tension, velocity (CONTRACTILE PARAMETERS)

↓

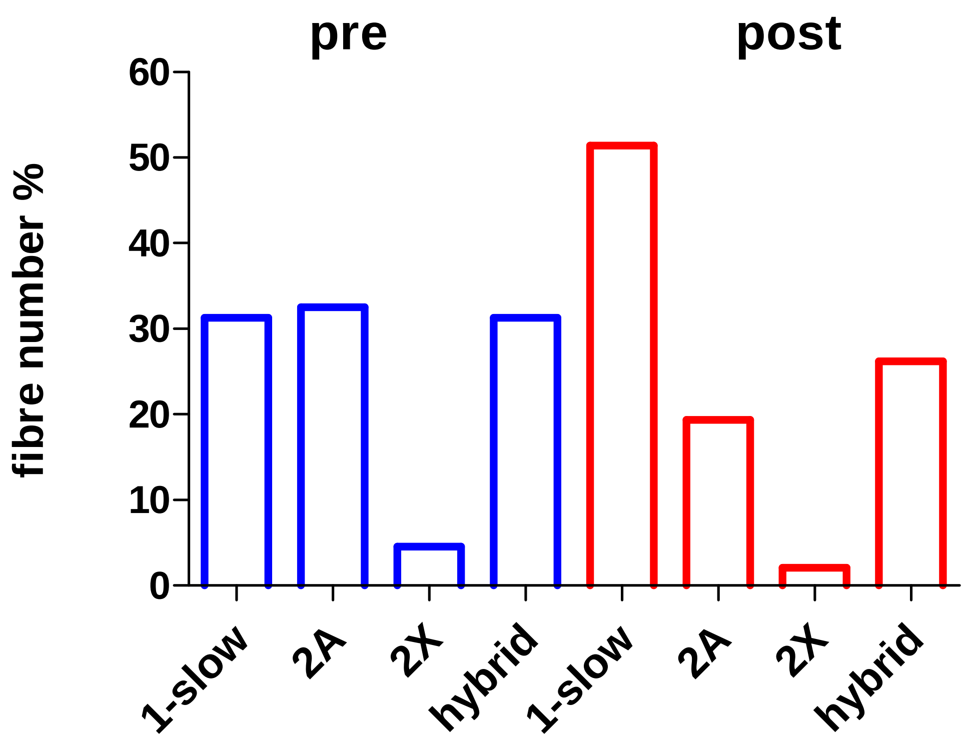
Muscle fibre lysis in sample buffer

↓

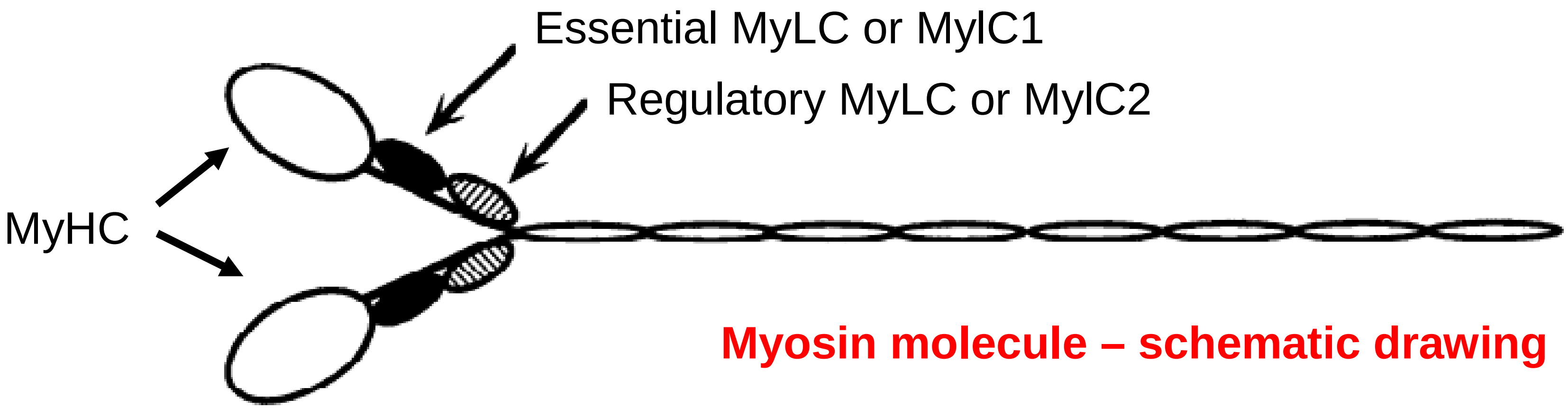
Electrophoresis and WB → Myosin isoform composition (FIBRE TYPE)

Distribution of fibres based on fibre type (myosin isoform composition)

Fast-to-slow shift in fibre type ?



> 200 individual muscle fibres were analysed from 5 subjects before and after expedition



Myosin molecule – schematic drawing

MyHC isoforms: slow or I

fast 2A

fast 2X

MyLC 2 isoforms: slow or 2s

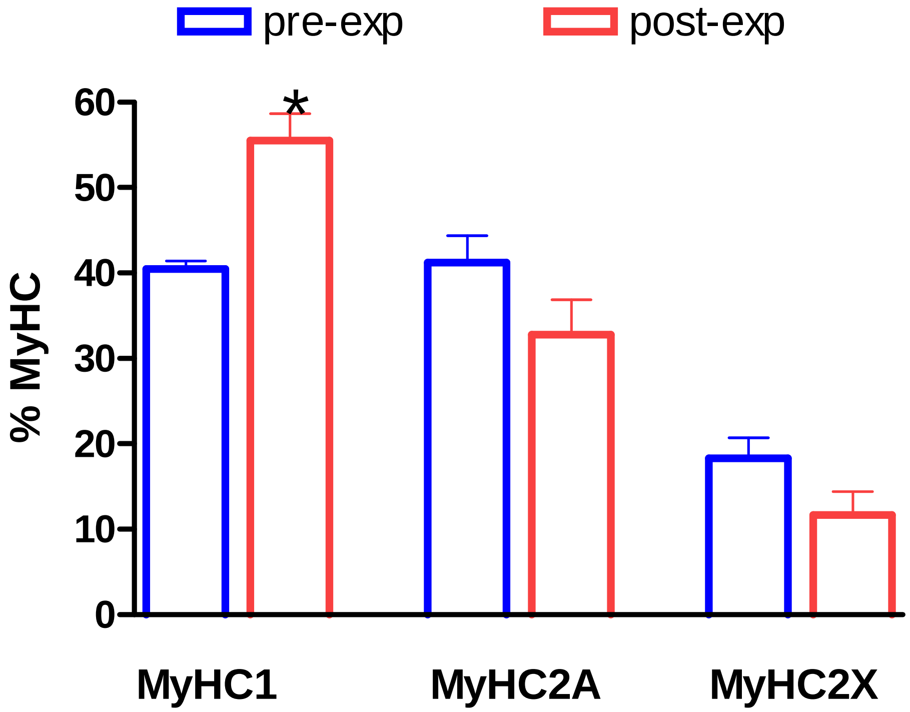
fast or 2f

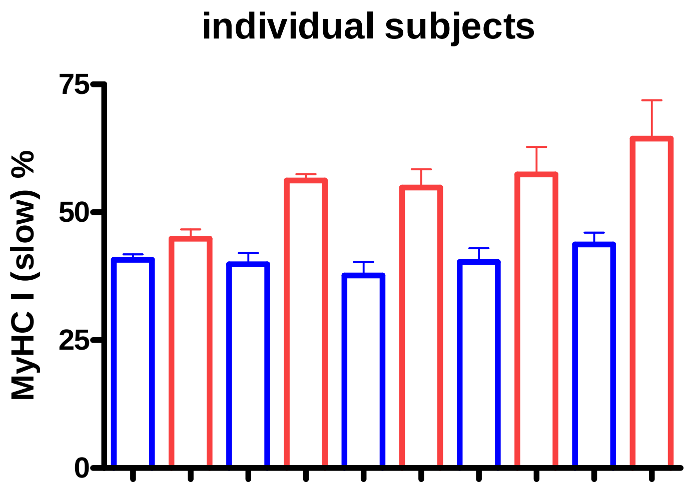
MyLC1 isoforms: slow or 1s

fast 1

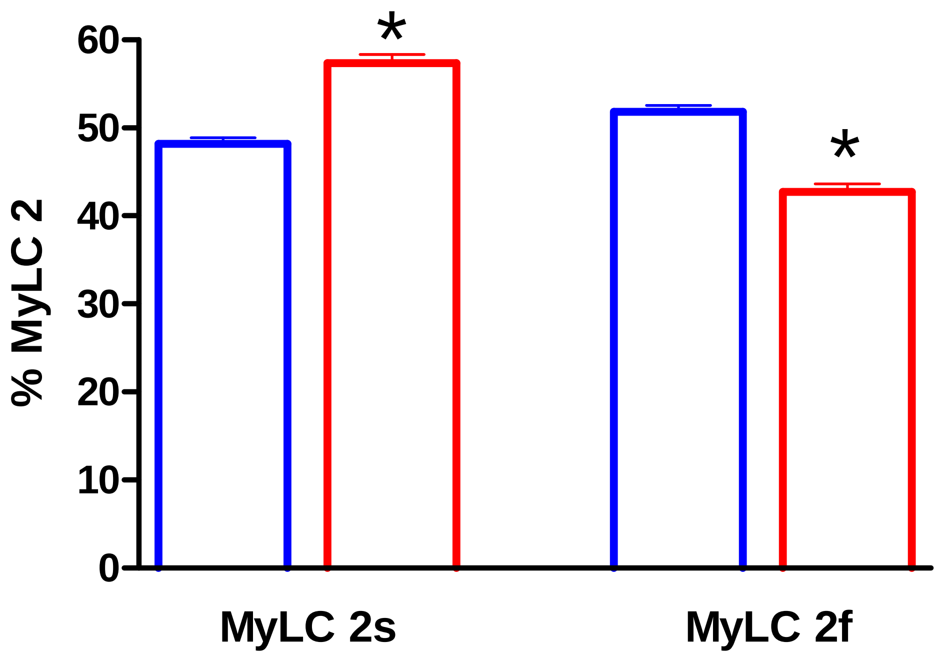
fast 3

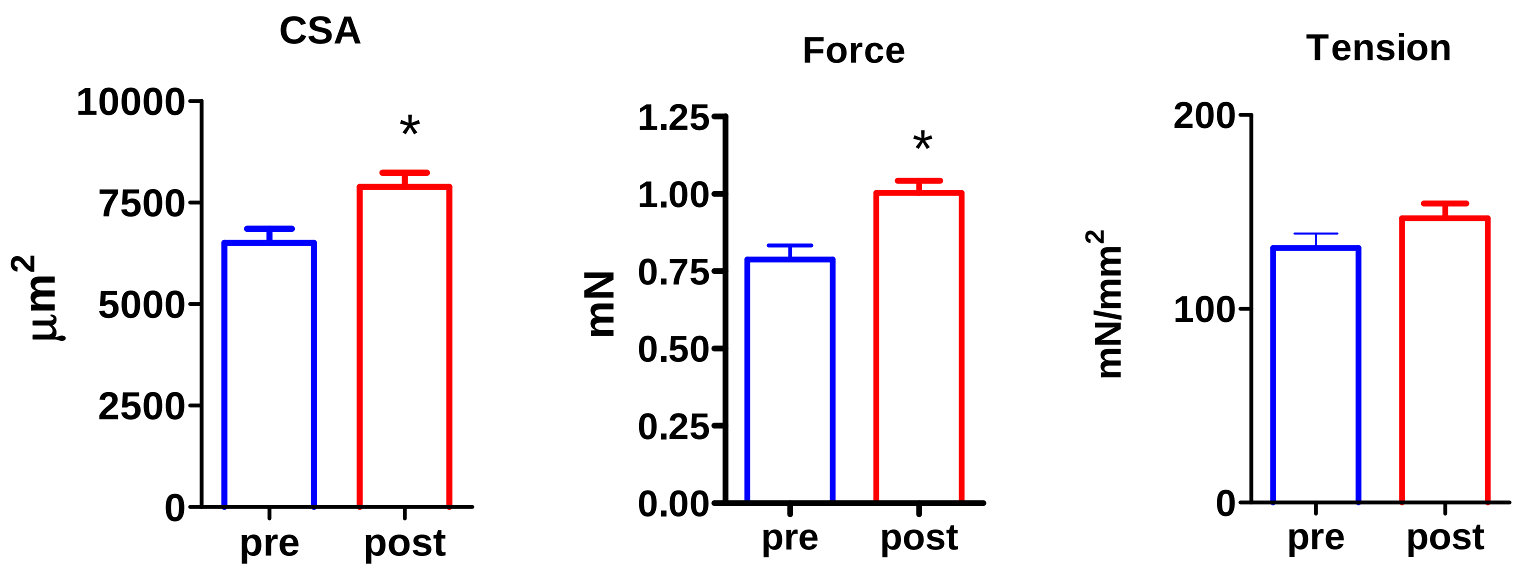
The fast-to-slow shift is confirmed by MyHC isoform quantification



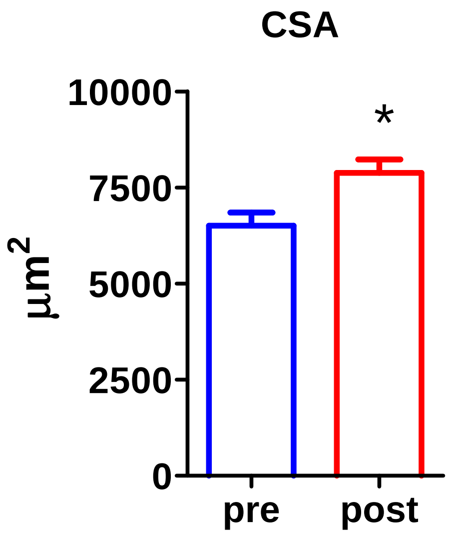


The shift fast-to-slow is confirmed by MLC2 isoform quantification





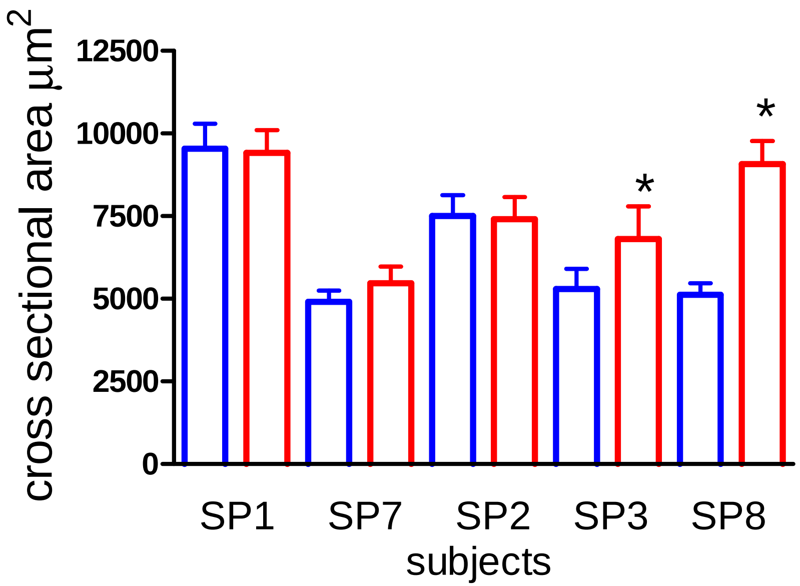
All fibres together: increase in CSA and Force, no change in Tension

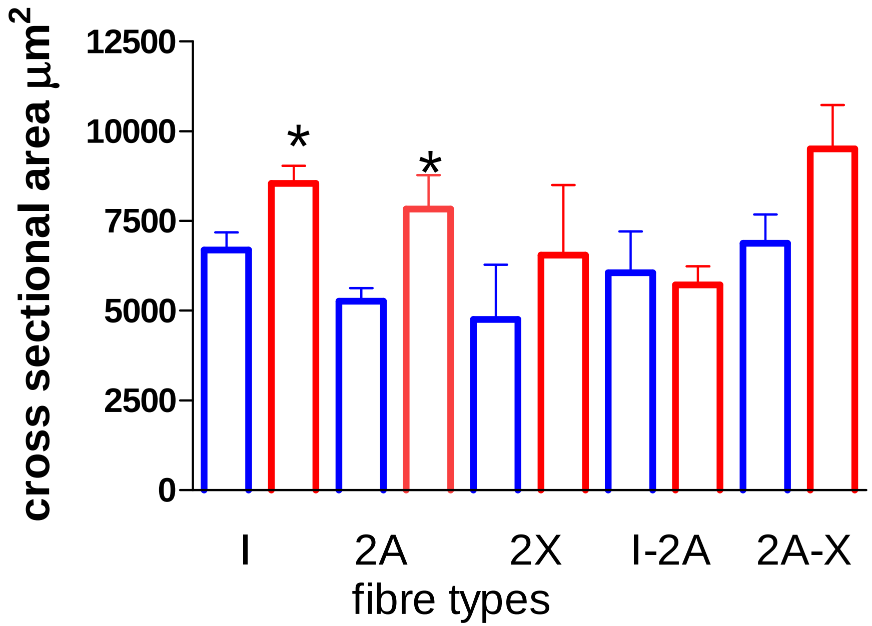


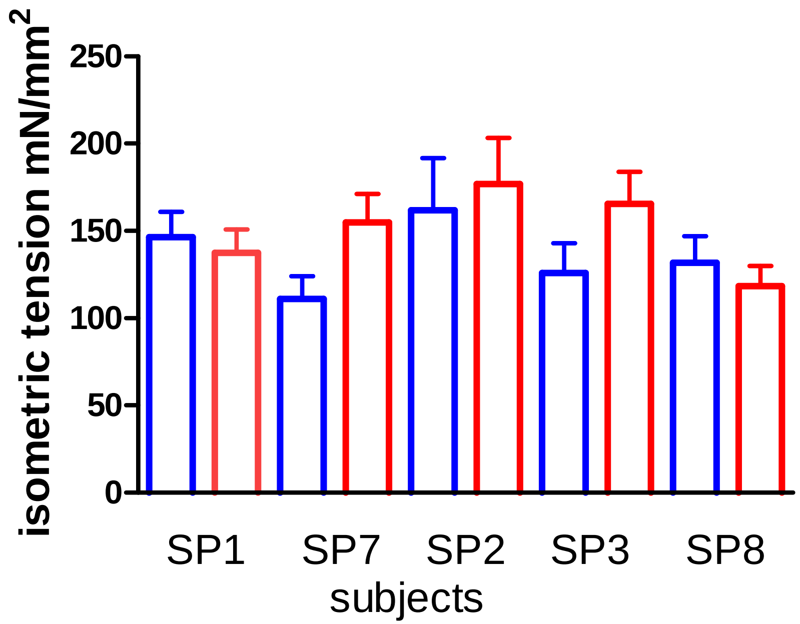
Fibre size significantly increased:

< slow and 2 fibres >

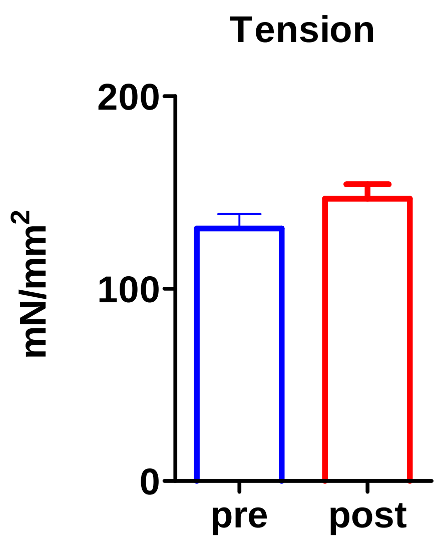
Note inter-individual diversity

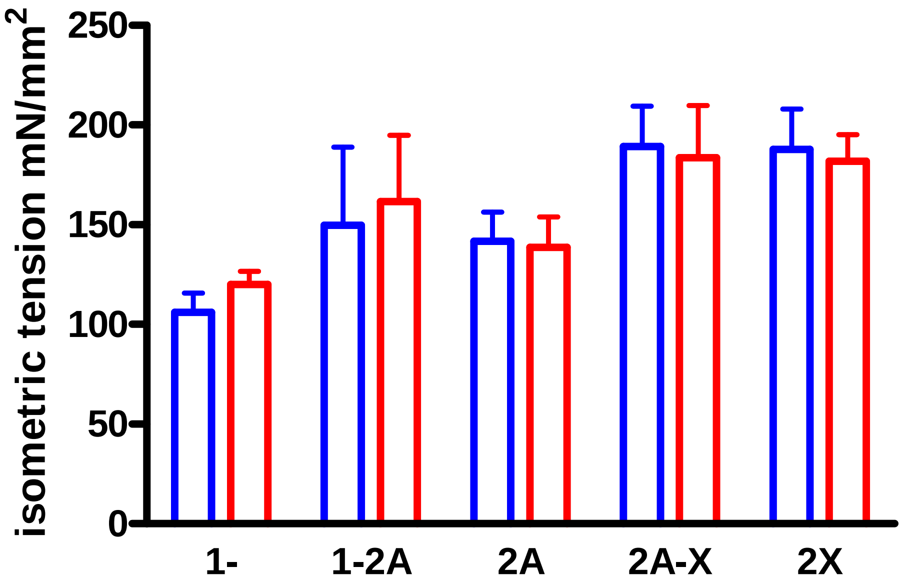


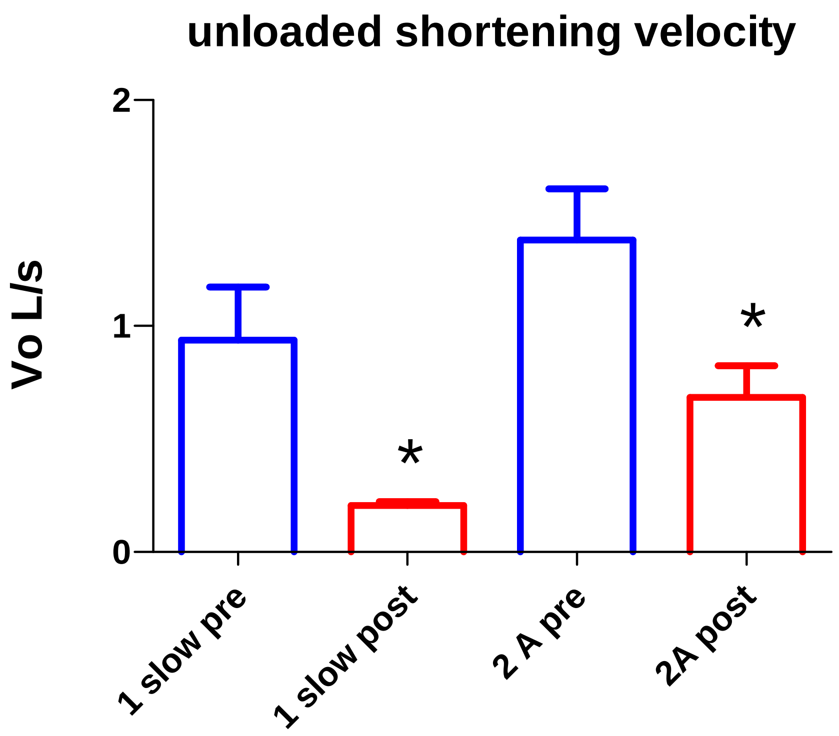




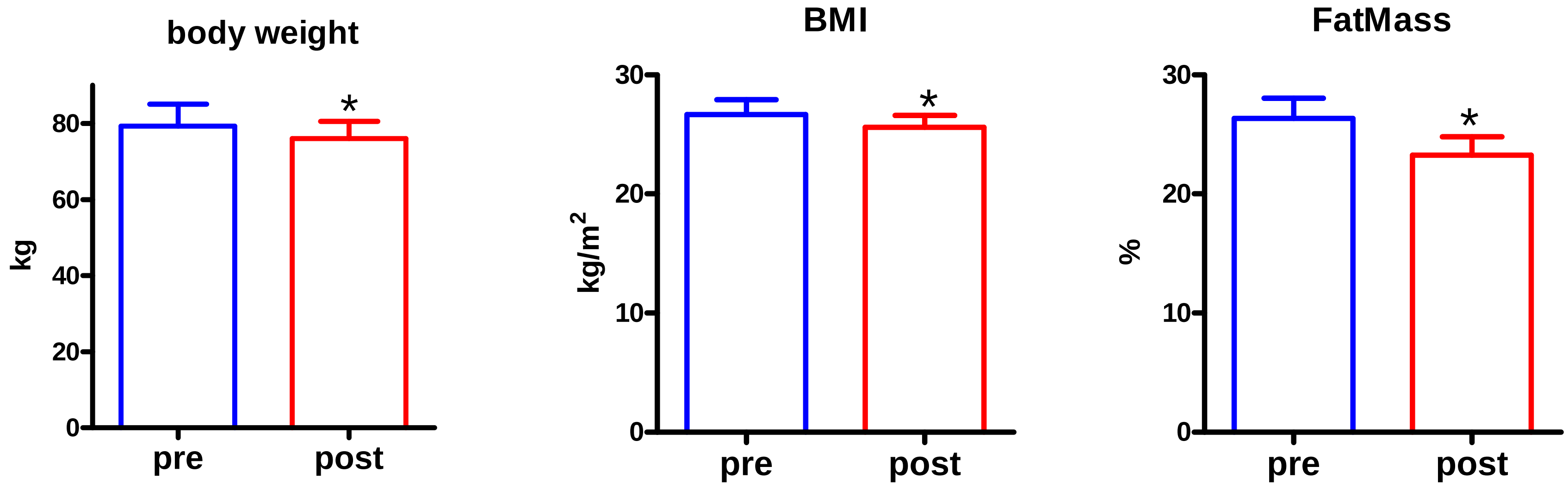
No change in isometric tension



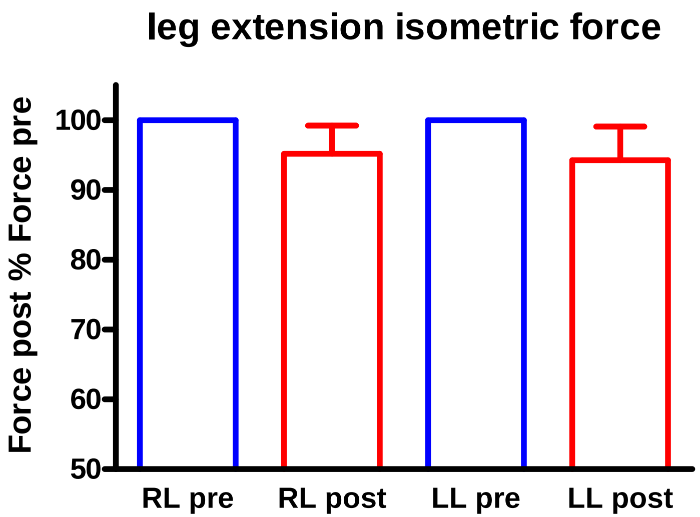




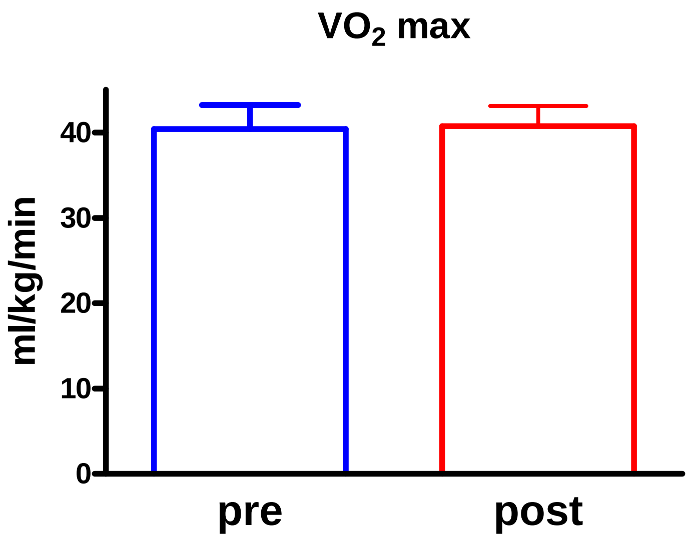
Significant reduction in unloaded shortening velocity of both slow and fast 2A fibres



body weight
kg
0
20
40
60
80
*
pre
post
BMI
kg/m2
0
10
20
30
*
pre
post
FatMass
%
0
10
20
30
*
pre
post



leg extension isometric force
Force post % Force pre
100
90
80
70
60
50
RL pre
RL post
LL pre
LL post



VO2 max
ml/kg/min
40
30
20
10
0
pre
post

Conclusions-1

- The comparison post- vs pre-expedition at single muscle fibre level shows
  - slow-to-fast transition
  - increase in fibre cross sectional area
  - preserved isometric tension (thus, increase in isometric force)
  - decrease in maximum shortening velocity

Conclusions-2

- The results obtained (preservation of mass and force, fast-to slow transition) are surprising and in contrast with data from previous studies

This is the first functional and molecular analysis on the effects of a prolonged sojour above 5000 m carried out on isolated single fibres

Acknowledgment

- Special thanks to the seven climbers and to dr. d'Amelio
- Thanks to Luana Toniolo and Lina Cancellara for myosin and single fibre analysis

- Experientia. 1990 Dec 1;46(11-12):1185-7.
- Human muscle structure after exposure to extreme altitude.
- Hoppeler H, Howald H, Cerretelli P.
- Muscle structural changes during typical mountaineering expeditions to the Himalayas were assessed on muscle biopsies. A significant reduction in muscle fiber size (-20%) and a loss of muscle oxidative capacity (-25%) were observed. The capillary network was not affected by catabolism. It is concluded that the oxygen supply to muscle mitochondria after high altitude exposure is thus improved.

Eur J Appl Physiol Occup Physiol. 1990;60(5):331-6. Hypoxia-induced fibre type transformation in rat hindlimb muscles. Histochemical and electro-mechanical changes.

Itoh K, Moritani T, Ishida K, Hirofuji C, Taguchi S, Itoh M. Laboratory of Applied Physiology, College of Liberal Arts and Sciences, Kyoto University, Japan.

Twelve male Sprague-Dawley rats (21 days old) were randomly assigned into two experimental groups: sea level control (CONT) and hypobaric hypoxia (HYPO). The HYPO rats were kept in an hypobaric chamber maintaining a simulated altitude of 4000 m (61.1 kPa). After 10 weeks of treatment, the rat hindlimb muscles [soleus (SOL) and extensor digitorum longus (EDL)] were subjected to histochemical and electro-mechanical analyses. Results indicated that compared to CONT the HYPO SOL muscle had a significantly greater relative distribution of fast-twitch-oxidative-glycolytic (FOG) fibres (28.9% SEM 2.0 vs 18.3% SEM 1.8, P less than 0.01) with a significant decrease in slow twitch oxidative fibre distribution (69.5% SEM 2.4 vs 82.9% SEM 3.1, P less than 0.01). Compared to CONT the HYPO EDL muscle also manifested a significant increase in FOG fibre distribution (51.6% SEM 0.8 vs 46.6% SEM 1.1, P less than 0.01), but this was accompanied by a significant decrease in fast twitch glucolytic fibres (44.3% SEM 0.9 vs 49.2% SEM 1.7, P less than 0.05). These histochemical fibre type transformations accompanied significant and expected changes in the electro-mechanical parameters tested in situ, e.g. maximal twitch force, maximal rate of force development, contraction time, half relaxation time, force: frequency curve, and fatigability. It was concluded that chronic hypobaric hypoxia could have a potent influence upon the phenotype expression of muscle fibres.

Ann Nutr Metab. 1996;40(6):315-24. Effect of altitude on body composition during mountaineering expeditions: interrelationships with changes in dietary habits.

Zamboni M, Armellini F, Turcato E, Robbi R, Micciolo R, Todesco T, Mandragona R, Angelini G, Bosello O. Institute of Internal Medicine, Policlinico di Borgo Roma, Italy.

Loss of body weight occurs during high mountain expeditions but whether it is due to inadequate diet or other factors is unknown. Moreover the composition of the weight loss is unclear. The aim of our study was to compare the nutritional, anthropometric and metabolic changes during a mountaineering expedition in two groups of climbers, whose dietary energy intake was ad libitum, one given a lacto-fish-ovo-vegetarian diet and one an omnivorous diet. The intake of various nutrients, body weight, body composition and metabolic variables were evaluated before and during high altitude exposure and after the return to low altitude. The two groups were matched for age, body mass index and gender. No significant differences were found for nutritional variables between the two groups. Energy, animal and vegetable protein and fiber intake were significantly lower at climbing quote than before the beginning of the expedition. Significant differences between before the beginning and base camp in all variables were found. Energy and animal protein intake, but not vegetable protein and fiber intake, were significantly lower at climbing quote than at base camp. All subjects significantly reduced body weight, body mass index, waist and hip circumferences but not fat-free mass and fat mass. Metabolic variables significantly improved after the mountaineering expedition. Our study seems to confirm that a mountaineering expedition decreases energy and protein intake, reduces body weight and improves metabolic variables. Because our subjects spontaneously tended to have the same food intake despite the different dietary recommendations, our study failed to observe any differences between the two groups. However, our study shows that a low protein diet, in which the type of protein is mostly vegetable protein, could be adapted for climbers determining only a small decrease of fat-free mass.

Int J Sports Med. 1990 Feb;11 Suppl 1:S10-4. Effect of chronic hypoxia on muscle enzyme activities. Howald H, Pette D, Simoneau JA, Uber A, Hoppeler H, Cerretelli P. Research Institute, Swiss School for Physical Education and Sports, Magglingen. Biopsies from the vastus lateralis muscle of seven participants in the Swiss expedition to Mt. Everest and Lhotse in 1986 were taken before departure to and after return from high altitude, and used for measurements of maximal activities of 12 reference enzymes of anaerobic and aerobic-oxidative metabolic pathways. The results indicated that strenuous exercise at high altitude induced increases in enzyme activities of glycolysis and decreases in enzyme activities of terminal substrate oxidation (the citric acid cycle, fatty acid oxidation, ketone body utilization, respiratory chain). The decreases in enzyme activities of aerobic-oxidative metabolism were related to similar decrements in mitochondrial volume density, which suggests that the enzymic changes resulted from a loss of mitochondrial structure rather than from qualitative changes of the mitochondrial population. These changes indicated that strenuous exercise may intensify the stress of high-altitude exposure and, thus, induce an aerobic to anaerobic shift of muscle energy metabolism.

Int J Sports Med. 1990 Feb;11 Suppl 1:S3-9. Morphological adaptations of human skeletal muscle to chronic hypoxia. Hoppeler H, Kleinert E, Schlegel C, Claassen H, Howald H, Kayar SR, Cerretelli P. Department of Anatomy, University of Berne, Switzerland. Muscle structural changes during typical mountaineering expeditions to the Himalayas were assessed by taking muscle biopsies from 14 mountaineers before and after their sojourn at high altitude (greater than 5000 m for over 8 weeks). M. vastus lateralis samples were analyzed morphometrically from electron micrographs. A significant reduction (-10%) of muscle cross-sectional area was found on CT scans of the thigh. Morphologically this loss in muscle mass appeared as a decrease in muscle fiber size mainly due to a loss of myofibrillar proteins. A loss of muscle oxidative capacity was also evident, as indicated by a decrease in the volume of muscle mitochondria (-25%). In contrast, the capillary network was mostly spared from catabolism. It is therefore concluded that oxygen availability to muscle mitochondria after prolonged high-altitude exposure in humans is improved due to an unchanged capillary network, supplying a reduced muscle oxidative capacity.

Experientia. 1990 Jul 15;46(7):672-6. Muscle lipofuscin content and satellite cell volume is increased after high altitude exposure in humans. Martinelli M, Winterhalder R, Cerretelli P, Howald H, Hoppeler H. Department of Anatomy University of Bern, Switzerland. Muscle ultrastructural changes during a typical expedition to the Himalayas were analyzed by taking muscle biopsies from seven climbers before and after their sojourn at high altitude (over 5000 m for 8 weeks). M. vastus lateralis samples were analyzed morphometrically from electron micrographs. A quantitative evaluation was made of lipofuscin, satellite cells and myonuclei. Significant increases of the volume densities of lipofuscin (+ 235%) and satellite cells (+ 215%) were observed.

J Appl Physiol. 1989 May;66(5):2454-61. Operation Everest II: adaptations in human skeletal muscle. Green HJ, Sutton JR, Cymerman A, Young PM, Houston CS. Department of Kinesiology, University of Waterloo, Ontario. Adaptations in skeletal muscle in response to progressive hypobaria were investigated in eight male subjects [maximal O2 uptake = 51.2 +/- 3.0 (SE) ml.kg-1.min-1] over 40 days of progressive decompression to the stimulated altitude of the summit of Mt. Everest. Samples of the vastus lateralis muscle extracted before decompression (SL-1), at 380 and 282 Torr, and on return to sea level (SL-2) indicated that maximal activities of enzymes representative of the citric acid cycle, beta-oxidation, glycogenolysis, glycolysis, glucose phosphorylation, and high-energy phosphate transfer were unchanged (P greater than 0.05) at 380 and 282 Torr over initial SL-1 values. After exposure to 282 Torr, however, representing an additional period of approximately 7 days, reductions (P less than 0.05) were noted in succinic dehydrogenase (21%), citrate synthetase (37%), and hexokinase (53%) between SL-2 and 380 Torr. No changes were found in the other enzymes. Capillarization as measured by the number of capillaries per cross-sectional area (CC/FA) was increased (P less than 0.05) in both type I (0.94 +/- 0.8 vs. 1.16 +/- 0.05) and type II (0.84 +/- 0.07 vs. 1.05 +/- 0.08) fibers between SL-1 and SL-2. **This increase was mediated by a reduction in fiber area**. **No changes were found in fiber-type distribution (type I vs. type II).** These findings do not support the hypothesis, at least in humans, that, at the level of the muscle cell, extreme hypobaric hypoxia elicits adaptations directed toward maximizing oxidative function.

Acta Physiol Scand. 1991 Jul;142(3):421-7. Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude. MacDougall JD, Green HJ, Sutton JR, Coates G, Cymerman A, Young P, Houston CS. Department of Physical Education, McMaster University, Hamilton, Ontario, Canada.

Alterations in skeletal muscle structure were investigated in 6 male subjects who underwent 40 days of progressive decompression in a hypobaric chamber **simulating an ascent to the summit of Mount Everest**. Needle biopsies were obtained from vastus lateralis of 5 subjects before and immediately after confinement in the chamber, and were examined for various structural and ultrastructural parameters. In addition, total muscle area was calculated in 6 subjects from CT scans of the thighs and upper arms. Muscle area at these sites was found to decrease significantly (by 13 and 15%) as a result of the hypobaric confinement. This was substantiated by significant (25%) decreases in cross sectional fibre areas of the Type I fibres and 26% decreases (non significant) in Type II fibre area. Capillary to fibre ratios remained unchanged following hypoxia as did capillary density although there was a trend (non significant) towards an increase in capillary density. There were no significant increases in mitochondrial volume density or other morphometric parameters. These data indicate that chronic, severe hypoxia on its own does not result in an increase in absolute muscle capillary number or a de novo synthesis of mitochondria. The trends toward an increase in capillary density and mitochondrial volume density were interpreted as being secondary occurrences in response to the pronounced muscle atrophy which occurred.

Int J Sports Med. 1993 Jul;14(5):244-7. Body composition and maximum alactic anaerobic performance during a one month stay at high altitude. Kayser B, Narici M, Milesi S, Grassi B, Cerretelli P. Département de Physiologie, CMU, Genève, Switzerland. Prolonged altitude exposure usually leads to considerable weight loss of which a large part is from muscle tissue. This loss reduces maximum alactic anaerobic muscle power. It was hypothesized that most of the weight loss may simply be the result of malnutrition due to lack of palatable food in an uncomfortable environment. To test this hypothesis eight healthy male subjects (age 33.7 +/- 4.6 S.C. yr), well acclimatized to prevent symptoms of acute mountain sickness, were exposed for 4 weeks to an altitude of 5050 m with access to a large choice of palatable food in comfortable conditions. Body weight (with a scale), body composition (from skinfolds), arm muscle plus bone cross-sectional area (Am + b) and muscle plus bone leg volume (Vm+b) (from skinfolds and circumferences), maximum voluntary contraction force of the elbow flexors (MVC, with a load cell) and maximum jumping height (Hmax, on a platform) were measured before departure (SL) and in the first (ALT1), second (ALT2) and fourth week (ALT4) of their altitude sojourn. Three-day dietary records were obtained at SL and at ALT4. Body mass had decreased significantly at ALT2 (-3.8%) and at ALT4 (-4.6%) likely reflecting changes in body water homeostasis. No changes were found in %fat, Am+b, Vm+b, MVC or Hmax. Average dietary intake at SL was 8.96 +/- 1.45 MJ and had increased to 13.59 +/- 3.07 MJ at ALT4. **In conclusion, up to an altitude of 5050 m loss of body mass from fat and muscle tissue, and hence impairment of maximum anaerobic muscle power (alactic) appears to be avoidable by food intake matched to energy expenditure.** The latter may be achieved simply by proper acclimatization, sufficient comfort and availability of palatable food.