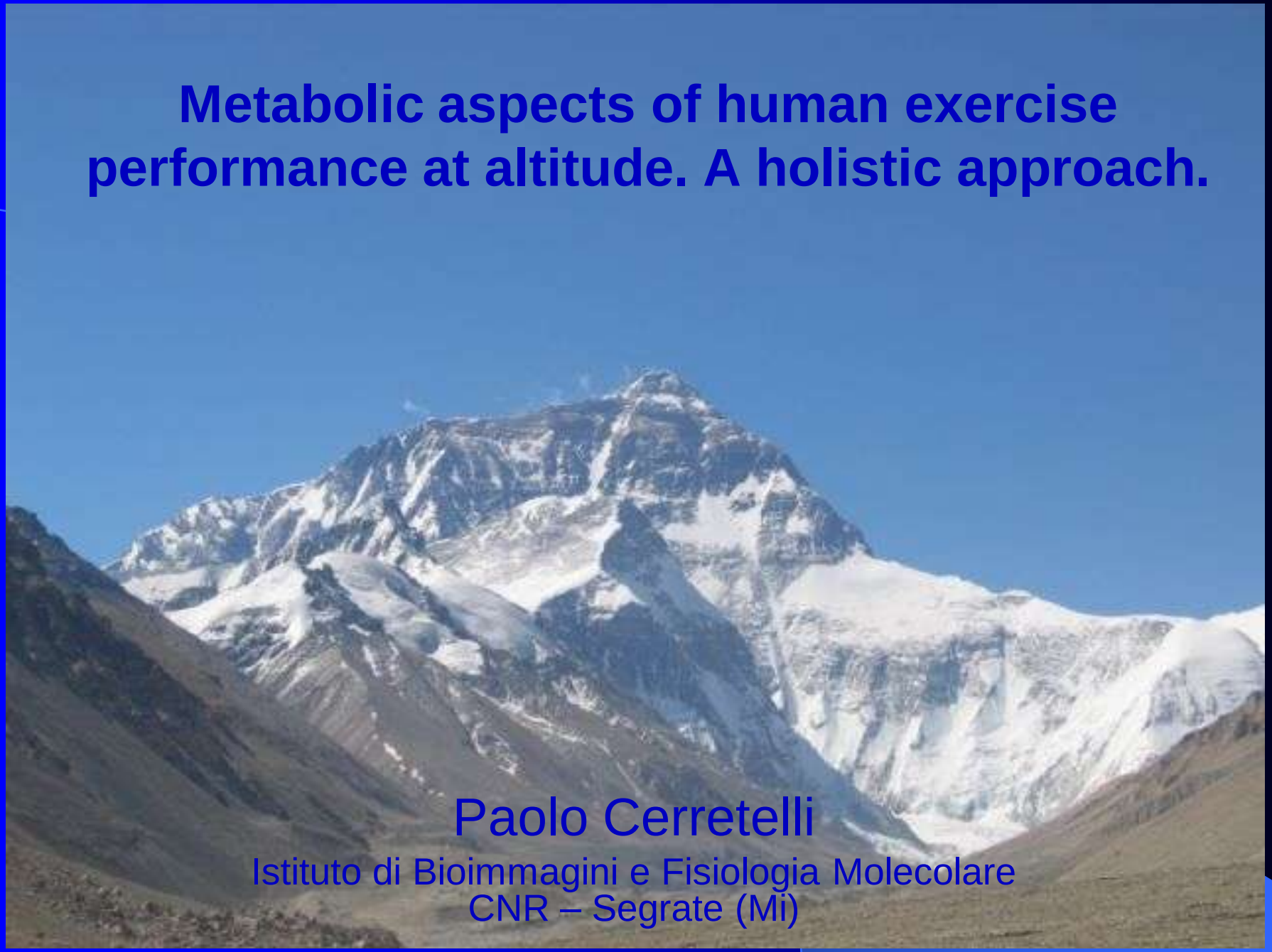
Metabolic aspects of human exercise performance at altitude. A holistic approach.



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Rovereto, November 12, 2015

The effects of Hypoxia on physical performance have been assessed as functions of:

a) Exposure duration

- < 10 days : acute and subacute
- 1-3 months : subchronic and chronic
- > 12 months : partial adaptation
- From birth : full adaptation

b) Metabolic level

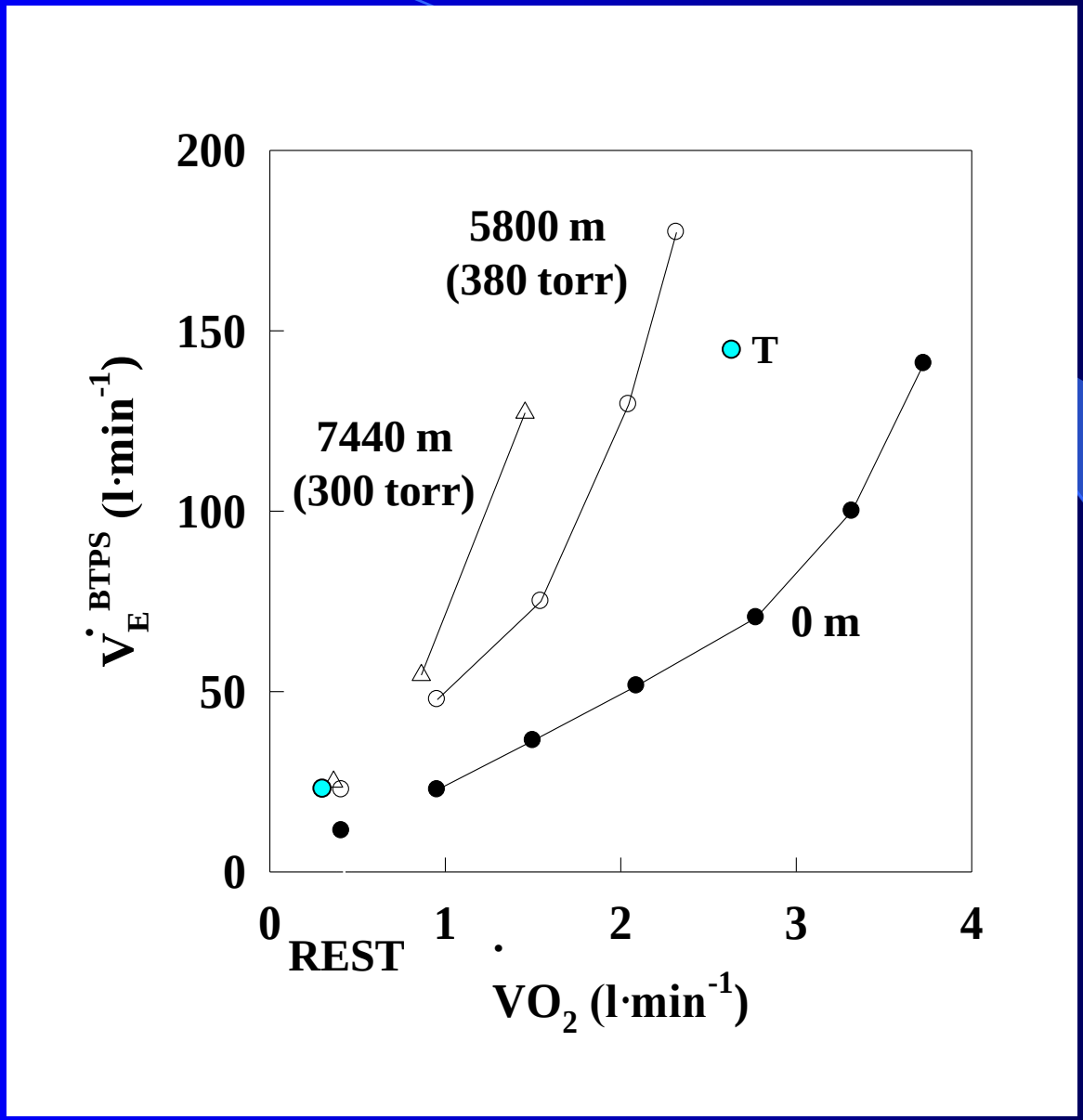
- Resting
- Submaximal workload
- Maximal workload ($\dot{V}O_2max$)
- Supramaximal workload (>$\dot{V}O_2max$ up to “peak”)

at the integrative, at the cellular and at the molecular level

A) The integrative level

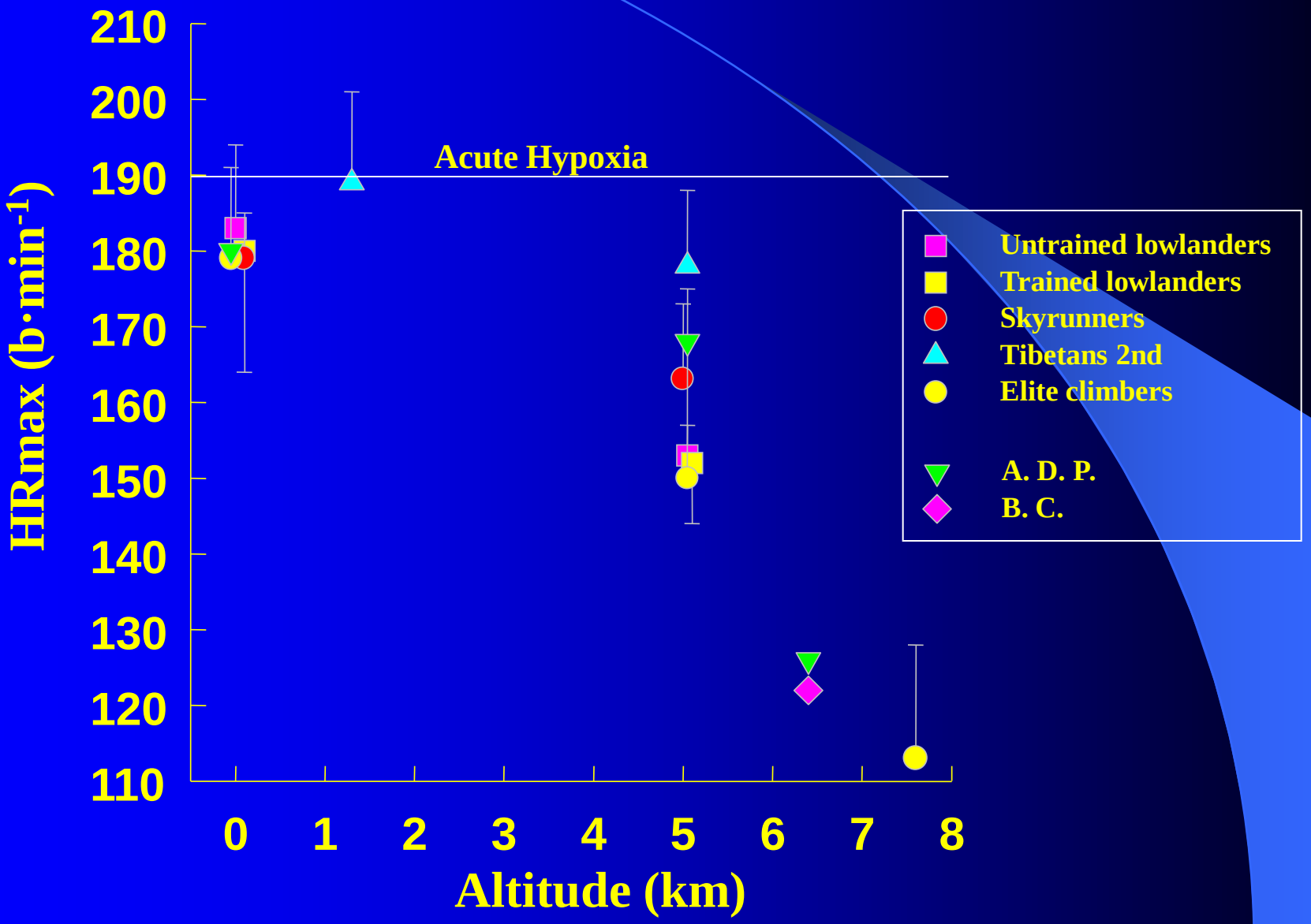
A1. Maximal and submaximal aerobic performance

Maximal pulmonary ventilation

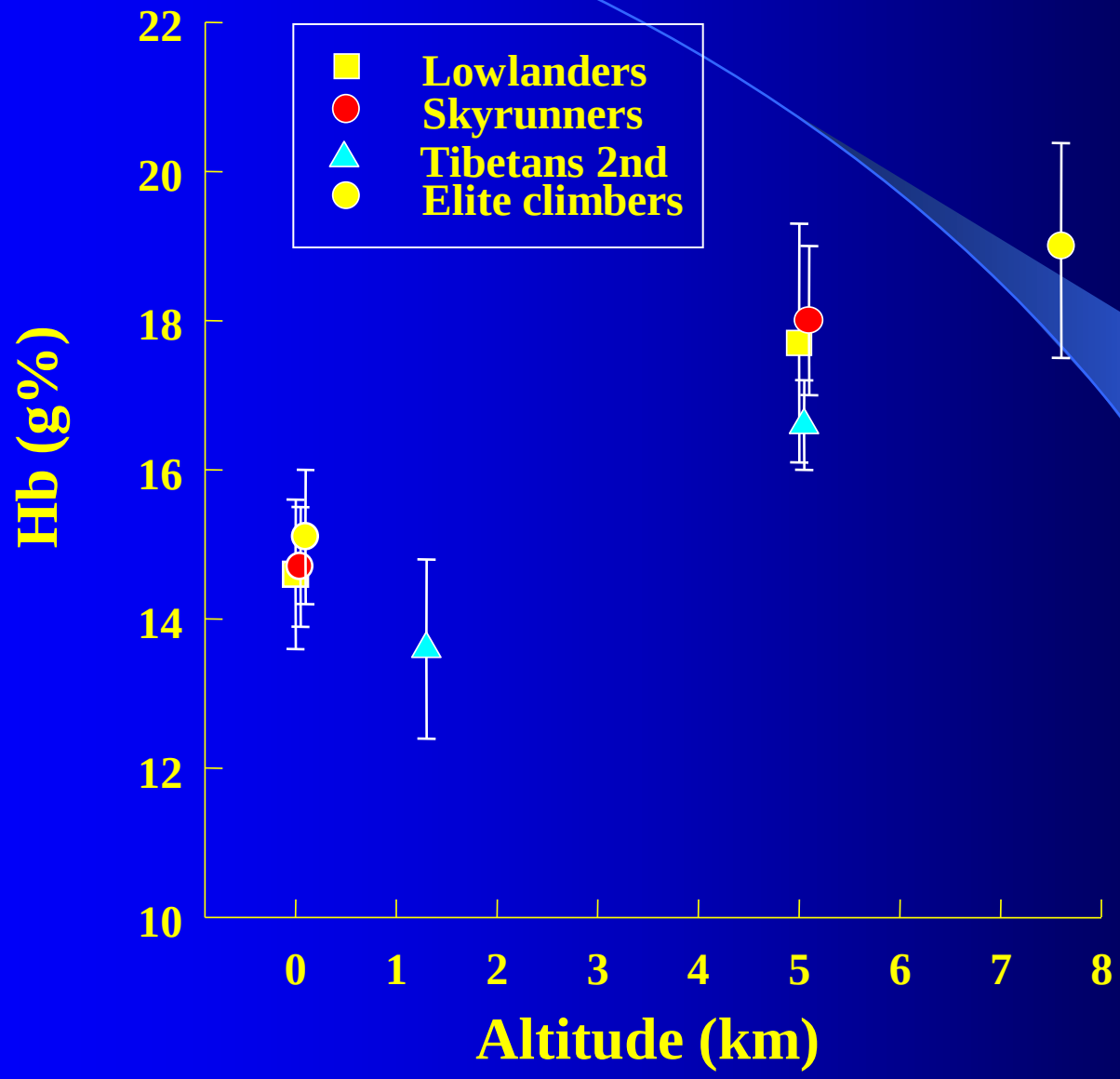


(from Pugh et al., 1964)

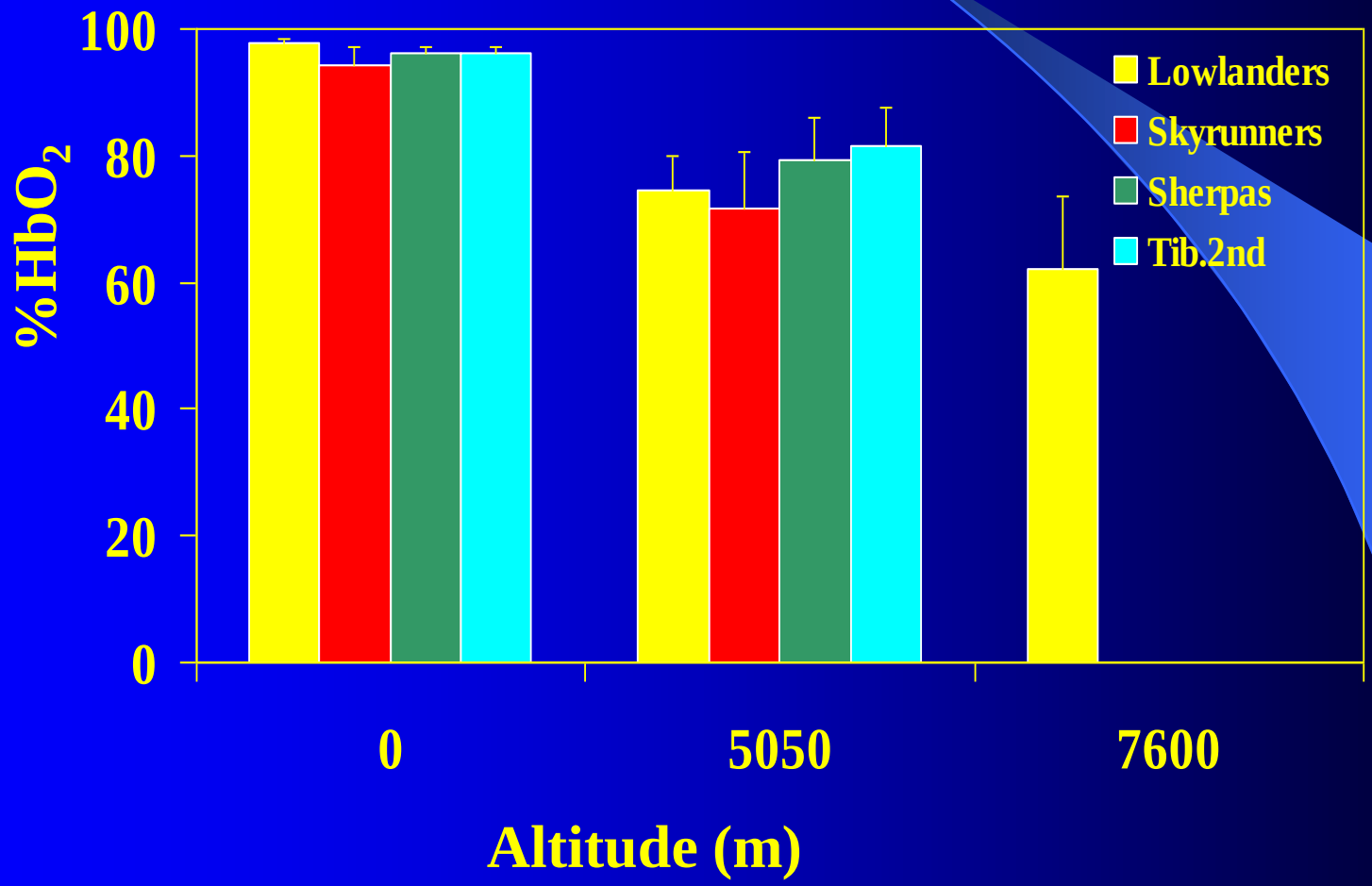
Maximal Heart Rate



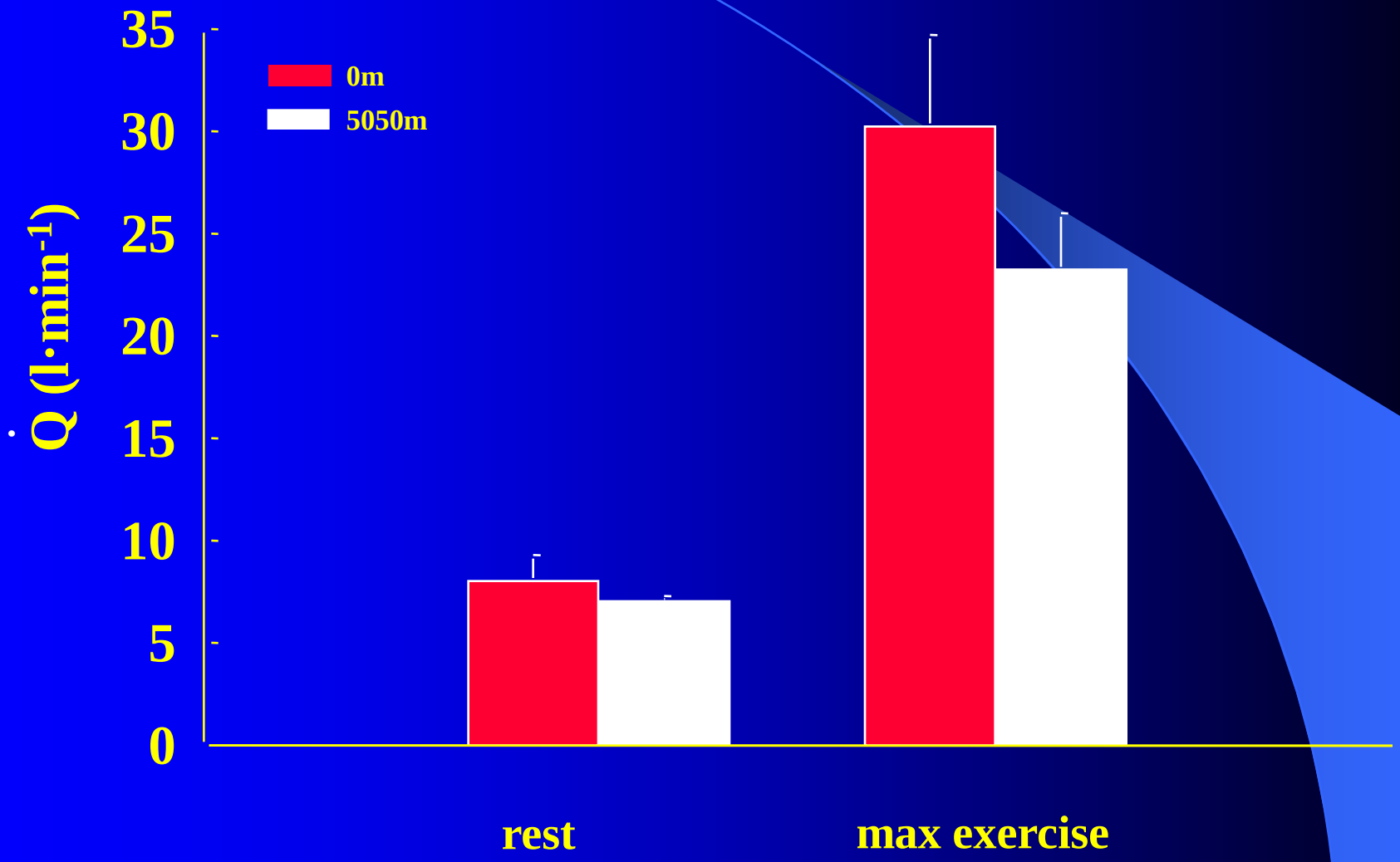
Hb concentration as a function of altitude



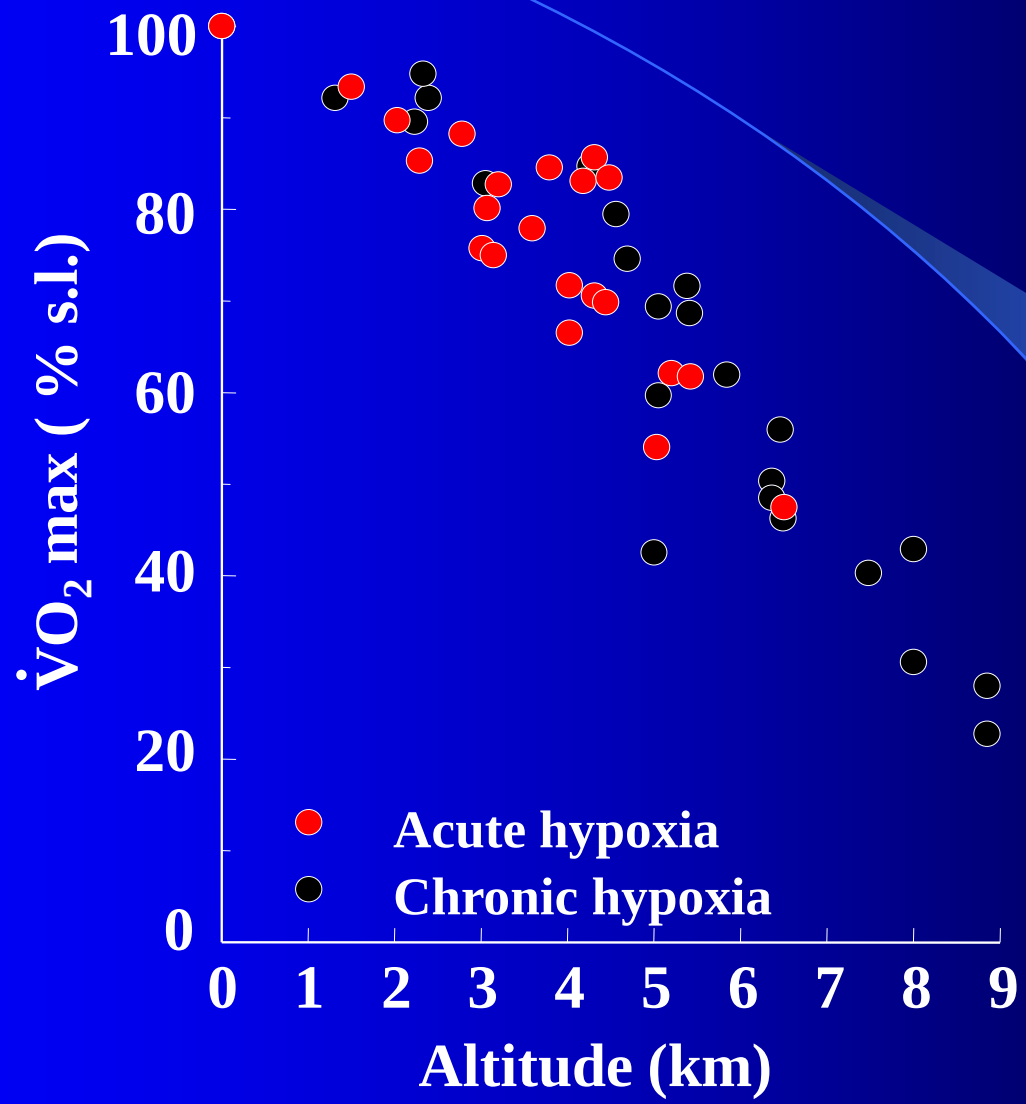
% Hb oxygen saturation at exhaustion



Cardiac output in élite climbers ★

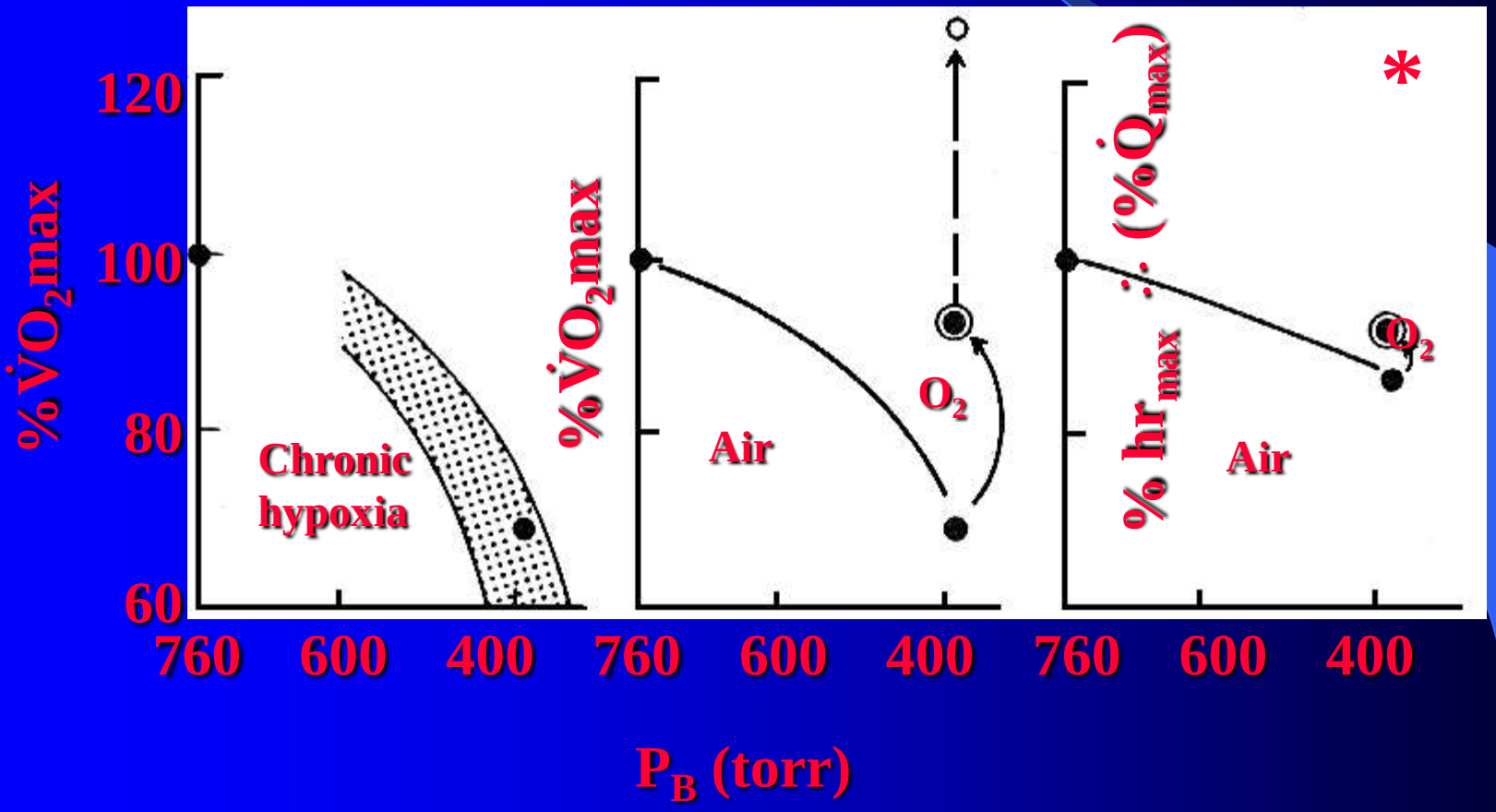


$\dot{V}O_2max$ as a function of altitude



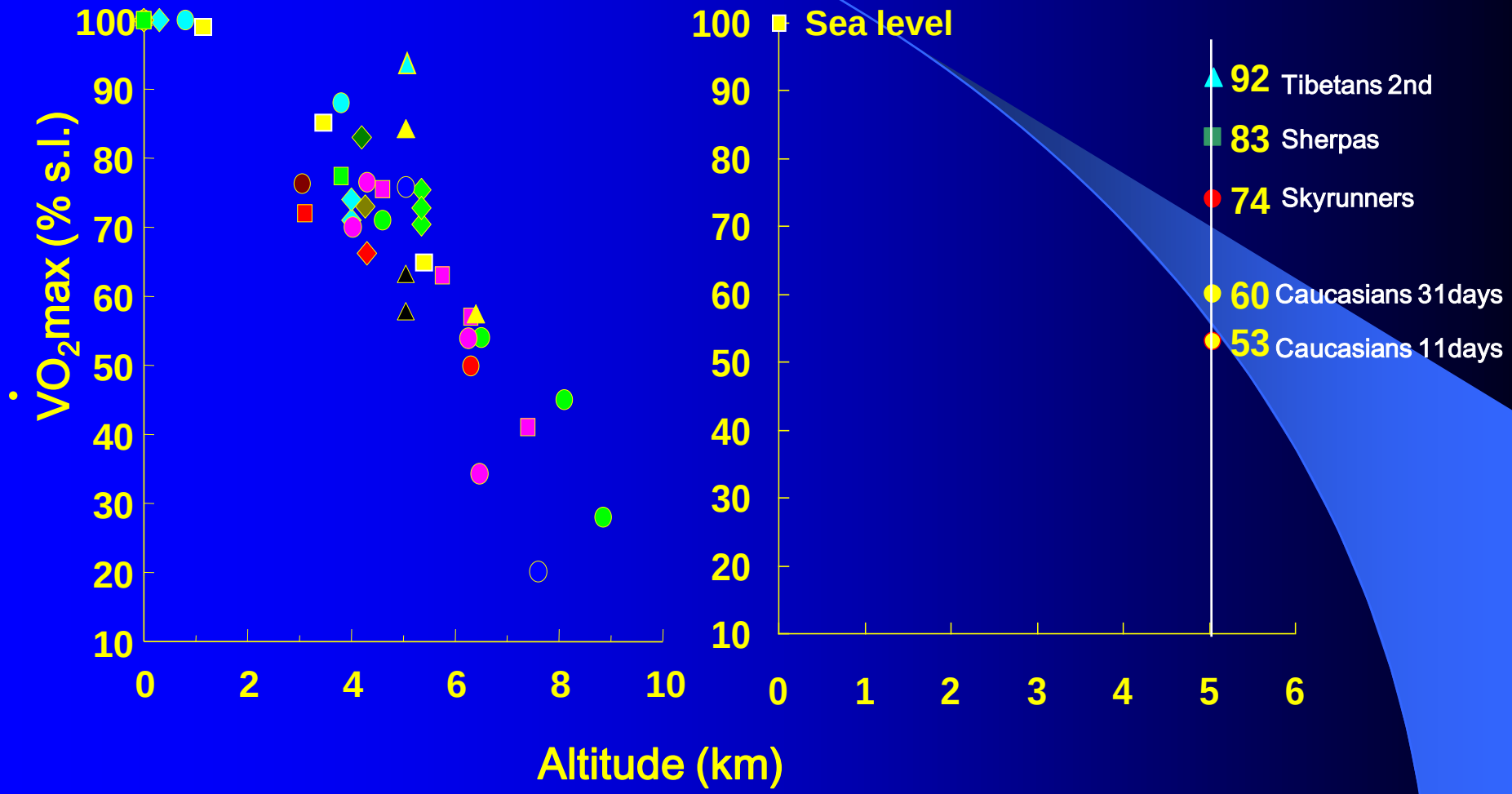
(From Cerretelli and Hoppeler, Handbook of Physiology, APS, 1996)

Effects of rapid reoxygenation on $\dot{V}O_2max$ of acclimatized Caucasians at Mt Everest base camp (5450 m)

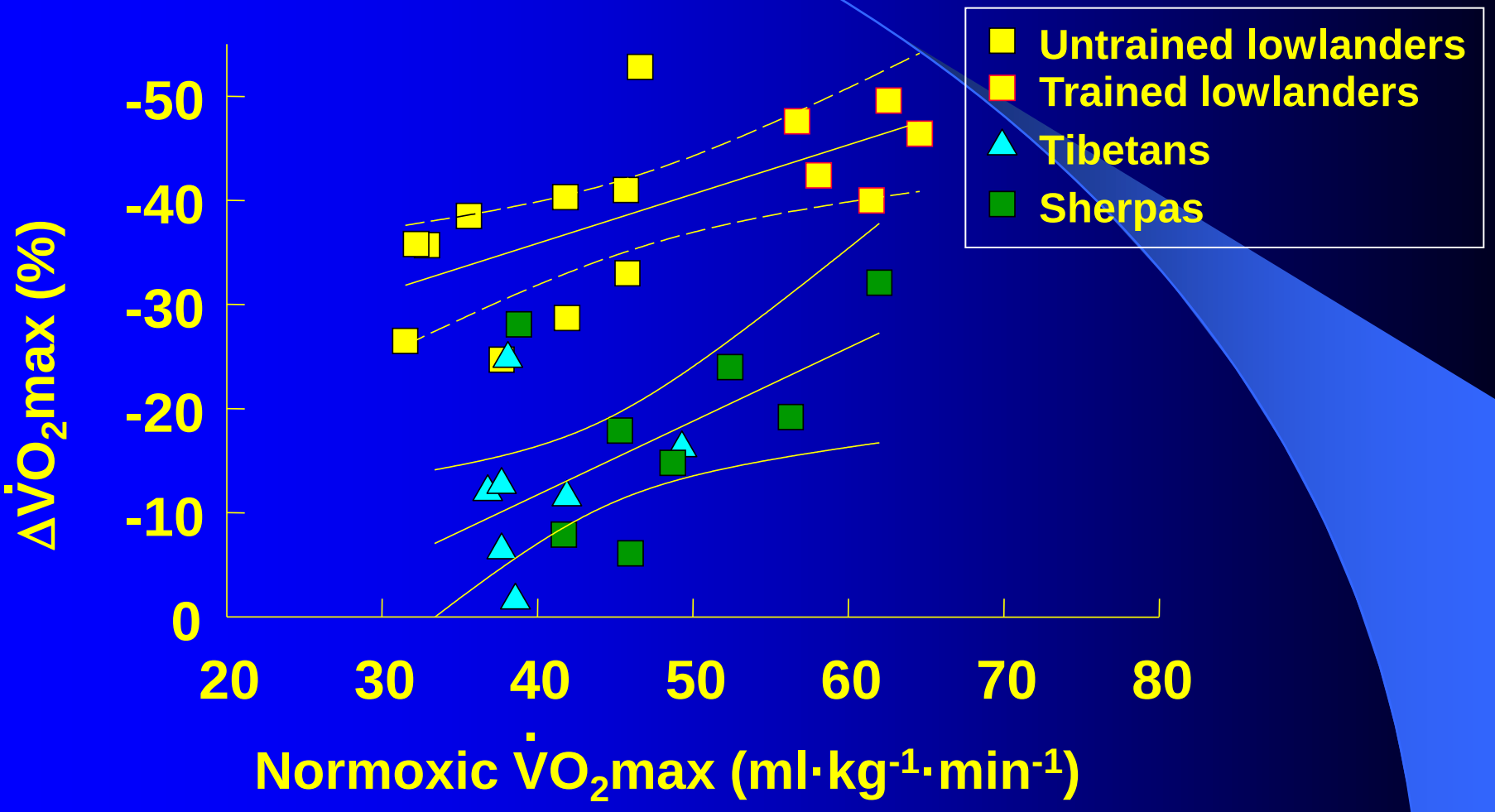


(from Cerretelli, J.Appl.Physiol., 1976)

$\dot{V}O_2max$ as a function of altitude: training and ethnic variability

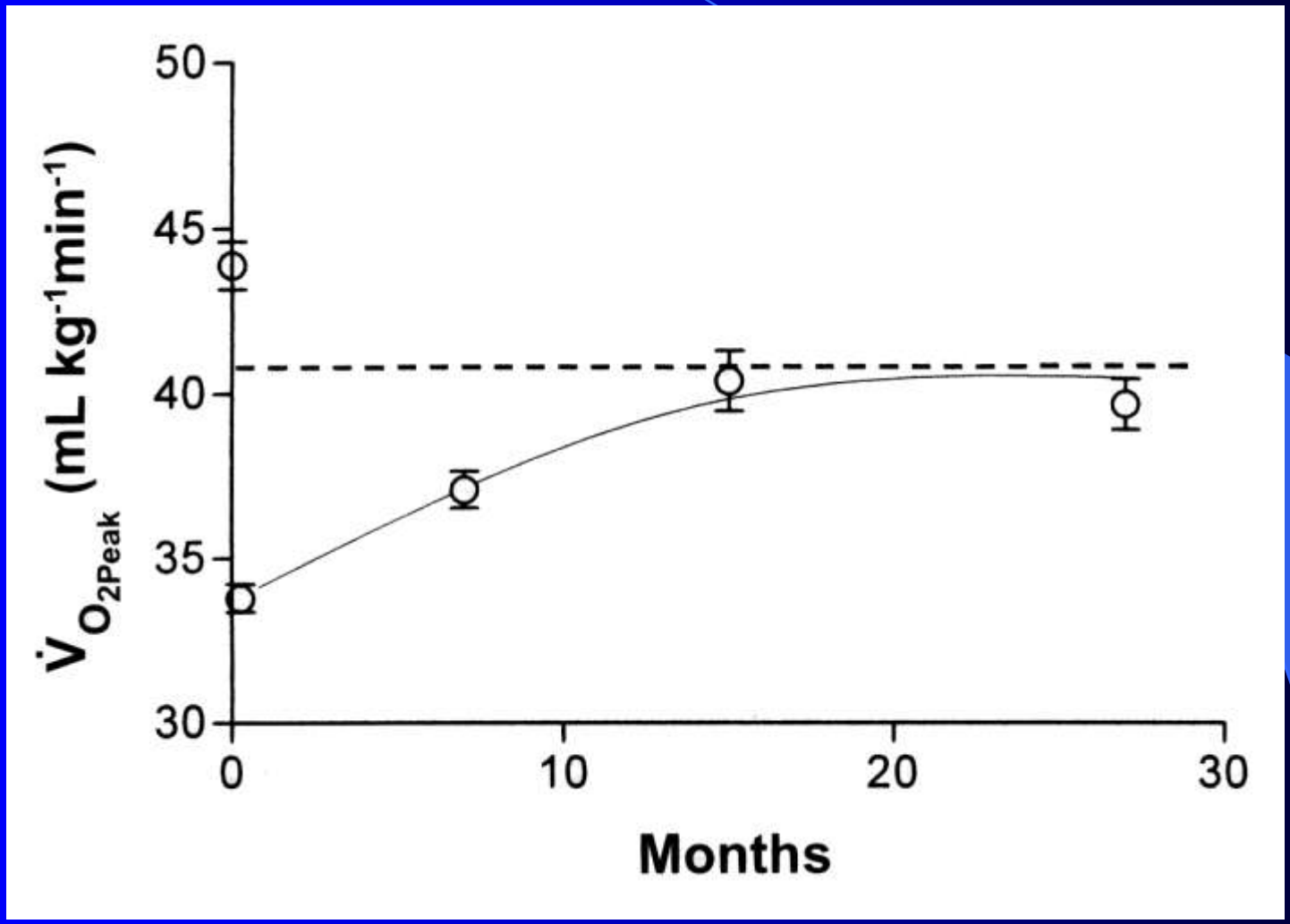


$\Delta \dot{V}O_2max$ at 5050 m. as a function of initial maximal aerobic power



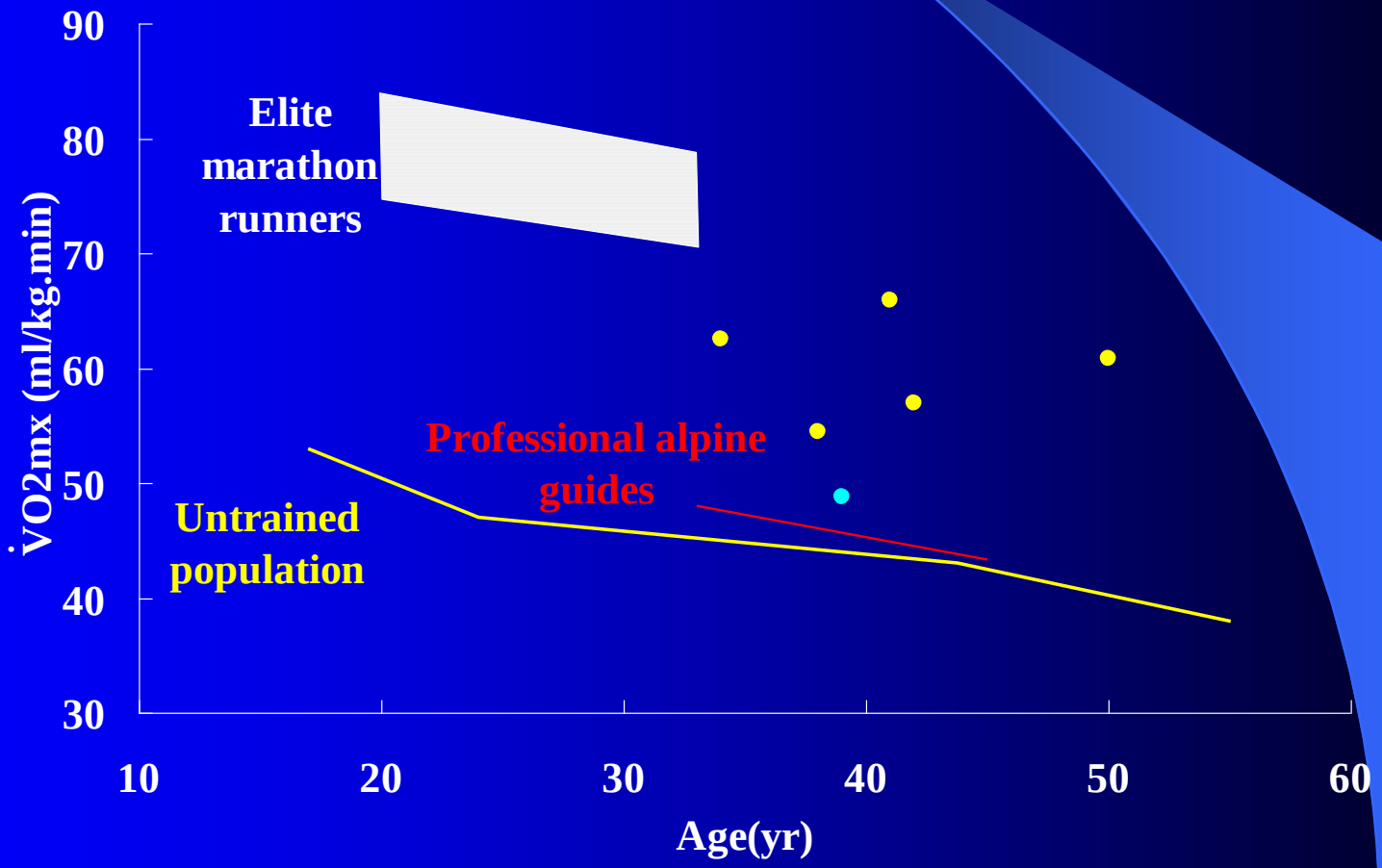
(From Marconi et al., J.Physiol. (London), 2004)

Evolution of $\dot{V}O_2$max during prolonged altitude exposure



(from Marconi et al., 2006)

$\dot{V}O_2$ max in élite himalayan climbers



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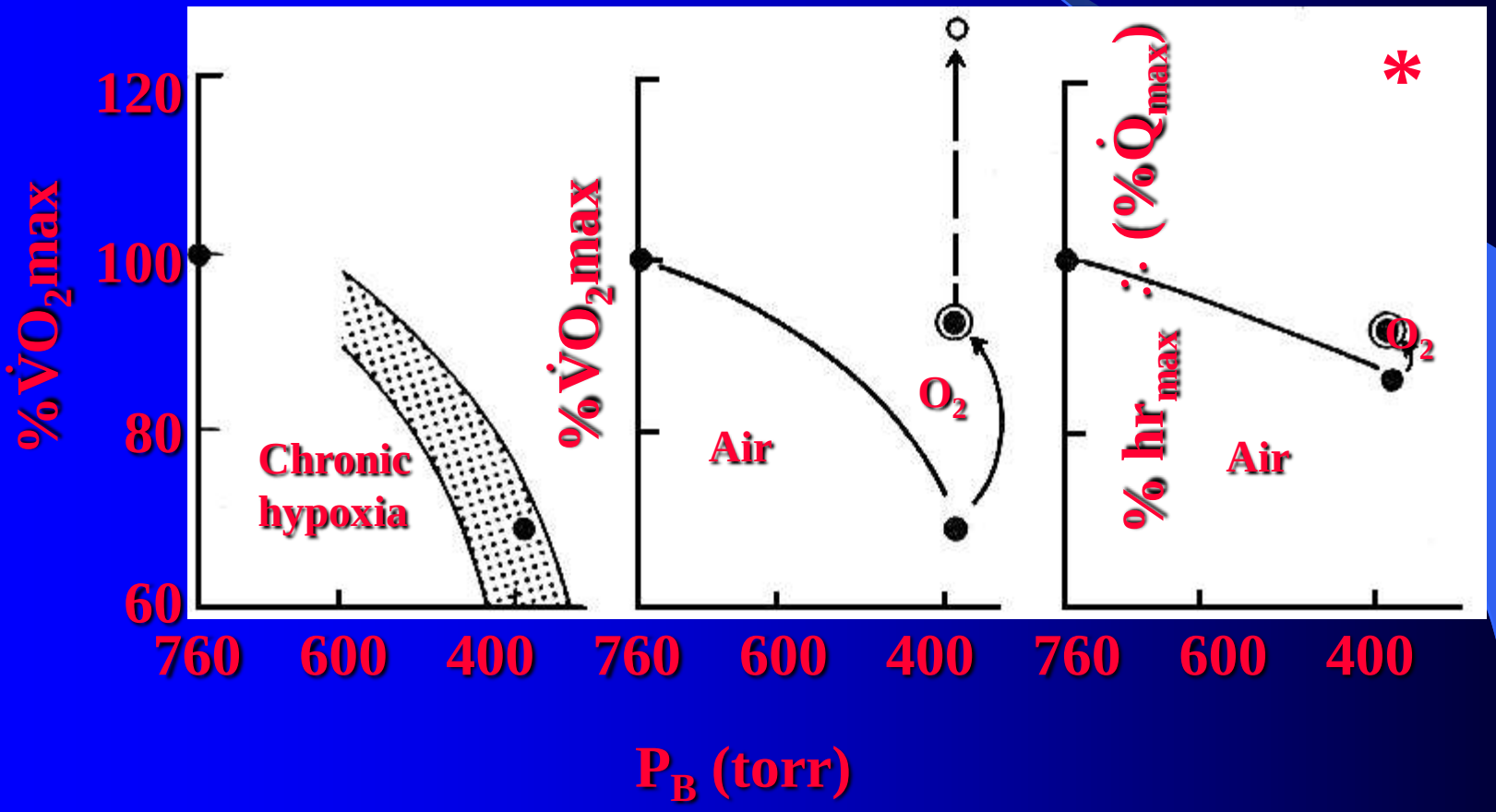
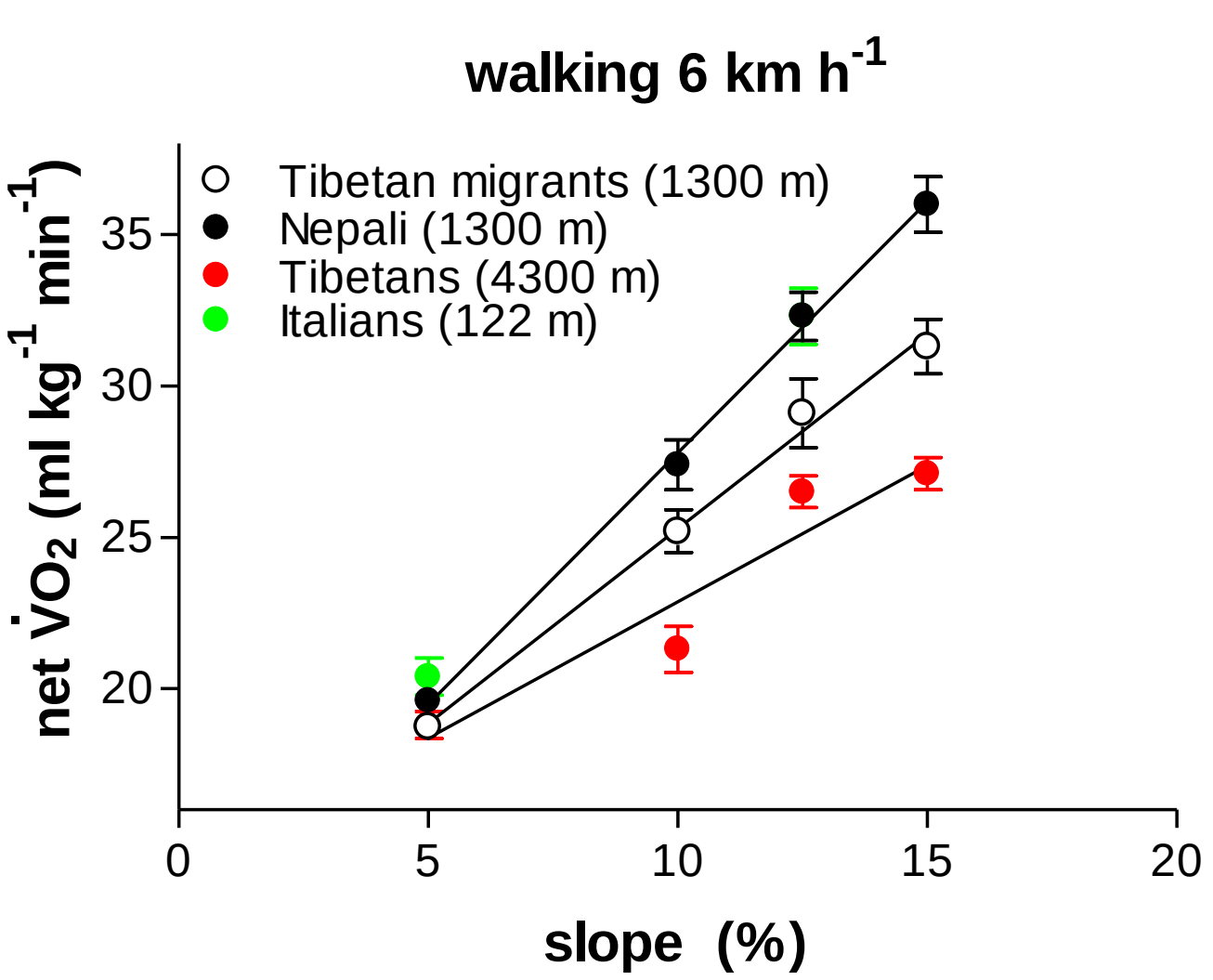


(from Cerretelli, J.Appl.Physiol., 1976)

Walking economy



(from Marconi et al., in preparation)

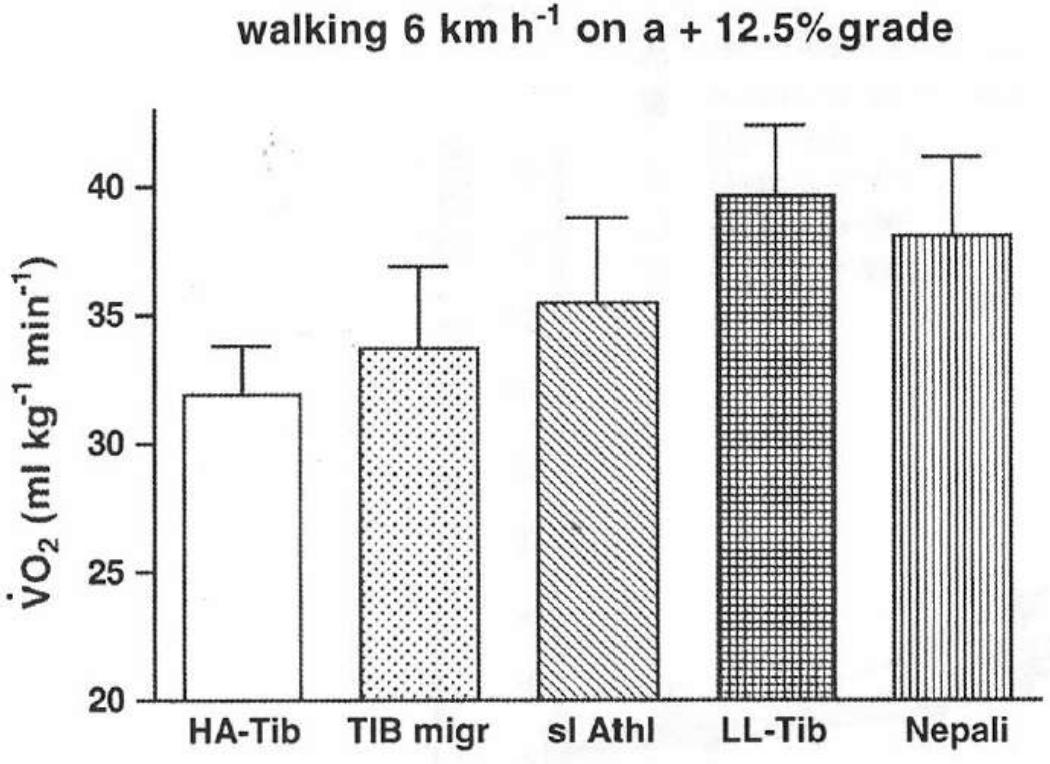


FIG. 2. Gross steady-state oxygen consumption during treadmill walking in Tibetans at 4300 m (HA-Tib, n = 18), altitude Tibetans recently migrated to 1300 m (TIB migr, n = 6), Caucasian middle-distance runners at 120 m (sl Athl, n = 4), second generation Tibetans born and living at 1300 m (LL-Tib, n = 6), and control subjects at 1300 m (Nepali, n = 10) (Data are mean ± SD).

(from Cerretelli, High Altitude Medicine & Biology., 2009)

Conclusions A 1

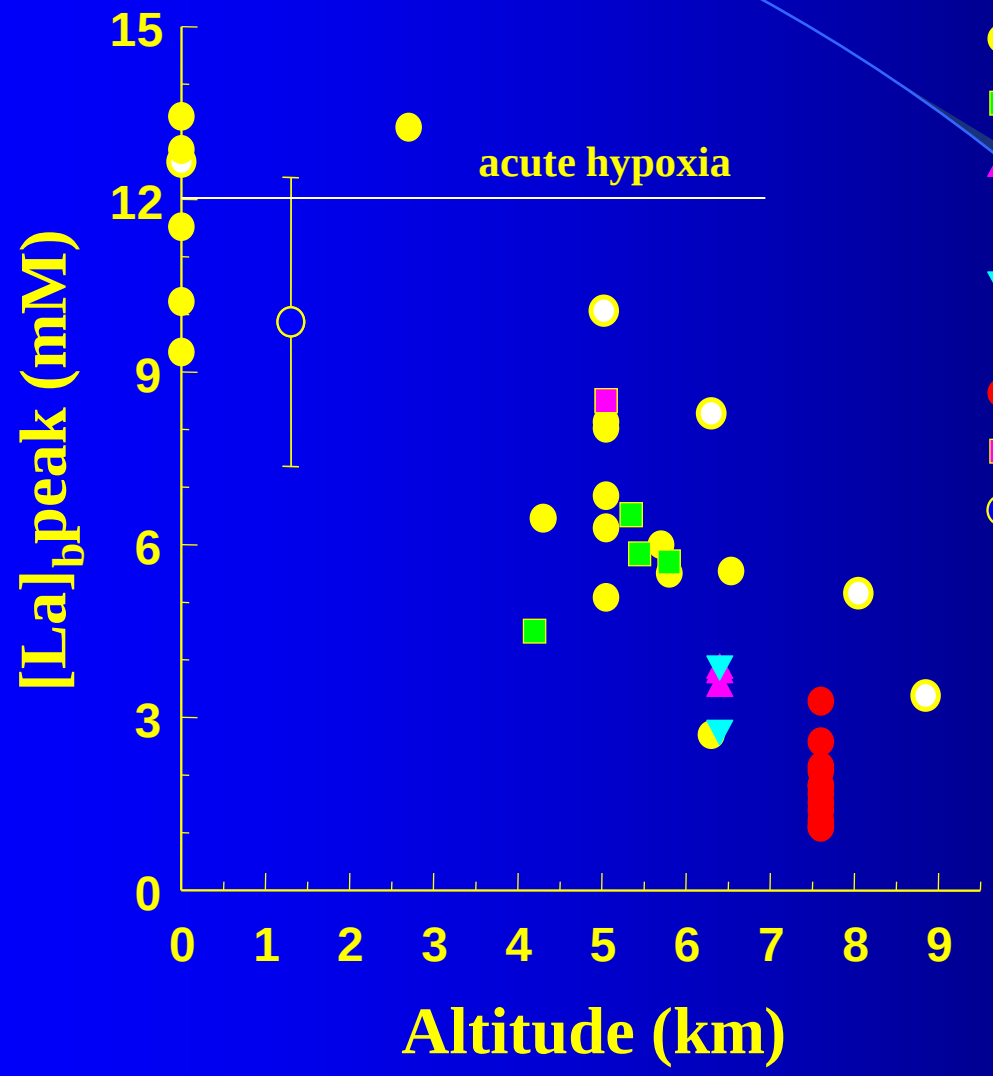
- $\dot{V}O_2$max decreases as a parabolic function of altitude.The rate of decrease is surprisingly very similar in acute and chronic hypoxia. Peripheral factors, possibly at the muscle level, appear to play a major role in chronic conditions.
- Altitude natives are characterized by higher $\dot{V}O_2$max than acclimatized lowlanders at any given altitude. However, altitude exposure for over two years tends to reduce the gap.
- There is a large scatter among various groups in the drop of $\dot{V}O_2$max as a function of altitude.
- Within any given ethnic group, individuals with greater maximum aerobic power undergo at 5050 m. a larger drop of $\dot{V}O_2$max.
- Elite himalayan climbers are not characterized by particularly high $\dot{V}O_2$max absolute levels.
- Sudden reoxygenation does not allow to resume initial normoxic $\dot{V}O_2$max.
- Walking economy is greater in altitude natives thanks to higher efficiency of oxidative phosphorylation

A 2. Maximal anaerobic performance

Extreme Altitude Survival Test 1 and 2 (1994-1997)

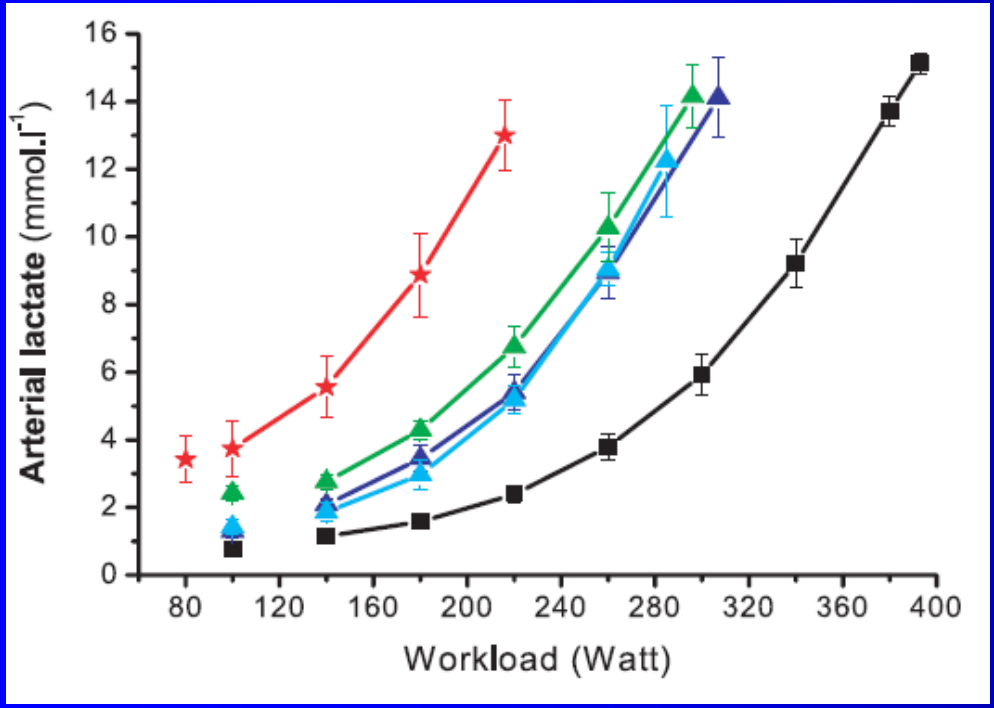
Mt Everest advanced base camp (6400 m)

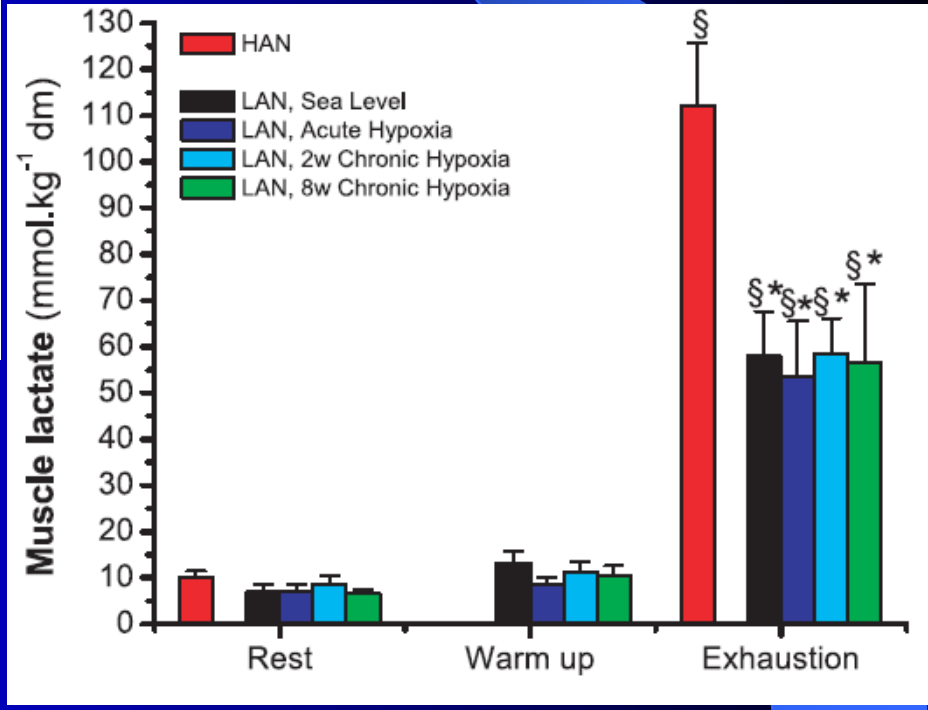
$La_{[max]}$ as a function of altitude



- Operation Everest II
- Caucasian lowlanders
- Altitude natives
- Caucasian lowlanders (personal observation, 1994)
- Sherpas (personal observation, 1994)
- EAST 1997
- 2nd generation Tibetans
- HA Tibetan refugees

Arterial lactate concentration and *vastus lateralis* lactate content: denial of the "lactate paradox"





from Van Hall et al., J Physiol (London), 2009

LDH activity in muscle in acute and chronic hypoxia

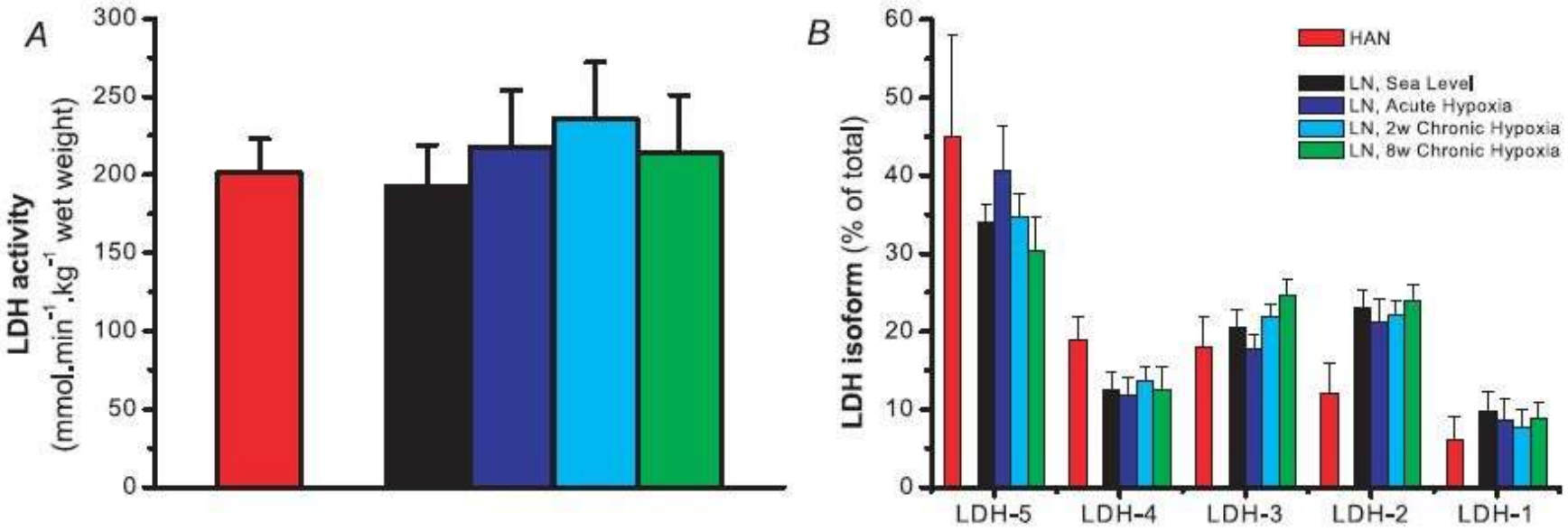


Figure 4. Vastus lateralis lactate dehydrogenase activity (*A*) and relative isoform content (*B*) in HAN and LN in the course of acclimatization to high altitude
Values are mean ± S.E.M. of LN ($n = 6$) and HAN ($n = 7$). No significant differences were found either between conditions in LN or between LN and HAN.

from Van Hall et al., J Physiol (London), 2009

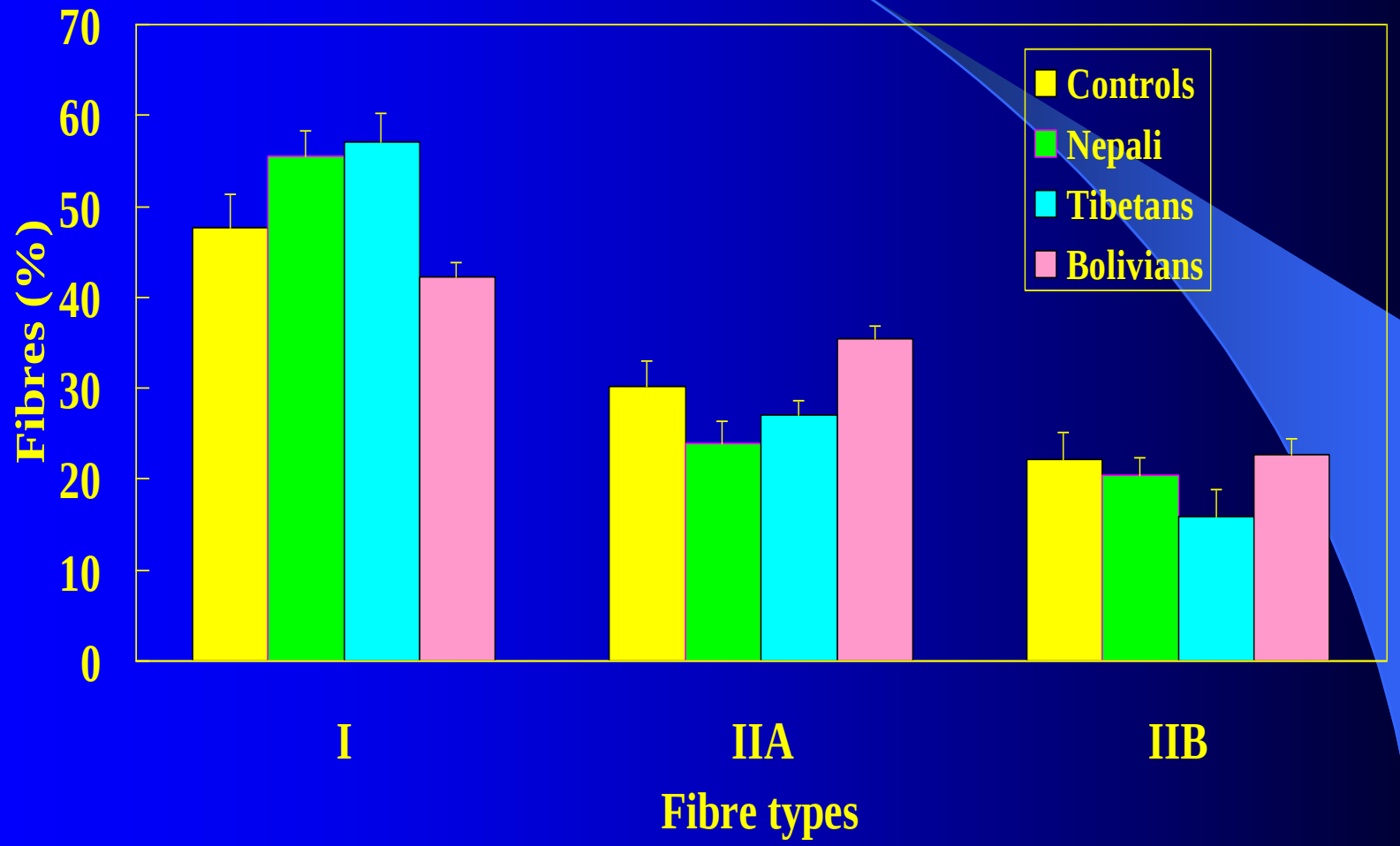
Conclusion A 2

- Is there a "*lactate paradox*"?

* The data of the preceding figure are the main basis of the so-called "lactate paradox", i.e. the apparent decrease of the subject's " maximal glycolytic capacity" in acclimatized lowlanders and altitude natives.
The above definition has been recently challenged since it is based on blood lactate data. In fact, muscle lactate determinations do not evidence impairment of anaerobic glycolysis in altitude adapted individuals: whence the recent contention by Van Hall et al.(2009) that the lactate paradox does not exist. The discrepancy between muscle and blood lactate levels at exhaustion could be the consequence of an impaired function of the lactate transporters in the sarcolemma .

B) The cellular and subcellular level

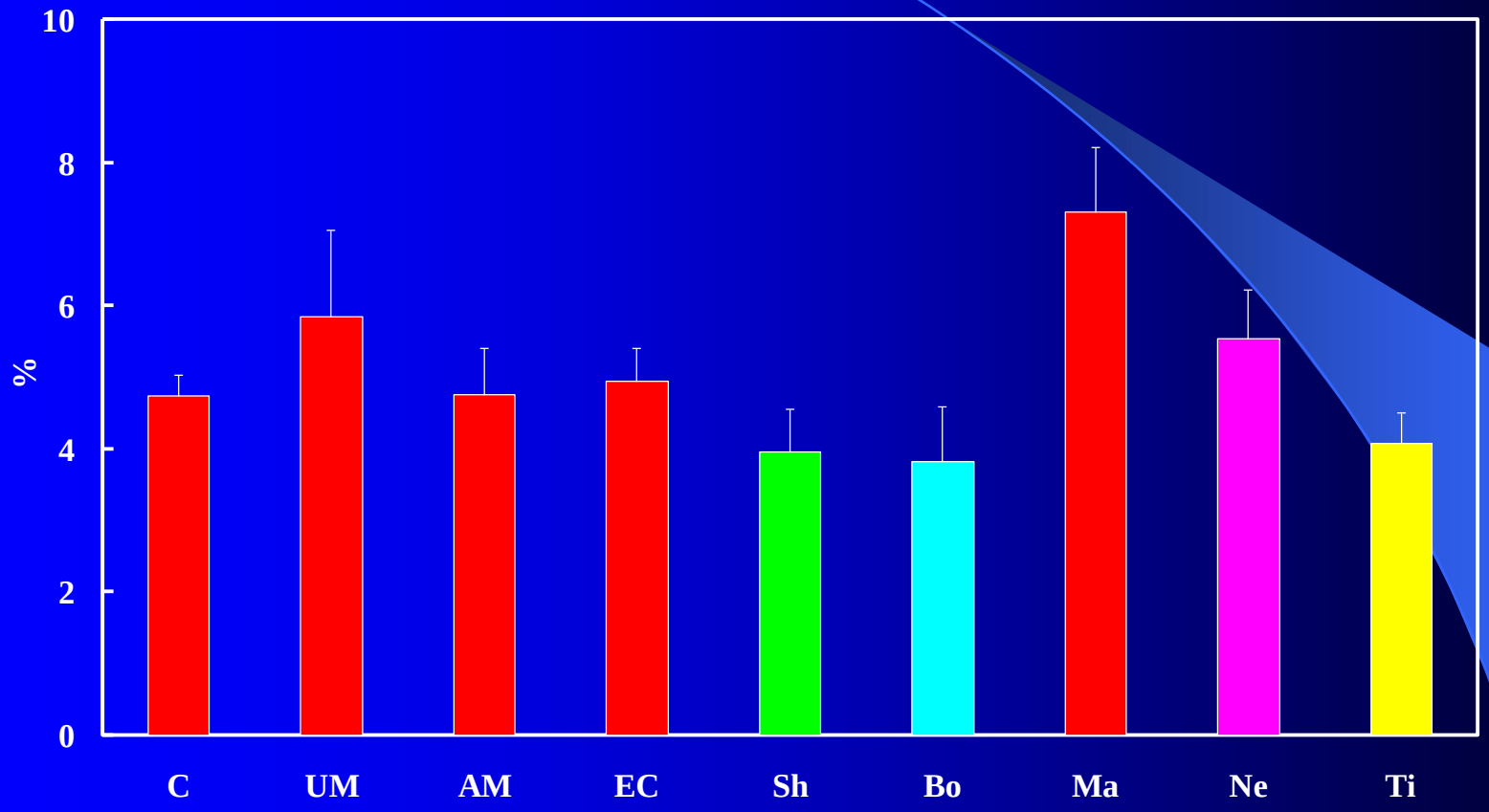
Fiber types distribution



Morphometry and Enzymes in muscle after the 1986 Swiss Mt. Everest expedition (n=7subjects)

VARIABLE	*% change*
Muscle mass	-11
Fiber diameter	-15
(central)	-55
Mitochondrial volume density (total)	-26
(sub-sarcolemmal)	-18
HK	-8
PFK	+6
LDH	0
CS (citric acid cycle)	-23
MDH	-20
CYTOX (respiratory chain)	-23
HADH (beta-oxidation of fatty acids)	-27
HBDH (utilization of ketone bodies)	-27

Mitochondrial volume density in various altitude and sea level populations



from Cerretelli, Textbook of Exercise Physiology, SEU, Roma 2001

Conclusion B

- Muscle fiber types distribution is the same in altitude natives and in lowlanders and is independent of ethnicity.
- Oxidative enzymes activity is reduced in acclimatized subjects.
- Mitochondrial volume density is low in altitude natives, independent of their ethnic background. In Caucasians, it undergoes reduction in the course of acclimatization.

C) The molecular level

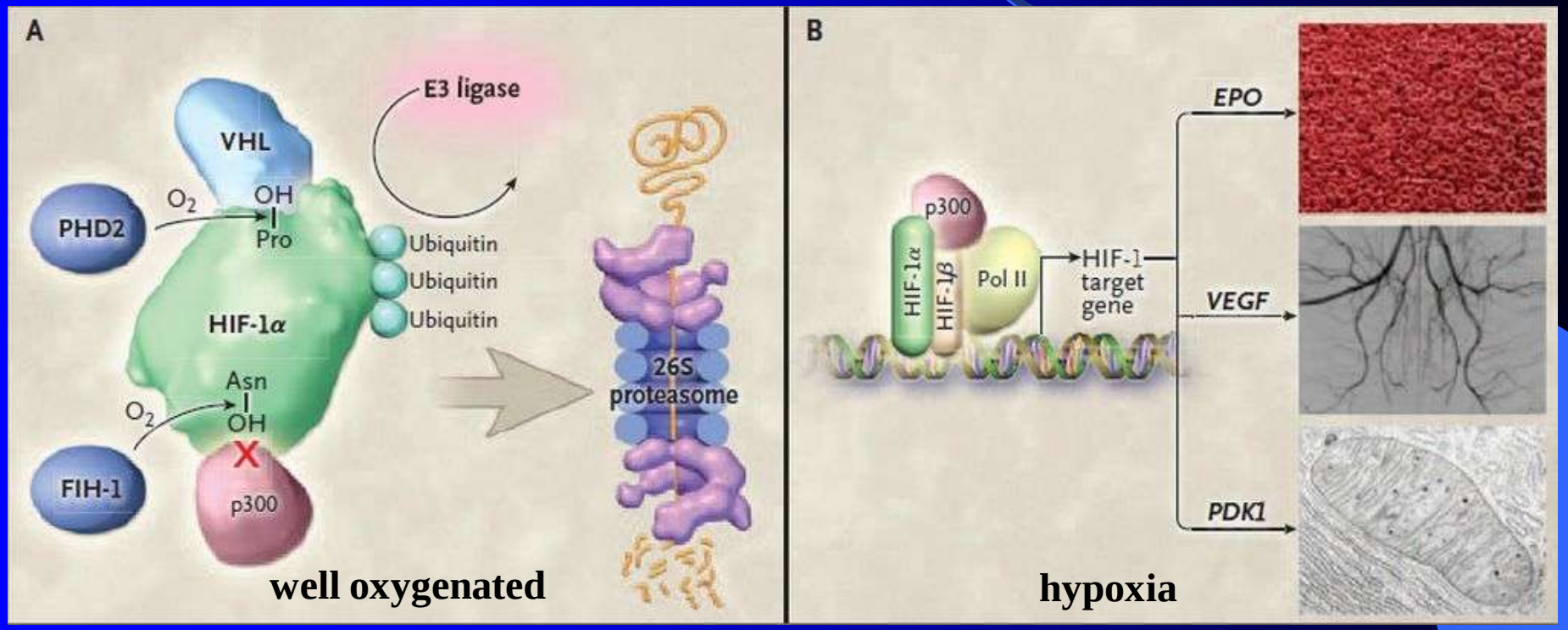
C1) The role of the Hypoxia Inducible Factor (HIF-1)

The interpretation of most functional responses of metazoan organisms to decreased oxygen partial pressure is supported and implemented by the discovery of a number of adaptive mechanisms for oxygen sensing and signal transduction promoted by a protein, the **Hypoxia Inducible Factor** (HIF-1). HIF-1, a dimer α and β, is expressed in all cell types and has been identified in all species suggesting that its appearance represented an **adaptation** essential to metazoan evolution. HIF-1 is a **transcription** factor regulating the expression of hundreds of genes in response to changes in oxygen availability. **The HIF-1α subunit of the dimer is continuously synthesized and is eliminated by proteasomal degradation under well oxygenated conditions**

HIF-1 : a Master Regulator of oxygen homeostasis

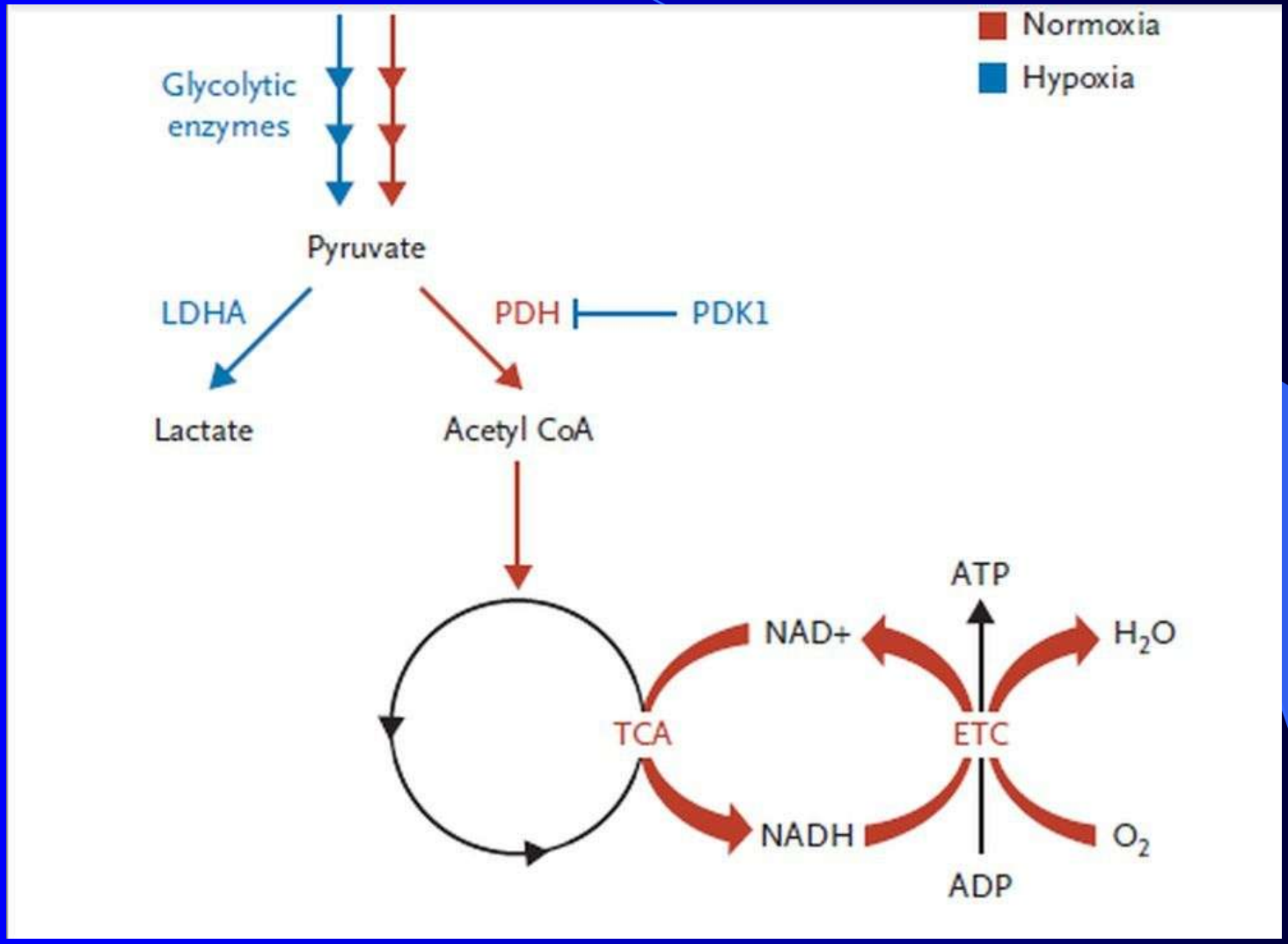
- Regulates erythropoiesis (EPO) and vascularization (VEGF).
- Activates transcription of genes encoding glucose transporters and glycolytic enzymes.
- Activates transcription of the PDK 1 gene shunting pyruvate away from mitochondria.
- Represses mitochondrial biogenesis and respiration thus preventing increased levels of reactive oxygen species and consequent cell dysfunction.
- Increases mitochondrial autophagy
- Coordinates a switch in the composition of cytochrome c oxidase (COX) increasing the efficiency of the latter under hypoxic conditions.

Oxygen sensing, gene expression, and adaptive responses to hypoxia



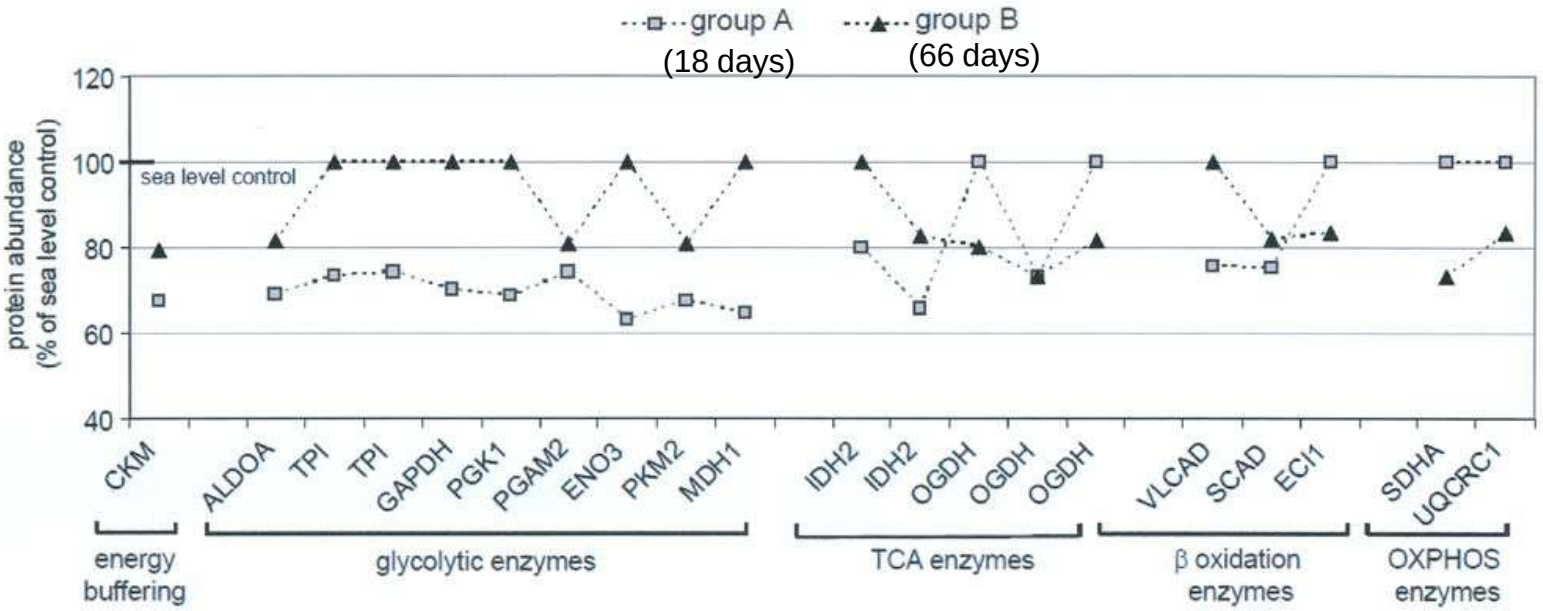
From Semenza, 2011

Regulation of glucose metabolism in response to changes in cellular oxygen levels



From Semenza, 2011

V.L. enzyme profiles after progressive increase of altitude exposure



CKM creatine kinase M type

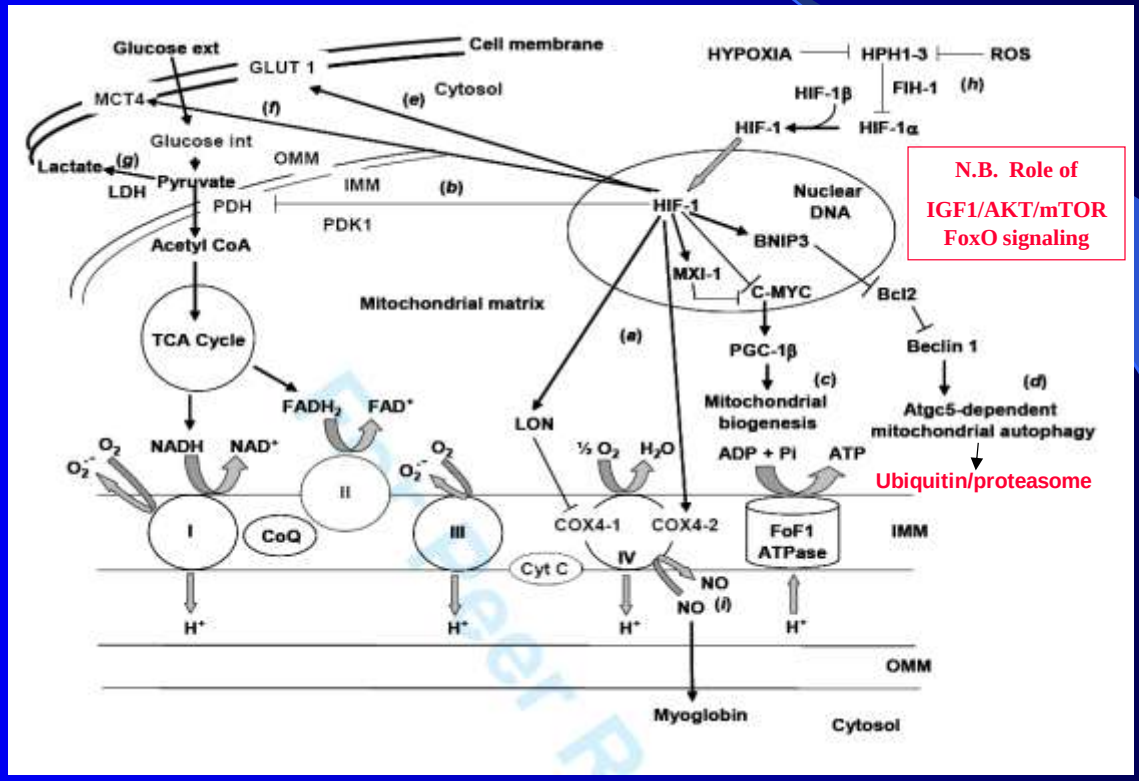
ALDOA fructose-bisphosphate aldolase A
TPI triosephosphate isomerase
GAPDH glyceraldehyde 3 phosphate dehydrogenase
PGK1 phosphoglycerate kinase 1
PGAM2 phosphoglycerate mutase 2
ENO3 beta enolase
PKM2 pyruvate kinase
MDH1 malate dehydrogenase, cytoplasmic

IDH2 isocitrate dehydrogenase
OGDH 2 oxoglutarate dehydrogenase

VLCAD very long chain specific acyl CoA dehydrogenase
SCAD short chain specific acyl CoA dehydrogenase
ECI1 enoyl CoA isomerase

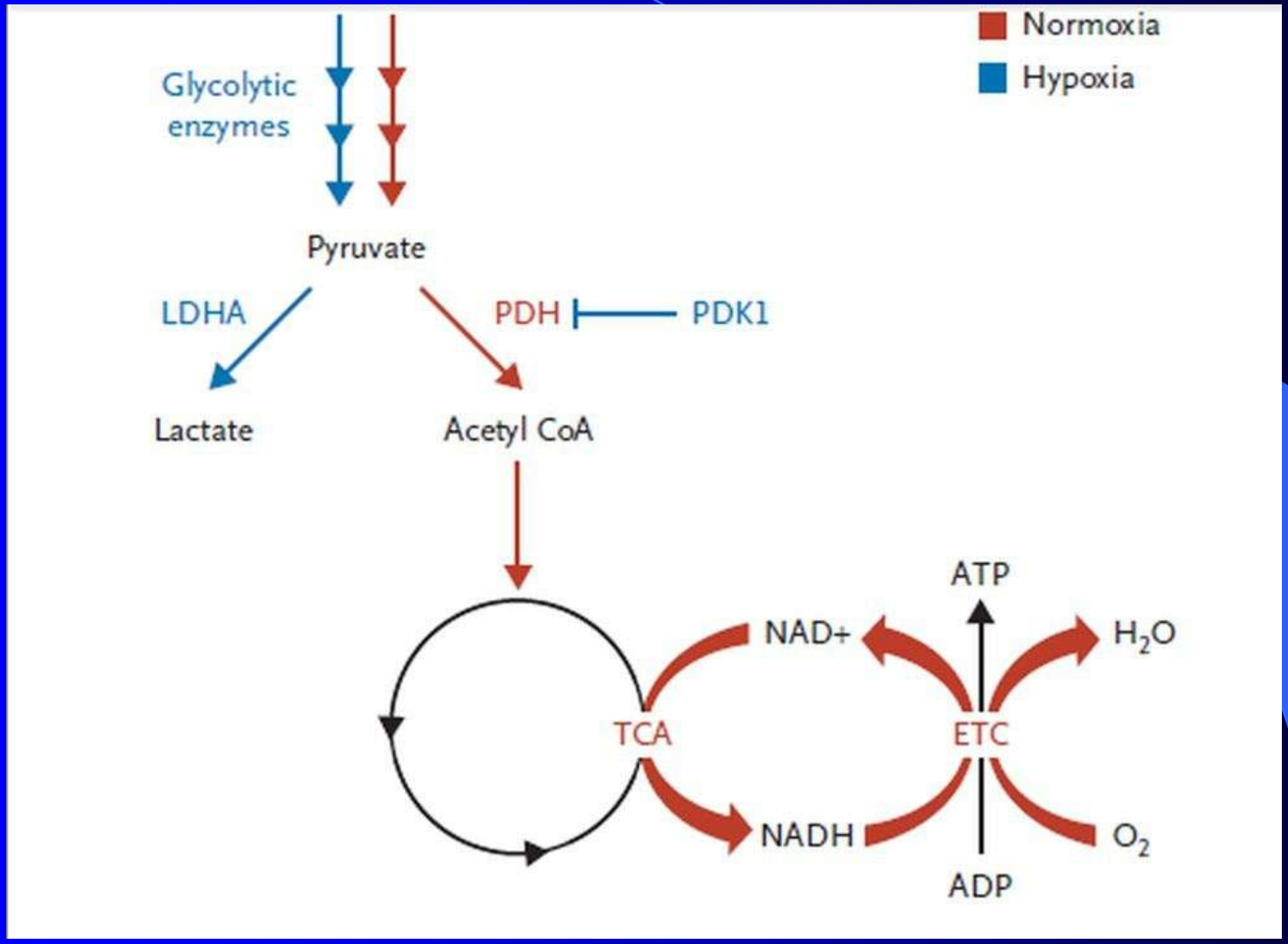
SDHA succinate dehydrogenase
UQCRC1 cytochrome b-c1 subcomplex subunit 1

Hypoxia and reactive oxygen species (ROS) prevent proteasomal degradation of HIF-1α, resulting in increased levels of HIF-1 (see *h*). The latter regulates transcription of genes enhancing a number of metabolic adaptations: *a*) a switch from COX4-1 to COX4-2 subunit, thereby increasing the efficiency of oxidative phosphorylation (the latter may depend also on the complex interaction among myoglobin, nitric oxide, and COX, see *i*);
b) inactivation of pyruvate dehydrogenase (PDH), induced by PDK1 (a gene expressing PDH kinase);
c) inhibition of mitochondrial biogenesis;
d) increased mitochondrial autophagy;
e) activated transcription of genes encoding glucose transporter GLUT 1;
f) activated transcription of genes encoding plasma membrane lactate transporter 4 (MCT4);
g) increased activity of lactate dehydrogenase (LDH).



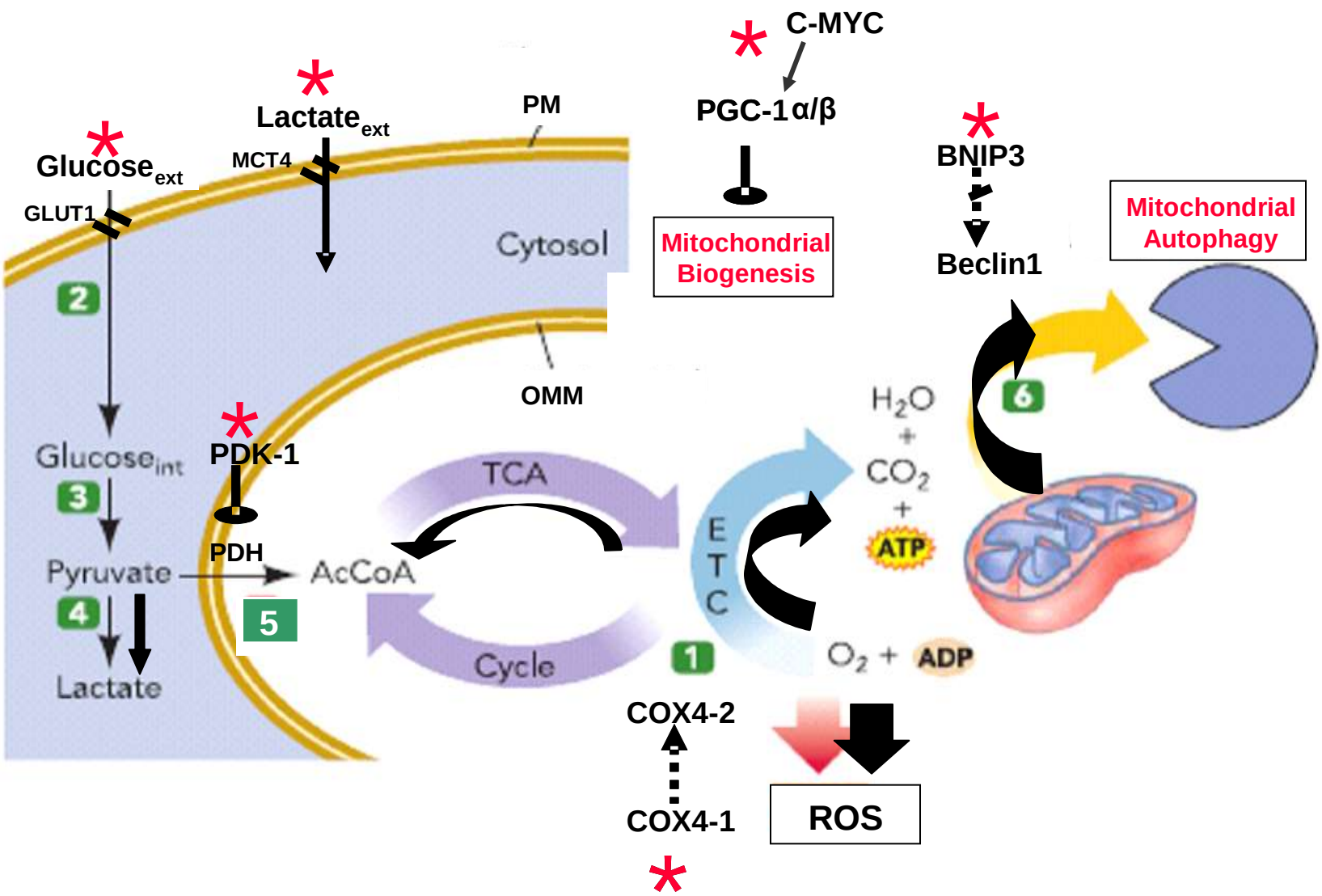
IMM and OMM refer to the inner and outer mitochondrial membrane, respectively; FIH 1 is a factor inhibiting HIF-1; BNIP3 is a cell death-related gene; Bcl2 and Beclin 1 are proteins involved in the regulation of macroautophagy; C-MYC is a transcription factor promoting mitochondrial biogenesis; MXI-1 is a gene competing with C-MYC; PCG-1β is a transcription factor involved in mitochondrial biogenesis; LON gene encodes a protease required for the degradation of the subunit COX4-1; Fo and F1 ATPase are the rotary motors driving ATP synthase; NO is nitric oxide; CoQ is Coenzyme Q10, an electron carrier in the mitochondrial respiratory chain. (For more details, see Semenza , 2007; Zhang et al, 2007 and 2008).

Regulation of glucose metabolism in response to changes in cellular oxygen levels

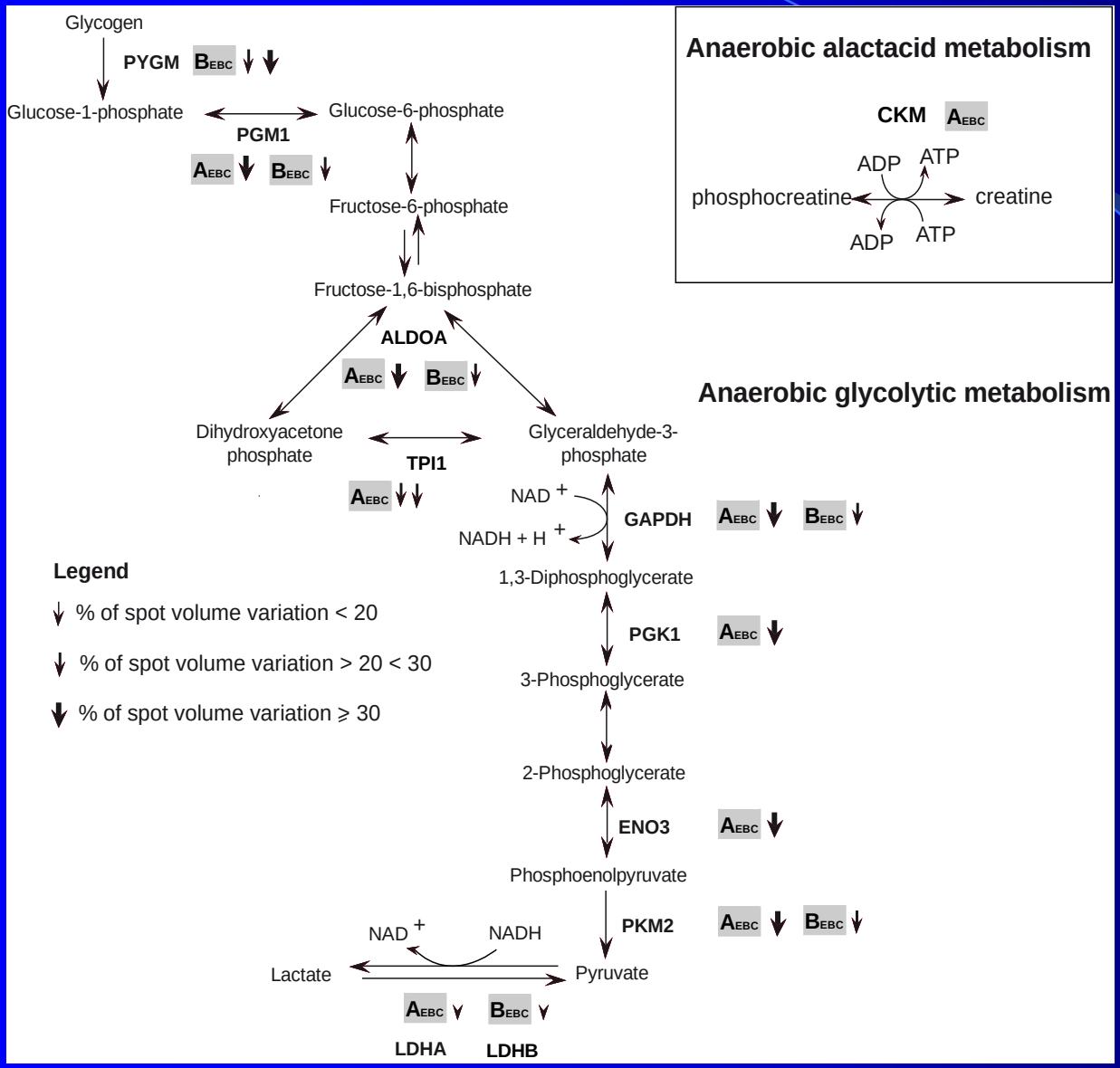


From Semenza, 2011

The regulation of energy metabolism in hypoxia (modified from Semenza, 2009)



Schematic representation of proteomic results of anaerobic (alactacid and glycolytic) metabolisms in *vastus lateralis* muscle.



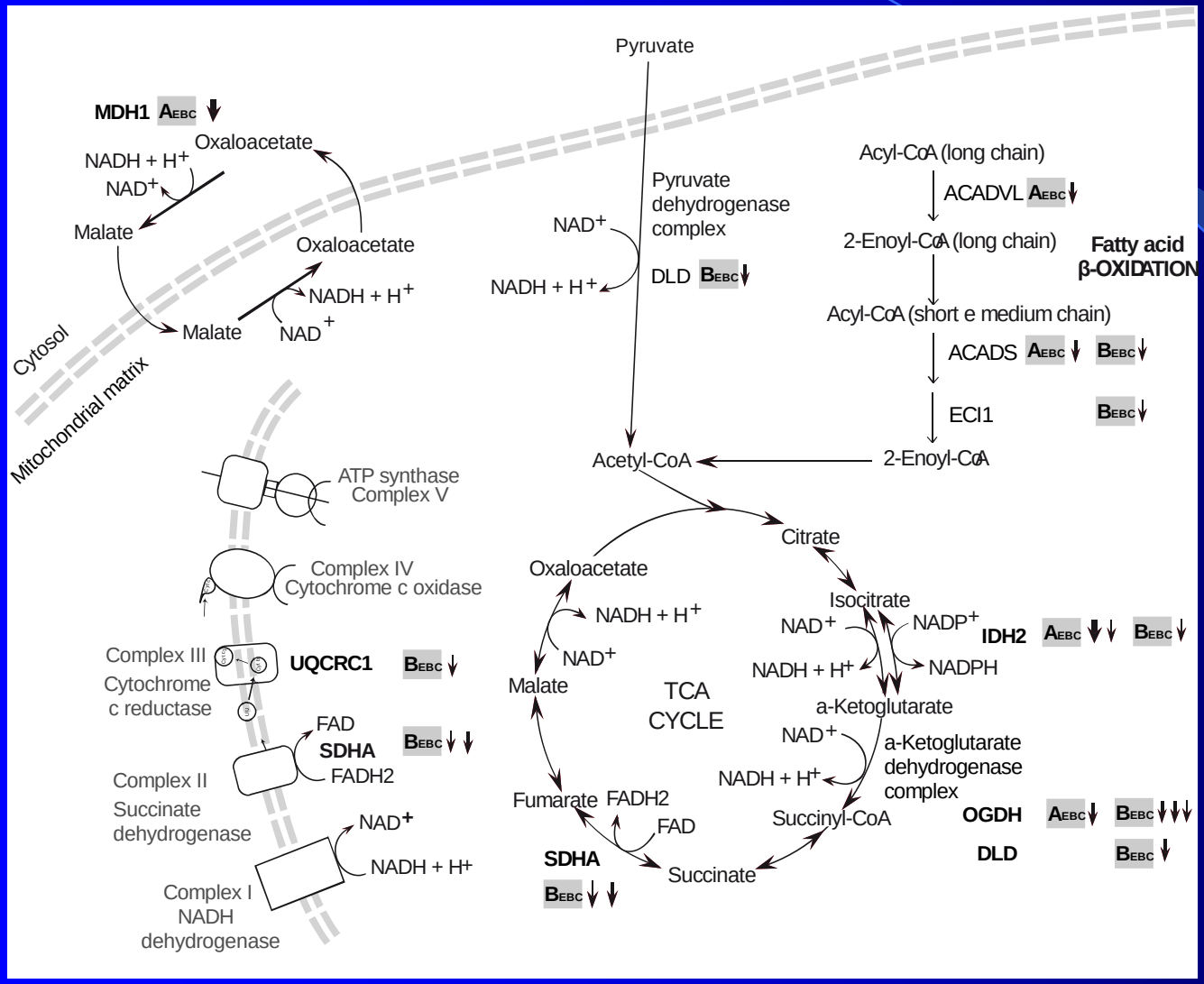
Group A: Base Camp laboratory staff
n = 5, two females, three males)
sojourning at EBC for the duration of the expedition

Group B: climbers
n = 6, males
who ascended higher on Mount Everest

CKM, creatine kinase
PYGM, glycogen phosphorylase
PGM1, phosphoglucomutase
ALDOA, bisphosphate aldolase A
TPI1, triosephosphate isomerase
GAPDH, glyceraldehyde-3-phosp dehyd
PGK1, phosphoglycerate kinase 1
ENO3, beta-enolase
PKM2, pyruvate kinase
LDHA, lactate dehydrogenase A

(from Levett et al., Proteomics 2015)

Schematic representation of proteomic results of aerobic metabolisms in vastus lateralis muscle.



Group A: Base Camp laboratory staff
n = 5, two females, three males)
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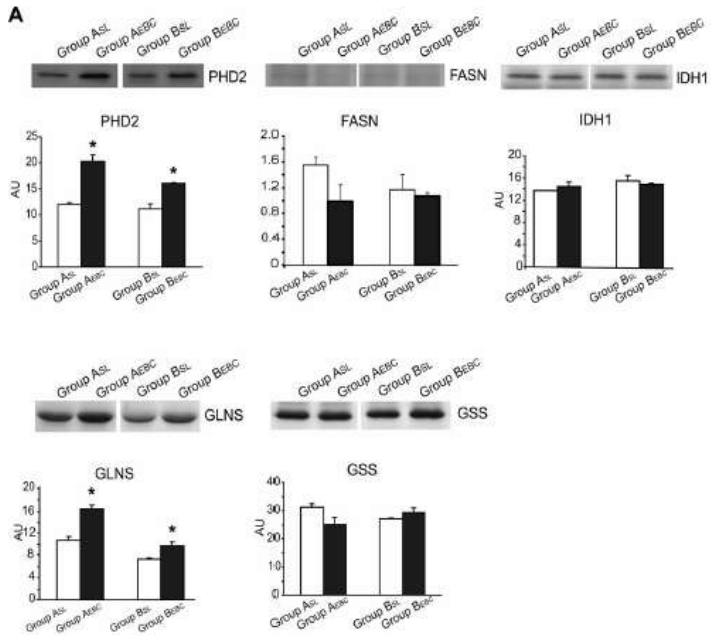
Group B: climbers
n = 6, males
who ascended higher on Mount Everest

MDH1, cytosolic malate dehyd
DLD, dihydrolipoyl dehyd
ACADVL, very long-chain acyl-CoA
ACADS, short-chain acyl-CoA dehy
ECI1, 3,2-transenoyl-CoA isomerase
IDH2, isocitrate dehydrogenase 2
OGDH, 2-oxoglutarate) dehyd
SDHA, succinate dehydrogenase
UQCRC1, cytochrome b-c1 complex

(from Levett et al., Proteomics 2015)

Schematic representation of α-ketoglutarate metabolic pathway

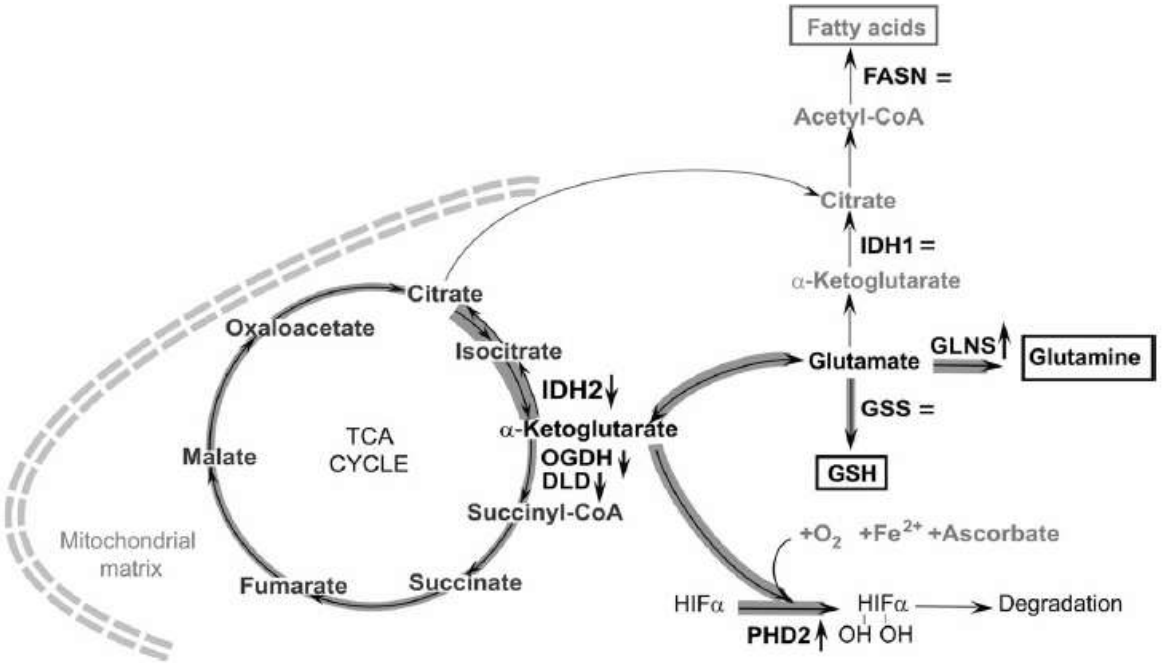


Group A: Base Camp laboratory staff
n = 5, two females, three males)
sojourning at EBC for the duration of the expedition
A_{SL}, group A sea level
A_{EBC}, group A Everest Base Camp

Group B: climbers
n = 6, males
who ascended higher on Mount Everest
B_{SL}, group B sea level
B_{EBC}, group B Everest Base Camp

PDH2, prolyl hydroxylase 2
FASN, fatty acid synthase
IDH1, isocitrate dehydrogenase 1
GLSN, glutamine synthetase
GSS, glutathione synthetase

↑, increase
↓, decrease
=, absence of variation



(from Levett et al., Proteomics 2015)

Acknowledgments

- **Dr. Mauro Marzorati & Dr. Claudio Marconi contributed a great deal of work on Himalayan natives and acclimatized Caucasians in the Pyramid laboratory at Lobuche (m.5050), Nepal.**

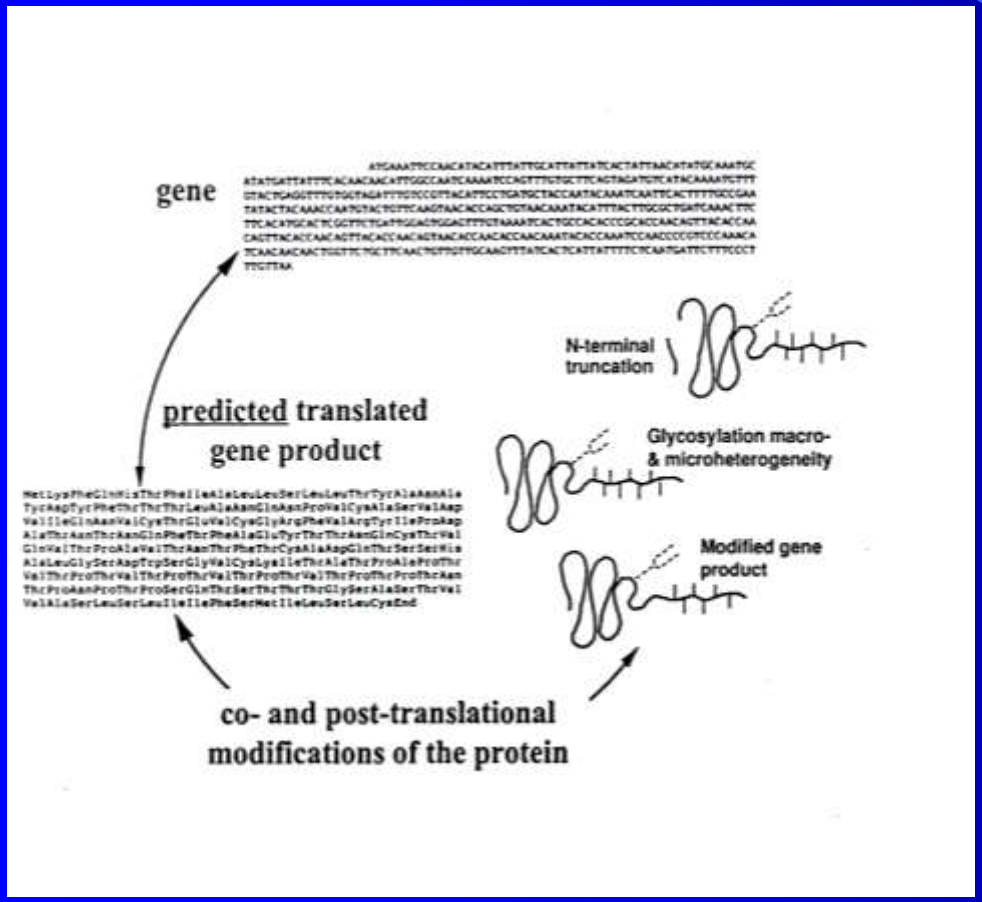
- **Prof. Cecilia Gelfi and Dott.ssa Manuela Moriggi developed muscle proteomics in humans and applied it to high altitude studies.**

The proteomic contribution in the study of man at altitude

The definition of proteome

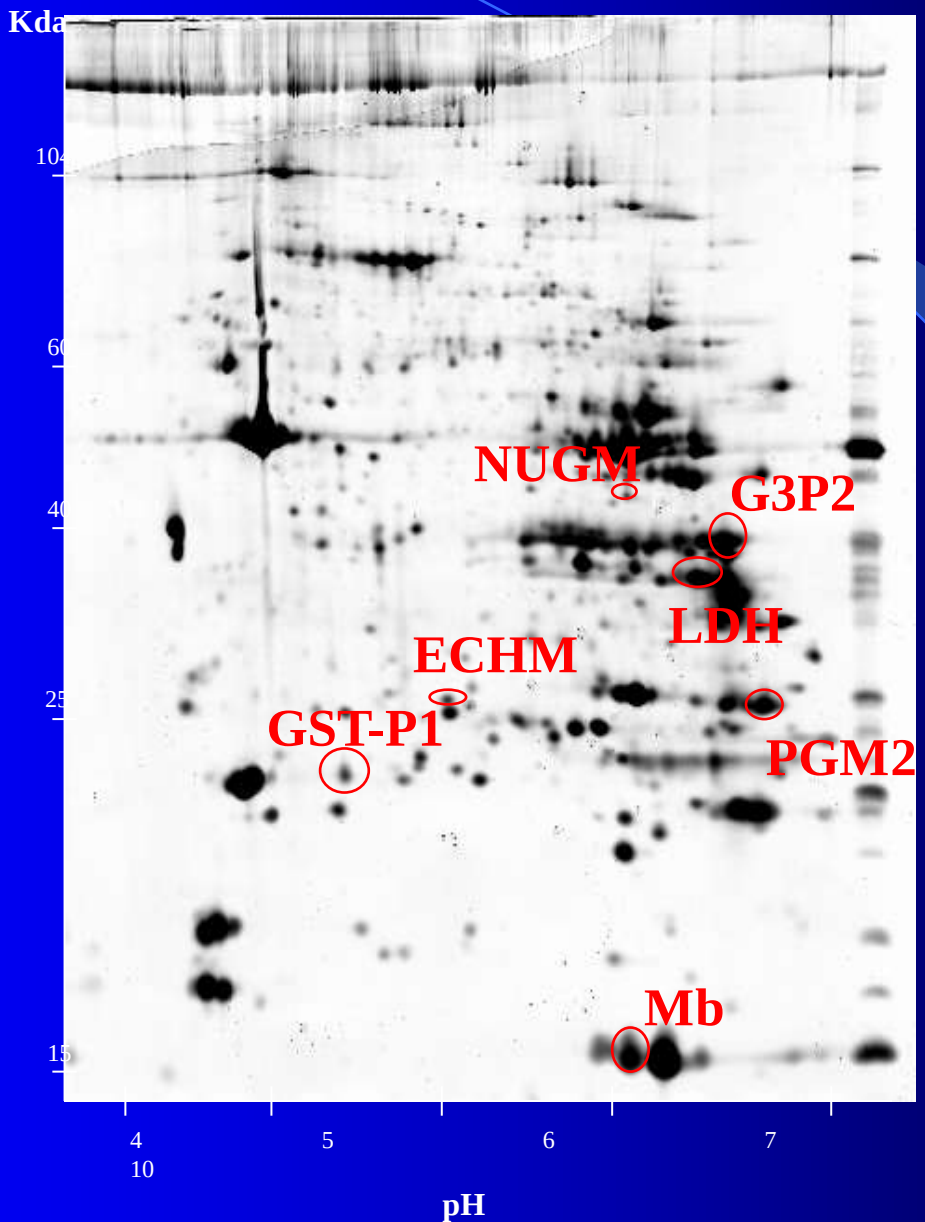
- **The proteome is defined by all proteins expressed by the genome in a given space (the cell) at a given time**

Why the proteome?

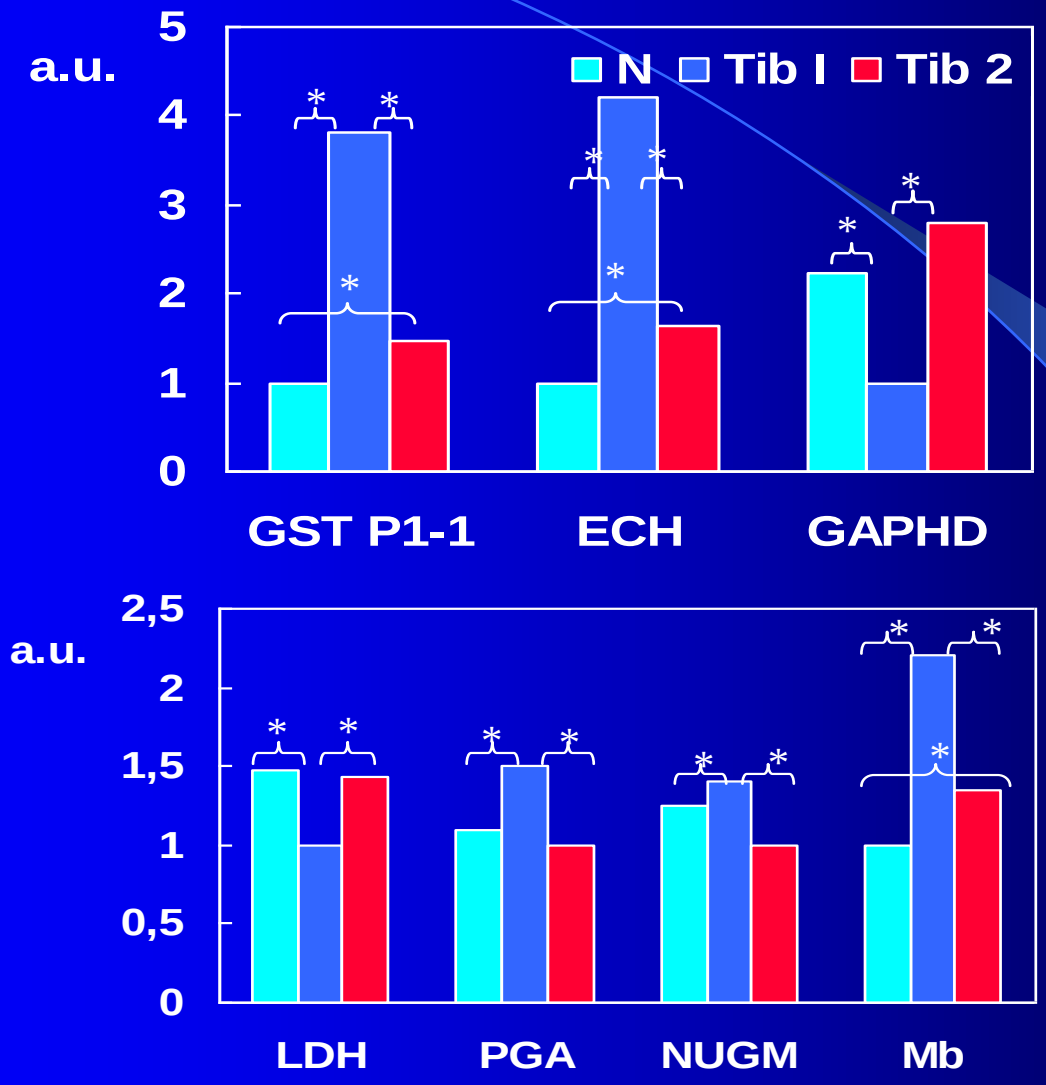


- The proteome is the protein complement of a genome representing its end product.
- The proteome is in a highly dynamic state of synthesis and degradation also as a consequence of environmental changes.
- The proteome does include also post -translational modifications.

C2) High altitude Sherpas vs. lowlanders: **“differential proteomics”**



Results



(Gelfi et al., FASEB J., 2004)