Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin?

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TERRADOS, N., E. JANSSON, C. SYLVÉN, AND L. KAIJSER. *Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin?* J. Appl. Physiol. 68(6): 2369–2372, 1990.—To compare two situations with similar magnitudes of mitochondrial substrate flux but different blood oxygen contents, one-legged training was employed. Ten healthy subjects trained one leg under normobaric conditions and the other under hypobaric conditions. At each session the subjects trained each leg for 30 min. The absolute work intensity was the same for both legs and was chosen to correspond to 65% of the average (right and left) pretraining one-legged maximal work capacity. There were three to four training sessions per week for 4 wk. Muscle biopsies from each leg were taken before and after training and analyzed for fiber types, capillaries, myoglobin, and oxidative and glycolytic enzymes. The most striking finding was a greater increase of citrate synthase activity under hypobaric conditions than under normobaric conditions. In addition, the myoglobin content increased in the leg trained under hypobaric conditions, whereas it tended to decrease in the normobarically trained leg. Because both legs were trained at the same intensity, the oxygen turnover and the substrate flux through the carboxylic acid cycle and the respiratory chain must have been of similar magnitude. Thus a difference in substrate flux is less likely to have caused the differences in enzyme activities and myoglobin content between training under normobaric and hypobaric conditions. Instead, the stimulus seems to be related to the blood oxygen content or tension.

physical training; high altitude; skeletal muscle; human; citrate synthase; creatine kinase; lactate dehydrogenase

ENDURANCE TRAINING increases the activities of mitochondrial enzymes in the working muscles (12, 32). Different stimuli have been considered to be involved in this adaptation. The increased substrate flux during the exercise bouts may be a stimulus (for review see Ref. 23). Local hypoxia has been proposed as an alternative stimulus, based mainly on studies of patients with intermittent claudication. Mitochondrial enzyme levels in calf muscles of these patients have been reported to be increased despite their moderate level of muscle use (13, 25).

To obtain an experimental model in which enzyme adaptation could be compared between two situations, with similar magnitude of substrate flux through the oxidative system but different blood oxygen content, one-legged training was employed. One leg was trained under normobaric conditions, and the other leg was trained under hypobaric conditions at the same absolute work intensity. Muscle biopsy specimens taken before and after the training period were biochemically analyzed for mitochondrial enzymes and certain cytoplasmic enzymes in the ATP-regenerating pathways as well as for myoglobin. Histochemical analyses for muscle fiber types and capillarization were also performed.

METHODS

Subjects. Ten healthy male subjects without any prior history of regular physical training volunteered for this study. Their mean age, height, and weight were 25 ± 2 yr, 182 ± 8 cm, and 74 ± 2 (SD) kg, respectively. They were fully informed about the nature and requirements of the study, which was approved by the Ethics Committee of the Karolinska Hospital. All were familiarized with the equipment, testing procedure, and one-legged cycling before the onset of the study.

Exercise tests. Under normobaric conditions, an incremental cycle ergometer test (20-W increase every 2 min) to exhaustion was performed with each leg to determine one-legged maximal work capacity (Wmax), which was defined as the maximal intensity that could be performed for 2 min. A toe clip and a heel strap held the foot of the exercising leg to the pedal. Heart rate was recorded at 1-min intervals. The average of left and right leg Wmax was used to calculate the training intensity. There was no systemic difference between right- and left-leg Wmax.

To evaluate the effect of training on work capacity under normobaric conditions, the subjects performed one-legged exercise tests with both legs to fatigue before and after the training period at a load corresponding to 80% of pretraining one-legged Wmax (100–160 W). Work capacity was expressed as time to fatigue. Heart rate was monitored at 1-min intervals throughout the exercise. The pedaling rate was 60 revolutions per minute, and fatigue was defined as the point when the subject could no longer keep up that rate. During tests and training sessions, the nonworking leg rested on the cycle ergometer between the pedals.

Training program. All training was done in a pressure chamber under normobaric or hypobaric conditions. Each subject trained one leg under normobaric conditions and the other leg under hypobaric conditions (572 Torr, corresponding to 2,300 m above sea level). Half of the group trained the left leg under normobaric and the right leg under hypobaric conditions; the other half of the group trained the right leg under normobaric and the

left leg under hypobaric conditions. There were three to four training sessions per week for 4 wk. At each training session the subjects trained each leg for 30 min, and the order of the legs was alternated from one session to the next. The intensity was chosen to correspond to 65% of the average (right and left) pretraining *W*max. The intensity was increased by the investigators over the 4 wk as the performance increased (10–15 W/wk).

Muscle biopsies and analyses. Muscle biopsy specimens from both legs were taken before the training period and 1–2 days after the last training session by the percutaneous needle biopsy technique (2). At each biopsy two specimens were taken. One was frozen in isopentane, precooled with liquid nitrogen, and used for the histochemical analysis. Serial cross sections (10 μm) were cut at −20°C and stained for myofibrillar adenosinetriphosphatase activity with preincubation at different pHs to distinguish type I, type IIa, type IIb, and type IIc fibers (3). To determine the number of capillaries, the cross sections were stained by the amylase-periodic acid-Schiff reaction (1).

The second muscle specimen was immediately frozen in liquid nitrogen and stored at −80°C for the quantitative enzyme analysis. All enzyme analyses of specimens from before and after training were performed on the same day. The activities of different enzymes were measured by fluorometric or spectrophotometric methods after the muscle specimen was freeze-dried and homogenized in a 0.1 M phosphate buffer, pH 7.7. The following enzymes were assessed: phosphofructokinase (PFK) (7), lactate dehydrogenase (LD), aspartate aminotransferase (ASAT) (24), citrate synthase (CS) (27), 3-hydroxyacyl-CoA dehydrogenase (HAD) (7), and creatine kinase (CK, *N*-acetyl-cysteine activated CK, Boehringer Mannheim Biochemicals, FRG) and its isozyme CK-MB (chromatographic separation, Boehringer Mannheim, FRG). Myoglobin was determined by a radioimmunoassay.

Statistics. All results are given as means ± SD. Intraindividual differences were tested by paired Student's *t* test.

RESULTS

The exercise time to fatigue before training was 28.3 ± 10.4 min. After training, the time to fatigue for the normobarically trained leg was significantly increased to 96.8 ± 27.0 min. The time to fatigue increased significantly more in the hypobarically trained leg, to 116.8 ± 40.4 min. Heart rate after 15 min of exercise before training was 166 ± 15 beats/min. It decreased to 146 ± 18 beats/min after normobaric training and significantly more, to 140 ± 20 beats/min, after hypobaric training. The maximal heart rate at the end of exercise before training was 176 ± 14 beats/min. It decreased to 165 ± 20 after normobaric training and similarly to 161 ± 22 beats/min after hypobaric training.

The mitochondrial enzyme activities (CS, HAD, ASAT) increased in both the normobarically and the hypobarically trained leg (Table 1). The increase was significantly larger for CS and tended to be larger for HAD and ASAT in the hypobarically trained leg than the normobarically trained leg. The myoglobin concentration decreased in the normobarically trained leg, whereas it increased in the hypobarically trained leg (Table 1). PFK activity did not change significantly in either of the legs (Table 2). LD activity decreased significantly in the hypobarically trained leg, whereas no significant change was found in the normobarically trained leg.

Total CK activity decreased in both legs with no significant difference between the legs. On the other hand, CK-MB activity increased in both legs without any significant difference between the legs. Fiber types, number of capillaries, and mean fiber area did not change significantly with training in either of the legs (Tables 3 and 4).

DISCUSSION

Both normobaric and hypobaric training substantially increased exercise time to fatigue. However, the most striking finding was the greater increase in CS activity in leg musculature trained under hypobaric than under normobaric conditions. CS is considered to be a flux-generating enzyme in the carboxylic acid cycle (20) and is used together with cytochrome oxidase as a conventional marker of oxidative capacity. In addition, the myoglobin concentration increased in the leg trained under hypobaric conditions, whereas it decreased in the leg trained under normobaric conditions. In keeping with the improved conditions for both tissue transport and utilization of oxygen, the one-legged working capacity, retested under normobaric conditions in both legs, was most increased in the hypobarically trained leg. Although the time to fatigue after training was far longer than before training and performance capacity may have been determined partly by other factors, the aerobic factors are still major determinants (10, 23).

Both legs were trained at the same intensity for the same period of time. The oxygen turnover and the substrate flux through the enzymes in the carboxylic acid cycle and the respiratory chain must therefore have been of approximately the same magnitude (26). Thus a difference in substrate flux is not a likely explanation of

TABLE 1. *Mitochondrial enzyme activities and myoglobin content before and after training*

Training Conditions	CS		HAD		ASAT		Myoglobin	
	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric
B	0.58±0.16[a]	0.55±0.13[b]	0.52±0.15[c]	0.50±0.11[b]	5.5±2.5	5.3±2.0[a]	25.0±3.6[c]	23.6±2.1[a]
A	0.65±0.13[d]	0.71±0.16	0.59±0.12	0.61±0.12	6.3±2.7	6.7±2.4	23.4±3.6[d]	25.5±3.9
A − B	0.07±0.08[d]	0.16±0.09	0.07±0.10	0.11±0.06	0.9±1.7	1.4±1.7	−1.6±1.4[e]	1.9±2.4

Values are means ± SD in μkat/g dry muscle for enzymes and mg/g dry muscle for myoglobin. B, before; A, after. Between B and A: [a] $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.1$. Between normobaric and hypobaric: [d] $P < 0.05$; [e] $P < 0.01$.

TABLE 2. *Cytoplasmic enzyme activities before and after training*

Training Conditions	PFK		LD		CK		CK-MB	
	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric
B	0.92±0.20	0.94±0.23	34±11	37±10[a]	513±94[b]	513±84[a]	9.3±1.4[a]	9.7±2.4[c]
A	0.93±0.18[d]	1.01±0.20	32±14	31±15	473±77	477±62	10.5±2.5	11.4±2.5
A − B	0.01±0.12	0.07±0.14	−2±6	−6±7	−40±26	−26±34	1.2±1.4	1.7±2.4

Values are means ± SD in μkat/g dry muscle. B, before; A, after. Between B and A: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.1$. [d] $P < 0.1$ between normobaric and hypobaric.

TABLE 3. *Muscle fiber types before and after training*

Training Conditions	Type I		Type IIa		Type IIb		Type IIc	
	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric
B	45±12	43±13	39±14	37±15	12±10	18±16	4±4	2±2
A	49±15	45±13	37±14	39±9	12±12	15±13	2±2	1±1

Values are means ± SD in percent. B, before; A, after.

TABLE 4. *Capillaries and mean fiber area before and after training*

Training Conditions	Cap/Fiber		Cap/mm^2		Mean Fiber Area, μm^2	
	Normobaric	Hypobaric	Normobaric	Hypobaric	Normobaric	Hypobaric
B	1.82±0.42	1.69±0.32	317±56	310±52	5,642±1,242	5,541±1,179
A	1.81±0.25	1.79±0.50	332±47	315±61	5,629±1,480	5,725±1,337

Values are means ± SD. Cap, capillaries; B, before; A, after.

the differences in enzyme activity increase between training under normobaric and hypobaric conditions. Arterial hypoxia produced during hypobaric conditions may possibly reduce muscular P_{O_2} as suggested by the regular finding of lowered muscle venous P_{O_2} under these conditions (16, 26). Thus the present data support the alternative hypothesis that hypoxia is a stimulus for enzyme synthesis. However, the adequate stimulus could be other factors related to arterial hypoxia, such as altered local energy balance or sympathoadrenal activity.

A lowered P_{O_2} may increase ADP and NADH concentrations, which stimulates cellular respiration to avoid a decrease in oxygen turnover rate (9, 33). A side effect of this may be a stimulation of glycolysis and lactate production. Such a change of local energy balance may add an extra "training effect" on mitochondrial enzymes during hypoxic conditions. An increased amount (activity) of mitochondrial enzymes may reduce the ADP concentration necessary to elicit a certain oxygen turnover rate, which in turn would reduce the stimulation of glycolysis (12). The oxygen debt at the onset of exercise is also known to be increased during exercise under hypobaric conditions (22), which probably contributes to the higher blood lactate concentrations found during this condition (16, 30). Increased activity of mitochondrial enzymes might lead to a faster acceleration of the cellular respiration, thereby facilitating oxygen diffusion by a faster increase in the blood-to-mitochondrion P_{O_2} gradient (for further discussion see Refs. 4, 5, and 23). Thus the greater increase in mitochondrial enzymes after training during hypobaric conditions may counteract a more pronounced disturbance of local energy balance at exercise during hypobaric conditions.

Exercise under hypobaric conditions has been shown to increase the concentration of catecholamines in the blood more than normobaric exercise (6). β-Receptor stimulation via its second messenger, adenosine 3′,5′-cyclic monophosphate, has been suggested to increase the synthesis of mitochondrial enzymes (11, 15, 18). Although these findings are controversial (8, 17, 28), it cannot be ruled out that an increased β-receptor stimulation contributed the more pronounced increase in CS activity during training under hypobaric than under normobaric conditions in the present study.

Earlier two-legged training studies in humans did not show an increase in myoglobin content under either normobaric (14, 29) or hypobaric conditions (31), although an increase has been shown in the rat (21). Furthermore, patients with intermittent claudication have normal levels of myoglobin, although mitochondrial enzymes are increased (13). The stimulus in the present study may have been stronger than that in earlier studies. First, the arterial oxygen content was decreased during the hypobaric conditions, and second, the exercise intensity per kilogram of leg muscle was higher than the intensity that can be achieved during two-legged exercise of the same duration or in patients with claudication.

In conclusion, whereas both legs performed the same amount of exercise during the training period, the increase in CS activity in the leg trained under hypobaric conditions was twice that in the leg trained under normobaric conditions. Substrate flux is therefore a less-likely candidate as a stimulus for the enzyme synthesis. Instead, the stimulus seems to be related to the lowered blood oxygen content or tension.

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