Muscle tissue adaptations of high-altitude natives to training in chronic hypoxia or acute normoxia


Muscle tissue adaptations of high-altitude natives to training in chronic hypoxia or acute normoxia. J. Appl. Physiol. 81(5): 1946–1951, 1996.—Twenty healthy high-altitude natives, residents of La Paz, Bolivia (3,600 m), participated in 6 wk of endurance exercise training on bicycle ergometers, 5 times/wk, 30 min/session, as previously described in normoxia-trained sea-level natives (H. Hoppeler, H. Howald, K. E. Conley, S. L. Lindstedt, H. Claassen, P. Vock, and E. R. Weibel. J. Appl. Physiol. 59: 320–327, 1985). A first group of 10 subjects was trained in chronic hypoxia (HT; barometric pressure = 500 mmHg; inspired O2 fraction = 0.209); a second group of 10 subjects was trained in acute normoxia (NT; barometric pressure = 500 mmHg; inspired O2 fraction = 0.314). The workloads were adjusted to ~70% of peak O2 consumption (\(V_{\text{O2peak}}\)) measured either in hypoxia for the HT group or in normoxia for the NT group. \(V_{\text{O2peak}}\) determination and biopsies of the vastus lateralis muscle were taken before and after the training program. \(V_{\text{O2peak}}\) in the HT group was increased (14%) in a way similar to that in NT sea-level natives with the same protocol. Moreover, \(V_{\text{O2peak}}\) in the NT group was not further increased by additional \(O_{2}\) delivery during the training session. HT or NT induced similar increases in muscle capillary-to-fiber ratio (26%) and capillary density (19%) as well as in the volume density of total mitochondria and citrate synthase activity (45%). It is concluded that high-altitude natives have a reduced capillarity and muscle tissue oxidative capacity; however, their training response is similar to that of sea-level residents, independent of whether training is carried out in hypobaric hypoxia or hypobaric normoxia.

MATERIALS AND METHODS

Subjects. The experiments were carried out on 20 healthy male subjects, residents of La Paz, Bolivia (3,600 m altitude). According to anthropological studies, these high-altitude natives ranged from American to European, most of them being mestizos (10). They were never exposed to a low altitude for >1 mo within 3 yr before the study. Moreover, they were not engaged in a training program during the preceding months. Their weekly exercising time averaged 4 ± 0.5 h, with two subjects reporting no physical activity at all. The subjects were fully informed about the possible risks involved in the experiment. Their main physical characteristics are given in Table 1.

Performance tests. Peak O2 consumption (\(V_{\text{O2peak}}\)) was measured on a mechanically braked Fleisch bicycle ergometer with a conventional open-circuit system, as previously described (8). The workload was increased stepwise by 30 W every 4 min until the subjects were unable to maintain the pedaling rate at 70 revolutions/min. The criterion for having reached \(V_{\text{O2peak}}\) was attaining a plateau in O2 consumption (~150 ml O2 consumption increase) with an increase in power output, blood lactate > 7 mM, and a respiratory quotient >1.1. During the test and training sessions in acute normoxia, the gas mixture was coming from a vacuum cleaner providing air to which O2 was added from a tank through a valve (Hans Rudolph model 2700). Each subject completed water and subsequently to a loss of muscle and fat mass (4) is often described as a consequence of hypoxia.

As exercise training in environmental hypoxia is often used as an ergogenic agent, one wonders about the mechanisms by which the combination of mild permanent hypoxia with training could induce organism adaptations favoring athletic performance. Recently, Levine et al. (21) suggested that an optimal strategy to improve performance at sea level would entail low-altitude training with residency at high altitude. The latter would improve cardiovascular O2 delivery due to an increased hematocrit, whereas the former would allow for maintaining high training intensities and thus stress on muscle tissue. To explore the contention that reduced muscle stress during endurance training in hypoxia could limit muscle adaptations, we hypothesized that high-altitude residents subjected to a training with supplementary O2 should show larger muscle biochemical and structural adaptations than high-altitude residents subjected to the same training protocol in hypoxia.
two incremental cycle ergometric tests separated at least by 24 h, inhaling either ambient hypoxic gas (Pb = 500 mmHg; FIO2 = 0.209) or normoxic gas (Pb = 500 mmHg; FIO2 = 0.314). Training program. These 20 high-altitude natives underwent a training program as previously described (9). A 6-wk exercise program was carried out by a first group of 10 subjects on bicycle ergometers in chronic hypoxia (HT; Pb = 500 mmHg; FIO2 = 0.209). Five training sessions were performed per week for 30 min each. The same training protocol was performed by a second group of 10 subjects but in acute normoxia (NT; Pb = 500 mmHg; FIO2 = 0.314). Workloads were adjusted when necessary to maintain a workload corresponding to ~70% of VO2peak measured either in hypoxia for the HT group or in normoxia for the NT group. Muscle biopsies and analysis by electron microscopy. Muscle biopsies of the vastus lateralis were taken at the midthigh level by using the technique of Bergström (1). A fraction of the muscle tissue was processed for electron microscopy. The tissues were immersion fixed in a 6.25% solution of glutaraldehyde in 0.1 M sodium cacodylate (adjusted to 430 mosmol for 70% of peak O2 consumption (VO2peak) in chronic hypoxia; NT, training at 70% of VO2peak in acute normoxia. *Significantly different before and after training. †Significantly different from normoxia (P < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>VO2peak, l/min</th>
<th>Normoxia</th>
<th>Hypoxia</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>HT</td>
<td>2.64 ± 0.10</td>
<td>3.02 ± 0.13*</td>
<td>2.42 ± 0.10†</td>
</tr>
<tr>
<td>NT</td>
<td>2.46 ± 0.10</td>
<td>2.94 ± 0.15*</td>
<td>2.31 ± 0.11†</td>
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</tbody>
</table>

Values are means ± SE; n, no. of subjects. HT, training at 70% of peak O2 consumption (VO2peak) in chronic hypoxia; NT, training at 70% of VO2peak in acute normoxia. *Significantly different before and after training. †Significantly different from normoxia (P < 0.05).

Statistical analysis. All data are expressed as means ± SE. A multifactorial analysis of variance was used for intergroup comparisons. The Fisher paired least significant difference was used to identify specific mean differences. In all cases, the level of significance was set at P < 0.05.
Table 2. Percent distribution and area of fiber types in vastus lateralis muscle of highlanders before and after HT and NT

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>percent Distribution of Fibers</th>
<th>Fiber Area, µm²</th>
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<tbody>
<tr>
<td></td>
<td>HT Before</td>
<td>HT After</td>
</tr>
<tr>
<td>Type I</td>
<td>50.8 ± 2.9</td>
<td>45.3 ± 4.7</td>
</tr>
<tr>
<td>Type IIA</td>
<td>30.3 ± 3.1</td>
<td>36.5 ± 3.6</td>
</tr>
<tr>
<td>Type IIA'B</td>
<td>2.9 ± 0.7</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>Type IIB</td>
<td>15.4 ± 3.2</td>
<td>15.5 ± 3.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects/group. *Significantly different between HT and NT fibres before training (P < 0.05).

There was a statistically significant difference in the percent distribution of type I and IIA fibers between the two groups before training; however, neither fiber type distribution nor fiber cross-sectional area changed significantly with either mode of training (Table 2). Capillary density increased significantly in both groups (21% in HT and 17% in NT; Table 3). This increase was due to a significant increase in the capillary-to-fiber ratio (26% in both groups) at an unchanged fiber cross-sectional area (Tables 2 and 3). The volume density of total mitochondria was increased similarly (by 47 and 43% in HT and NT groups, respectively; Table 3). However, HT induced a greater increase in subsarcolemmal than in interfibrillar mitochondria (109 vs. 38%), whereas NT had similar effects on subsarcolemmal (58%) and interfibrillar (41%) mitochondria. A significant decrease in myofibrils (-7%) occurred after training with both exercise conditions, whereas the volume density of lipids remained constant (Table 3). Both training programs induced a significant 45% increase in CS activity but not in the activity of HAD (Table 4). The PFK activity also remained unchanged.

**DISCUSSION**

We investigated the muscle structural composition and activity of key metabolic enzymes in 20 young lifelong residents of mixed ethnic origin of La Paz (3,600 m) before and after 6 wk of endurance exercise training either in environmental hypoxia or in artificial normoxia. Most of the subjects were medical or physical education students at the University of La Paz. They led an active lifestyle but were not enrolled in any systematic endurance exercise program previous to our study.

Comparing the ultrastructural composition of their vastus lateralis muscles to previous studies, we note most importantly that muscle oxidative capacity measured as volume density of total mitochondria or as CS activity was found to be reduced by some 20% compared with lowlanders of similar age and socioeconomic background (13, 14, 17). This finding is consistent with a number of studies that reported significantly reduced muscle tissue oxidative capacities in native highland populations (18, 19, 26) as well as in lowlanders after prolonged exposure to simulated or real hypoxia (11, 14, 23). The only study to our knowledge reporting on an increased muscle tissue oxidative capacity in high-altitude residents dates back to the 1960s (24). The results of that study reporting significantly higher activities of cytochrome c reductase in highlanders have been questioned, however, on the fact that physically active highlanders were compared with sedentary lowlanders. It therefore seems that a prominent and consistent effect of a hypoxic environment on human skeletal muscle tissue is a reduction in muscle oxidative capacity, i.e., peripheral O₂ demand. Also noteworthy and consistent is the finding of a much reduced intramyocellular lipid content in biopsies from untrained permanent high-altitude residents (18, 19). Lipid substrate stores in muscle fibers of highlanders are barely one-half of those seen in young untrained lowlanders (13). Maintaining energy balance in chronic hypoxia seems difficult (4, 20). It is well known that altitude can lead to weight loss and possibly to sub-

Table 3. Muscle volume densities, capillary-to-fiber ratio, capillary density, and fiber area before and after HT and NT

<table>
<thead>
<tr>
<th></th>
<th>HT Before</th>
<th>HT After</th>
<th>NT Before</th>
<th>NT After</th>
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<tbody>
<tr>
<td>Muscle volume density, %</td>
<td>3.61 ± 0.17</td>
<td>4.98 ± 0.13*</td>
<td>3.28 ± 0.27</td>
<td>4.61 ± 0.28*</td>
</tr>
<tr>
<td>Interfibrillar mitochondria</td>
<td>0.55 ± 0.10</td>
<td>1.15 ± 0.11*</td>
<td>0.43 ± 0.07</td>
<td>0.68 ± 0.11*</td>
</tr>
<tr>
<td>Subsarcolemmal mitochondria</td>
<td>4.17 ± 0.21</td>
<td>6.13 ± 0.13*</td>
<td>3.71 ± 0.33</td>
<td>5.29 ± 0.37*</td>
</tr>
<tr>
<td>Total mitochondria</td>
<td>0.19 ± 0.05</td>
<td>0.37 ± 0.06</td>
<td>0.20 ± 0.04</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>Lipids</td>
<td>81.2 ± 0.8</td>
<td>75.0 ± 1.1*</td>
<td>84.1 ± 0.06</td>
<td>78.4 ± 0.9*</td>
</tr>
<tr>
<td>Myofibrils</td>
<td>1.39 ± 0.06</td>
<td>1.77 ± 0.10*</td>
<td>1.42 ± 0.07</td>
<td>1.79 ± 0.12*</td>
</tr>
<tr>
<td>Capillaries/fiber</td>
<td>395 ± 24</td>
<td>476 ± 22*</td>
<td>414 ± 19</td>
<td>484 ± 24*</td>
</tr>
<tr>
<td>Capillary density, mm²²</td>
<td>3,575 ± 160</td>
<td>3,732 ± 151</td>
<td>3,438 ± 141</td>
<td>3,734 ± 229</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects/group. *Significantly different before and after training (P < 0.05).
Table 4. Muscle enzyme activities before and after HT and NT

<table>
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<tr>
<th></th>
<th>HT</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>PFK</td>
<td>43.9±5.7</td>
<td>48.0±6.7</td>
<td>45.4±8.1</td>
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</tr>
<tr>
<td>CS</td>
<td>10.0±0.7</td>
<td>9.9±1.0</td>
<td>14.4±1.0*</td>
<td>14.7±1.4*</td>
</tr>
<tr>
<td>HAD</td>
<td>7.3±0.9</td>
<td>6.5±1.1</td>
<td>7.8±0.6</td>
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</tbody>
</table>

Values are means ± SE for 10 subjects/group. PFK, phosphofructokinase; CS, citrate synthase; HAD, 3-hydroxyacyl-CoA dehydrogenase. *Significantly different before and after training (P < 0.05).

strate depletion in muscle cells. An increased reliance of muscle cells on glucose metabolism has been previously described at rest as well as during exercise (3). The low muscle lipid content in the group of young students investigated in this study seems related more to hypoxia than to diet because they were from a privileged socioeconomic background and can hardly have been described as malnourished. As shown in a previous paper (10; see Table 1), the anthropometric data of our population show that height, weight, body mass index (considered as a relatively good index of nutritional status), and percent fat are within the standard range.

With regard to the capillary supply of muscle fibers of high-altitude-exposed subjects, the literature data are varied. Some of this variability must be attributed to the technical difficulties involved in measuring fiber cross-sectional area in muscle biopsies when both the degree of fiber contraction and the alignment of muscle fibers cannot be controlled (33). By using young untrained lowlanders as a comparison (13), the present study indicates that muscle capillarity is reduced in close proportion to the reduction in oxidative capacity, whereas the reduction in capillary density is brought about by a reduction in the capillary-to-fiber ratio at a constant fiber size. Similar results have been obtained in a group of Quechuas, also natives of the high Andes (26), and in Sherpas native to the Himalayas (18). In permanent high-altitude residents, we thus find that the number of capillaries supplying similar-sized muscle fibers is reduced in proportion to the reduction of mitochondria within these fibers. In contrast, lowlanders exposed to simulated or real hypoxia are capable of improving muscle tissue O₂ supply (11, 14, 23). This is achieved by maintaining a constant capillary-to-fiber ratio when fiber size is reduced. As a consequence, capillary density as well as capillary length per volume of mitochondria is significantly increased in lowlanders after long-term exposure to hypoxia (14).

Fiber type distribution as determined from ATPase staining characteristics showed a significantly lower value for type I fibers in one of the experimental groups (NT). This finding is likely a chance result. Taken together, both groups are well within fiber type distributions reported for the untrained human vastus lateralis muscle (8).

When subjected to exercise training for 6 wk on a bicycle ergometer, HT and NT subjects were able to improve their VO₂peak similarly by close to 15% whether measured in hypoxia or in normoxia [for a detailed analysis of the functional results, see Favier et al. (9)]. This increase in VO₂peak is of the same magnitude as that observed in untrained lowlanders subjected to a similar training regimen under near sea-level conditions (13, 25). In particular, it appears that training in acute normoxia, i.e., at 19% higher absolute exercise intensity, did not convey any functional advantage.

In neither of the groups did we observe an increase in the type I fiber population and a decrease in the type IIB population as previously reported for this type of training in lowlanders (16). Because short-term endurance exercise training is not consistently observed to lead to fiber type shifts, we would not attribute much significance to the lack of fiber type plasticity in this study. Fiber size did not change significantly in either group whether measured globally (morphometry of electron micrographs) or by fiber type (morphometry of histochemistry). The difference in fiber size between measurements taken from fixed or frozen tissue is related to tissue shrinkage incurred when the tissue is processed for electron microscopy (7).

Whatever the O₂ availability during training, we observed a similar increase in muscle tissue oxidative capacity of close to 45% in both groups whether measured morphometrically (volume density of total mitochondria) or biochemically (CS activity). These changes are entirely consistent with those observed in lowlanders subjected to the same training paradigm (13, 25). Supplementary O₂, enabling the NT subjects to train at higher absolute workloads, did not seem to add to the training effect. Note that the NT group was always subjected to the environmental hypobaric hypoxia characteristic of La Paz except during the training periods. As protein synthesis is known to be sensitive to the intracellular oxygenation state (27, 30, 32), this may have affected muscle cell remodeling in the recovery period between exercise sessions for both groups. Acute normoxia during training did affect the response of specific subpopulations of mitochondria, however. A much larger increase in subsarcolemmal mitochondria could be observed in the HT group (109%), very much in line with the results obtained in lowlanders (13). In NT subjects, both subsarcolemmal and interfibrillar mitochondria increased in similar proportions. Because the functional significance of the subsarcolemmal vs. the interfibrillar location of mitochondria is currently being debated (5, 15), our findings merely support the concept that these two populations should indeed be distinguished, at least based on their capacity to respond differently to extrinsic stimuli.

Neither of the two experimental groups increased the volume density of intracellular lipid stores significantly with exercise training. Lipid concentrations in excess of 1% of the fiber volume are a consistent finding in lowlanders subjected to the same 6-wk training protocol as the subjects in this study (13). The lack of a structural adaptation of the lipid substrate stores in muscle cells is all the more surprising because pretraining values were very low indeed. As previously mentioned, either dietary influences or hypoxia-induced
shifts in muscle fiber metabolism may be responsible for these findings, which need to be confirmed and expanded. An important finding in this context is the lack of an increase in the capacity to oxidize free fatty acids, as indicated by an unchanged HAD activity; this enzyme is usually observed to increase its activity with endurance exercise training (12). The increase in CS in parallel with total mitochondrial volume density supports the morphometric data. No change occurred in muscle glycolytic capacity as estimated by an unchanged activity of PFK.

The results of this study clearly suggest that, although NT subjects were subjected to a 19% higher absolute training intensity, there were no functional or structural improvements over those seen in the HT group. The hypothesis of this study, namely, that training in hypoxia reduces stress on muscle, thus limiting muscle tissue adaptations, therefore has to be rejected. However, because improvement of athletic performance as a consequence of high-altitude training possibly has multiple causes, it does not mean that the contention that it may be beneficial for athletes to live at altitude but to train at sea level is necessarily wrong (21). For one, the major gain in $V_{\text{O}_2}\text{peak}$ and performance in lowland athletes was brought about by an increase in hematocrit due to altitude exposure, whereas in high-altitude residents, the increase in $V_{\text{O}_2}\text{peak}$ was the consequence of exercise training at a constant but elevated hematocrit in both NT and HT (9).

It is further worth mentioning that this study was carried out on untrained subjects. It cannot be excluded that untrained subjects are capable of maximally increasing structural and functional performance parameters. Hence training at a reduced exercise intensity at altitude may, in fact, be detrimental with regard to maintaining optimal structural muscle capacities for aerobic metabolism in athletes. This remains to be proven, however, with direct observations. With regard to exercise intensity, it should also be considered that the reduction in $V_{\text{O}_2}\text{peak}$ and sustainable work intensity is substantially larger in lowlanders exposed to acute hypoxia than the gain in aerobic performance and $V_{\text{O}_2}\text{peak}$ of permanent high-altitude residents on exposure to acute normoxia (10). This would result in a larger penalty of lowlanders training in hypoxia compared with a smaller advantage of highlanders training in acute normoxia.

In conclusion, this study lends further support to previous observations that skeletal muscle tissue consistently responds to permanent hypoxia by a decrease in oxidative capacity. In contrast to lowlanders exposed to acute hypoxia, high-altitude residents seem to reduce capillarity in proportion to oxidative capacity. The training response of highlanders to a standard endurance exercise protocol is similar to that of the lowlanders with regard to improving $V_{\text{O}_2}\text{peak}$, mitochondrial content, oxidative enzyme activity, and capillary supply. In contrast, neither HAD enzyme activity nor the low intramyocellular lipid stores are increased with training in highlanders. Supplementary $O_2$ during training, allowing subjects to work at higher absolute workloads, has no effect on the magnitude of the training-induced functional or structural adaptations. The hypothesis that reduced muscle stress in hypoxia could limit muscle adaptations in high-altitude training is therefore rejected for a population of previously untrained permanent high-altitude residents.

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