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Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions

M. VOGT,[1] A. PUNTSCHART,[1] J. GEISER,[2] C. ZULEGER,[2,†]
R. BILLETER,[1] AND H. HOPPELER[1]
[1]*Institute of Anatomy, University of Bern, 3012 Bern; and* [2]*Institute of Physiology, University of Fribourg, 1700 Fribourg, Switzerland*

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Vogt, M., A. Puntschart, J. Geiser, C. Zuleger, R. Billeter, and H. Hoppeler. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J Appl Physiol* 91: 173–182, 2001.—This study was performed to explore changes in gene expression as a consequence of exercise training at two levels of intensity under normoxic and normobaric hypoxic conditions (corresponding to an altitude of 3,850 m). Four groups of human subjects trained five times a week for a total of 6 wk on a bicycle ergometer. Muscle biopsies were taken, and performance tests were carried out before and after the training period. Similar increases in maximal O_2 uptake (8.3–13.1%) and maximal power output (11.4–20.8%) were found in all groups. RT-PCR revealed elevated mRNA concentrations of the α-subunit of hypoxia-inducible factor 1 (HIF-1) after both high- (+82.4%) and low (+78.4%)-intensity training under hypoxic conditions. The mRNA of HIF-1α^{736}, a splice variant of HIF-1α newly detected in human skeletal muscle, was shown to be changed in a similar pattern as HIF-1α. Increased mRNA contents of myoglobin (+72.2%) and vascular endothelial growth factor (+52.4%) were evoked only after high-intensity training in hypoxia. Augmented mRNA levels of oxidative enzymes, phosphofructokinase, and heat shock protein 70 were found after high-intensity training under both hypoxic and normoxic conditions. Our findings suggest that HIF-1 is specifically involved in the regulation of muscle adaptations after hypoxia training. Fine-tuning of the training response is recognized at the molecular level, and with less sensitivity also at the structural level, but not at global functional responses like maximal O_2 uptake or maximal power output.

hypoxia training; gene expression; oxygen sensor system

EXPOSURE TO ALTITUDE HAS SPECIFIC biological effects in humans. Continuous residence at moderate heights (2,000–2,500 m) improves the oxygen transport capacity by an erythropoietin-induced increase in the hematocrit (6). An increase in the hemoglobin concentration has been shown to augment maximal O_2 consumption ($\dot{V}O_{2\,max}$) and to enhance exercise performance (10). Because a 2- to 3-wk exposure to such altitudes is sufficient to elicit these adaptations, competitive endurance athletes often live and train at moderate altitudes to attain peak performance at altitude (1, 4). Although improved endurance performance is achieved under altitude conditions, the effects of altitude training on sea-level performance show contradictory results. Acute mountain sickness, problems with acclimatization, and detraining due to decreased intensity are believed to influence the effectiveness of altitude training (4).

To evoke an increase in the hemoglobin concentration without incurring the deleterious effects of altitude exposure, some athletes sleep and live at moderate altitudes but train near sea level. Levine and Stray-Gundersen (23) have shown that 4 wk of "living high-training low" improves sea-level running performance in practiced runners because of increases in red cell mass and $\dot{V}O_{2\,max}$, whereas "living high-training high" or "living low-training low" for similar periods elicits no such improvement in running performance.

On the other hand, when training alone is performed under hypoxic conditions (e.g., "living low-training high"), increased mitochondrial densities, capillary-to-fiber ratios, and fiber cross-sectional areas have been observed (9). Other studies that used similar training protocols have demonstrated significant increases in the activities of oxidative enzymes and in capillary density (13, 27, 43). In each of these investigations, the activity of citrate synthase was elevated to a greater extent after training at the same level of intensity under hypoxic than under normoxic conditions. One study revealed a significantly higher myoglobin (Mb) protein content only after training in hypoxia (43). After living low-training high, endurance performance and $\dot{V}O_{2\,max}$ are improved when tested in normoxia and in hypoxia (27, 38, 44). The results of these studies suggest that exercise under hypoxic conditions could possibly induce muscular and systemic adaptations, which are either absent or found to a lesser degree after training under normoxic conditions.

We speculate that hypoxia training induces specific molecular adaptations in human muscle tissue. In cell culture experiments, hypoxia has been shown to activate a transcription factor, hypoxia-inducible factor 1

†Deceased.

Address for reprint requests and other correspondence: M. Vogt, Univ. of Bern, Institute of Anatomy, Buehlstrasse 26, 3012 Bern, Switzerland (E-mail: vogt@ana.unibe.ch).

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(HIF-1), which binds to a specific enhancer sequence of the erythropoietin gene for transcriptional activation (42). HIF-1 is expressed in all mammalian tissues thus far analyzed, including skeletal muscle (51). It is a dimeric protein composed of the regulatory subunit HIF-1α, which is encoded by 15 exons (20), and the constitutively expressed subunit HIF-1β, which is identical to the aryl hydrocarbon receptor nuclear translocator (ARNT) (48). Transactivation of HIF-1 is necessary for the induction of several genes, such as those encoding the glycolytic enzymes, the vascular endothelial growth factor (VEGF), and the GLUT-1, as well as other metabolic proteins (50). These and other data indicate that HIF-1 is involved in the cellular oxygen-sensing system (6). Activation of HIF-1 appears to trigger adaptations, which diminish the negative effects of chronic exposure to hypoxia. Experiments with HIF-1α-deficient mice exposed to chronic hypoxia for 6 wk have revealed delayed hypertrophy of the right ventricle and reduced body weights compared with normal mice (54).

We hypothesize that HIF-1 might play a key role in mediating the hypoxia-specific adaptations reported in a number of publications (9, 13, 27, 38, 43, 44). We postulate that, in human skeletal muscle, gene expression depends both on training intensity and on the presence or absence of hypoxia during the training session. To test these hypotheses, we compared the effects of 6 wk of endurance training at two levels of intensity under hypoxic and normoxic conditions in human subjects. Performance tests were conducted before and after the training period. With the use of biopsies of skeletal muscle, morphometric analyses were performed, and changes in the levels of various mRNAs were quantified by RT-PCR.

METHODS

Subjects, Training Protocol, and Exercise Testing

Thirty untrained male volunteers participated in this study (see Table 1 for anthropometric data), with informed written consent being obtained in each case. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Bern, Switzerland.

The subjects were randomly assigned to one of four groups (Table 1). They trained for 30-min periods five times a week for a total of 6 wk on an electrically braked precision ergometer. Two groups trained at a high-intensity level (training blood lactate levels: 4–6 mM): one under normoxic (Nor-high) and the other under normobaric hypoxic conditions (Hyp-high). The other two groups trained at a low-intensity level (training blood lactate levels: 2–3 mM): again one under normoxic (Nor-low) and the other under normobaric hypoxic conditions (Hyp-low). Hyp-high and Nor-low groups trained at the same percentage of normoxic maximal power output ($\dot{W}_{max}$) (e.g., similar absolute values of ATP-turnover). High-intensity training groups and low-intensity training groups trained at the same percentage of $\dot{W}_{max}$ in each condition (e.g., similar relative values of ATP turnover). Normobaric hypoxic conditions, corresponding to an altitude of 3,850 m (inspired P_{O_2} of 89 Torr), were simulated by diluting ambient air with nitrogen. This was achieved by injecting nitrogen into a 200-liter mixing chamber through which a constant, desired, expected inspired P_{O_2} was achieved, which was monitored with a Taylor Servomex. The subjects training under hypoxic conditions breathed through face masks connected to the mixing chamber via the appropriate tubing.

Table 1. *Anthropometric data and training conditions*

Group	Nor-high	Hyp-high	Nor-low	Hyp-low
No. of subjects	8	7	8	7
Age, yr	25 ± 3	23 ± 2	29 ± 13	23 ± 2
Height, cm	181 ± 5	180 ± 6	181 ± 6	177 ± 4
Body weight, kg	75.9 ± 8.8	75.1 ± 9.2	75.5 ± 13.4	79.4 ± 13.5
$\dot{V}O_{2max}$, ml·min^{-1}·kg	50.7 ± 5.1	51.0 ± 7.0	48.4 ± 8.0	47.7 ± 6.0
Training intensity	high	high	low	low
Training lactate, mM	4–6	4–6	2–3	2–3
Training altitude, m	600	3,850	600	3,850
$P_{I_{O_2}}$, Torr	138	89	138	89
$\dot{W}_{absolute}$, %	67.0	54.3	57.8	42.7
$\dot{W}_{relative}$, %	67.0	65.6	57.8	52.4

Nor-high and Nor-low: groups that trained at a high- and low-level of intensity under normoxic conditions, respectively; Hyp-high and Hyp-low: groups that trained at a high- and low-level of intensity under normobaric hypoxic conditions, respectively; $\dot{V}O_{2max}$, maximal O_2 uptake; $P_{I_{O_2}}$, inspired P_{O_2}; $W_{absolute}$, training intensity expressed as a percentage of the maximal power output under normoxic conditions; $W_{relative}$, training intensity expressed as a percentage of the maximal power output in the group-specific training environment under normoxic or hypoxic conditions. $\dot{V}O_{2max}$, body weight, age, and height were determined initially before the onset of the study (means ± SE). To achieve the expected training intensity, lactate was monitored and workload was adjusted periodically during the training period.

Before and after the 6-wk training period, $\dot{V}O_{2\,max}$ and $\dot{W}_{max}$ were determined by means of incremental step tests (to exhaustion) for each subject under normoxic and hypoxic conditions. The exercise began at a level of 100 W, which was increased every 2 min by 30 W until the subject could no longer maintain a cadence over 60 rpm, despite verbal encouragement.

Muscle Biopsy Sampling

Using the Bergstrom et al. (3) technique, biopsies were taken at midthigh level from vastus lateralis muscle after at least 24 h without any exercise activity before and after the 6-wk training period. For mRNA analyses, about one-half of the muscle tissue was immediately frozen in isopentane, cooled by liquid nitrogen, and then stored in the latter until required for analyses. The remaining muscle tissue was fixed in buffered glutaraldehyde solution for electron microscopy (18).

Morphometric Analysis

Fixed muscle biopsy samples were processed and sectioned according to standard protocols (14, 18). Tissue blocks were sectioned using an isotropic uniform random method so as to obtain an unbiased estimation of capillary length and fiber size (for details, see Ref. 47). For the ultrastructural analysis of muscle fibers, point counts were taken from 40 micrographs (at a final magnification of ×24,000), whereas, for the determination of capillary length density and fiber size, they were taken from 16 (at a final magnification of ×1,800).

RNA Extraction and Reverse Transcription

Total RNA was prepared by using the acid-phenol method (8), as described by Puntschart et al. (30). The total RNA

obtained was reverse transcribed with Superscript RNase H-RT (GIBCO BRL, Life Technologies), by means of random hexamer priming in accordance with the manufacturer's specifications. After 1 h at 37°C, the enzyme was heat inactivated (at 95°C) for 10 min. The incubation medium was then diluted to 200 μl with TE solution (10 mM Tris and 1 mM EDTA, pH 7.4), which was used directly for PCR. To correct for differences in the amount of total RNA, the amount of each PCR product was normalized by the amount of 28S rRNA, as described previously (32).

Primers and Oligodeoxynucleotides

The locations of the primers used for PCR and of the biotin-labeled nested oligodeoxynucleotides employed for the ELISA quantification are depicted in Table 2.

PCR

Specific mRNAs (Table 2) were quantified by using a statistical PCR approach, as previously described (31). An updated and detailed account of this method has been published (32). Owing to the statistical approach adopted, small differences of down to 30% for a specific mRNA were detectable (31, 32). One of the most important requisites for the successful quantification of relative differences in mRNA content among samples is that all samples be processed in parallel at each stage (RNA extraction, RT-PCR, and ELISA quantification). For a specific sample, some differences in the yield of the PCR product were found from one PCR to another, whereas only small discrepancies were detected within the same PCR (31). Due to the limitations of our equipment, we were unable to process samples for each of the four groups together; however, all of those derived from one group (e.g., pre- and posttraining) were treated and analyzed in parallel at all stages, and hence with the same efficiency. This design limitation rendered impossible a comparison of absolute mRNA values among groups. For this reason, pretraining values were normalized, and relative changes were determined.

Setup PCR runs were performed to determine the specific conditions for PCR master mix and temperature and cycle profile of each mRNA to be quantified. For each mRNA, at least three PCR runs were performed. For each run, a master mix, containing buffer, primers, nucleotides, digoxigenin-dUTP (PCR DIG labeling mix, Roche Diagnostics) and Dynazyme DNA polymerase (Finnzymes, Bioconcept), was prepared. Two microliters of each RT mix (undiluted for all PCRs except 28S, in which case a further dilution of 1:10 was necessary) were transferred to 200-μl wells of a Thermo-Fast 96-well plate (Biolabo), to which 38 μl of the master mix were then added. The plate was transferred to a preheated (95°C) thermocycler with a heated lid (UNO-Thermoblock, Biometra), and PCR steps run according to the conditions specified for each mRNA in the setup PCR runs.

ELISA Quantification of PCR Products

PCR products were quantified by hybridizing them to specific biontinylated probes and were detected by means of the ELISA, as previously described (32). Absorbance was determined 30–120 min after incubation with *p*-nitrophenyl phosphate (Roche Diagnostics).

Statistics

The statistical analysis was performed by using a statistic software package (Statistica 5.1 for Windows, Statsoft). Differences between values obtained pre- and posttraining for a particular group were analyzed by using Student's paired *t*-test. The effects of hypoxia or training intensity on differences among the four groups were assessed by two-way ANOVA. Data are presented as means ± SE. Percent changes in means (post- vs. pretraining) are given, with the pretraining values being normalized to 1. For the comparison of changes in mitochondrial and nuclear-coded mRNAs, data relating to cytochrome oxidase 1 (COX-1) and NADH dehydrogenase subunit 6 (NADH6), plus COX-4 and succinate dehydrogenase (SDH), were averaged. Because of large differences in the absolute concentration of these mRNAs, values are expressed as means of the individual percent changes. Differences were considered to be significant for $P < 0.05$ and to be a tendency for $P < 0.10$.

Table 2. *Gene bank accession code, product length, primer, and oligodeoxynucleotide location*

Sequence	Gene Bank Access Code	Primer Location		Product Length	Oligo Location
		5′ end	3′ end		
28S	M11167	4,535–4,564	4,628–4,667	132	4,583–4,606
COX-1	M10546	2,361–2,390	2,527–2,554	194	2,418–2,442
COX-4	M34600	322–349	463–489	168	421–445
SDH	D90047	278–303	450–476	199	369–393
NADH6	X62996	13,889–13,862	13,651–13,622	268	13,810–13,786
VEGF	M32977	140–166	270–296	157	215–234
Mb	X00373	297–323	618–643	347	378–397
HIF-1α	U22431	2,308–2,332	2,533–2,552	245	2,372–2,391
ARNT	M69238	1,190–1,215	1,479–1,505	316	1,271–1,290
Hifdel	U22431	2,210–30/2,358–63	2,533–2,552	216	2,372–2,391
HSP70	M15432	924–948	1,196–1,225	302	1,109–1,135
PFK	U24183	644–670	825–851	208	741–760
MCAD	M16827	1,043–1,063	1,242–1,265	223	1,137–1,157

COX, cytochrome oxidase; SDH, succinate dehydrogenase; NADH6, NADH dehydrogenase subunit 6; VEGF, vascular endothelial growth factor; Mb, myoblogin; HIF, hypoxia-inducible factor; ARNT, aryl hydrocarbon receptor nuclear translocator; Hifdel, HIF-1α without the 127 bp of exon 14; HSP70, heat shock protein 70; PFK, phosphofructokinase; MCAD, medium chain acyl dehydrogenase; oligo, oligodeoxynucleotide. The 5′ primer for Hifdel PCR was specifically designed to amplify mRNAs for HIF-1α lacking the segment from 2,211 to 2,357 (e.g., exon 14 of HIF-1α). The primer was composed of a part located upstream of the 5′ end and a part located downstream of the 3′ end of this 127-bp exon, respectively. Therefore, primer binding is possible only to the Hifdel mRNA.

RESULTS

$\dot{V}O_{2\,max}$ and $\dot{W}_{max}$

$\dot{V}O_{2\,max}$ and $\dot{W}_{max}$ were measured under normoxic (600 m) and under simulated hypoxic conditions (3,850 m) before and after the training period (Table 3). Training led to significant increases in $\dot{V}O_{2\,max}$ in all groups (8.3–13.1%, $P < 0.05$), except for the Nor-low group tested under hypoxic conditions. Although mean increases in $\dot{V}O_{2\,max}$ were higher in both of the hypoxic groups, i.e., Hyp-high and Hyp-low, these values were not significantly different from those in subjects training under normoxic conditions. $\dot{W}_{max}$ was also significantly increased in all four training groups (11.4–20.8%, $P < 0.05$). When tested under normoxic conditions, the high-intensity training groups had a significantly higher change in $\dot{W}_{max}$ than did the low-intensity training groups, whereas testing under hypoxic conditions yielded significantly higher values for groups training in hypoxia. The highest relative increases in $\dot{V}O_{2\,max}$ and $\dot{W}_{max}$ were found for the Hyp-high group (13.1 and 20.8%, respectively). Overall, the Nor-low group of subjects, who trained at the same percentage of normoxic $\dot{W}_{max}$ as the Hyp-high one, manifested the smallest changes.

Mitochondrial Density, Capillary Length Density, and Fiber Cross-Sectional Area

As shown in Table 4, total mitochondrial density increased significantly after training under hypoxic conditions (Hyp-high: +55.2%, Hyp-low: +24.1%) and to a lesser extent after high-intensity training under normoxic conditions (Nor-high: +17.0%, $P < 0.05$). No significant change in this parameter was found after low-intensity training under normoxic conditions (Nor-low: +9.6%). Interfibrillar mitochondrial density increased significantly after high-intensity training (Hyp-high: +39.3%, Nor-high: +22.6%) and after low-intensity training under hypoxic conditions (Hyp-low: +11.8%). The subsarcolemmal mitochondrial fraction increased after training under hypoxic conditions, irrespective of training intensity (Hyp-high: +130.1%, $P < 0.05$; Hyp-low: +100.0%, $P < 0.10$). No change was observed for either of the normoxia training groups.

Capillary length density increased only after high-intensity training under hypoxic conditions (Hyp-high: +18.7%, $P < 0.05$). The ANOVA among groups revealed capillary growth to be related to hypoxia ($P < 0.05$) and to a lesser extent to training intensity ($P < 0.10$). Training under normoxic conditions, but not in hypoxia, led to increases in fiber cross-sectional areas (Nor-high: +42.9%, $P < 0.05$; Nor-low: +45.4%, $P < 0.10$).

Table 3. *$\dot{W}_{max}$ and $\dot{V}O_{2max}$ measured in normoxia and hypoxia before and after the 6-wk training period*

	Nor-high	Hyp-high	Nor-low	Hyp-low
$\dot{W}_{max}$, W				
Normoxia				
Pre	294 ± 14	294 ± 12	301 ± 6	299 ± 10
Post	346 ± 19*	355 ± 13*	341 ± 9*	333 ± 11*
Hypoxia				
Pre	235 ± 15	247 ± 8	255 ± 7	241 ± 8
Post	264 ± 15*	290 ± 9*	279 ± 7*	274 ± 5*
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}				
Normoxia				
Pre	50.7 ± 1.9	51.0 ± 2.9	48.4 ± 3.3	47.7 ± 2.3
Post	55.5 ± 2.0*	57.7 ± 3.2*	52.4 ± 2.6*	52.6 ± 2.3*
Hypoxia				
Pre	38.3 ± 1.6	42.0 ± 1.5	39.7 ± 2.5	37.6 ± 1.8
Post	40.4 ± 1.0*	45.6 ± 1.8*	41.1 ± 2.2	40.6 ± 2.0*

Values are means ± SE. $\dot{W}_{max}$, maximal power output; pre, before; post, after. Significant difference, pre- vs. posttraining values, *$P < 0.05$.

Table 4. *Morphometric data before and after the 6-wk training period*

	Nor-high	Hyp-high	Nor-low	Hyp-low
Vv(mc, f), %				
Pre	4.65 ± 0.29	4.48 ± 0.28	4.59 ± 0.29	4.49 ± 0.27
Post	5.70 ± 0.22*	6.24 ± 0.29*	5.26 ± 0.46	5.02 ± 0.17*
Vv(ms, f), %				
Pre	1.52 ± 0.26	0.93 ± 0.22	1.01 ± 0.16	0.74 ± 0.20
Post	1.53 ± 0.27	2.14 ± 0.52*	0.88 ± 0.18	1.48 ± 0.31*
Vv(mt, f), %				
Pre	6.18 ± 0.52	5.40 ± 0.48	5.60 ± 0.39	5.23 ± 0.36
Post	7.23 ± 0.37*	8.38 ± 0.73*	6.14 ± 0.59	6.49 ± 0.40*
J_v(c, f), mm/mm^3				
Pre	779 ± 36	701 ± 56	729 ± 31	644 ± 42
Post	749 ± 41	831 ± 46*	655 ± 52	658 ± 23
a(f), μm^2				
Pre	3,257 ± 326	4,168 ± 483	3,269 ± 212	4,767 ± 843
Post	4,656 ± 414*	3,836 ± 218	4,754 ± 690†	4,301 ± 375

Values are means ± SE. Vv(mc, f), volume density of central (interfibrillar) mitochondria; Vv(ms, f), volume density of subsarcolemmal mitochondria; Vv(mt, f), volume density of total mitochondria; J_v(c, f), length density of capillaries; a(f), fiber cross-sectional area. Significant difference between pre- and posttraining values, *$P < 0.05$. Difference between pre- and posttraining values is considered to be a tendency, †$P < 0.10$.

PCR Quantification

A summary of the normalized values obtained for each mRNA, as determined by RT-PCR, before and after the 6-wk training is given in Table 5.

Oxygen sensor system (HIF-1). Analysis of PCR products derived from the regulatory subunit of HIF-1α mRNA revealed two additional, weaker bands below and above the expected fragment (Fig. 1). Sequencing of the lower isolated band revealed this to match the known HIF-1α, but without the 127 bp of exon 14. This variant of HIF-1α, which we refer to as Hifdel, was present within each muscle-tissue sample tested. PCR quantification (Fig. 1) revealed the level of HIF-1α mRNA to be increased after training under hypoxic conditions, irrespective of training intensity (Hyp-high: + 82.4%, $P < 0.10$; Hyp-low: +58.2%, $P < 0.05$). Similar results were found for Hifdel mRNA (Hyp-high: +78.4%, $P < 0.05$; Hyp-low: +82.2%, $P = 0.12$). ANOVA revealed an effect neither of training intensity nor of hypoxia. No statistically significant changes were found after training under normoxic conditions

Table 5. *Molecular data: RT-PCR results of posttraining values in relation to normalized pretraining values*

	Nor-high	Hyp-high	Nor-low	Hyp-low
COX-1	1.38 ± 0.13†	1.42 ± 0.14†	1.00 ± 0.09	1.15 ± 0.14
NADH6	1.69 ± 0.32†	1.40 ± 0.08*	1.01 ± 0.09	1.24 ± 0.16
COX-4	1.73 ± 0.20*	1.45 ± 0.20*	0.88 ± 0.08	1.35 ± 0.16†
SDH	1.25 ± 0.11	1.47 ± 0.12*	0.83 ± 0.14	1.42 ± 0.17†
PFK	1.51 ± 0.17*	1.85 ± 0.26*	0.78 ± 0.11†	1.00 ± 0.13
MCAD	1.26 ± 0.10†	1.49 ± 0.31†	1.50 ± 0.22*	1.12 ± 0.14
HIF-1α	1.25 ± 0.17	1.82 ± 0.31†	1.23 ± 0.13	1.58 ± 0.15*
Hifdel	1.01 ± 0.20	1.78 ± 0.32*	1.03 ± 0.09	1.82 ± 0.35
ARNT	1.06 ± 0.14	1.07 ± 0.10	1.04 ± 0.28	1.10 ± 0.10
VEGF	1.13 ± 0.12	1.72 ± 0.27*	0.95 ± 0.10	1.17 ± 0.20
Mb	1.17 ± 0.33	1.52 ± 0.11†	0.88 ± 0.19	0.91 ± 0.14
HSP70	2.47 ± 0.49*	2.38 ± 0.21*	0.90 ± 0.23	1.21 ± 0.34

Values are means ± SE. Mean values after training are presented in relation to pretraining values when pretraining values are normalized to 1 SE. Significant difference between pre- and posttraining values $*P < 0.05$. Difference between pre- and posttraining values is considered to be a tendency, $†P < 0.10$.

for HIF-1α (Nor-high: +24.9%, $P = 0.32$; Nor-low: +23.4%, $P = 0.19$) or for Hifdel mRNA (Nor-high: +1.3%, $P = 0.91$; Nor-low: +2.6%, $P = 0.84$). The level of mRNA for the HIF-1 β-subunit (ARNT) remained unchanged in all four training groups.

Oxygen transport (Mb and VEGF). As shown in Fig. 2, the level of both mRNAs increased only in the Hyp-high group (Mb: +72.2%, $P = 0.06$; VEGF: +52.4%, $P < 0.05$). Those for all other training groups did not change.

Oxidative enzymes. mRNA concentrations of COX-1 and COX-4 increased after high-intensity training under normoxic conditions (COX-1: +38.1%, $P < 0.10$; COX-4: +73.2%, $P < 0.05$) and under hypoxic conditions (COX-1: +42.3%, $P = 0.10$; COX-4: +45.4%, $P < 0.05$). mRNA concentrations of NADH6 and SDH were significantly higher after high-intensity training under hypoxic conditions (NADH6: +39.7%; SDH: +46.9%; $P < 0.05$). NADH6 manifested a tendency to increase after high-intensity training under normoxic conditions (+68.7%, $P < 0.10$). No statistically significant changes were revealed for any of the mRNAs after low-intensity training, although trends for increased levels of COX-4 and SDH were apparent under hypoxic conditions (COX-4: +35.0%; SDH: +41.7%; $P < 0.10$). Changes for averaged mitochondrial-coded mRNAs (COX-1 and NADH6) and nuclear-coded ones (COX-4 and SDH) are represented in Fig. 3. After high-intensity training, the levels of both mitochondrially coded (Nor-high: +67.8 ± 24.4%, $P < 0.10$; Hyp-high: +79.6 ± 30.7%, $P < 0.05$) and nuclear-coded (Nor-high: +64.0 ± 22.4%, $P < 0.05$; Hyp-high: +58.3 ± 18.3%, $P < 0.05$) mRNAs were elevated. No changes were found for the Nor-low group. The levels of nuclear-coded mRNAs tended to be higher in the Hyp-low group (+45.9 ± 17.1%, $P < 0.10$).

Glycolysis and β-oxidation (phosphofructokinase and medium chain acyl dehydrogenase). Mean values for phosphofructokinase (PFK) mRNA increased significantly after high-intensity training (Nor-high: +51.4%; Hyp-high: +84.9%; $P < 0.05$), exhibited a tendency to

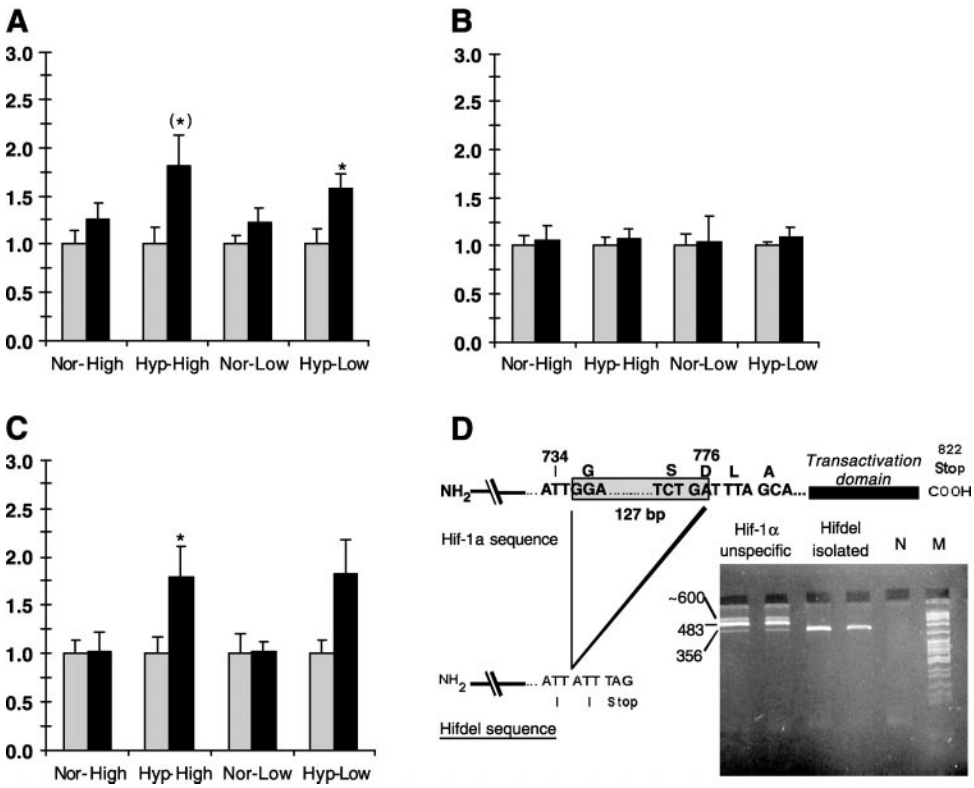

Fig. 1. Relative changes in mRNAs coding for hypoxia-inducible factor 1 α-subunit (HIF-1α; *A*), β-subunit (aryl hydrocarbon receptor nuclear translocator; *B*), and the HIF-1α splice variant HIF-1α^{736} (Hifdel; *C*). Subject groups are as follows: Nor-high and Nor-low, high- and low-intensity level of training under normoxic conditions, respectively; Hyp-high and Hyp-low, high- and low-intensity level of training under normobaric hypoxic conditions, respectively. Values are means ± SE. Significant difference, pre- vs. posttraining values, $*P < 0.05$; difference between pre- and posttraining values is considered to be a tendency, $^{(*)}P < 0.10$. *D*: schematic illustration of the HIF-1α splice variant Hifdel and its effect on the transactivation domain of HIF-1α. RT-PCR result showing the expected HIF-1α product with the exon 14 (483 bp) as well as a weak lower one (356 bp). Another yet-unidentified product (~600 bp) was coamplified but was not further analyzed. 5′ And 3′ primers for this experiment were selected from *positions 2,071–2,095* and from *positions 2,543–2,553* of the HIF-1α sequence. N, negative control; M, length marker (pBR322xHpaII).

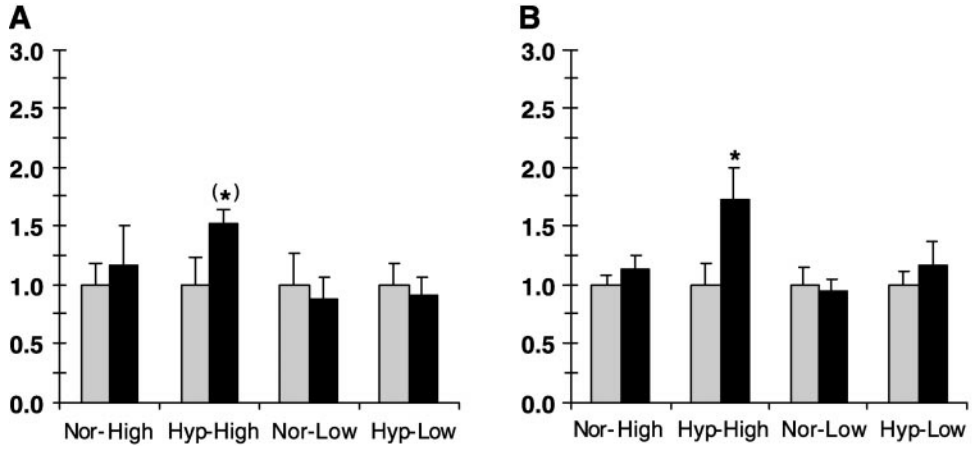

Fig. 2. Relative changes in mRNAs coding for myoglobin (*A*) and vascular endothelial growth factor (*B*). Values are means ± SE. Significant difference, pre- vs. posttraining values, $^{*}P < 0.05$; difference between pre- and posttraining values is considered to be a tendency, $^{(*)}P < 0.10$.

decrease in the Nor-low group (−21.5%, $P < 0.10$), and did not change at all in the Hyp-low (Table 5). Changes were thus dependent on training intensity and significantly augmented by hypoxia (ANOVA; $P < 0.05$). Medium chain acyl dehydrogenase (MCAD) mRNA was the only to one to be significantly increased in the Nor-low group (+50.5%, $P < 0.05$). High-intensity training under normoxic and hypoxic conditions elicited a tendency for an mRNA increase (Nor-high: +25.6%; Hyp-high: +48.9%; $P < 0.10$).

Stress protein response (heat shock protein 70). The stress of high-intensity (as compared with low-intensity) training was reflected in the increased content of heat shock protein 70 (HSP70) mRNA (Nor-high: +146.5%; Hyp-high: +137.7%; $P < 0.05$); its level in low-intensity training groups did not change significantly.

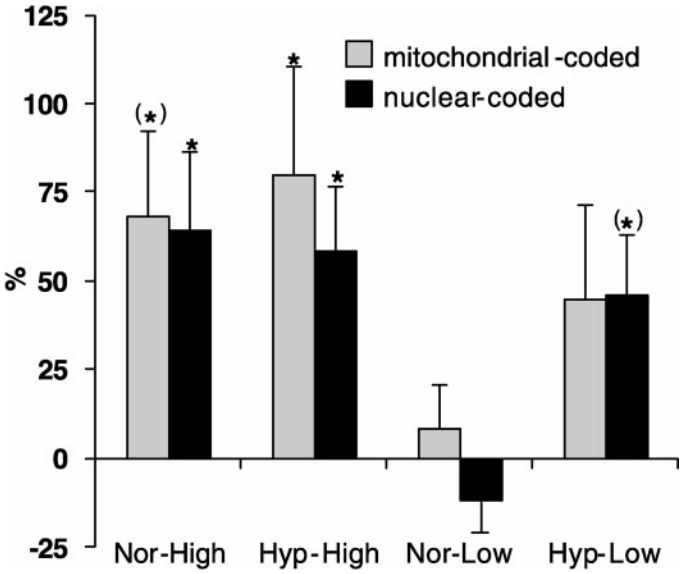

Fig. 3. Combined changes in either mitochondrial-coded (cytochrome oxidase 1, NADH dehydrogenase subunit 6) or nuclear-coded (cytochrome oxidase 4, succinate dehydrogenase) mRNAs of oxidative enzymes. Values are means ± SE in percent changes. Significant percent change between averaged pre- and posttraining values, $^{*}P < 0.05$; difference between pre- and posttraining values is considered to be a tendency, $^{(*)}P < 0.10$.

DISCUSSION

For the group of 12 selected mRNAs, the present study demonstrates that the combination of two different levels of training intensity with normoxia or hypoxia elicits distinct molecular adaptive changes in human skeletal muscle. Quantification of the mRNAs encoding proteins and enzymes involved in different cellular energy turnover processes suggests that, in addition to the mechanical training stimuli, hypoxia seems to be responsible for some of these adaptational specificities. In this context, we showed increased levels of HIF-1α and Hifdel mRNA only after training in hypoxia. With respect to molecular and structural parameters, overall results indicate that high-intensity training under hypoxic conditions (Hyp-high) induces the most profound adaptations, whereas training at the same percentage of $\dot{W}_{max}$ under normoxic conditions (Nor-low) elicited the smallest changes. According to Neufer et al. (29), transcriptional activation of genes is transient, with the net long-term changes reflecting the cumulative effects of intermittent transient changes in the expression of a certain gene. As biopsies were taken at least 24 h after an exercise bout, it could be expected that our results demonstrate the result of net long-term changes of mRNAs (i.e., resting mRNA levels). In the case of normoxic low-intensity training, it is likely that the stimuli failed to induce an increase in transcription sufficient enough to generate an increase in mRNA content that persisted until the next training bout. In contrast to molecular and structural adaptations, only small differences in the changes in $\dot{V}O_{2\,max}$ and $\dot{W}_{max}$ were found among the four groups. This discrepancy between global physiological and local structural and molecular changes may be explained by the circumstance that exercise performance depends on multiple central and peripheral factors (53).

Oxygen Sensing

The level of HIF-1α mRNA increased after training under hypoxic conditions, irrespective of the level of training intensity (Fig. 1). We propose that this elevation in the concentration of the regulatory α-subunit of

the HIF-1 is a key factor in hypoxia-related specificity. HIF-1 was first described by Semenza and Wang in 1992 (42). They reported that, under hypoxic conditions, the transcriptional activation of erythropoietin depends on the binding of HIF-1 to the enhancer of the erythropoietin gene. Subsequent experiments have revealed that HIF-1 is also expressed in skeletal muscle (51). Activation of HIF-1 leads to cellular adaptations, which counteract the effects of reduced oxygen supply to cells under hypoxic conditions. These include improved oxygen transport capacity in the blood due to an erythropoietin-induced increase of the hematocrit (50, 54), induction of neovascularization by an enhanced expression of the VEGF (11), more efficient utilization of oxygen due to an increase in glucose oxidation induced by activation of glycolytic enzymes (50), and possibly also a reduction of negative effects on tissue growth and body weight during chronic exposure to hypoxia (54). From these studies, it can be inferred that activation of HIF-1 might lead to adaptations, which improve oxygen transport, substrate oxidation, and probably tissue growth, adaptations that are also known to influence exercise performance capacity in humans (53).

In experiments with HIF-1α-deficient mice (54), HIF-1 activity has been shown to induce compensatory mechanisms, which lead to a diminution of the "negative" effects found on muscle tissue after permanent hypoxia exposure (17). In our study, an activation of the HIF-1 system was indicated by the increase in HIF-1α mRNA concentration after the 6-wk training period. We hypothesize that, if hypoxia is applied only during the training session itself, HIF-1-dependent pathways will be activated during the subsequent normoxic recovery period. This would explain why we observed more pronounced adaptations, at least on the molecular level, after training under hypoxic than under normoxic conditions.

It is questioned whether HIF-1 activity is regulated transcriptionally or posttranscriptionally (22, 48, 50). An early study suggests the former (48), but a more recent one reports that, under normoxic conditions, HIF-1α protein is degraded within several minutes by the ubiquitin-proteasome pathway, which indicates that regulation is at the posttranscriptional level (22). Under hypoxic conditions, ubiquitination decreased drastically and led to an accumulation of HIF-1α protein (22). In our study, the increased concentration of mRNA for the regulatory α-subunit of HIF-1 (as well as for all other mRNAs monitored) reflect persistent adaptations to a 6-wk training period and not the effects of an immediate hypoxic training stimulus. As mentioned above, the measurements could, therefore, represent the result of a cumulative process over the 6-wk period (29); as such, they do not shed further light on the level at which HIF-1 is regulated. Regular activation and increased turnover of proteins over a period of time are proposed to lead to an increased steady-state level of mRNAs (29, 30). In this context, an increase in the steady-state level of HIF-1α mRNA after a 6-wk training period under hypoxic conditions indicates an adaptation of the hypoxia sensor system.

In our experiments, we detected a variant of the HIF-1α mRNA, which we refer to as Hifdel (Fig. 1). PCR amplification and sequence analysis revealed that Hifdel lacks a 127-bp sequence corresponding to the exon 14 of human HIF-1α (20). This deletion leads to a shift in the reading frame of the HIF-1α mRNA, resulting in the generation of a stop codon at amino acid 736. Native Hifdel thus lacks the transactivation domain at the carboxyl end, thereby giving rise to a new variant, which can dimerize with HIF-1β (ARNT) and bind to the target DNA sequence but may be unable to confer transcriptional activation (24). Quantification of Hifdel mRNA revealed that changes in its levels parallel those in HIF-1α (Fig. 1). An alternative splice variant of HIF-1α (HIF-1α^{736}), which is identical to Hifdel, has recently been identified in several human cell lines and skin fibroblasts, but not in rodents (12). Our study demonstrates that HIF-1α^{736} is also expressed in human skeletal muscles. The function of HIF-1α^{736} remains unclear, but an involvement in the modulation of gene expression on hypoxia has been postulated (12). Our own data offer no suggestions as to its function.

Capillary Growth Factor

From experiments conducted in vitro and in vivo, it is known that the capillary growth factor VEGF is an HIF-1-regulated gene (11, 41, 50). In accordance with an HIF-1-dependent regulation of VEGF, increased levels of the mRNAs for VEGF and HIF-1α mRNA were found after high-intensity training under hypoxic conditions (Fig. 2). No change in the concentrations of VEGF mRNA was observed after training under normoxic conditions or after low-intensity training under hypoxic conditions, even though an increase in the concentrations of HIF-1α mRNA was detected for the latter. This apparent discrepancy might be explained by the additive effect of mechanical training stimuli and hypoxia on the VEGF response (15). Metabolic stress, like glucose deprivation, is also known to induce VEGF expression (40, 41). Evidence in support of high-metabolic stress is afforded by the increased levels of HSP70 mRNA that were found after high-intensity training. Expression of HSP70 is known to be induced by various cellular stresses, including glucose deprivation (7, 26). It can thus be assumed that the combination of exercise intensity and (local) hypoxia leads to an increased steady-state level of VEGF mRNA in the Hyp-high group after the 6-wk training period.

The VEGF mRNA data are supported by structural analyses, which revealed an increase in capillary-length density only after high-intensity training under hypoxic conditions (Table 4). On the other hand, our results are at variance with other studies, which show that capillarity is increased after endurance training (9, 16, 28) or after a single bout of exercise (34, 37) under normoxic conditions. With regard to the complexity of VEGF regulation (11), differences in the training protocol or previous fitness level might affect

the response of a regulatory gene like VEGF differently (35).

Intramyocellular Oxygen Transport

Increased levels of Mb mRNA were found only after high-intensity training under hypoxic conditions (Fig. 1). No changes were detected in the other groups. With respect to humans, the present study and the work of Terrados et al. (43) represent the only investigations that demonstrate increased Mb mRNA or protein levels after endurance training, but only under hypoxic conditions. Changes in the concentration of skeletal muscle Mb mRNA are known to be proportional to the Mb protein content, and the regulation of Mb gene expression is known to be pretranslational (46, 49). We, therefore, assume that the increased level of Mb mRNA found in our study is associated with an increased Mb content within the muscles of subjects in the Hyp-high group. Because slow-twitch type I muscle fibers have a higher Mb protein content than fast-twitch type II ones (21, 55), it would be valuable to know whether the detected increases in mRNA levels are specific to a certain fiber type. Preliminary in situ hybridization experiments on muscle sections derived from Hyp-high group subjects have revealed no signs of a fiber-type-specific change in Mb mRNA (data not presented). The mechanism underlying the induction of Mb gene expression is unknown. That comparable changes in the level of mRNA for Mb and VEGF were found in the present study, taken together with their known functions, suggests that Mb might be regulated, at least partially, via oxygen-sensing pathways and the HIF-1 system.

Oxidative Phosphorylation

Overall, our analysis revealed increased levels of a selection of mRNAs encoding oxidative enzymes after high-intensity training under normoxic and hypoxic conditions, whereas for low-intensity training only minor changes were found (Table 5, Fig. 3). Changes in the combined levels for nuclear- and mitochondrial-coded mRNAs paralleled those in mitochondrial density. In an earlier study, the mRNA content of oxidative enzymes in endurance-trained athletes has been shown to be twice as high as that in untrained individuals (30). Assuming that these athletes, after several years of training, had reached the limit of their adaptive potential, our results show that 6 wk of intense endurance training is sufficient to exhaust ~50% of the adaptive potential for mRNAs of oxidative enzymes.

Combination of the average changes in either nuclear- (COX-4, SDH) or mitochondrial-coded mRNAs (COX-1, NADH6) yielded results that are compatible with the concept of coordinated regulation of nuclear and mitochondrial gene expression (Refs. 30, 52; Fig. 3). Nuclear respiratory factor 1 (NRF-1) and NRF-2 are held to be responsible for the synchronization of nuclear and mitochondrial gene expression (52). If hypoxia influences the adaptation of oxidative enzymes and mitochondria, as suggested by our own data and those of other authors (19, 40, 43, 44, 52), it can be hypothesized that hypoxia, in combination with exercise, should influence NRF-1 and NRF-2. Whether there is cross talk between these factors and hypoxia-dependent signal transduction pathways is not known. However, support for such a concept is furnished by our observation that subsarcolemmal fractions of mitochondria increased in parallel to the changes in HIF-1α mRNA after training under hypoxic but not under normoxic conditions. An increased subsarcolemmal mitochondrial content diminishes the distance for intracellular oxygen transport. Hence, high-subsarcolemmal mitochondria content, in addition to improved capillary density and higher muscular Mb content, decreases the limitation for tissue oxygen diffusion, and could, therefore, increase muscular oxygen consumption and performance under hypoxic conditions.

Glycolytic and β-Oxidation Pathways

Our study demonstrates an exercise-intensity-dependent increase in the mRNA for PFK, a key enzyme in the glycolytic pathway, which was augmented by the hypoxic stimulus (Table 5). PFK is known to be inducible by HIF-1 activity (40, 50). It can, therefore, be assumed that high-intensity training under normoxic and even more so under hypoxic conditions stimulates glucose-dependent metabolic pathways to a greater extent than does low-intensity training. Our results are in accordance with those addressing very-high-intensity training, which report increased activities of PFK (36) and other glycolytic enzymes (5, 45). The mRNA of MCAD, a key enzyme of β-oxidation, was the only RNA analyzed whose level increased in the group at low-intensity levels under normoxic conditions (Table 5). Changes in the level of PFK and MCAD mRNAs led us to speculate on the specificity of the training adaptation on substrate pathways.

Stress Protein Response

From cell culture experiments, it is known that hypoxia can induce expression of the HSP70 (2). In the present study, we quantified the mRNA for the inducible form of HSP70. Significantly increased mRNA levels were recorded after high-intensity training under normoxic and hypoxic conditions but not after low-intensity training (Table 5). Hence, training intensity (metabolic stress?) rather than hypoxia appears to be responsible for the detected adaptations. Several studies in humans and animals have demonstrated increased levels of HSP70 mRNA or protein after a single bout of exercise (26, 33, 39). In the present study, it is shown for the first time that the mRNA for the inducible form of HSP70 is constitutively increased after 6 wk of intense endurance training. In accordance with our result, Liu et al. (25) recently showed the protein levels of HSP70 to be increased after intensified training periods in rowers. The enhanced levels of HSP70 were believed to be attributed to high lactate level during exercise training. In our study, high-inten-

sity training, which led to increased HSP70 mRNA levels, was performed at blood lactate concentrations between 4 and 6 mM. Training at low-intensity, which elicited blood lactate levels between 2 and 3 mM, did not affect the concentration of HSP70 mRNA.

Conclusion

Changes of HIF-1α and Hifdel (HIF-1α^{736}) mRNA indicate that training under hypoxic conditions, independent of exercise intensity, elicits specific effects at the molecular level of human skeletal muscle compared with similar training under normoxic conditions. Whereas these adaptations are independent of exercise intensity, adaptations of mRNAs coding for VEGF, Mb, and PFK seem to be influenced by the oxotensic conditions as well as by exercise intensity. Overall, the most pronounced adaptations occur after high-intensity training under hypoxic conditions (Hyp-high), whereas training at the same percentage of $\dot{W}_{max}$ under normoxic conditions (Nor-low) elicited the smallest changes. Our results reveal that high-intensity training in hypoxia elicits molecular and structural adaptations favoring oxygen transport and utilization in human skeletal muscle under oxygen-restricted conditions. Hence, we speculate that high-intensity training in hypoxia might enhance muscle and exercise performance at altitude.

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REFERENCES

1. **Bailey DM and Davies B.** Physiological implications of altitude training for endurance performance at sea level: a review. *Br J Sports Med* 31: 183–190, 1997.
2. **Benjamin IJ, Kröger B, and Williams RS.** Activation of the heat shock transcription factor by hypoxia in mammalian cells. *Biochemistry* 87: 6263–6267, 1990.
3. **Bergstrom J, Hermansen L, Hultman E, and Saltin B.** Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140–150, 1967.
4. **Boning D.** Altitude and hypoxia training: a short review. *Int J Sports Med* 18: 565–570, 1997.
5. **Boros-Hatfaludy S, Fekete G, and Apor P.** Metabolic enzyme activity patterns in muscle biopsy samples in different athletes. *Eur J Appl Physiol* 55: 334–338, 1986.
6. **Bunn HF and Poyton RO.** Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 76: 839–885, 1996.
7. **Burel C, Mezger V, Pinto M, Rallu M, Trigon S, and Morange M.** Mammalian heat shock protein families. Expression and functions. *Experientia* 48: 629–634, 1992.
8. **Chomczynski P and Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159, 1987.
9. **Desplanches D and Hoppeler H.** Effects of training in normoxia and normobaric hypoxia on human muscle ultrastructure. *Pflügers Arch* 425: 263–267, 1993.
10. **Ferretti G, Kayser B, Schena F, Turner DL, and Hoppeler H.** Regulation of perfusive O_2 transport during exercise in humans: effects of changes in haemoglobin concentration. *J Physiol (Lond)* 455: 679–688, 1992.
11. **Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, and Semenza GL.** Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 16: 4604–4613, 1996.
12. **Gothie E, Richard DE, Berra E, Pages G, and Pouyssegur J.** Identification of alternative spliced variants of human hypoxia-inducible factor-1α. *J Biol Chem* 275: 6922–6927, 2000.
13. **Green H, MacDougall J, Tarnopolsky M, and Melissa NL.** Downregulation of Na^+-K^+-ATPase pumps in skeletal muscle with training in normobaric hypoxia. *J Appl Physiol* 86: 1745–1748, 1999.
14. **Hoppeler H.** Exercise-induced ultrastructural changes in skeletal muscle. *Int J Sports Med* 7: 187–204, 1986.
15. **Hoppeler H.** Vascular growth in hypoxic skeletal muscle. *Adv Exp Med Biol* 474: 277–286, 1999.
16. **Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, and Weibel ER.** Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol* 59: 320–327, 1985.
17. **Hoppeler H, Kleinert E, Schlegel C, Claassen H, Howald H, Kayar SR, and Cerretelli P.** Morphological adaptations of human skeletal muscle to chronic hypoxia. *Int J Sports Med* 11, *Suppl* 1: S3–S9, 1990.
18. **Hoppeler H, Luthi P, Claassen H, Weibel ER, and Howald H.** The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. *Pflügers Arch* 344: 217–232, 1973.
19. **Howald H, Pette D, Simoneau JA, Uber A, Hoppeler H, and Cerretelli P.** Effect of chronic hypoxia on muscle enzyme activities. *Int J Sports Med* 11, *Suppl* 1: S10–S14, 1990.
20. **Iyer NV, Leung SW, and Semenza GL.** The human hypoxia-inducible factor 1 alpha gene: HIF1α structure and evolutionary conservation. *Genomics* 52: 159–165, 1998.
21. **Jansson E and Sylven C.** Myoglobin concentration in single type I and type II muscle fibres in man. *Histochemistry* 78: 121–124, 1983.
22. **Kallio PJ, Wilson WJ, O'Brien S, Makino Y, and Poellinger L.** Regulation of the hypoxia-inducible transcription factor 1 alpha by the ubiquitin-proteasome pathway. *J Biol Chem* 274: 6519–6525, 1999.
23. **Levine BD and Stray-Gundersen J.** "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 83: 102–112, 1997.
24. **Li H, Ko HP, and Whitlock JP.** Induction of phosphoglycerate kinase 1 gene expression by hypoxia. Roles of Arnt and HIF1 alpha. *J Biol Chem* 271: 21262–21267, 1996.
25. **Liu Y, Mayr S, Opitz-Gress A, Zeller C, Lormes W, Baur S, Lehmann M, and Steinacker JM.** Human skeletal muscle HSP70 response to training in highly trained rowers. *J Appl Physiol* 86: 101–104, 1999.
26. **Locke M, Noble EG, and Atkinson BG.** Exercising mammals synthesize stress proteins. *Am J Physiol Cell Physiol* 258: C723–C729, 1990.
27. **Melissa L, Macdougall JD, Tarnopolsky MA, Cipriano N, and Green HJ.** Skeletal muscle adaptations to training under normobaric hypoxic versus normoxic conditions. *Med Sci Sports Exerc* 29: 238–243, 1997.
28. **Mizuno M, Juel C, Bro-Rasmussen T, Mygind E, Schibye B, Rasmussen B, and Saltin B.** Limb skeletal muscle adaptation in athletes after training at altitude. *J Appl Physiol* 68: 496–502, 1990.
29. **Neufer PD, Ordway GA, and Williams RS.** Transient regulation of c-fos, αB-crystallin, and hsp70 in muscle during recovery from contractile activity. *Am J Physiol Cell Physiol* 274: C341–C346, 1998.
30. **Puntschart A, Claassen H, Jostarndt K, Hoppeler H, and Billeter R.** mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. *Am J Physiol Cell Physiol* 269: C619–C625, 1995.
31. **Puntschart A, Jostarndt K, Hoppeler H, and Billeter R.** An efficient polymerase chain reaction approach for the quantitation of multiple RNAs in human tissue samples. *PCR Methods Appl* 3: 232–238, 1994.
32. **Puntschart A and Vogt M.** A stochastic PCR approach for RNA quantification in multiple samples. In: *Methods in Molecular Medicine. Quantitative PCR Protocols*, edited by Kochanowski B and Reischl U. Totowa, NJ: Humana, 1998, vol. 26, chapt. 19, p. 277–287.

33. **Puntschart A, Vogt M, Widmer HR, Hoppeler H, and Billeter R.** Hsp70 expression in human skeletal muscle after exercise. *Acta Physiol Scand* 157: 411–417, 1996.
34. **Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA, and Wagner PD.** Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. *Am J Physiol Heart Circ Physiol* 277: H2247–H2252, 1999.
35. **Richardson RS, Wagner H, Mudaliar SR, Saucedo E, Henry R, and Wagner PD.** Exercise adaptation attenuates VEGF gene expression in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 279: H772–H778, 2000.
36. **Roberts AD, Billeter R, and Howald H.** Anaerobic muscle enzyme changes after interval training. *Int J Sports Med* 3: 18–21, 1982.
37. **Roca J, Gavin TP, Jordan M, Siafakas N, Wagner H, Benoit H, Breen E, and Wagner PD.** Angiogenic growth factor mRNA responses to passive and contraction-induced hyperperfusion in skeletal muscle. *J Appl Physiol* 85: 1142–1149, 1998.
38. **Roskamm H, Landry F, Samek L, Schlager M, Weidemann H, and Reindell H.** Effects of a standardized ergometer training program at three different altitudes. *J Appl Physiol* 27: 840–847, 1969.
39. **Salo DC, Donovan CM, and Davies KJA.** Hsp70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. *Free Radic Biol Med* 11: 239–246, 1991.
40. **Semenza GL.** Transcriptional regulation by hypoxia-inducible factor 1: molecular mechanisms of oxygen homeostasis. *Trends Cardiovasc Med* 6: 151–157, 1996.
41. **Semenza GL, Agani F, Iyer N, Kotch L, Laughner E, Leung S, and Yu A.** Regulation of cardiovascular development and physiology by hypoxia-inducible factor 1. *Ann NY Acad Sci* 874: 262–268, 1999.
42. **Semenza GL and Wang GL.** A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 12: 5447–5454, 1992.
43. **Terrados N, Jansson E, Sylven C, and Kaijser L.** Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin? *J Appl Physiol* 68: 2369–2372, 1990.
44. **Terrados N, Melichna J, Sylven C, Jansson E, and Kaijser L.** Effects of training at simulated altitude on performance and muscle metabolic capacity in competitive road cyclists. *Eur J Appl Physiol* 57: 203–209, 1988.
45. **Tremblay A, Simoneau JA, and Bouchard C.** Impact of exercise intensity on body fatness and skeletal muscle metabolism. *Metabolism* 43: 814–818, 1994.
46. **Underwood LE and Williams RS.** Pretranslational regulation of myoglobin gene expression. *Am J Physiol Cell Physiol* 252: C450–C453, 1987.
47. **Vock R, Weibel ER, Hoppeler H, Ordway G, Weber JM, and Taylor CR.** Design of the oxygen and substrate pathways. V. Structural basis of vascular substrate supply to muscle cells. *J Exp Biol* 199: 1675–1688, 1996.
48. **Wang GL and Jiang BH.** Hypoxia-inducible factor 1 is a basic-helix-loop-helix-Pas heterodimer regulated by cellular O_2 tension. *Genetics* 92: 5510–5514, 1995.
49. **Weller PA, Price M, Isenberg H, Edwards YH, and Jeffreys AJ.** Myoglobin expression: early induction and subsequent modulation of myoglobin and myoglobin mRNA during myogenesis. *Mol Cell Biol* 6: 4539–4547, 1986.
50. **Wenger RH and Gassmann M.** Oxygen(es) and the hypoxia-inducible factor-1. *Biol Chem* 378: 609–616, 1997.
51. **Wiener CM, Booth G, and Semenza GL.** In vivo expression of mRNAs encoding hypoxia-inducible factor 1. *Biochem Biophys Res Commun* 225: 485–488, 1996.
52. **Wiesner RJ.** Adaptation of mitochondrial gene expression to changing cellular energy demands. *News Physiol Sci* 12: 178–184, 1997.
53. **Wilmore JH and Costill DL.** *Physiology of Sport and Exercise*. Champaign, IL: Human Kinetics, 1994.
54. **Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT, and Semenza GL.** Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 α. *J Clin Invest* 103: 691–696, 1999.
55. **Zheng A, Rahkila P, Vuori J, Rasi S, Takala T, and Vaananen HK.** Quantification of carbonic anhydrase III and myoglobin in different fibre types of human psoas muscle. *Histochemistry* 97: 77–81, 1992.