Pflugers Arch - Eur J Physiol (2003) 446:742–751
DOI 10.1007/s00424-003-1133-9

SKELETAL MUSCLE

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Effects of low-resistance/high-repetition strength training in hypoxia on muscle structure and gene expression

Received: 13 February 2003 / Revised: 14 May 2003 / Accepted: 10 June 2003 / Published online: 12 July 2003

Abstract To test the hypothesis that severe hypoxia during low-resistance/high-repetition strength training promotes muscle hypertrophy, 19 untrained males were assigned randomly to 4 weeks of low-resistance/high-repetition knee extension exercise in either normoxia or in normobaric hypoxia (FiO_2 0.12) with recovery in normoxia. Before and after the training period, isokinetic strength tests were performed, muscle cross-sectional area (MCSA) measured (magnetic resonance imaging) and muscle biopsies taken. The significant increase in strength endurance capacity observed in both training groups was not matched by changes in MCSA, fibre type distribution or fibre cross-sectional area. RT-PCR revealed considerable inter-individual variations with no significant differences in the mRNA levels of hypoxia markers, glycolytic enzymes and myosin heavy chain isoforms. We found significant correlations, in the hypoxia group only, for those hypoxia marker and glycolytic enzyme mRNAs that have previously been linked to hypoxia-specific muscle adaptations. This is interpreted as a small, otherwise undetectable adaptation to the hypoxia training condition. In terms of strength parameters, there were, however, no indications that low-resistance/high-repetition training in severe hypoxia is superior to equivalent normoxic training.

Keywords Human skeletal muscle · Fibre types · Myosin heavy chain mRNA · Hypoxia markers

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Introduction

Chronic exposure to high altitude leads to a reduction of muscle cross-sectional area (MCSA) and a decrease in muscle fibre size in humans. It is possible that hypoxia per se is responsible for this atrophy [29], but malnutrition and reduced activity levels have also been identified as possible causes for the loss of muscle [17, 19, 23, 24, 28]. Such muscle loss has not been found in studies using intermittent hypoxia, i.e. in studies in which the subjects exercised under hypoxic conditions but lived in normoxia in between the training sessions. On the contrary, a significant increase in mean fibre cross-sectional area (FCSA) in the human vastus lateralis muscle is seen after 3 weeks of endurance training in severe, progressive, normobaric hypoxia (equivalent to altitudes of 4,100–5,700 m) with recovery in normoxia [15]. Intermittent hypoxia was also suggested as an additional hypertrophic stimulus in a further study that showed increases in the MCSA of biceps brachii, brachialis and triceps brachii muscles after 16 weeks low-intensity resistance training [30–50% of the one repetition maximum (1RM)] combined with moderate vascular occlusion [38]. It was suggested that the restricted blood circulation causes a hypoxic and acidic muscular environment, which induces the recruitment of additional motor units and leads to greater hypertrophy. Thus chronic severe hypoxia seems to have different effects on protein metabolism in muscle tissue than intermittent severe hypoxia. Moreover, hypoxia could be a beneficial stimulus for the development of muscle hypertrophy, at least during low-resistance/high-repetition exercise, if recovery takes place in normoxia.

Exercise training in hypoxia is thought not only to modulate the training response, but also to elicit specific

adaptations: in biopsies taken after high-intensity endurance training in normobaric hypoxia with recovery in normoxia, phosphofructokinase (PFK) mRNA is increased in comparison to training in normoxia and the mRNAs of vascular endothelial growth factor (VEGF) and of myoglobin are increased only after high-intensity endurance training in hypoxia [42].

Low-resistance/high-repetition (strength endurance) training, although widely used in the practice of training for many types of sports, has been much less investigated than e.g. endurance or maximal strength training. Remarkably little is known about its effects on the molecular, cellular and structural changes in skeletal muscle. Two systems which are likely to be involved are glycolysis and fibre types [12, 35]. Interactions of hypoxia with this type of training have not been investigated so far.

It has long been known that MCSA, together with the ability to activate maximal numbers of motor neurons, is the major determinant of maximal contractile force in human skeletal muscle. More recent work has shown that the proportions of different fibre types, and thus the myosin heavy chain (MHC) composition of the muscle, have significant effects on strength. There is a close relationship between the proportion of fast-twitch type-II fibres and maximal knee extension torque [1]. In addition, training-induced adaptations of MHC gene expression have been well documented [6, 31, 45, 46]. Changes in the expression of MHC isoform mRNAs precede changes in accumulation of the corresponding myosins in the muscle fibres; the muscle myosin proteins are thought to have slower turnover rates than their mRNAs [4]. Most studies on the effects of quadriceps strength training and fibre types have found an increase in type-IIA fibres and MHC IIa protein/mRNA with a concomitant decrease in type-IIX fibres [3, 18, 22, 25, 37]. To our knowledge, no studies have been conducted on the effects of training in hypoxia on MHC gene expression in human skeletal muscle.

The main purpose of the present study was to test the hypothesis that strength endurance training performed in severe hypoxia with recovery in normoxia would lead to enhanced adaptation at the functional, structural and molecular level of human skeletal muscle. MCSA, FCSA, fibre type distribution and the relative contents of specific mRNAs in the quadriceps muscle were investigated before and after 4 weeks low-resistance/high-repetition strength training in severe normobaric hypoxia (equivalent to an altitude of 4,500 m above sea level) as well as before and after equivalent normoxic training. In particular, we wanted to find out if intermittent hypoxia

i. induces muscle hypertrophy,
ii. leads to specific hypoxia adaptations, detectable as changes in specific marker mRNAs,
iii. leads to enhanced changes in the proportions of fibre types.

Materials and methods

Subjects

Nineteen male subjects volunteered for the study. They were either completely untrained or recreationally active and had not participated in any strength training for at least 1 year prior to the study. Written, informed consent was obtained in each case. The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg, Germany.

During the early phase of strength training, increases in strength can mainly be attributed to neuromuscular adaptations [27] with most structural changes in the muscle becoming evident subsequently. As these structural changes were the focus of the present study, the subjects went through a 3-week lead-in-phase, during which they were familiarized with the testing and training procedures and performed low-intensity/high-repetition knee extension exercise 3 times a week in normoxia. Thereafter, they were assigned randomly to 4 weeks strength endurance training of the quadriceps muscles in normoxia (NORM, n=9, 24.3±2.5 years, 179.3±8.4 cm, 72.9±9.0 kg) or in hypoxia (HYP, n=10, 25.1±2.9 years, 183.5±5.1 cm, 77.0±9.0 kg). The subjects maintained their habitual activity level during the experimental period.

Training protocol

Both groups performed a supervised, one-leg knee-extension exercise on a leg curler (M3, Schnell, Peutenhausen, Germany) in sitting position 3 times weekly (Monday, Wednesday, Friday). After a standardized warm-up program, they had to complete 6 sets of 25 repetitions, either in normoxia or hypoxia. Resistance was set at 30% of their one-repetition maximum (1RM), determined in either normoxia or hypoxia. Each set had to be completed within 45 s. After 2 weeks of training, 1RM was measured again to adjust the load. Both legs were trained, always starting with the right side. During the 1-min rest periods between sets for the right leg, the left leg was exercised and vice versa. After finishing the knee extensions, the knee flexors were trained in a similar manner to prevent muscular dysbalance. Altogether, one exercise bout lasted about 40 min.

All training sessions took place in a room that enabled normobaric hypoxia to be generated by diluting ambient air with nitrogen. The N_2-enriched air supply was controlled by an O_2-sensor-driven inlet valve; N_2 enrichment was achieved by an O_2 separation system using compression (AGA, Hamburg, Germany). For training in hypoxia, the ambient inspiratory oxygen fraction (FiO_2) was set at 0.12, which is equivalent to an altitude of about 4,500 m above sea level. Ambient O_2 and CO_2 were continuously monitored, as was the arterial oxygen saturation (SaO_2) with the help of pulse oxymetry. SaO_2 never dropped below 70%. Immediately following each exercise bout, the subjects left the hypoxia room and returned to ambient conditions. For training in normoxic conditions, ambient O_2 and CO_2 fractions were not manipulated at a natural altitude of about 120 m.

Testing procedures

Strength tests, magnetic resonance imaging (MRI) and muscle biopsies were performed in the week before and after the 4-week training period. Since we wanted to investigate steady-state adaptations and not acute exercise effects, the second muscle biopsy was taken 5 days after the last exercise bout.

Strength tests

Strength tests of the quadriceps muscles were conducted on an isokinetic device (System3Pro, Biodex Medical Systems, New York, N.Y., USA) in sitting position for both legs separately in normoxia. The subjects were seated 5° reclined and strapped firmly

at shoulders, hip and thigh. Maximal strength was determined as the peak torque from three maximal attempts at an angular velocity of 60° s^{-1}. After a short rest, strength endurance capacity was determined as the work performed during 50 repetitions at an angular velocity of 180° s^{-1}. During these tests, the subjects were continuously encouraged to exercise with maximal effort. Both tests were performed within a 90–180° range of limb excursion. The recorded torque-angle curves were not corrected for the effect of gravity on the lower leg.

Magnetic resonance imaging

An MRI scan of both thighs was performed in the supine position using a 1.5-T system (Symphony, Siemens, Erlangen, Germany) with a T2 weighted sequence (TSE, repetition time 3100 ms, echo time 119 ms, turbo factor 17). The field of view was 45 cm. MCSA of the quadriceps femoris was measured in the proximal, middle and distal third of both thighs 10, 15 and 20 cm from the very distal part of the os pubis before and after the training period. A computerized digitiser with a trackball was used to trace each area as displayed on the computer's monitor by using software provided by the manufacturer. Measurements were made in randomised order by two investigators; the mean values of both measurements were used for statistical analysis. Our original attempt to determine the MCSA of the vastus lateralis muscle on its own had to be abandoned because, especially in the proximal axial MRI scans, the facial boundaries between the lateral and deep vastus muscles could often not be identified. The MCSA of the whole quadriceps femoris muscles was determined instead.

Muscle biopsy sampling

Before and after the 4-week training period, muscle biopsy samples were taken from the same region at mid-thigh level of the vastus lateralis muscle under local anaesthesia, using the Bergström technique [8]. The second biopsy was taken 5 days after the last training session to ensure that we were determining long term steady-state changes in the mRNAs chosen, thus avoiding the influence of short-term transient changes after an exercise bout, which have been demonstrated for various skeletal muscle mRNAs [21]. The muscle pieces were immediately frozen in isopentane, cooled by liquid nitrogen, and then stored at −80 °C.

Histochemistry and morphometry

Serial transverse sections (6 µm) were cut in a cryotome at −20 °C and stained for myofibrillar ATPase after preincubation at pH 4.35 (5 min, room temperature), 4.6 (5 min, room temperature) or 10.5 (15 min, 37 °C) [11]. Four fibre types (I, IIA, IIAX, IIX) could be distinguished after preincubation at pH 4.6 and three fibre types (I, IIC, II) after preincubation at pH 10.5, based on their staining intensities. Biopsies with less than 100 fibres were not analysed, which led to subjects with too small biopsies being excluded from the statistical analysis. On average, 385±228 fibres were classified in each of the remaining samples.

Microscopic images of the ATPase-stained cross-sections (pH 4.6) were recorded with a video camera (Olympus HCC-3600 P high-gain) and digitised on a personal computer equipped with an image analysis system (VIBAM 0.0-VFG 1), as described earlier [26]. FCSA was measured at 200×. Since the number of the hybrid fibre types IIC and IIAX was very small, reliable statistical comparison of changes in their FCSA was not possible. The statistical analysis was therefore performed only for the major fibre types. For the analysis of fibre type distribution and percentage fibre type area, the (few) type IIC fibres were added to type I and the type IIAX to type IIA fibres. The fibre type-specific cross-sectional areas could not be determined in all muscle samples, as in a few of them insufficient numbers of fibres with perpendicular cross-sections could be found.

RNA extraction

For the extraction of total RNA, a modification of the mini-protocol (Quiagen, Hilden, Germany) for heart, skeletal muscle and skin was used [47]. Briefly, about 10 mg of a biopsy (estimated by planimetry) were cut into 25-µm sections in a cryotome at −20 °C and stored at −80 °C. After adjusting to −20 °C, the cut tissue was homogenized in 333 µl lysis buffer (RLT buffer, Quiagen). After threefold dilution with water, 30 mAU proteinase K was added and proteins digested for 1 h 45 min. Thereafter, one volume each of RLT and ethanol were added and the RNA bound to a Quiagen mini column, washed twice with buffer RW 1 and subjected to DNAse I digestion on the column. This step, as well as the following washes and elution of the RNA, were done according to the manufacturer's instruction. From 10 mg of human adult skeletal muscle the yield was about 1 µg total RNA.

Reverse transcription

The RNA pellet was dissolved in 11 µl water. Aliquots (5 µl) of the RNA were reverse transcribed with help of the Superscript II (Invitrogen, Paisley, UK) in a 20-µl reaction according to the manufacturer's specifications, using random hexamer priming. After 50 min at 42 °C, the enzyme was inactivated by incubation at 70 °C for 15 min and the tubes subsequently cooled on ice for 2 min or longer. The resulting DNA was then diluted to 200 µl in TRIS-EDTA buffer (TE, 10 mM TRIS, 1 mM EDTA) and aliquoted for direct use in PCR. Aliquots (1 µl) of each RNA were also processed in a 5-µl reactions under identical conditions, but without the reverse transcriptase, as negative controls.

PCR and Primers

PCR was quantified using a real-time cycler (LightCycler, Roche, Mannheim, Germany), with SyBr green detection, with the exception of the 18S cDNA, for which we used the TaqMan probe and the primers contained in the TaqManRibosomal RNA Control Reagents (Applied Biosystems, Foster City, Calif., USA). For every PCR run, a master mix was prepared using the reagents of the LightCycler Fast Start DNA Master SYBR Green I according to the manufacturer's instructions. Aliquots (1 µl) of the cDNAs (diluted as given above) were combined with 9 µl of master mix in the LightCycler capillaries. For all transcripts, three independent measurements were performed for each cDNA sample and their values averaged and related to the values for 18S cDNA to correct for variations in input total cDNA. Primers and PCR conditions for each assay are given in Table 1. Ten of the experimental cDNA samples were chosen as reference standards and were measured in each run. Relative quantification was performed with help of the "fit-point" method using the preinstalled software program of the LightCycler. For each run, ratios relative to each of the reference standards were determined based on the respective changes in cycle thresholds (ΔC_Ts) and an average efficiency (determined graphically, SD 2–3%). These ratios were then averaged over all three runs and related to the average content of 18S cDNA in each sample. The obtained "mRNA values" are therefore relative values based on total RNA content.

Statistics

Data were analysed using the software programs Sigmastat 2.0 and Sigmaplot 2001 for Windows (Jandel Scientific). Data are presented as means±SD. Statistical analyses were performed by utilizing a 2×2 repeated measures ANOVA [group (HYP, NORM)×test (pre-training, post-training)]. All data were tested for normal distribution and, if necessary, transformed to make them suitable for ANOVA. Significant between-test differences were determined involving the post-hoc Tukey test. Additionally, differences between values obtained pre- and post-training for

Table 1 Primers and PCR conditions for RT-PCR quantitation of mRNAs (see Materials and methods). The 18S PCR was run for 35 cycles with an annealing temperature of 60 °C in the reagents of the Fast Start DNA Master Hybridisation kit (*MHC* myosin heavy chain, *VEGF* vascular endothelial growth factor, *PFK* phoshofructokinase, *LDH* lactate dehydrogenase)

cDNA probed for	Gene bank access code	Primer location		Annealing temperature (°C)	Number of cycles	Source
		Forward	Reverse			
MHC I	M58018	5895–5914	5972–5991	55	35	[43]
MHC IIA	AF111784	5829–5845	5926–5949	46	40	[43]
MHC IIX	AF111785	5819–5844	5882–5907	46	40	[43]
VEGF	M32977	140–166	270–296	70	35	[42]
Myoglobin	NM005368	32–54	159–181	57	35	Own design
PFK	NM000289	587–613	768–794	58	35	Own design
LDH A	X02152	1143–1164	1264–1286	58	37	Own design
LDH B	BC015122	32–53	142–165	58	35	Own design

each group were analysed by using Student's paired *t*-test. Correlations between selected parameters were analysed by linear regression. The level of significance was set at $P<0.05$.

Results

Strength endurance capacity, maximal strength and MCSA

Since all muscle biopsies were taken from the right vastus lateralis muscle, only the results of the right leg are discussed.

No significant group×test interactions and no significant group effects were observed for strength endurance capacity, maximal strength and MCSA. There was a significant ($F=12.048$, $P=0.003$, power=0.894) test effect for strength endurance capacity: the latter was increased significantly (by about 9%) in both groups after 4 weeks strength endurance training (Table 2).

Table 2 Strength endurance capacity, maximal strength and muscle cross-sectional area (*MCSA*). Means±SD before and after 4 weeks of low-resistance/high-repetition strength training in normoxia or normobaric hypoxia

	Test	Hypoxia	Normoxia
Strength endurance capacity (J/kg)	before	56.0±11.4	59.6±7.3
	after	60.5±10.6*	64.7±7.2*
Maximal strength (Nm/kg)	before	2.7±0.4	2.8±0.4
	after	2.8±0.5	2.9±0.3
MCSA (cm^2)	before	91.2±11.2	87.4±14.2
	after	91.4±11.7	88.4±15.0

*$P<0.05$ vs. before training

Table 3 Fibre type distribution and fibre cross-sectional area. Means±SD before and after 4 weeks of low-resistance/high-repetition strength training in normoxia or normobaric hypoxia

Fibre types	Test	Hypoxia	Normoxia
Fibre type distribution (%)			
Type I	before	47.5±21.8	50.0±12.4
	after	45.1±20.5	50.5±9.4
Type IIC	before	0.8±1.1	0.1±0.1
	after	0.2±0.3	0.1±0.2
Type IIA	before	28.4±10.5	33.1±6.6
	after	35.2±11.5	38.0±6.7
Type IIAX	before	1.9±2.3	3.4±3.4
	after	3.0±3.6	0.9±1.4
Type IIX	before	20.4±15.2	13.4±9.9
	after	16.6±16.5	10.8±7.1
Fibre cross-sectional area (μm^2)			
Type I	before	4368±1097	4298±1710
	after	4445±947	5525±428
Type IIA	before	5517±890	5458±2536
	after	5227±735	6193±1598
Type IIX	before	5004±1062	4282±2219
	after	4632±576	4804±720
Mean	before	4727±854	4631±1937
	after	4606±744	5709±1380

Fibre type distribution

For the fibre type-related parameters, no significant differences were detected. Neither significant group×test interactions, nor significant group or test effects were observed for percentages of the different fibre types, for the FCSA of the different fibre types or mean FCSA (Table 3), or for the fibre type areas (data not shown).

mRNAs of MHC isoforms, glycolytic enzymes, VEGF and myoglobin

The relative contents of all the mRNAs determined showed considerable inter-individual variations between the biopsies of the different subjects. Substantial variability was also seen between the values from a given person's biopsy taken before the training and the values after the training period. This is illustrated in Figs. 1 and 2. This inter-individual variability was most conspicuous for the relative values of MHC IIx mRNA. In six out of eight hypoxia-trained subjects, increases of 16–427% were seen, in the other two, MHC IIx mRNA decreased by 68 and 78%. The range in the normoxia group was a 32–634% increase in four out of six subjects and 78 and 92% decreases in the remaining two.

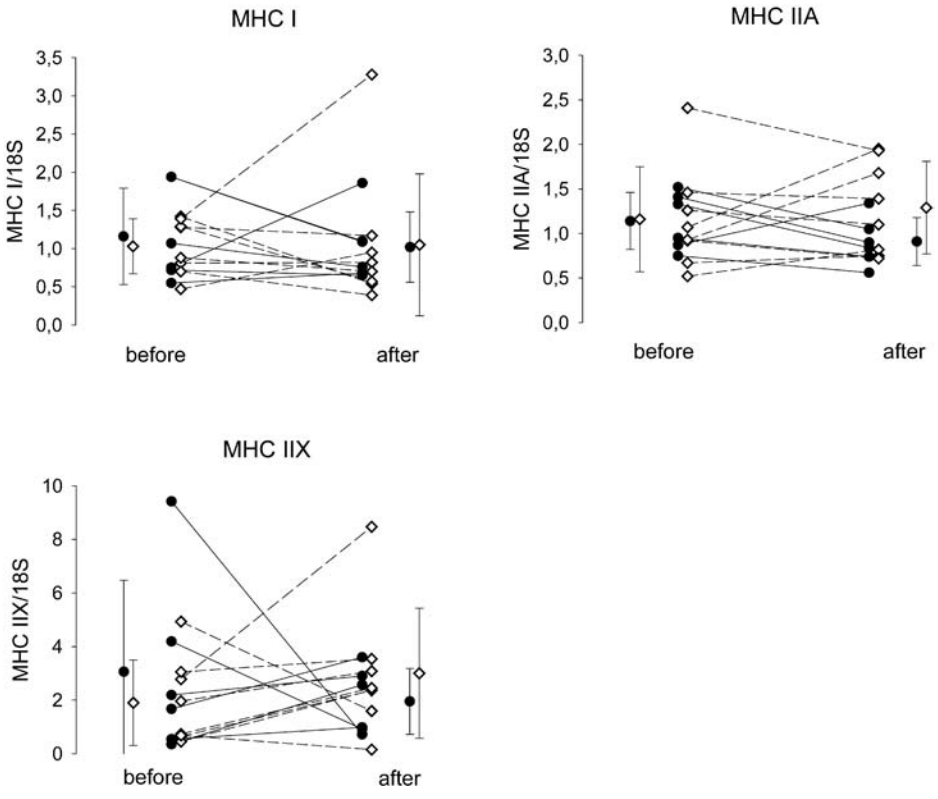

Fig. 1 mRNAs coding for myosin heavy chain (*MHC*) isoforms I, IIA, IIX. The data (relative fluorescence signals, corrected for 18S cDNA input) are shown as individual values as well as means±SD before and after 4 weeks of low-resistance/high-repetition strength training in normoxia (●) or normobaric hypoxia (◇)

Table 4 Correlations (correlation coefficient *R*, probability *P*) between mRNAs of hypoxic markers (VEGF, myoglobin) and glycolytic enzymes (PFK, LDH A, LDH B). The ratios between the values obtained before and after 4 weeks of low-resistance/high-repetition strength training were analysed for the hypoxia and the normoxia training group

mRNA		Myoglobin	PFK	LDH A	LDH B
Hypoxia					
VEGF	*R*	0.971	0.826	0.680	0.753
	P	<0.001	0.011	0.064	0.031
Myoglobin	*R*		0.887	0.629	0.847
	P		0.003	0.095	0.008
PFK	*R*			0.505	0.717
	P			0.202	0.045
LDH A	*R*				0.186
	P				0.659
Normoxia					
VEGF	*R*	0.775	0.137	0.130	0.791
	P	0.070	0.796	0.805	0.06
Myoglobin	*R*		–0.067	0.313	0.865
	P		0.900	0.546	0.026
PFK	*R*			0.018	0.034
	P			0.974	0.949
LDH A	*R*				–0.186
	P				0.724

Neither significant group×test interactions nor significant group or test effects were found for any of the mRNAs determined. In the hypoxia group, PFK mRNA tended to increase (P=0.123, increased in six out of eight subjects). The analysis of correlations between the different mRNAs and structural parameters, however, revealed significant interactions: For the pre- to post-training ratios of the mRNAs of VEGF, myoglobin, PFK and LDH B, significant correlations were found, but only in the group that trained in hypoxia. In the normoxia group, there was only one significant correlation, viz. that between the relative values of myoglobin and LDH B (Table 4). Highly significant correlations were also found between the proportions of fibre types and the different MHC mRNAs plus the two LDH isoenzymes (Fig. 3).

Discussion

In this study, muscular adaptations to strength endurance training in human vastus lateralis muscle were investigated to answer the question if hypoxia during the training sessions promotes hypertrophy. The mode of training (low-resistance/high-repetition) was chosen similar to two other studies that suggested a beneficial effect of hypoxic exercise conditions on muscle mass [15, 38]. A first biopsy was taken after a 3-week lead-in phase in normoxia, the period in which most of the "neural" adaptation to the exercise should have taken place [27]. The second biopsy was obtained after 4 weeks of further training in either hypoxia or normoxia. Four weeks of hypoxia training have been shown previously to induce hypoxia-specific adaptations in the protein contents of citrate synthetase, myoglobin and the LDH activity in the human vastus lateralis muscle [39]. The second biopsy was taken 5 days after the last training session to ensure long-term changes in the steady state of the mRNAs in

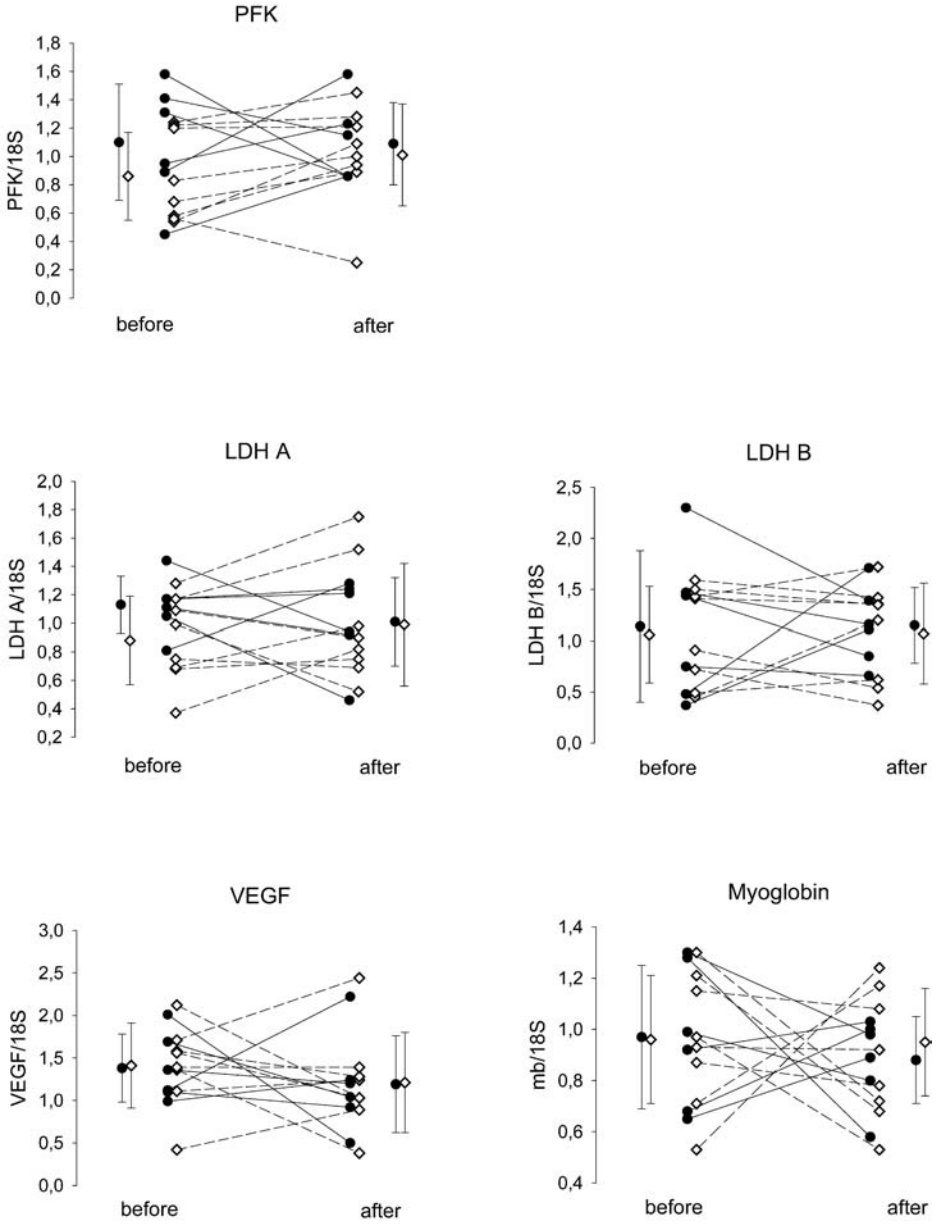

Fig. 2 mRNAs of the glycolytic enzymes phosphofructokinase (*PFK*), lactate dehydrogenase (*LDH*) A and LDH B and of the hypoxia markers vascular endothelial growth factor (*VEGF*) and myoglobin (*mb*). The data (relative fluorescence signals, corrected for 18S cDNA input) are shown as individual values as well as means±SD before and after 4 weeks of low-resistance/high-repetition strength training in normoxia (●) or normobaric hypoxia (◇)

question were determined, with minimal interference from transient regulatory phenomena.

For a number of mRNAs involved in metabolism or contraction, transient increases have recently been shown to occur in the recovery hours after a bout of exercise, e.g. VEGF mRNA [34], selected mRNAs of enzymes involved in the glucose and lipid metabolism [32], mRNAs of lipid metabolism [40] and, probably, MHC [46]. These conspicuous transient increases are thought to produce small increases in the steady-state levels of the mRNAs, too small to be individually detected, but their cumulative effect after repeated bouts during a training program is thought to result eventually in significantly increased steady-state levels [44]. It is these long-term steady states that correlate best with the biochemical and structural adaptations seen in exercising muscles [9].

To our knowledge, data on transient post-exercise changes in metabolic or structural mRNAs are scarce, data on the longevity of mRNA steady states produced by exercise programs practically nonexistent. Our 5-day span between the last training session and the biopsy is derived from Vestergaard et al. [41] who recommended a 4- to 5-day break from exercise, on the basis of current knowledge about the post-exercise modulation of the glucose uptake system, which probably is the best-characterised post-exercise muscle response to date. The fact that we found significant increases in the mRNAs of MHC IIa and LDH A in a study involving 4 weeks of strength training with eccentric overload in the biopsies taken 5 days after the last training session (B. Friedmann, R. Kinscherf, S. Borisch, G. Richter, P. Bärtsch, R. Billeter, unpublished data) argues against these cumulative steady-state effects

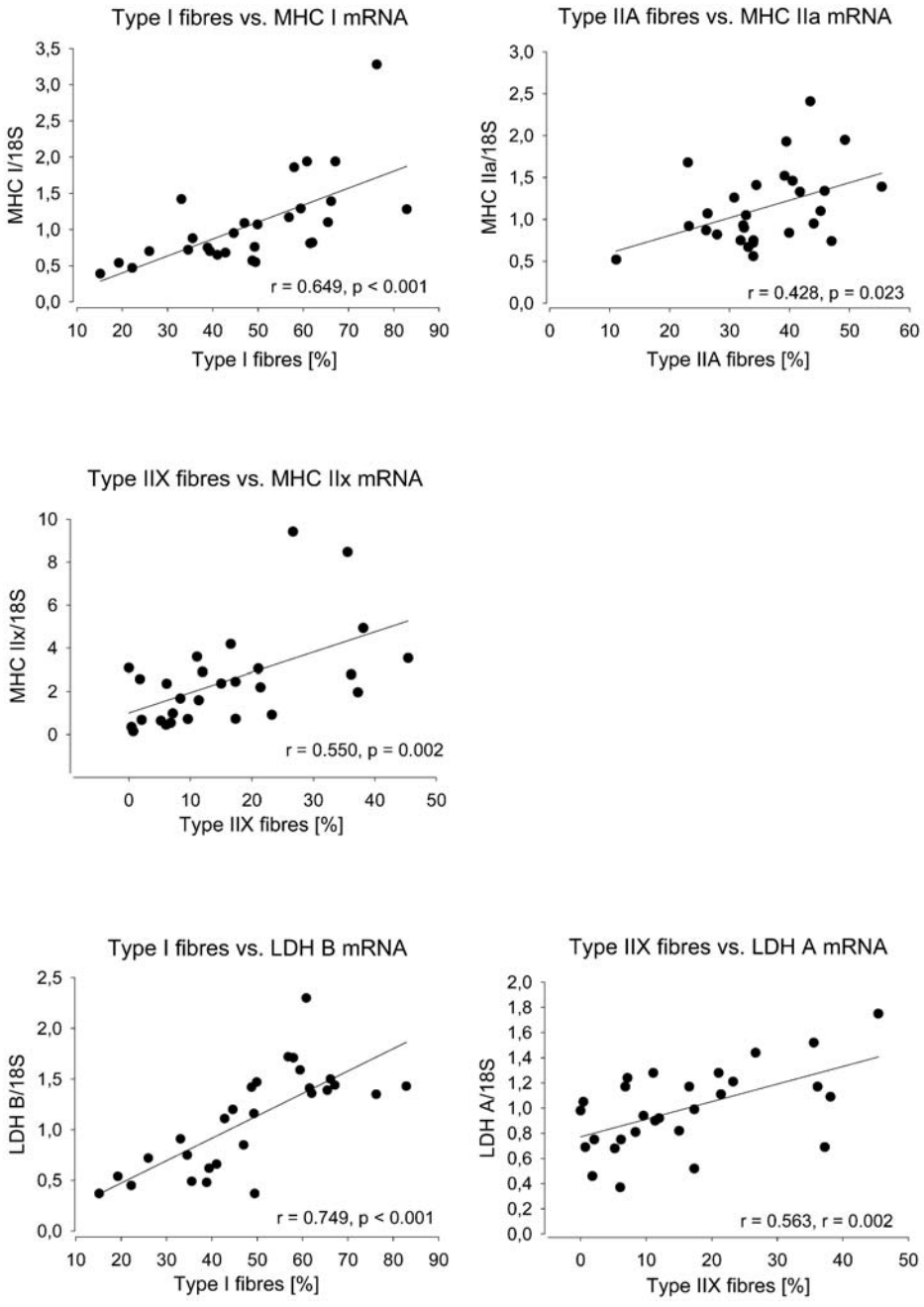

Fig. 3 Correlations between the proportions of fibre types and the values of their corresponding myosin heavy chain (MHC) mRNAs or lactate dehydrogenase (LDH) mRNAs. The mRNA values are relative PCR signals corrected for 18S cDNA input for each of the biopsies analysed

reversing rapidly, which is also the case for the significant correlations among the "hypoxia marker" mRNAs discussed below. Significant changes in MHC mRNAs in response to 3 months strength training were also noted in a study in which the biopsies were taken 6 days after the last training session [6]. In addition, training-induced changes in the protein levels of myoglobin [30], LDH and PFK [14, 36] have been shown to last for many weeks, which is unlikely to have happened with their mRNAs back at untrained levels. We therefore argue that our data reflect the training-adapted long-term steady states of the measured mRNAs.

The training protocol in our study was similar to the strength endurance training protocols used by competitors in many different sports, except for the O_2 content of inspired air in the hypoxia group, equivalent to 4,500 m above sea level. As expected, strength endurance capacity rose by about 9% during the training period (Table 2). However, neither maximal strength nor MCSA in the quadriceps muscles (which normally correlate well [2, 27]) changed significantly. Concomitantly, no changes in fibre diameters were found, either when averaged over all fibres measured, or when the diameters of fibres of a given type were compared. In addition, no significant changes could be detected in the proportions of fibres,

either in the training responses of the hypoxia or the normoxia groups or after pooling the data from both groups.

Our data therefore neither provide evidence for the hypothesis that hypoxia promotes hypertrophy, nor do they indicate that it positively influences the development of strength endurance capacity or maximal strength. Hypoxia did, however, have an effect on the intracellular environment in these muscles. This is illustrated by the pre-/post-training ratios of those mRNAs that code for proteins of systems known to adapt to hypoxia. They were significantly correlated in the hypoxia but not in the normoxia group (Table 4). For the mRNAs of VEGF, myoglobin and PFK, increases have been demonstrated for (high-intensity) endurance training in temporary hypoxia [42]. To our knowledge, there are no data available for LDH B mRNA changes during training in a hypoxic environment. The results of our study (positive correlation of LDH B and PFK) are in agreement with the finding of reduced lactate production after altitude acclimatisation [7]. The mRNA ratios of VEGF, myoglobin, PFK and LDH B were all positively correlated, an indication for concomitant hypoxia adaptations to individually variable extent which become manifest as significant correlations, but not as significant differences. Distinct inter-individual variability in the response to training and recovery at altitude has also been reported in long-distance runners [13].

In the present study, we found high inter-individual variability in the values of all mRNAs measured (Figs. 1 and 2). These figures over-represent the variability to a small extent, because the scatter introduced by the reverse transcriptase (RT) reaction cannot be accounted for. The SD of RT is 10–20% in our hands, as derived from cDNA array studies ([47] and M. Wittwer, M. Flück, H. Hoppeler, S. Müller, D. Desplanches, R. Billeter, unpublished results). This high variability in our mRNA data is in agreement with many studies on gene expression in human muscle biopsies, where similar variability was found, e.g. for the mRNAs of PFK [41], VEGF [20] and MHC (estimated SDs from [45]). The data in [41] were derived from Northern blots, not from RT-PCR, indicating that such variability is not due to the RT-PCR procedure, except for the small contribution of the RT. Interestingly, their PFK protein values derived from Western blots show less variability, which might well illustrate a general feature of human muscle biopsies. Recent microarray studies ([5] and M. Wittwer, R. Billeter, H. Hoppeler, M. Flück, unpublished data) also point towards high variability in the relative levels of many, if not most mRNAs in human muscle biopsies, which is as large or larger than the one observed in this study. Nevertheless, our data suggest that these mRNA values are good indicators of the cellular events underlying the structural and functional characteristics of these biopsies. This is illustrated by significant correlations between the proportions of fibres of a given type and the mRNAs of their MHC isoforms and the two isoforms of LDH, respectively (Fig. 3). Fibre types and glycolysis are the systems most likely to change during strength endurance training [12, 35]. We did not find any significant shifts in the mRNAs coding for proteins involved in these systems, however.

The present data can be interpreted in two ways. On one hand, the expression of the tested markers could have remained principally unchanged. In this case, the gain in strength endurance capacity would likely have been a consequence of neuromuscular adaptation. On the other hand, since strength endurance training represents a compromise between two adaptation extremes [35, 44], these markers could reflect the effect of individually diverging adaptations, with the vastus muscles of some subjects adapting towards an endurance pattern, those of others towards more strength. On the basis of the present data, it is not possible to decide between these two hypotheses. In the light of the good correlations between fibre type proportions and the MHC and LDH mRNAs, the remarkably diverse changes for a given mRNA between the pre- and post-training biopsies would argue for the latter. Additional support for this interpretation comes from the "HERITAGE Family Study", in which wide ranging responses were found for several parameters in response to carefully standardised endurance training programmes [10]. However, the correlation analysis of the overall pre- to post-training ratios of MCSA or FCSA with the various mRNAs in our study did not indicate individuals who experienced strength adaptation (data not shown).

There remains the distinct possibility, however, that much of the observed variability is a consequence of normally occurring variations between biopsy sites in human vastus lateralis muscle (and other human muscles). The fibre type composition of this muscle is non-uniform, to the extent that different biopsies from the same muscle can have quite different fibre type compositions [16]. Variations similar to this study have also been found for the mRNA of cytochrome oxidase subunit IV in a previous study [33], where three vastus lateralis muscle biopsies were taken from five persons within 3 h. While the average relative contents of cytochrome oxidase IV mRNA were constant, the values of individual biopsies from a given subject differed up to 2.5-fold. It is not possible to decide if the data in our present study represent sampling variability on a constant expression level or diverging changes between the muscles of different individuals, superimposed on the variability from the biopsy sampling.

In conclusion, after 4 weeks of low-resistance/high-repetition strength training, the significant increases in strength endurance capacity in this study were not matched by changes in MCSA, fibre type distribution, FCSA or fibre type area. Severe normobaric hypoxia, equivalent to an altitude of about 4,500 m during the exercise bouts, did not induce the development of muscle hypertrophy. Indications of a specific adaptation to the hypoxic training conditions were detected in the form of significant positive correlations between the relative mRNA levels of the hypoxia markers VEGF and myo-

globin and the glycolytic enzymes PFK and LDH B. The inter-individual variability in the changes of the mRNA levels was remarkably large. The question remains as to whether this is a consequence of individually diverging muscle adaptations or a consequence of variability introduced by the multiple biopsy sampling.

Acknowledgements The authors thank Silke Vorwald for expert assistance with tissue preparation and histochemistry and Judith Schoenith for excellent assistance with RT-PCR. They also gratefully acknowledge the invaluable help of Karel Kucera and Helmut Müller from the Olympic Training Centre Heidelberg with the design and conduction of strength training and testing. In addition, they thank Gunther Erb for assistance on the measurement of muscle cross sectional area in the MRI scans. This investigation was supported by grants from the Deutsche Forschungsgemeinschaft (FR 1262/3-1) and from the Bundesinstitut für Sportwissenschaft (VF 0407/01/04/98).

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