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SKELETAL MUSCLE

Silvia Schmutz · Christoph Däpp · Matthias Wittwer
Michael Vogt · Hans Hoppeler · Martin Flück

Endurance training modulates the muscular transcriptome response to acute exercise

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Abstract We hypothesized that in untrained individuals ($n=6$) a single bout of ergometer endurance exercise provokes a concerted response of muscle transcripts towards a slow-oxidative muscle phenotype over a 24-h period. We further hypothesized this response during recovery to be attenuated after six weeks of endurance training. We monitored the expression profile of 220 selected transcripts in muscle biopsies before as well as 1, 8, and 24 h after a 30-min near-maximal bout of exercise. The generalized gene response of untrained *vastus lateralis* muscle peaked after 8 h of recovery ($P=0.001$). It involved multiple transcripts of oxidative metabolism and glycolysis. Angiogenic and cell regulatory transcripts were transiently reduced after 1 h independent of the training state. In the trained state, the induction of most transcripts 8 h after exercise was less pronounced despite a moderately higher relative exercise intensity, partially because of increased steady-state mRNA concentration, and the level of metabolic and extracellular RNAs was reduced during recovery from exercise. Our data suggest that the general response of the transcriptome for regulatory and metabolic processes is different in the trained state. Thus, the response is specifically modified with repeated bouts of endurance exercise during which muscle adjustments are established.

Keywords Endurance exercise · Gene expression · Skeletal muscle · Structure · Time course

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S. Schmutz · C. Däpp · M. Wittwer · M. Vogt · H. Hoppeler
M. Flück (✉)
Department of Anatomy, University of Bern,
Baltzerstrasse 2, 3000 Bern 9, Switzerland
E-mail: flueck@ana.unibe.ch
Tel.: +41-31-6314619
Fax: +41-31-6313807

Introduction

Skeletal muscle tissue shows a remarkable malleability to remodel its structural make-up and to adapt functionally in response to contractile stimuli. For instance, endurance training is known to lead to specific improvements of oxidative metabolism [14, 16, 28, 37], capillarity [1, 8, 28] and occasionally a shift towards a slower contractile phenotype [15]. Several studies also demonstrate an increase in the content of intramyocellular lipids (IMCL) [14, 32], while the size of muscle fibers seems to remain essentially unchanged with endurance training [6, 14].

Concerted changes in the concentration of expressed messenger ribonucleic acids (mRNAs), i.e., the transcriptome, have been identified as a major molecular strategy of muscle for governing structural and functional adaptations with exercise training [4, 12, 13, 21]. A match between the quantity of multiple mitochondrial transcripts, both coded on the nuclear and mitochondrial genome, and mitochondrial volume density was found in highly endurance-trained subjects [24, 25, 31]. Thus, coordinated adaptations of the concentration of mitochondrial transcripts appear to underlie the improvement of oxidative metabolism in the endurance-trained state. However, the involvement of other gene families and the time course of the response of the transcriptome remain largely unknown.

In untrained *vastus lateralis* muscle, a single bout of intense or prolonged ergometer exercise is known to induce transient expressional adaptations of gene ontologies involved in lipid metabolization (LPL), mitochondrial biogenesis (TFAM, PGC-1α), redox regulation (UCP3, HO1), carbohydrate metabolism (GLUT4, HKII, PDK4) and angiogenesis (VEGF) within the first hours of recovery [17, 22, 23, 26, 33, 34, 36]. Repetitive exercise bouts have further been shown to enhance the basal concentration of factors of oxidative (FAT, CPT1) and glycolytic metabolism (GAPD) after weeks to years of endurance training [22, 36, 39]. This

indicates that the accumulation of specific gene transcripts in skeletal muscle due to the repetitive action of exercise stimuli may contribute to the functional improvements that are typical of the endurance-trained state [10, 12, 25, 31]. Recent evidence further suggests that the transcriptional response of metabolic factors (HKII, TFAM, PPARα, CPT1, FAT) may be different in the trained compared to the untrained state [23, 36]. However, the knowledge on how short-term transcriptional changes relate to long-term adjustments of the transcriptome with exercise stimuli and whether they reflect the time course of improvements of muscular performance with training [30] is limited.

In order to gain information on the coordination of the gene response during endurance exercise-induced alterations, we carried out a gene expression profiling study. Biopsies were taken from *vastus lateralis* muscle during the first 24 h of recovery from a single bout of intense ergometer cycling before and after 6 weeks of endurance training. The samples were subjected to analysis on custom-designed microarrays. This allowed for parallel analysis of transcript levels of gene ontologies involved in metabolic, contractile and regulatory muscle function as well as in the adjustment of the interstitial compartment. It was hypothesized that the acute alterations of the transcriptome of sedentary subjects to a single bout of endurance exercise should encompass the set of genes responsible for the structural and biochemical muscle adjustments occurring with training. Secondly, it was hypothesized that the acute transcript-level adaptations of the trained muscle to a single bout of exercise at the same relative intensity would be qualitatively similar to those seen in the untrained state, but reduced in quantity due to the expected increase in steady-state concentration of mRNA.

Materials and methods

Subjects

Six healthy, not systematically trained men gave their written consent to participate in the study. The study was conducted with permission of the Ethics Committee of Bern, Switzerland, in compliance with the Helsinki Convention for Research on human subjects. Anthropometric parameters (age, height, body mass, and percent body fat) were determined at the beginning of the study and after the 6 weeks' training period. Percent body fat was determined by a seven-point skin fold measurement using a calibrated skin fold caliper (GPM, Switzerland).

Endurance training

During the 6 weeks, the subjects trained five times per week for 30 min at an intensity of approximately 65% of the maximal workload (P_{max}). The training intensity was monitored and adjusted by heart rate. Training workload was increased as necessary to maintain a constant individual training heart rate which corresponded to $83 \pm 1\%$ of the maximal heart rate in the first training week and to $90 \pm 2\%$ in the sixth training week.

Exercise test

The subjects were advised to stop extra physical activity 4 weeks before the start of the study (see Fig. 1). All subjects were familiarized with the test equipment 2 weeks before the initial exercise single bout. At the same time, an exercise test was carried out to determine the admissible intensity of the initial exercise bout. A second exercise test was carried out after the 6 weeks' training period. Exercise tests were carried out on a bicycle ergometer (Ergoline 800S, Ergoline GmbH, Bitz, Germany). Expired air was analyzed with breath-by-breath measurements (Oxycon alpha, Jäger GmbH, Würzburg, Germany). Starting with 40 watts, the workload was increased by 30 watts every 2 min until the subjects could no longer maintain a cadence of more than 60 rpm.

Single endurance exercise bout

Before and after the endurance-training period, the subjects performed a single bout of bicycle exercise at a low intensity (approximately 40% of P_{max}) for 10 min followed by 30 min of high-intensity exercise (approximately 65% of P_{max}) on the ergometer. After the

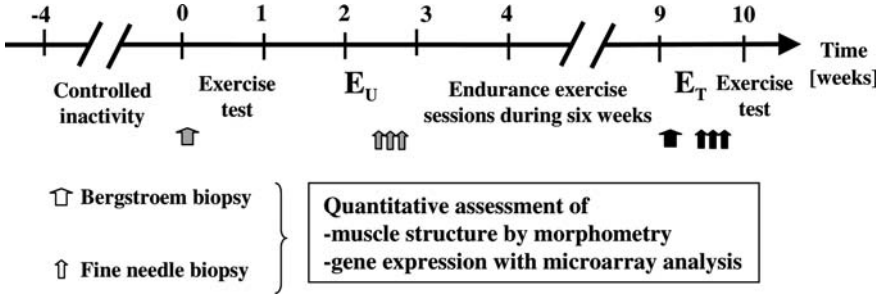

Fig. 1 Experimental protocol time line. Muscle biopsies were taken before and after a single bout of ergometer exercise in the untrained state (E_U) and after 6 weeks of endurance training (E_T)

6 weeks' training period, the relative intensity was 6% higher and the absolute intensity was 21% higher than before (Table 1).

Muscle biopsies

Using the Bergstroem technique [2], resting needle biopsies were taken from the *vastus lateralis* muscle 3 days before the first exercise test and 3 days after the training period. A fraction of the muscle tissue was fixed in glutaraldehyde and subsequently embedded in Epon for microscopical analyses as described earlier [14]. For measuring the capillary density, 1 μm cross-sections were cut with an ultramicrotome from two tissue blocks randomly chosen from each biopsy. Sections were stained with toluidin blue and pictures were obtained by light microscopy at a final magnification of ×1,850. On average 117 fibers were analyzed per muscle biopsy. The number of capillaries was counted directly. For mean fiber cross-sectional area, point counting was performed in consecutive corners of the frames on 100-square mesh grids on the same sections.

The mitochondrial volume density was measured as described on electron micrographs performing point counting with a B36 grid with 144 test points at a final magnification of ×24,000 [14].

For additional analysis of the molecular time course, fine needle biopsies (14 Gauge single use; Medilink S.A., Pregassona, Switzerland) were taken 1, 3, 8 and 24 h after the first and second single bout of exercise alternately from the left and right leg. For each biopsy a fresh incision was made at a distance of at least 1.5 cm from any previous biopsy of the same leg. These biopsies were frozen in isopentane cooled by liquid nitrogen and subsequently stored in liquid nitrogen.

Table 1 Subject characteristics

	Untrained	Trained	*P*
Age (years)	28 (21;38)		
Height (cm)	182 (172;189)		
Body mass (kg)	75.8 (65.0;101.8)	74.8 (65.8;99.3)	0.92
Body fat (%)	12.1 (6.0;35.4)	12.9 (5.2;34.0)	0.60
$\dot{V}O_{2\,max}$(ml/min/kg)[a]	44.6 (26.8;56.2)	50.2 (28.5;58.7)	0.03
4 mmol/l Lactate threshold (watt)[a]	199 (149;291)	228 (180;312)	0.03
P_{max} (watt)[a]	285 (226;371)	316 (253;403)	0.03
$P_{exercise\ bout}$ (%) low[a]	39 (35;40)	42 (39;49)	0.03
$P_{exercise\ bout}$ (%) high[a]	62 (57;65)	66 (62;75)	0.03
$P_{exercise\ bout}$ (watt) low[a]	105 (90;145)	138 (100;195)	0.03
$P_{exercise\ bout}$ (watt) high[a]	170 (140;235)	205 (165;300)	0.03
Vv(mt, f) (%)[a]	4.1 (2.8;5.8)	6.0 (3.7;7.3)	0.05
N_N(c,f)	1.8 (1.5;2.0)	1.8 (1.4;2.1)	0.60
N_A(c,f) (mm^{-2})	528 (455;634)	686 (457;803)	0.07
a(f) (μm^2)[a]	3426 (2605;4084)	2848 (2499;3847)	0.03
Fiber type (% type II)	46.5 (39,9;56,9)	45.4 (39,5;54,1)	0.92

Median and range of the six subjects. Systemic and ultrastructural changes in response to 6 weeks of endurance training
$\dot{V}O_{2\,max}$ maximal oxygen consumption, P_{max} maximal power output, $P_{exercise\ bout}$ (%) load of the single exercise bout relative to P_{max}, Vv(mt,f) total mitochondrial volume per fiber volume, N_N(c,f) number of capillaries per number of fibers, N_A(c,f) number of capillaries per fiber area, a(f) fiber cross sectional area
[a]Significantly changed ($P < 0.05$) relative to the untrained state

Fiber typing

Immunohistochemical determination of fast, slow, and hybrid fiber types was carried out as described on 12-μm cryosections using monoclonal antibodies specific for fast myosin (My-32) or slow myosin (MAB1628) [11]. Type II fiber percentage was determined by counting all fibers on the cross-sections of the biopsies.

Microarray analysis

Total RNA was isolated from 25 μm cryosections of the muscle biopsies and quantified as described previously [40]. Subsequently, microarray experiments and signal quantification using custom-designed low-density Atlas® cDNA expression arrays (BD Biosciences, Allschwil, Switzerland) was carried out basically as described previously [7]. The filter membrane held 229 double-spotted probes of human cDNAs associated with particular aspects of skeletal muscle functioning (see supplemental online Fig. 1). Additionally, cDNA probes for the internal reference, 18S rRNA, were included on the nylon membrane. ^{32}P dATP-labeled cDNA was generated from 0.8 μg of total RNA by using the 229 gene-specific primers supplied. Probe synthesis for the measurement of the internal 18S rRNA reference was carried out in parallel. 0.3 μg of total RNA of each sample was always run for the generation of ^{32}P dATP-labeled cDNA with a specific primer for 18S rRNA. Filters were hybridized with a mix of total cDNA and 18S cDNA diluted 1:1,800 and washed as described [7]. The microarray experiment was designed to minimize variability of the time course data for individual subjects. Therefore, samples from the pre, 1, 8, and 24 h biopsies of any subject were processed in parallel for reverse transcription and array hybridization from the same master mix. Due to space limitations during the washing step, we had to process biopsies of the subjects taken after 3 h of recovery from exercise separately. As a consequence, we found a low variability for the time course in each subject as well as a low variability in the dataset for the 3-h recovery. Due to the fact that the 3-h recovery data was generated separately, we did not include these data in the time course of each subject.

Array evaluation

Raw signals were determined from the average signal intensities of the two corresponding dots as described previously [7]. The background was estimated using the Grid Background Dots mode for 54 dots. Transcripts

were considered as detected if the corresponding signal intensity was 30% above background in at least four of six possible filter hybridizations for one time-point (see supplemental online Fig. 1). This led to the inclusion of 112 (untrained state) and of 104 (trained state) out of 229 possible transcript signals in the statistical evaluation.

Statistics

Data are presented as median and range. Differences between values obtained before and after 6 weeks of training were statistically evaluated using the paired Wilcoxon test (Statistica 6.1; StatSoft (Europe), Hamburg, Germany).

Raw signals of the detected transcripts were background-corrected. Negative values were set to zero and a pixel count of one was added to each value. The corrected transcript signals were logarithmized to the base of 2 and standardized to 18S rRNA by subtraction. Standardization to 18S was chosen since ribosomal RNA represents a major portion of the total RNA (i.e., ~27%) [18]. To analyze the overall effect of training on the mRNA levels in both training states, an ANOVA with repeated measurements was carried out with all 702 time courses (117 transcripts of six subjects).

Afterwards, transcripts were divided into different gene ontologies according to the literature. Then, the same analysis was done for each ontology. To determine which mRNA signals were significantly different ($P < 0.05$) throughout the time course, a Friedman ANOVA with repeated measurements was used for each transcript (Statistica 6.1). The nonparametric Friedman ANOVA was used because it is not possible to reliably test for distribution with six observations. Then, to locate differences between before and after the single exercise bout, the paired Wilcoxon test was applied. No adjustments were made for multiple testing.

Verification of the array results with RT-PCR

The levels of seven transcripts (LPL, CPT1, PFKM, PPARG, MCT1, MYH1, MYH2) were verified by means of RT-PCR as previously described [31]. For this purpose, RNA from pre and 8-h biopsies was used from those four subjects where sufficient total RNA was available and compared to the corresponding four array measurements. New primers were designed with the Primer Express software (PE Biosystems, Rotkreuz, Switzerland) for MYH1 (5′-primer: ggaggaacaatccaacgtcaa, 3′-primer: tgacctgggactcagcaatg), MYH2 (5′-primer: caatctagctaaattccgcaagc, 3′-primer: tcacttatgacttttgtgtgaacc) and MCT1 (5′-primer: ccaaggcagggaaagataagtct, 3′-primer: atctttttttcacaccagattttcca). Relative cDNA amounts to 18S were calculated using the comparative CT method (threshold cycle for target amplification) according to user bulletin no. 2 of the ABI Prism 7700 Sequence Detection System (PerkinElmer) with the modification that the relative efficiency of each primer pair was included in the calculation. To test for a trend of transcript level alterations a paired Wilcoxon test at a significance level of $P < 0.1$ was applied on log-transformed 18S-normalized values.

Results

Anthropometry

All six subjects completed the 6 weeks of endurance training and the two acute bouts of ergometer exercise. The subjects performed 92 ± 4% of the 30 possible training sessions. Neither body mass nor body fat was altered after training (see Table 1). Training improved the maximal power output by 11%, the maximal oxygen consumption by 13% and the 4 mmol/l lactate threshold by 15%.

Morphometry and fiber typing

Ultrastructural muscle analysis revealed an increase in the mitochondrial density by 46% after training. The mean fiber area was reduced by 17%. The capillary density showed a shift towards an increase (30%). The fiber type distribution remained unchanged (see Table 1).

Training effects on steady-state transcript levels

The steady-state mRNA concentration of 26 transcripts was altered. Transcripts involved in oxidative metabolism were increased after 6 weeks of endurance training (Fig. 2). The number of transcripts of several matrix proteins (MMP8, MMP9, MMP15, PLAT, TIMP2, COL1A1, COL4A4) was also increased (data not shown).

Variability for microarray experiments

The separate analysis of the 3 h biopsies indicated an important assay-to-assay variability for microarray experiments. Handling the 3 h biopsies on 2 consecutive days resulted in a coefficient of determination (r^2) of the transcripts of 0.95 of this particular time point. This is significantly better than the r^2 of transcripts of the other time points ($r^2 = 0.79$), when biopsies were processed on 6 different days.

Acute effects on muscle transcriptome

In the untrained state, a 30-min bout of exercise at 62% of P_{max} led to an upregulation of the concentration of 23 out of 112 detected transcripts. The concentration of three mRNAs was downregulated. These adaptations

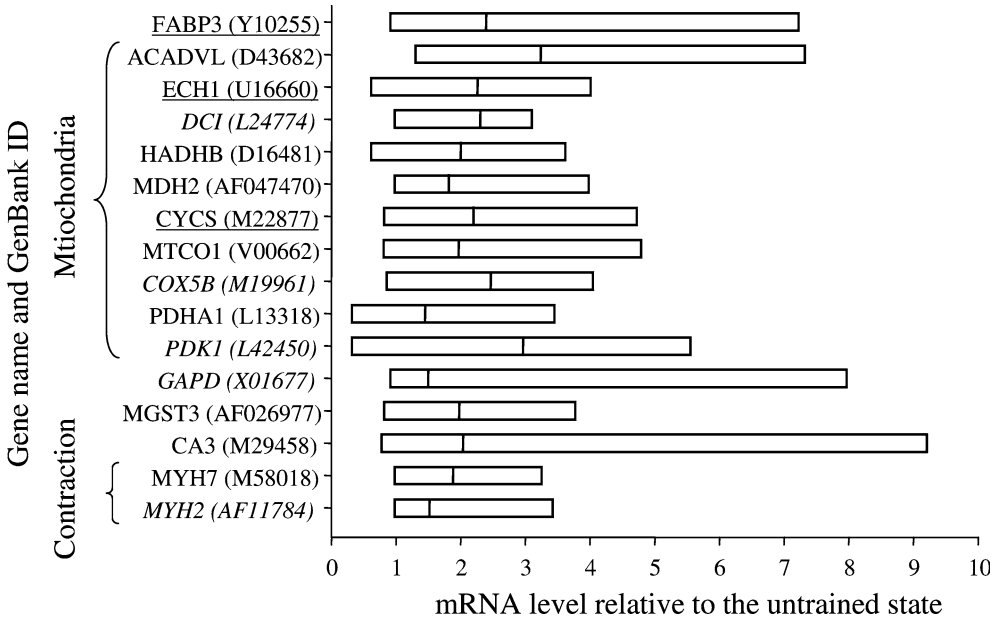

Fig. 2 Changes in the steady-state mRNA levels. Median and range of 18S standardized mRNAs with significantly altered steady-state levels after 6 weeks of training relative to the untrained state. *Underlined* gene names and *italic letters* represent those transcripts which were significantly ($P < 0.05$) or tendentiously ($P < 0.1$) altered in response to a single bout of endurance exercise in the untrained state

concerned different gene ontologies. The largest differences were evident after 8 h of recovery (Fig. 3, Table 2). Transcript levels of several glycolytic and oxidative metabolic pathways were significantly enhanced. Additionally, three transcripts coding for myogenic factors and the myosin heavy polypeptide 4 (MYH4) were increased. The pooled effect on angiogenic mRNA concentrations was a reduction after one hour of recovery independent of the training state. However, only one single factor (KDR) was significantly downregulated at this time point. Likewise, transcripts for regulatory factors collectively showed a transient reduction after one hour (Fig. 3; supplemental online Table 1).

After 6 weeks of ergometer training, 16 of 104 detected transcripts were affected during recovery from the single bout of endurance exercise even though the intensity was higher both in absolute and relative terms than before training. Most of them were downregulated between 1 and 8 h after the cessation of endurance training. In general, the response after 6 weeks of training was less pronounced than in the untrained state (Fig. 3). Compared to the single bout in the untrained state, three transcripts showed a similar regulation (PDK1, SLC16A1 and TUBA1; Table 2). 21 of the 23 previously upregulated transcripts were not significantly changed. Additionally, 11 mRNA levels were significantly altered after the single bout in the trained state (Table 2).

PCR verification

Due to the insufficient amount of muscle tissue, PCR verification could only be carried out on four biopsies. Because of the few biopsies analysed, the level of statistical significance cannot be reached with the Wilcoxon test applied. We therefore tested for trends ($P < 0.1$). Real-time RT-PCR experiments confirmed the trend for an alteration of PFKM, CPT1, MCT1 at 8 h of recovery from a single bout of endurance exercise in the untrained muscle. No differences in mRNA levels were found in either experiment for three additionally tested transcripts (MYH1, MYH2, PPARG; Table 3).

Discussion

Endurance exercise leads to specific phenotypical adaptations of muscle tissue. The known adjustments to 6 weeks of endurance training on a bicycle ergometer involve an increase in mitochondrial content and capillarity and occasionally a fast-to-slow muscle fiber type shift [12]. Marked differences of the training response found between endurance-trained and untrained subjects suggest that the adaptive processes induced by exercise stimuli critically depend on the training state [30]. Our longitudinal microarray study demonstrates that the rapid transcriptome response of human skeletal muscle during the recovery from a single endurance exercise bout is biphasic for some gene ontologies and modulated by endurance training. The observed alterations point to the cellular processes, which are induced by bouts of endurance exercise and underlie the specific structural adjustments of muscle tissue to endurance exercise. Finally, our results provide the molecular rationale for the observed decrease in the adaptive potential in trained compared to sedentary populations [30].

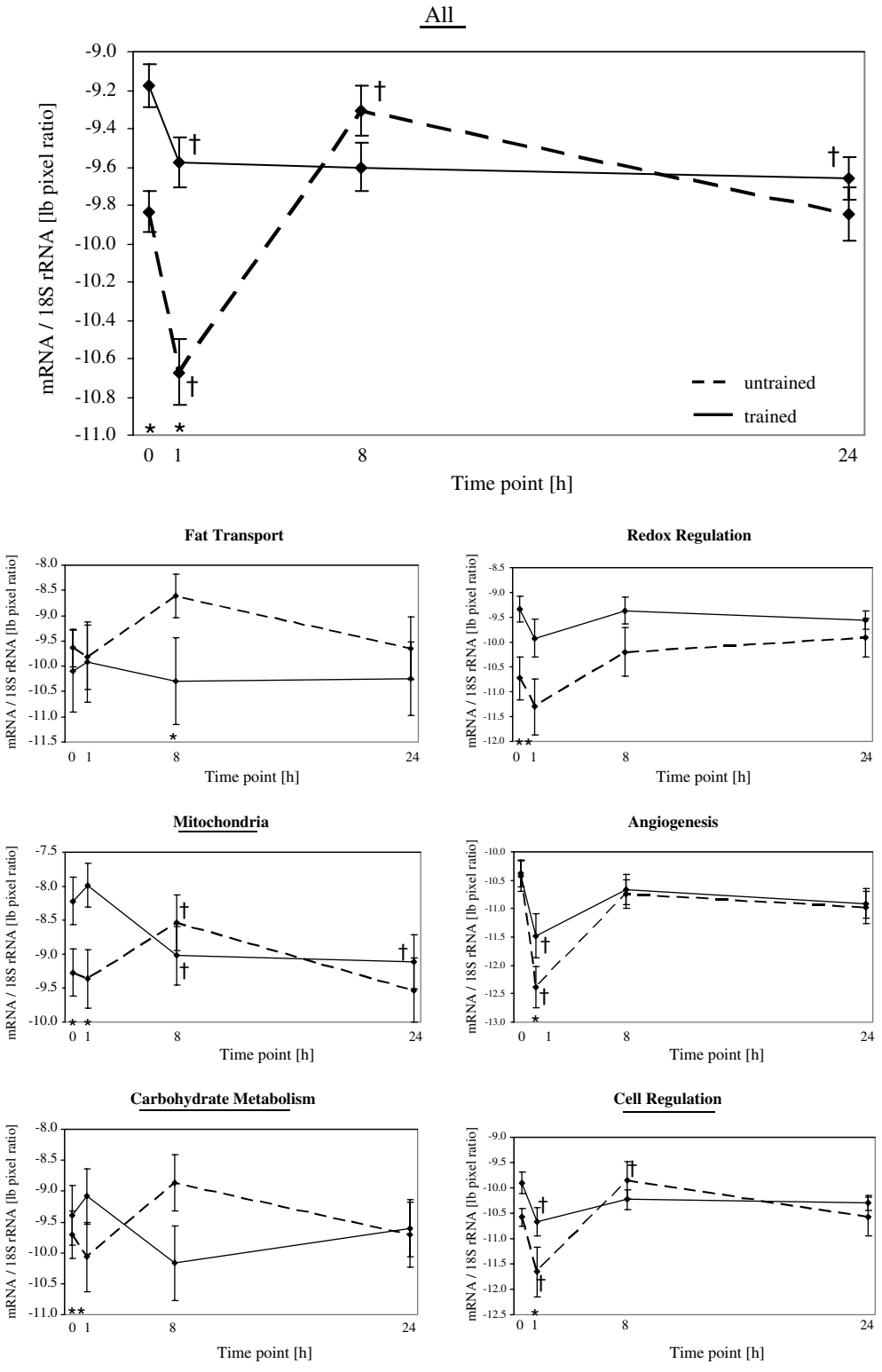

Fig. 3 Differences in the gene response after 6 weeks of endurance training. Mean values and standard error bars of logarithmized and 18S standardized mRNA levels grouped into gene ontologies. *Underlined titles* indicate a significantly different response of the untrained (*dashed line*) compared to the 6 weeks trained state (*solid line*; $P < 0.001$). Bottom of each graph, *asterisk* symbol indicates significant ($P < 0.05$) difference between the same time point of the two states. *dagger* symbol indicates significant ($P < 0.05$) difference between the time point and the pre biopsy of the same training state. The transcripts included in this analysis are listed in supplemental online Table 1

Elevated steady-state mRNA levels after endurance training

The prominent, twofold enhancement of steady-state levels of mRNAs encoding factors for fatty acid transport and mitochondrial respiratory functions points to the coincident alterations of multiple aspects of the oxidative metabolism (Fig. 2). The changes indicate that transcript concentrations of the myocellular fatty acid transport (FABP3), import of pyruvate in the citrate cycle (PDHA1, PDK1), beta oxidation (ECH1, HADHB, ACADVL, DCI), citrate cycle (MDH2), respiration (CYCS, MTCO1, COX5B) and redox regulation (CA3) are subject to concurrent regulation (Fig. 2). The training-enhanced GAPD mRNA levels are in line with the increased level of this transcript in professionally trained cyclists [39]. Our observations thus extend previous findings on elevated levels of other mitochondrial and glycolytic factors (HKII, GAPD) in endurance-trained human skeletal muscle [23, 25, 31, 36, 39]. The novel observation on significantly increased mRNA concentrations of fast IIA (MYH2) and slow type I myosin heavy chain (MYH7) provide evidence that transcriptional alterations in contractile make-up are molecular manifestations of the occasionally observed shift towards a slower contractile phenotype and the loss of type IIB muscle fibers with 6 weeks of endurance training (Fig. 2; [15]). No significant shift towards type I fibers could be observed in our study as estimated from immunohistochemistry, indicating that corresponding mRNA shifts towards the slower fiber types are more sensitive to training interventions than overt fiber type transitions.

Only three of the RNA species of metabolic factors for which steady-state concentrations were significantly increased after 6 weeks of training showed an increase after the single bout of endurance exercise in the untrained state (Table 2, Fig. 2) (FABP3, ECH1, CYCS). As most of the permanently increased mRNAs are not significantly affected after a single bout of exercise, transcriptional microadaptations need to accumulate with repetition of the endurance exercise stimuli. This may be the cause for the increased pre-exercise concentrations of several mRNAs in the trained state as previously suggested [22, 36, 39].

Limitations in repeated biopsies

Any biopsy sampling scheme may influence transcript levels and thus interfere with the interpretations on the exercise-dependence of mRNAs during a time course of recovery from exercise [38]. Repeated sampling of muscle tissue has been shown to provoke a regenerative response in a small percentage (2% of total muscle area) of muscle fibers [20]. In the present study, we have used minimally traumatic fine-needle biopsies in combination with alternate sampling at both legs to limit possible bias due to the biopsy intervention. Hence, the 1-h biopsy is taken from muscle that had not been traumatized by biopsies and can thus be regarded as not being affected by such biopsy-dependent micro-lesions. With regard to the transcript alterations observed in the untrained state that relate to muscle regeneration, i.e., IGFBP6 and IGF1, we cannot exclude interference from biopsy damage. The fact that these transcripts were not found to be altered in the trained-state using the same biopsy scheme is an argument against the biopsy procedure producing significant alterations in these (and possibly other) transcript levels. Moreover, other significantly altered transcripts involved in muscle regeneration, i.e., IL6 and MYOD1 have been reported to be elevated after cycling or resistance exercise in previously not biopsied legs [3, 35]. This indicates that some muscle regeneration is part of the normal response to exercise.

Limitations due to technical variability

We abstained from integrating the separately processed 3 h time-point data into the set of time course data as this inclusion massively added noise to the time course data. Further, this type of technical noise inhered in array processing possibly prevented the identification of transcript to structure relationships such as those previously identified in RT-PCR studies [24, 31].

Acute transcriptome response to a single bout of endurance exercise in the untrained state

To the best of our knowledge, this is the first exploratory study to show concerted alterations of multiple gene transcripts of important metabolic pathways during recovery from endurance exercise in humans. This transcriptional response induced by a single bout of endurance exercise in sedentary subjects included multiple steps of myocellular oxidative, glycolytic and redox metabolism (Fig. 3, Table 2).

The quantified mRNA alterations of different gene ontologies point to a general adaptive response in the recruited muscle of sedentary subjects in the first hours after a single bout of exercise (see Fig. 3). We assume that the upregulation of the concentration of transcripts involved in mitochondrial respiration is essential for the increase of the mitochondrial density.

Difference in the transcriptome response between the untrained and the trained state

Our study indicates that several aspects of transcript level adaptations to a single bout of endurance exercise are modified with endurance training. The dependence of transcript level changes on the training state is shown by a less-pronounced response of the trained muscle throughout recovery (Fig. 3). In contrast to the untrained state, the mRNA concentrations were decreased

Table 2 Time course of mRNA levels. Median values of 18S standardized transcript levels relative to the pre biopsy levels after a single bout in the untrained and in the trained state. *P*-values resulting from the Friedman ANOVA are indicated. Significantly upregulated and downregulated transcripts at $P < 0.05$ are colored in black or grey, respectively (Wilcoxon test). *n.d.* mRNA not detected. If the transcript could not be detected in the pre biopsy, the ratios of the time course are italicized

		Category	Gene	GenBank ID	untrained p	untrained 1h vs pre	untrained 8h vs pre	untrained 24h vs pre	trained p	trained 1h vs pre	trained 8h vs pre	trained 24h vs pre
Metabolism	CHO metabolism	CHO transport	GLUT4	M20747	0.022	*n.d.*	***2.70***	*1.12*	0.615	*1.23*	*n.d.*	*n.d.*
		Glycolysis	GAPD	X01677	0.086	1.53	1.78	2.00	0.284	1.14	1.46	1.40
			ALDOA	M11560	0.072	1.02	1.88	1.44	0.572	0.95	1.07	0.92
			ALDOC	AF054987	0.015	1.36	**3.88**	1.29	0.050	0.97	0.48	0.32
			LDHB	Y00711	-	*n.d.*	*n.d.*	*n.d.*	0.015	***1.59***	*n.d.*	***n.d.***
			PFKFB3	D49817	0.038	*n.d.*	***3.99***	*n.d.*	0.002	*1.28*	*n.d.*	*n.d.*
	Fat transport		FABP3	Y10255	0.035	1.60	**2.90**	2.84	0.532	1.08	1.42	1.10
			LPL	M15856	0.020	*2.36*	***4.62***	*2.19*	0.055	1.00	0.55	0.42
	Mitochondria	Beta oxidation	ECH1	U16660	0.020	n.d.	**2.05**	n.d.	0.102	1.27	0.81	0.70
			ACADL	M74096	0.024	1.56	**2.94**	1.06	0.072	0.97	0.62	0.36
			ACADM	M16827	0.060	1.73	2.54	0.97	0.145	1.42	0.93	1.03
			CPT1	D87812	0.024	0.96	**2.55**	1.38	0.392	0.83	0.45	0.57
			DCI	L24774	0.086	1.50	2.15	1.76	0.978	0.92	0.79	0.86
		Citrate cyclus	PDHA1	L13318	0.172	*2.27*	*3.25*	*n.d.*	0.017	1.19	**n.d.**	**0.40**
			PDHA2	M86808	0.122	2.00	1.99	0.97	0.024	1.24	n.d.	n.d.
		Oxidative Phosphorylation	PDK1	L42450	0.024	n.d.	n.d.	n.d.	0.007	**0.65**	**0.53**	**n.d.**
			COX5B	M19961	0.086	1.07	1.98	1.55	0.572	1.32	1.27	0.98
			CYCS	M22877	0.038	1.14	**2.18**	1.91	0.334	1.27	1.36	1.31
			UCP3	AF011449	0.102	1.28	2.36	1.11	0.004	0.80	**0.31**	**0.24**
	Radical metabolism		SOD1	M13267	0.029	*n.d.*	*2.49*	***3.71***	0.133	*1.29*	*1.36*	*1.18*
			SOD3	J02947	0.010	1.15	**1.95**	1.31	0.172	0.66	0.60	0.28
	Detoxification		APEX1	X59764	0.060	*n.d.*	*1.23*	*n.d.*	0.706	0.67	n.d.	n.d.
			GPX5	AJ005277	0.615	0.96	1.57	1.39	0.008	0.66	**0.38**	**0.22**
	Acid-base equilibrium		SLC16A1	L31801	0.017	*n.d.*	***2.99***	***4.34***	0.035	*n.d.*	***2.98***	***2.72***
	Ketone bodies		ACAT1	D90228	0.145	*1.57*	*2.73*	*n.d.*	0.050	1.55	0.84	n.d.
Muscle fiber	Contraction		MYH2	AF111784	0.086	1.18	1.76	1.39	0.261	0.82	0.98	0.87
			MYH4	AF111783	0.020	n.d.	**2.73**	n.d.	0.284	n.d.	n.d.	0.96
			TNNC1	X07897	0.093	0.94	1.24	0.98	0.145	0.77	0.81	0.71
	Cytoskeletal protein		TUBA1	K00558	0.035	n.d.	**2.15**	3.01	0.038	*0.89*	*1.57*	*1.32*
			VIM	X56134	0.094	1.26	1.18	1.17	0.172	0.85	1.04	1.06
Cell regulation	Myogenesis		MYOD1	X56677	0.015	1.34	**2.74**	1.13	0.457	1.00	0.82	0.36
			MEF2B	X63380	0.020	n.d.	**1.08**	n.d.	0.308	0.76	0.84	0.59
			MEF2C	L08895	0.050	n.d.	n.d.	1.01	0.334	*0.73*	*n.d.*	*1.14*
	Cell cycle regulation		IL6	X04602	0.006	1.62	**2.36**	2.11	0.094	0.67	0.87	0.62
			IGFBP1	M31145	0.615	n.d.	2.14	n.d.	0.060	0.78	n.d.	n.d.
			IGFBP5	M65062	0.072	0.79	1.30	0.91	0.102	0.63	0.96	0.85
			IGFBP6	M62402	0.007	n.d.	**2.19**	0.74	0.204	0.72	1.03	0.63
			IGF1	M27544	0.007	*n.d.*	***5.73***	*n.d.*	-	*n.d.*	*n.d.*	*n.d.*
			IGF2	M29645	-	*n.d.*	*n.d.*	*n.d.*	0.024	**0.73**	1.50	**0.69**
	Transcription factor		PPARG	L40904	0.086	1.40	2.35	3.10	-	*n.d.*	*n.d.*	*n.d.*
			NCOA4	L49399	0.241	1.16	2.08	1.50	0.007	0.71	0.47	**0.29**
			ARNT	M69238	0.086	1.01	1.53	n.d.	0.334	0.81	n.d.	0.42
	Cell signaling		YWHAZ	M86400	0.066	*4.85*	*n.d.*	*n.d.*	-	*n.d.*	*n.d.*	*n.d.*
			VCAM1	X53051	0.006	**0.56**	1.00	**0.71**	0.060	0.66	n.d.	0.67
			JUN	J04111	0.392	0.98	0.87	1.43	0.086	0.95	0.72	0.82
Angiogenesis	Angiogenesis		TNC	X78565	0.079	1.27	2.18	1.07	0.392	0.93	0.84	0.82
			EPAS1	U81984	0.241	0.74	1.59	1.19	0.060	0.95	0.86	0.77
			VEGF A	M32977	0.102	1.21	1.78	1.43	0.013	1.67	1.50	0.78
			KDR	X61656	0.013	**n.d.**	**n.d.**	**n.d.**	-	*n.d.*	*n.d.*	*n.d.*
			LAMA4	X70904	0.094	*n.d.*	*2.42*	*4.00*	-	*n.d.*	*n.d.*	*n.d.*
			FGFR4	L03840	0.060	1.52	1.81	1.92	0.532	0.71	0.42	0.36
			ANGPT1	U83508	0.066	n.d.	n.d.	n.d.	-	*n.d.*	*n.d.*	*n.d.*
			ACE	J04144	0.029	0.92	2.10	1.05	0.079	1.05	n.d.	0.77
			ADORA1	S56143	0.102	1.12	2.71	1.32	0.014	**0.60**	1.13	0.92
Protein turnover	Matrix degradation		TIMP2	J05593	0.172	1.21	2.60	0.64	0.005	**0.53**	**0.50**	**n.d.**
			TIMP3	U14394	0.046	1.21	1.42	1.18	0.457	0.61	0.60	0.50
			MMP15	Z48482	0.086	n.d.	1.18	n.d.	0.172	0.70	0.78	0.48
			MMP14	D26512	0.072	1.25	2.56	0.64	0.457	0.63	0.92	0.38
			MMP8	J05556	0.060	0.62	1.11	n.d.	0.020	**0.90**	**0.53**	**0.37**
	Degradation		UBC	M26880	0.042	1.11	1.64	1.50	0.079	0.93	1.57	1.26
			RPL13A	X56932	0.086	0.98	1.66	1.46	0.457	0.94	1.03	0.90
			RPS9	U14971	0.172	*0.99*	*2.34*	*2.54*	0.015	*0.92*	***1.35***	***1.45***
			CCT1	X52882	0.204	n.d.	1.83	1.36	0.012	**0.85**	**n.d.**	n.d.
	Extracellular structure		COL6A1	X15880	0.007	*n.d.*	***2.75***	***5.01***	0.020	*n.d.*	*n.d.*	*1.11*
			COL1A1	K01228	0.060	1.27	2.54	2.28	0.334	0.75	1.25	0.80
	Diverse		ADMR	BC034761	0.067	1.12	1.20	1.24	0.145	0.66	0.46	0.33

Table 3 Array versus RT-PCR results. Median and range of the 8 h biopsy compared to the pre biopsy in the untrained state ($n=4$) of the array as well as of the RT-PCR experiments

Gene	Array 8 h/pre		RT-PCR 8 h/pre	
	Median	*P*	Median	*P*
LPL	3.8 (2.8;4.3)	0.07	1.1 (0.8;1.8)	0.47
PPARG	1.5 (0.6;2.3)	0.27	1.4 (0.8;2.0)	0.27
PFKM	1.9 (1.5;2.8)	0.07	1.4 (1.0;1.9)	0.07
MCT1	1.3 (1.1;2.8)	0.07	1.2 (1.1;3.0)	0.07
CPT1	2.7 (1.8;3.6)	0.07	1.4 (1.2;1.6)	0.07
MYH2	1.8 (0.8;2.4)	0.14	1.0 (0.7;1.3)	0.72
MYH1	1.4 (0.7;1.8)	0.27	1.3 (0.7;1.7)	0.27

throughout the first 24 h of recovery from the single bout of endurance exercise in the trained state (Fig. 3). Relative changes in mRNA concentrations were diminished after the 6 weeks of endurance training even though the absolute as well as the relative load was higher in the second single bout. The results are compatible with previous observations on an attenuation of exercise-induced changes of IL6 and HKII concentrations with endurance training [9, 23, 36]. However, the attenuation of the VEGF response due to training could not be confirmed [27] (Table 2). The lower gene response in the trained state could be a reason why both functional [30] and structural [14] adaptations are attenuated with ongoing training with the same relative intensity. Hence, adaptations may possibly level out when the training stimulus is not changed.

Adjustments of major extracellular matrix components, i.e., collagen (COL1A1, COL4A4) and metalloproteinases (MMP8, MMP9, MMP15), indicate an important remodeling of interstitial components with endurance training. The decrease in the concentration of these factors and several transcripts involved in angiogenesis and redox regulation throughout the 24 h after the single bout of exercise in the trained state awaits explanation (see below).

Regulatory implications

How can the apparent downregulation of multiple RNAs (see Table 2) in response to exercise in the untrained and trained state be explained? Our surprising findings may be related to the events governing rapid RNA degradation. The common pathway for degradation of normally poly-adenylated RNA species includes a translation-mediated degradation via binding of factors to AU-rich sequence elements in the 3′-untranslated region (3′UTR) of the RNA [29]. Inspection of the 3′UTRs in the up- and downregulated transcript species of our study does not indicate a strict association of the presence of AU-rich sequences and reduced mRNA levels after training for these mRNAs, which would have explained the observed phenomenon. We postulate that an additional mechanism could be responsible for the regulation of the observed drop after a single bout of endurance exercise in the trained state. For instance, changed interaction of mRNA with stabilizing proteins such as seen for cytochrome c and VEGF mRNA after increased contractile activity [41] or local hypoxia [12, 19] could be involved and needs investigation.

Remarkable findings

In contrast to similar studies, the capillary per fiber ratio was not significantly increased after 6 weeks of endurance training. Angiogenesis could have been expected in response to endurance exercise, since muscle blood flow and mechanical stress are increased [5] and capillary supply has previously been shown to be enhanced after endurance training of similar duration and intensity [1, 8, 28]. However, in keeping with the structural findings of an essentially unchanged capillarity we found a low incidence of transcriptional response of pro- and anti-angiogenic factors after the single bouts as well as due to 6 weeks of endurance training. It is unclear at present why this training study did not result in the expected improvement of capillarity.

Conclusions

An intense exercise bout induces a rapid, transient transcriptome response in untrained muscle. The repetitive impact of concentric endurance exercise stimuli leads to increased steady-state levels of transcripts mainly involved in mitochondrial metabolism, muscle contraction and extracellular matrix composition. These changes go along with an increased mitochondrial density but not with a shift towards a slow fiber phenotype. In the trained muscle, a lowered responsiveness of transcript levels to a single bout of endurance exercise and a different response between regulatory events which are downregulated and metabolic processes that were upregulated after one hour of recovery is apparent.

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References

1. Andersen P, Henriksson J (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J Physiol 270:677–690
2. Bergstrom J, Hermansen L, Hultman E, Saltin B (1967) Diet, muscle glycogen and physical performance. Acta Physiol Scand 71:140–150
3. Bickel CS, Slade J, Mahoney E, Haddad F, Dudley GA, Adams GR (2005) Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. J Appl Physiol 98:482–488

4. Booth FW, Baldwin KM (1996) Muscle plasticity: energy demanding and supply processes, Sect 12, Chap 24. In: Rowell LB, Shepherd JT (eds) Handbook of physiology. Oxford University Press, New York, pp 1075–1123
5. Brown MD, Hudlicka O (2003) Modulation of physiological angiogenesis in skeletal muscle by mechanical forces: involvement of VEGF and metalloproteinases. Angiogenesis 6:1–14
6. Carter SL, Rennie CD, Hamilton SJ, Tarnopolsky (2001) Changes in skeletal muscle in males and females following endurance training. Can J Physiol Pharmacol 79:386–392
7. Dapp C, Schmutz S, Hoppeler H, Fluck M (2004) Transcriptional reprogramming and ultrastructure during atrophy and recovery of mouse soleus muscle. Physiol Genomics 20:97–107
8. Denis C, Chatard JC, Dormois D, Linossier MT, Geyssant A, Lacour JR (1986) Effects of endurance training on capillary supply of human skeletal muscle on two age groups (20 and 60 years). J Physiol (Paris) 81:379–383
9. Fischer CP, Plomgaard P, Hansen AK, Pilegaard H, Saltin B, Pedersen BK (2004) Endurance training reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle. Am J Physiol 287:E1189–E1194
10. Fluck M (2004) Exercise-modulated mitochondrial phenotype; sensors and gene regulation. J Muscle Res Cell Motil 25:235–237
11. Fluck M, Chiquet M, Schmutz S, Mayet-Sornay MH, Desplanches D (2003) Reloading of atrophied rat soleus muscle induces tenascin-C expression around damaged muscle fibers. Am J Physiol 284:R792–R801
12. Fluck M, Hoppeler H (2003) Molecular basis of skeletal muscle plasticity—from gene to form and function. Rev Physiol Biochem Pharmacol 146:159–216
13. Hood DA (2001) Invited review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol 90:1137–1157
14. Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, Weibel ER (1985) Endurance training in humans: aerobic capacity and structure of skeletal muscle. J Appl Physiol 59:320–327
15. Howald H, Hoppeler H, Claassen H, Mathieu O, Straub R (1985) Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. Pflugers Arch 403:369–376
16. Ingjer F (1979) Capillary supply and mitochondrial content of different skeletal muscle fiber types in untrained and endurance-trained men. A histochemical and ultrastructural study. Eur J Appl Physiol Occup Physiol 40:197–209
17. Kraniou Y, Cameron-Smith D, Misso M, Collier G, Hargreaves M (2000) Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle. J Appl Physiol 88:794–796
18. Larson DE, Zahradka P, Sells BH (1991) Control points in eucaryotic ribosome biogenesis. Biochem Cell Biol 69:5–22
19. Levy NS, Chung S, Furneaux H, Levy AP (1998) Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. J Biol Chem 273:6417–6423
20. Malm C, Nyberg P, Engstrom M, Sjodin B, Lenkei R, Ekblom B, Lundberg I (2000) Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. J Physiol 529(Pt 1):243–262
21. Neufer PD, Ordway GA, Hand GA, Shelton JM, Richardson JA, Benjamin IJ, Williams RS (1996) Continuous contractile activity induces fiber type specific expression of HSP70 in skeletal muscle. Am J Physiol 271:C1828–C1837
22. Pilegaard H, Ordway GA, Saltin B, Neufer PD (2000) Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. Am J Physiol 279:E806–E814
23. Pilegaard H, Saltin B, Neufer PD (2003) Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol 546:851–858
24. Puntschart A (1994) Molekulare Adaptation des menschlichen Skelettmuskels an Ausdauertraining. Thesis/Dissertation. ETH Zürich
25. Puntschart A, Claassen H, Jostarndt K, Hoppeler H, Billeter R (1995) mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. Am J Physiol 269:C619–C625
26. Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA, Wagner PD (1999) Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. Am J Physiol 277:H2247–H2252
27. Richardson RS, Wagner H, Mudaliar SR, Saucedo E, Henry R, Wagner PD (2000) Exercise adaptation attenuates VEGF gene expression in human skeletal muscle. Am J Physiol 279:H772–H778
28. Rosler K, Hoppeler H, Conley KE, Claassen H, Gehr P, Howald H (1985) Transfer effects in endurance exercise. Adaptations in trained and untrained muscles. Eur J Appl Physiol Occup Physiol 54:355–362
29. Sachs AB (1993) Messenger RNA degradation in eukaryotes. Cell 74:413–421
30. Saltin B, Henriksson J, Nygaard E, Andersen P, Jansson E (1977) Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. Ann N Y Acad Sci 301:3–29
31. Schmitt B, Fluck M, Decombaz J, Kreis R, Boesch C, Wittwer M, Graber F, Vogt M, Howald H, Hoppeler H (2003) Transcriptional adaptations of lipid metabolism in tibialis anterior muscle of endurance-trained athletes. Physiol Genomics 15:148–157
32. Schrauwen-Hinderling VB, Schrauwen P, Hesselink MK, van Engelshoven JM, Nicolay K, Saris WH, Kessels AG, Kooi ME (2003) The increase in intramyocellular lipid content is a very early response to training. J Clin Endocrinol Metab 88:1610–1616
33. Seip RL, Angelopoulos TJ, Semenkovich CF (1995) Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue. Am J Physiol 268:E229–E236
34. Seip RL, Mair K, Cole TG, Semenkovich CF (1997) Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. Am J Physiol 272:E255–E261
35. Starkie RL, Arkinstall MJ, Koukoulas I, Hawley JA, Febbraio MA (2001) Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. J Physiol 533:585–591
36. Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, Cameron-Smith D (2002) Exercise training increases lipid metabolism gene expression in human skeletal muscle. Am J Physiol 283:E66–E72
37. Turner DL, Hoppeler H, Claassen H, Vock P, Kayser B, Schena F, Ferretti G (1997) Effects of endurance training on oxidative capacity and structural composition of human arm and leg muscles. Acta Physiol Scand 161:459–464
38. Vissing K, Andersen JL, Schjerling P (2005) Are exercise-induced genes induced by exercise?. FASEB J 19:94–96
39. Wittwer M, Billeter R, Hoppeler H, Fluck M (2004) Regulatory gene expression in skeletal muscle of highly endurance-trained humans. Acta Physiol Scand 180:217–227
40. Wittwer M, Fluck M, Hoppeler H, Muller S, Desplanches D, Billeter R (2002) Prolonged unloading of rat soleus muscle causes distinct adaptations of the gene profile. FASEB J 16:884–886
41. Yan Z, Salmons S, Jarvis J, Booth FW (1995) Increased muscle carnitine palmitoyltransferase II mRNA after increased contractile activity. Am J Physiol 268:E277–E281