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Review

Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli

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Summary

Biological systems have acquired effective adaptive strategies to cope with physiological challenges and to maximize biochemical processes under imposed constraints. Striated muscle tissue demonstrates a remarkable malleability and can adjust its metabolic and contractile makeup in response to alterations in functional demands. Activity-dependent muscle plasticity therefore represents a unique model to investigate the regulatory machinery underlying phenotypic adaptations in a fully differentiated tissue.

Adjustments in form and function of mammalian muscle have so far been characterized at a descriptive level, and several major themes have evolved. These imply that mechanical, metabolic and neuronal perturbations in recruited muscle groups relay to the specific processes being activated by the complex physiological stimulus of exercise. The important relationship between the phenotypic stimuli and consequent muscular modifications is reflected by coordinated differences at the transcript level that match structural and functional adjustments in the new training steady state. Permanent alterations of gene expression thus represent a major strategy for the integration of phenotypic stimuli into remodeling of muscle makeup.

A unifying theory on the molecular mechanism that connects the single exercise stimulus to the multi-faceted adjustments made after the repeated impact of the muscular stress remains elusive. Recently, master switches have been recognized that sense and transduce the individual physical and chemical perturbations induced by physiological challenges *via* signaling cascades to downstream gene expression events. Molecular observations on signaling systems also extend the long-known evidence for desensitization of the muscle response to endurance exercise after the repeated impact of the stimulus that occurs with training. Integrative approaches involving the manipulation of single factors and the systematic monitoring of downstream effects at multiple levels would appear to be the ultimate method for pinpointing the mechanism of muscle remodeling. The identification of the basic relationships underlying the malleability of muscle tissue is likely to be of relevance for our understanding of compensatory processes in other tissues, species and organisms.

Key words: exercise, endurance, hypoxia, gene, transcriptome, morphometry, microarray, PCR.

Introduction

Skeletal muscle's malleability, which enables remodeling of the muscle's structural makeup according to alterations in demand, is a particularly striking phenomenon in the animal kingdom. This plasticity is reflected by the pronounced adjustments seen in muscular force, endurance and contractile velocity of mammalian skeletal muscle as a result of an alteration in demand (Booth and Baldwin, 1996). This guise is widely recognized in sports, where distinct adaptation of muscle tissue after training in athletes leads to striking phenotypic modifications that maximize the specific performance of this contractile tissue.

One notable facet of skeletal muscle plasticity is the specificity of the adaptive response to a given stimulus (Fluck and Hoppeler, 2003), where the degree of loading and the number of muscular contractions appear to be the dominant stimuli for the muscular adaptations. For instance, highly repetitive, low-load exercise training will cause differentiation of muscle fibers towards a fatigue-resistance phenotype (Pette, 2002). This cellular specialization allows the recruited muscle fibers to sustain a high number of slow contractions. Conversely, exercise regimes involving a high degree of loading provoke an increase in force *via* fiber hypertrophy. By contrast, maintenance of both skeletal muscle mass and

oxidative capacity are dependent on the impact of contractile stimuli, as shown by the pronounced deconditioning of muscle function with inactivity. Thus the profile of muscle perturbation exerts essential control over the muscle phenotype. This review sets out our recent findings that build the case for the important involvement of gene expression in ameliorations of muscle function with repetitive exercise stimuli.

Mechanisms underlying myocellular adaptations to endurance training

The cellular and functional mechanisms underlying the particular adaptations of the composite muscle tissue to endurance exercise are now well understood. The cellular processes behind muscle plasticity involve qualitative and quantitative alterations in muscle fiber cells and associated structures. Alterations to endurance training over a period of weeks to months involve differentiation of the muscle fibers towards a phenotype with a high mitochondrial volume density (Fluck and Hoppeler, 2003). These myocellular improvements are assisted by an increase in capillary density and may involve a shift of the contractile character of the fibers towards a slow type *via* an exchange of sarcomere components (Fluck and Hoppeler, 2003). Collectively, these linked adjustments contribute towards maximization of substrate delivery, respiratory capacity and contractile parameters during the frequent slow contractions that occur with endurance-type exercise.

The regulatory mechanisms underlying the specific adjustments of muscular organelles to exercise are beginning to be unravelled. The data support the notion that gene expression underlies muscular adjustments in response to physical activity (Fig. 1). The model suggests that individual homeostatic perturbations provoked by exercise are integrated into alterations in expression levels of diffusible gene copies (i.e. mRNAs), leading to translation of the encoded proteins by the ribosomal machinery. Enhanced levels of gene transcripts would therefore support the synthesis of protein components and provoke structural remodeling and functional adjustments in the long term. Thus changes in mRNA act as a blueprint for adjustment of protein composition (for reviews, see Fluck et al., 2005a; Fluck and Hoppeler, 2003; Booth and Baldwin, 1996). In this manner, exercise is known to specifically affect the rate of synthesis (transcription) and degradation of gene transcripts (Yan et al., 1996; Fluck and Hoppeler, 2003). Gene expression is therefore an important layer of processing for integration of exercise stimuli into the adjustments of muscle makeup necessary to match muscle function to alterations in demand.

To test this basic concept we set out to investigate the post-transcriptional processes underlying the tuning of muscle metabolism upon endurance training. The focus of analysis was on key factors of carbohydrate and lipid metabolization, since these molecule classes constitute the main substrates of skeletal muscle (Holloszy and Coyle, 1984; van Loon et al., 2001). Both of these organic compounds are imported from the capillary bed *via* facilitative processes into the myocellular compartment. There they reside as myocellular stores until they are subjected to controlled metabolization to generate their energy equivalents (see Fig. 2). During the catabolic reaction, carbohydrates in the form of glucose are primarily degraded *via* anaerobic glycolysis to pyruvate, and eventual complete oxidative combustion in the mitochondria *via* the Krebs cycle. Similarly, triglyceride-derived free fatty acids are imported into mitochondria where they are combusted *via* beta-oxidation and the Krebs cycle. This latter process produces carbon dioxide and supplies reduction equivalents that lead to ATP production *via* coupling to oxidative phosphorylation. The ATP generated during mitochondrial respiration is then used to drive energy-dependent processes such as contractions (Fig. 2). From a calorific perspective, the aerobic processes within mitochondria are more efficient in generating ATP than

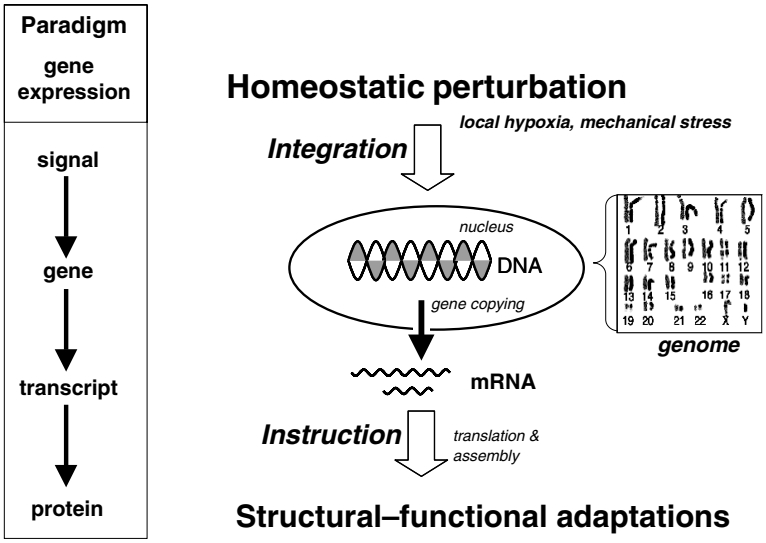

Fig. 1. Concept of the integration of physiological stimuli in phenotypic responses. Homeostatic perturbations such as those induced by exercise in muscle are integrated *via* signaling pathways into alterations in gene transcription. The diffusible gene copies produced then provide the message for the instruction of muscle tissue remodeling *via* translation and assembly of the encoded proteins. Based upon this relationship it is hypothesized that the systematic exploration of differences in transcript levels relative to phenotypic adjustments arising from the impact of exercise will reveal the strategy underlying muscle plasticity.

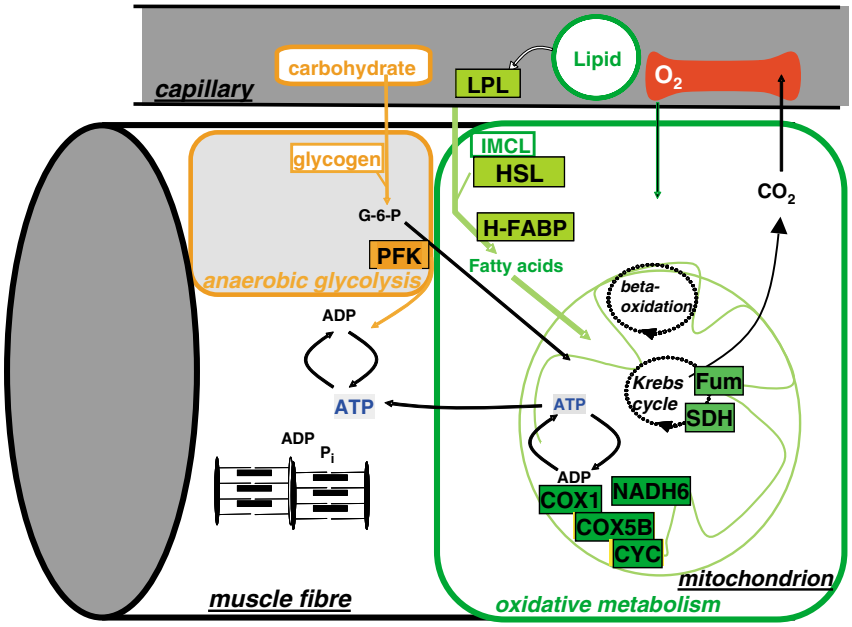

Fig. 2. Metabolic processes in muscle fibers. The main biochemical processes involved in energy generation in striated muscle involve the combustion of fatty acids and carbohydrates. Carbohydrates (orange) are imported *via* facilitative processes from the capillary supply lines to the myofibre, where they may be stored as intramuscular triglycerides or glycogen, respectively, for later combustion. Fatty acid metabolization (green box) is an obligatory aerobic process that takes place in mitochondria *via* beta-oxidation and the Krebs cycle. In contrast, the 'metabolic conversion' of carbohydrates *via* glycolysis in the cytoplasm (orange box) is oxygen-independent and is not necessarily coupled to mitochondrial respiration. This may lead to the production of the anaerobic end-product lactate. The decomposition of organic backbones in mitochondria produces reduction equivalents (and CO_2), the former of which drive the oxygen-dependent generation of ATP *via* coupling to respiratory chain. Boxed factors are the crucial proteins involved at successively aligned transport, storage and conversion steps of metabolic pathways in striated muscle and whose mRNA expression was investigated. Endothelial LPL is involved in transporting fatty acids (FA) from the vasculature through the interstitium into the myocellular compartment (Glatz and Storch, 2001; Jeukendrup, 2002). There H-FABP is believed to play a main role in the intramyocellular transport of free FA. HSL liberates free FA from IMCL for mitochondrial oxidation. CPT I is a key enzyme for the uptake of FA into the mitochondrial matrix. The Krebs cycle enzymes Fum and SDH and the constituents of the electron transport chain, NADH6, COX1 and COX4, are then responsible for oxygen-dependent ATP production during mitochondrial respiration. PFKM represents a main control step for entry of carbohydrates into the glycolytic pathway. For further explanation, see List of abbreviations.

the anaerobic processes. This relates to the principal implication of oxidative processes in energy allocation with sustained, submaximal types of exercise (Jeukendrup, 2002).

The strategy employed to unravel the regulation of metabolic processes involved parallel assessment of expression, structural and functional parameters in a major recruited muscle group in two 'steady states': endurance-trained and untrained subjects, in order to reveal the biological relationships that drive the muscle's response to repeated endurance exercise. Oxidative metabolism measurements included the determination of mRNA levels for factors necessary for relevant steps of mobilization and oxidative metabolization of fatty acid in mitochondria as well as mitochondrial volume densities (Fig. 2). Alterations characterized in the heavily recruited vastus lateralis muscle demonstrated that mitochondrial respiratory factors are concomitantly enhanced in endurance-trained athletes (Puntschart et al., 1995). These adjustments in transcript expression were in proportion to the augmented mitochondrial volume density and the increase in systemic maximal oxygen uptake seen in the athletic population compared to untrained controls (Fig. 3A). Note that both mitochondrial- and nuclear-encoded transcripts were increased in a corresponding fashion. Thus mRNA levels of major mitochondrial respiratory subunits are significantly correlated with mitochondrial volume density (Fig. 4), suggesting that local adaptations of mitochondrial transcript number in a major locomotory muscle group are co-regulated, and matched to maximal respiration of the system (i.e. V_{O2max}) during exercise.

Symmorphosis at the molecular level

Molecular examination of the processes underlying the amelioration of metabolic processes with repeated endurance exercise corroborated observations on the specific co-regulation of oxidative processes in skeletal muscle. Detailed investigation of tibialis anterior muscle in competitive duathletes and untrained male subjects revealed a concomitant enhancement of gene transcript levels for factors involved in mobilization of fatty acids. Furthermore, the study on this muscle group, which is mostly involved in body balance and foot control, confirmed the augmented gene message for the respiratory chain component, cytochrome *c* oxidase subunit I (COX1; Fig. 3B) (Schmitt et al., 2003). Further exploratory correlation analysis uncovered significant relationships between the two main lipases of skeletal muscle, hormone-sensitive lipase (HSL) and alkaline lipoprotein lipase (LPL), with functional elements of lipid metabolism (Fig. 4B). For instance, HSL mRNA was significantly correlated with the volume density of intramyocellular triglycerides and mitochondria, determined by electron microscopy. HSL is known to reside at the periphery of triglyceride droplets and liberates the entrapped triglycerides for mitochondrial oxidation (for reviews, see Schmitt et al., 2003; Donsmark et al., 2004). The findings now imply a relationship between the

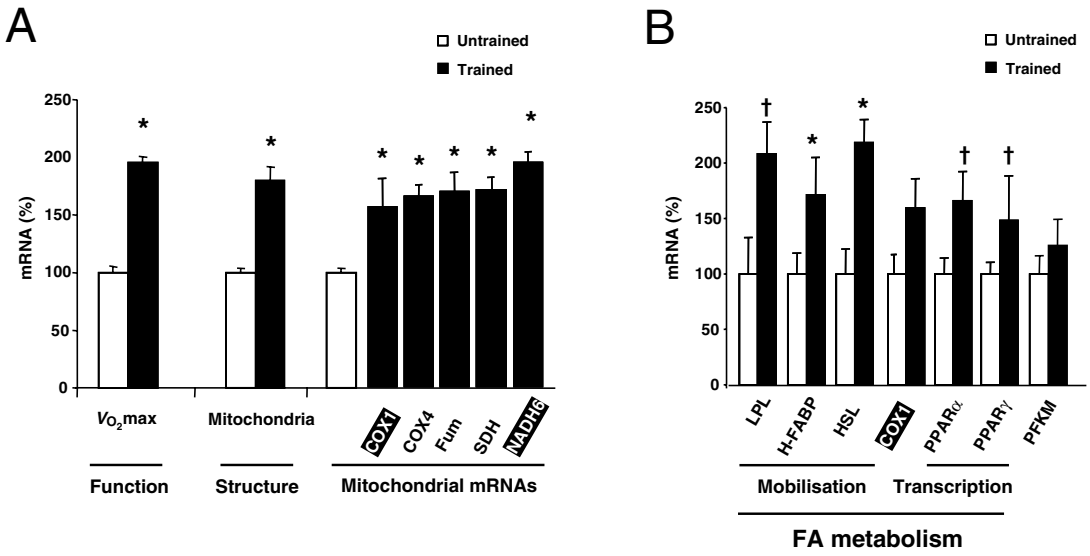

Fig. 3. Transcriptional basis of the tuning of oxidative capacity by endurance training. (A) Normalized maximal (V_{O2max}) consumption, mitchondrial volume and mitochondrial RNA levels of various respiratory proteins in m. vastus lateralis of endurance runners *vs* untrained subjects. mRNA levels are relative to levels of 28S rRNA. (B) Differences in concentration of transcripts in tibialis anterior muscle between untrained and endurance-trained subjects relative to 18S rRNA. Values are means ± s.e.m. Black boxes denote those mRNAs being encoded by mitochondrial DNA. Trained subjects had been exposed to years of endurance training and competition. Significant differences between values from endurance-trained *vs* untrained subjects are indicated: $^{*}P<0.05$; $^{\dagger}P<0.10$. For enzymes, see List of abbreviations.

flux of fatty acids liberated from intramyocellular lipids (IMCL) on the one hand and expression of the transcript encoding HSL on the other. Similarly, the transcript level of the LPL was found to correlate with the absolute rate of repletion of intramuscular triglyceride stores after exhaustive exercise (Schmitt et al., 2003). LPL hydrolyses fatty acids from lipoproteins and chylomicrons in the vasculature. The molecular information implies that endurance training does improve mitochondrial respiration along with an increase in the capacity to import blood-borne fatty acids. The match of expressional adjustments in sequential steps of the oxidative pathway in recruited muscle groups is interpreted to reflect the local level of coordination, which contributes to the systemic improvements of the oxygen pathway with endurance training (Weibel et al., 1991).

Expressional mechanisms underlying symmorphosis

The correspondence of gene expression changes with functional adjustments of the oxidative pathway after endurance training signals a distinct molecular circuitry in skeletal muscle that underlies improved fatigue resistance. This argument is supported by observations on an important correlation of the transcript levels for HSL, COX1 and fatty acid binding protein of the heart (H-FABP) in tibialis anterior muscle of untrained and endurance-trained subjects (Schmitt et al., 2003). This relationship calls for a specific mechanism capable of coordinating adjustments in gene expression dependent on muscle recruitment. The correspondence between two master transcriptional regulators of lipid metabolism is striking, i.e. the peroxisome proliferator-activated receptors α and γ (PPARα and γ), with HSL and LPL mRNA levels. The PPARs are nuclear receptors that control transcription of a battery of genes involved in fatty acid catabolism (Smith, 2002). Free fatty acids act as the main physiological substrates for the activation of PPARs and the downstream activation of gene expression. In this regard, the relationship between mitochondrial gene transcript number and the RNA concentration of major regulators of mitochondrial biogenesis is also striking. For instance, the mitochondrial transcription factor (Tfam) and peroxisome proliferator coactivator-1α (PGC-1α), known to lie up- and downstream of PPAR action (reviewed in Fluck and Hoppeler, 2003), are significantly correlated with COX1 and COX4 mRNA (Zoll et al., 2006). These inter-gene relationships indicate a major role of the PPAR-pathway in the control of mitochondrial phenotype by exercise. These steady-state adaptations of the PPAR-system relate to the increased flux of fatty acids in muscle tissue with a sustained increase in locomotion in man (van Loon et al., 2001). Consequently, the PPAR-system is seen to represent a major pathway that underlies sensing and integration of exercise stimuli into modifications of the oxidative pathway. This metabolically driven circuitry provides a rationale for the symmorphotic adjustment in skeletal muscle with endurance training and the suspected alteration in mitochondrial turnover (Weibel et al., 1991; Connor et al., 2000).

The muscular gene response and the repetition effect

By definition, training-induced muscle adjustments are the consequence of repetition of single-exercise stimuli.

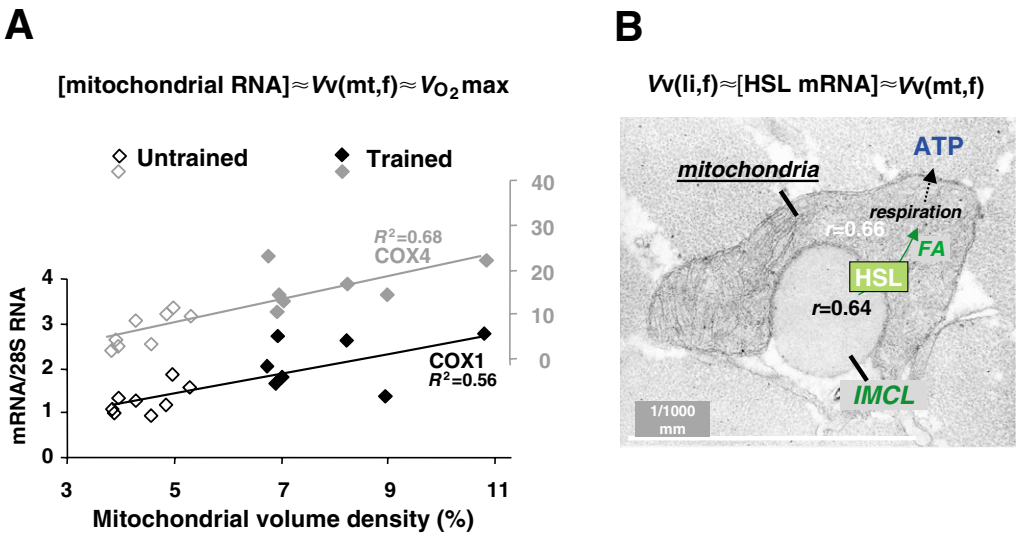

Fig. 4. Symmorphosis at the RNA level. Transcript–structure correlations bring about significant functional relationships. These concern the coordination of gene expression between the nuclear and mitochondrial genomes and the match of lipase expression to rate and capacity of fatty acid metabolism. (A) Correlation of mitochondrial-encoded COX1 and nuclear-encoded COX4 mRNA levels with mitochondrial volume density per fiber, i.e. *Vv*(mt,f). (B) Micrograph showing an intramyocellular droplet of lipid (IMCL) being enveloped by a mitochondrion. The pathway involved in the oxidative combustion of IMCL-derived fatty acids (FA) in mitochondria is indicated by a green arrow. The suspected localization of HSL and the correlation coefficients (*r*) between HSL mRNA with volume density of intramuscular lipids, *Vv*(li,f), and volume density of mitochondria, *Vv*(mt,f), are given in black and white font, respectively.

Adaptations of muscle tissue to increased contractile activity are proposed to be confined to the recovery phase from each fatiguing bout of exercise (Fluck, 2004; Pilegaard et al., 2000). This would allow an overshoot of cellular adaptations that support the accumulation of incremental remodeling responses after each session, and with repetition of the single-exercise stimuli this would support the enhanced endurance performance (Fig. 5). The extent to which gene expression processes underlie the continued build-up of muscle structure and performance with each bout of exercise remains to be explored.

To that end we hypothesized that a systematic exploration of changes in mRNA levels would reveal the molecular strategies underlying muscle plasticity. We employed microarray technology to test whether RNA adaptations in the recovery phase contribute to exercise-induced build-up of muscle tissue. This novel technology allows the parallel assessment of adjustments in the levels of hundreds to thousands of transcripts. Nylon filters holding 222 cDNA probes for muscle-relevant factors were custom-designed for the detection of reverse-transcribed RNAs after different recovery times from exercise (Fluck et al., 2005a).

Exploration of the muscular adjustments revealed a general trend for a transient upregulation 8 h after one bout of ergometer exercise (Fig. 6) (Schmutz et al., 2006). This main response to 30 min of bicycling concerned gene families

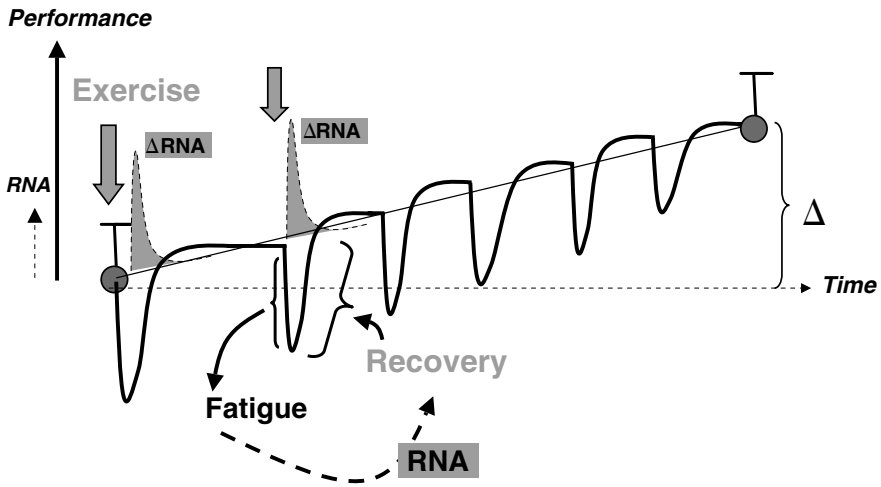

Fig. 5. Microadaptations of transcript expression relate to the training effect. Model of the increase in (mitochondrial) RNA and endurance performance with repetition of exercise. Each bout of exercise leads to an overshoot of transcript levels in the recovery phase from fatiguing exercise (ΔRNA), which leads *via* translation to a microadaptation of the encoded protein and related structure. This relays to the gradual accumulation (Δ) of mitochondrial volume density and the improved oxidative capacity with repetition of endurance exercise. A match of transcriptional, structural and functional parameters is observed in recruited muscle groups between untrained and endurance-trained steady states. Black stippled and solid lines indicate the evolution of RNA levels and performance, respectively, during the training.

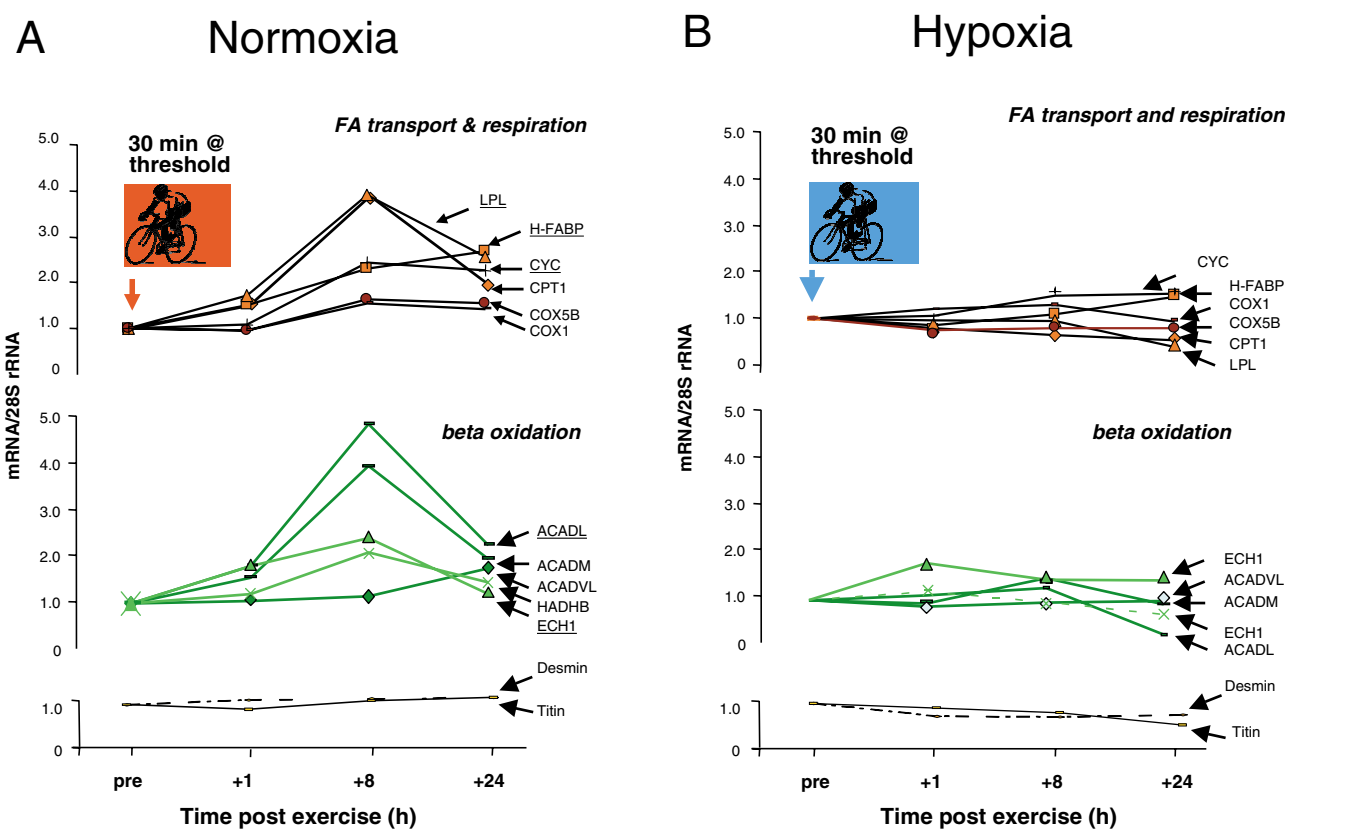

Fig. 6. Time-course of the muscular exercise response in (A) normoxia and (B) hypoxia. Untrained male subjects exercised for 30 min at the aerobic threshold on a bicycle ergometer while breathing normoxic (21% O_2) or hypoxic air (13% O_2, N=6/group). Biopsies were harvested during the time-course of recovery from this single bout of exercise. Total RNA was isolated and subjected to expression profiling using custom-made microarrays (Fluck et al., 2005a; Schmutz et al., 2006). Transcript signals were related to the internal 28S rRNA reference and analyzed for statistical significance using a Friedman ANOVA. The significant changes of selected transcripts related to the oxidative pathway, such as fatty acid transport, beta oxidation and mitochondrial respiration, are shown in A and B. Significantly altered mRNA levels throughout the time-course of recovery are underlined. Unaffected transcript levels of the main cytoskeletal factors titin and desmin demonstrate the specificity of effect.

implicated in the oxidative pathway. Multiple factors involved in the extracellular and myocellular mobilization of fatty acids as well as mitochondrial beta oxidation and the electron transport chain were affected. The acute adjustment of the muscle transcriptome related to the oxidative pathway to 6 weeks of training was found to recapitulate the known elevation with years of endurance training (Fig. 3) (Fluck and Hoppeler, 2003). The results support the concept that microadaptations in expression after each exercise bout instruct the structural–functional adjustments of oxidative muscle metabolism to each exercise bout which accumulate with repetition of exercise stimuli (Fig. 5).

Conversely, when typical muscle adjustments had been established after 6 weeks of endurance training this transcript response was specifically modified. In particular, the acute induction of most transcripts to a matched single exercise bout was blunted (not shown) (Schmutz et al., 2006), which can be explained by the increased steady-state mRNA levels and relates to the reduced adaptive potential in trained individuals (Saltin et al., 1977). These observations reveal that a multi-faceted and coordinated expression program underlies the specific muscular adjustments with the repeated impact of exercise with training and relates to the sensitivity of response.

Hypoxia as a stimulus of the exercise response

In the context of the stimuli that instruct muscle plasticity, local hypoxia has been postulated to constitute a main signal for muscular adjustments to endurance exercise (Hoppeler and Vogt, 2001), given that there is a dramatic drop in muscle oxygen tension with the onset of exercise (Richardson et al., 1995; Richardson et al., 2001). Similarly, ambient hypoxia reduces muscular oxygen levels and is known to amplify the exercise-induced local muscle hypoxia (Richardson et al., 1995; Hoppeler et al., 2003). This relates to the long-held theory of promotion of respiratory adjustments in chronic hypoxia and the hypoxia-induced shift away from the oxidative metabolisation of fatty acids towards increased utilization of carbohydrates *via* the glycolytic pathway (Reynafarje, 1962; Hoppeler et al., 2003). We therefore speculated that the addition of a defined normobaric hypoxic stress to the stimulus of a 30 min ergometer-bout would shift the acute muscular transcriptome response towards reduced level adjustments of

transcripts related to fatty acid metabolism. This assumption was tested in vastus lateralis muscle of untrained subjects, and a blunting of the exercise-induced transcript level response for factors of fatty acid transport in the recovery phase after exercise at simulated altitude was seen (Fig. 6B) (S. Schmutz, unpublished observations). Similarly, the transcript expression of mitochondrial respiratory factors was blunted during recovery from bicycling in hypoxia. The differentiation of the acute molecular response to exercise in untrained subjects by hypoxia highlights the physiological role of muscular oxygen tension for the adjustments in striated muscle. The blunting of the ATP-dependent mRNA response in untrained subjects probably relates to a sizable drop in free energy levels in recruited muscle with the extra muscle deoxygenation due to the hypoxic co-stimulus (Kammermeier, 1987). The appreciation of the hypoxia-specific modulation of human muscle's response to exercise and the functional consequences for muscle remodeling invite further exploration.

Hypoxia signaling in muscle

In subsequent experiments we aimed to explore the signaling mechanisms that drive hypoxia-inducible gene expression in skeletal muscle. For these investigations, we took advantage of the possibilities offered by transgenic mouse models, which allowed us to access the role of single factors in signaling processes *via* the visualization of phenotypic aberrations resulting from inactivation/activation of specific genes and products (Booth et al., 1998). The use of inbred rodent models

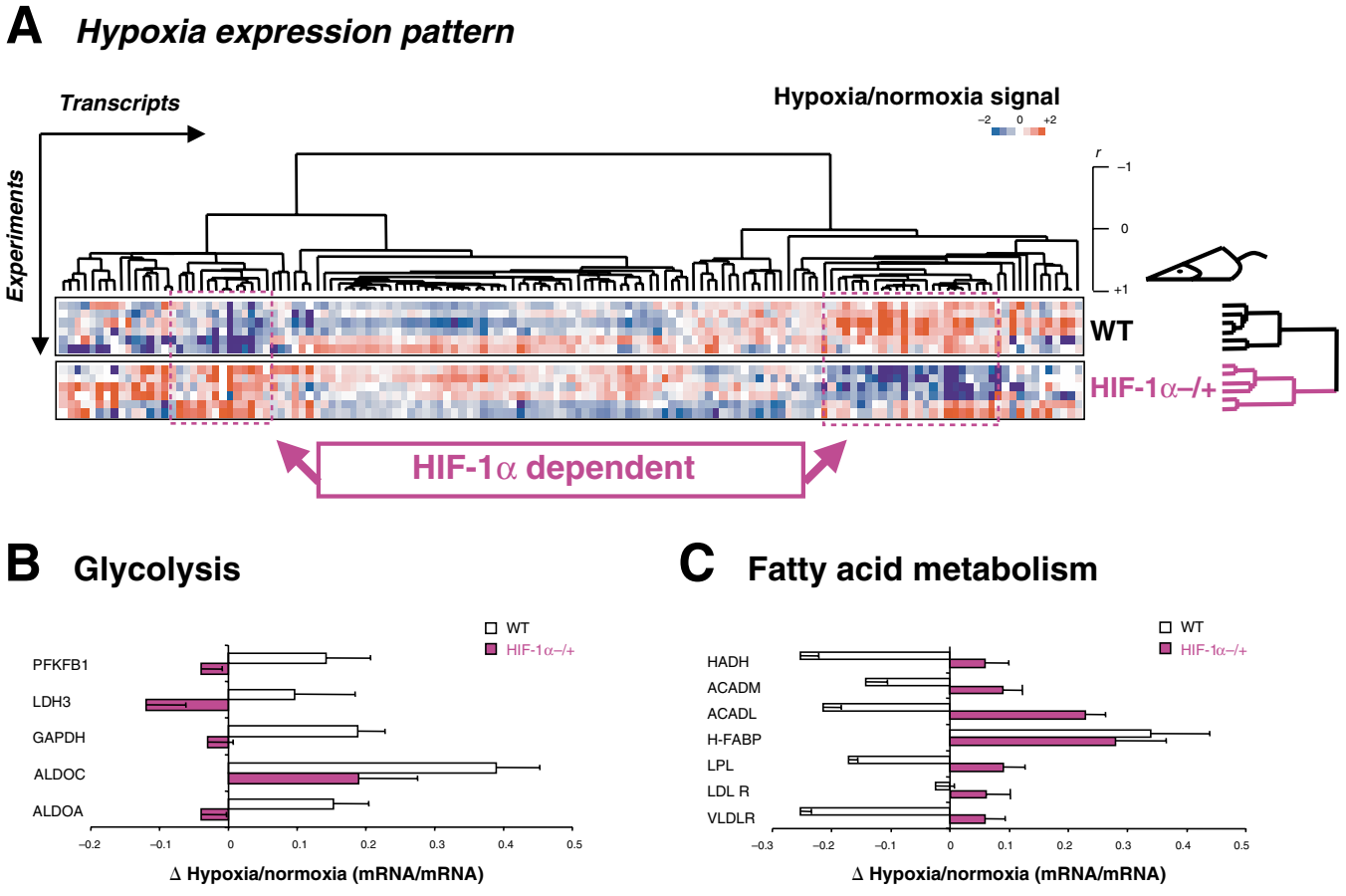

Fig. 7. The muscular hypoxia-response is HIF-1α dependent. Spontaneously active HIF-1α heterozygous-deficient mice (HIF-1α–/+) and wild-type mice (WT) were subjected to 24 h of normoxia (21% O_2) or hypoxia (10.5% O_2, *N*=6/group). M. solei were harvested from the four experimental groups and analyzed with custom-designed microarrays for expression of 222 muscle-relevant transcripts (Fluck et al., 2005a; Dapp et al., 2004). The hypoxia-to-normoxia signal ratio of 142 detected transcripts was assessed by descriptive cluster analysis (A) and probability testing (B,C) to identify genotype-dependent differences in the hypoxia response (Däpp et al., 2006). (A) Hierarchical cluster analysis visualizing the global pattern of hypoxia-induced expression changes for the different experiments. The correlations (*r*) of the transcript response in hypoxia are reflected by the line length in the dendrogram. Alterations of expression levels for each transcript and experiment are given in color coding (up, red; down, blue). The clustering was recalculated as described (Fluck et al., 2005b) for centered correlations from the data of (Däpp et al., 2006) using log-transformed and mean-centered data. Note*:* the hypoxia expression patterns group according to the respective genotype. Distinct clusters of HIF-1α-dependent transcripts, which demonstrate co-regulated level changes upon hypoxia and whose response was 'inverted' in HIF-1α deficient muscle, are boxed. (B,C) Mean and standard error of significant transcript level differences for factors involved in the successive steps of glycolysis (B) and fatty acid metabolism (C). Gene expression alterations in HIF-1α–/+ mice are shown in pink. Note*:* the muscular transcript response of glycolytic and oxidative pathways in hypoxia is reversed in HIF-1α-deficient mice.

also has obvious advantages as it permits control over experimental variables, reducing noise and variability and allowing maximization of the physiological input that drives muscle plasticity (for a review, see Wittwer et al., 2004). We applied this tool towards elucidation of the role of the alpha subunit of the hypoxia-inducible factor 1 (HIF-1α) in response to a reduction in ambient oxygen concentration. This factor acts as a regulatory switch for hypoxia sensing in various cellular systems (Semenza, 2000). In normoxia, HIF-1α is rapidly tagged for degradation. Conversely, it is stabilized in an organ-specific manner in hypoxia, permitting its association with the HIF-1β subunit to form the DNA-binding HIF-1 complex (Pisani and Dechesne, 2005; Stroka et al., 2001; Yu et al., 1998). This heterodimer initiates the transcription of various hypoxia-responsive genes of metabolic processes that would be advantageous under the constraint of reduced oxygen, such as capillary growth and glycolysis (for a review, see Hoppeler et al., 2003).

The specific experimental set-up to elucidate the role of HIF-1α in the muscular hypoxia response employed HIF-1α heterozygous-deficient mice exposed to hypoxic *vs* normoxic air (Fig. 7). Mice with one HIF-1α allele ablated (HIF-1α–/+) demonstrated a 30% lower level of HIF-1α mRNA in the anti-gravitational soleus muscle under study than control mice (Däpp et al., 2006). Such a partial HIF-1α deficiency has been shown before to interfere negatively with multiple systemic responses to hypoxia (Yu et al., 1999). To test this assumption, differences in hypoxia-induced adjustments in transcript levels in soleus muscle under spontaneous cage activity were compared between wild-type and HIF-1α heterozygous-deficient mice. Subsequently, genotype-dependent differences of the effect of a 24 h exposure to hypoxia were analyzed for major patterns using cluster analysis. This multi-correlation algorithm identified that the expressional response of muscle to hypoxia was distinct between the two genotypes (Fig. 7A). Detailed inspection of the indicated differences using probability testing demonstrated major shifts in hypoxia-induced adjustments in expression related to carbohydrate metabolism with a reduction of the HIF-1α mRNA level. In contrast, a general level of reduction of transcripts related to fatty acid metabolism was noted in hypoxia and reversed in the HIF-1α heterozygous-deficient mice (Fig. 7C). Conversely, hypoxia-induced mRNA levels of glycolytic factors were blunted in the mice with reduced HIF-1α levels (Fig. 7B). As local hypoxia is a suspected consequence of ambient oxygen concentration, the latter finding underscores the suspected role

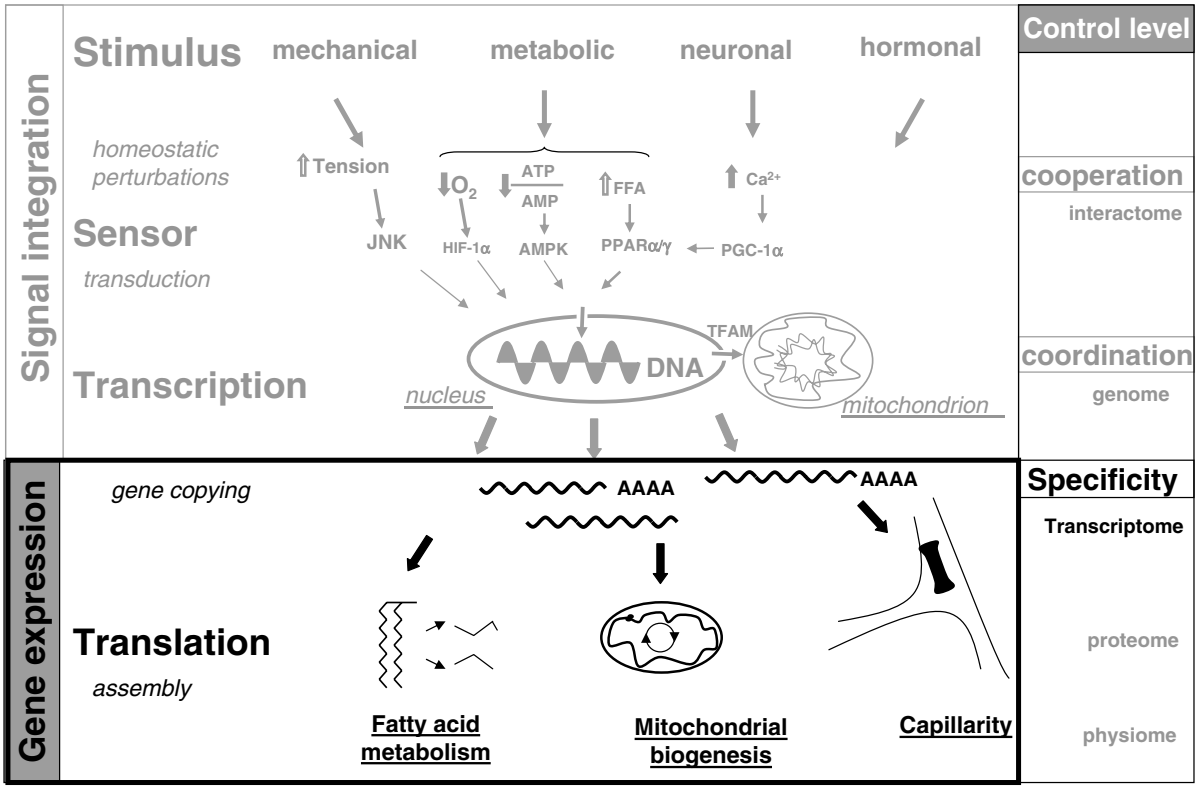

Fig. 8. Scheme visualizing the integration of the complex stimulus of exercise in recruited skeletal muscle. Different homeostatic perturbations, such as those related to metabolic flux, loading, hormonal and neuronal alterations, are converted by specific sensory molecules into the activation of signaling cascades. These ultimately control muscle fate *via* the regulation of gene expression. Distinct master switches evolve that relate to the main themes of the gene expressional response in striated muscle. These phenomena involve the cooperation of gene expressional regulation of metabolic pathways, the coordination between nuclear and mitochondrial genomes and the specificity of the muscular adaptation with respect to the 'composition' of the respective exercise stimulus. Consequently, gene expression represents an important layer of control for the processing of physiological information towards a biological outcome.

of hypoxia as a major regulator of the muscle phenotype. Meanwhile the results also highlight the importance of HIF-1α in the opposing regulation of carbohydrate- and fat-metabolizing processes in muscle.

Signal integration

Within a historical perspective our results extend the biochemical and cellular exploration of the paradigm of muscle plasticity to show that transcript level adjustments underlie the tuning of the biological processes by exercise. The current data support the concept that the structural/functional adjustments seen on training reflect the accumulation of transient adjustments in gene expression after repetition of exercise stimuli during training (Fig. 5). The complex stimulus of exercise provokes a series of homeostatic perturbations in recruited muscle (Fig. 8). These are sensed by distinct signaling processes and transduced to downstream activation of gene transcription or a stabilization of transcripts. Known perturbations include alterations of metabolic, mechanical, hormonal and neuronal factors. With regard to the involvement of metabolic alterations, a drop in oxygen tension, as pointed out in here, an increased flux in free fatty acids and a drop in the AMP/ATP ratio have evolved as the main components of the phenotypic active stimuli in muscle (Fig. 8) (Fluck and Hoppeler, 2003). Interaction of these signaling events must be considered in order to explain the complex physiological outcome of endurance exercise when combined with co-stimuli such as hypoxia.

Conclusions

Taking all the above findings together, we infer that a complex gene response reflects the specificity of the muscular adaptation to different types of exercise. Co-regulation appears to exist across the oxidative pathway, chromosomes and genomes. The apparent correlation within gene families and structure–function relationships reveals that a distinct molecular circuitry underlies symmorphosis of the pathway of oxygen, suggesting the involvement of master switches in the coordination of the local training response. The members of these pathways that integrate the homeostatic perturbations in exercised muscle tissue into specific remodeling of muscle organelles begin to be identified. The well-described phenomenology of skeletal muscle plasticity and the unique features of this tissue behavior such as specificity, reversibility, desensitization and accessibility, put muscle into a unique position for future studies on the biological principles underlying cell plasticity *in vivo*.

List of abbreviations

ACADL	long-chain acyl-CoA dehydrogenase
ACADVL	very-long-chain acyl-CoA dehydrogenase
ACADM	medium-chain specific acyl-CoA dehydrogenase
ALDOA	aldolase A
ALDOC	aldolase C
AMPK	5′AMP-activated protein kinase
CPT1	carnitine palmitoly transferase 1
CYC	cytochrome *c*
COX1	cytochrome *c* oxidase subunit 1
COX4	cytochrome *c* oxidase subunit 4
COX5B	cytochrome *c* oxidase subunit 5B
ECH1	enoyl-CoA hydratase
FFA	free fatty acids
Fum	fumarase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
G-6-P	glucose-6-phosphate
HADH	3-hydroxyacyl-CoA dehydrogenase type II
HADHB	3-hydroxyacyl-CoA dehydrogenase B
H-FABP	fatty acid binding protein of the heart
HIF-1α,β	hypoxia-inducible factor 1α,β
HSL	hormone-sensitive lipase
IMCL	intramyocellular lipids
PFK	phosphofructokinase
JNK	c-jun N-terminal kinase
LDH3	lactate dehydrogenase 3
LDL R	low-density lipoprotein receptor
LPL	alkaline lipoprotein lipase
NADH	nicotinamide adenine dinucleotide dehydrogenase
PFKM	phosphofructo-kinase muscle-type
PFKFB1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1
PPARα	peroxisome proliferator-activated receptor α
PPARγ	peroxisome proliferator-activated receptor γ
SDH	succinate dehydrogenase
Tfam	mitochondrial transcription factor
PGC-1α	peroxisome proliferator coactivator-1α
VLDLR	Very low-density lipoprotein receptor
WT	wild type

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