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ORIGINAL ARTICLE

J. Zoll · R. Steiner · K. Meyer · M. Vogt
H. Hoppeler · M. Flück

Gene expression in skeletal muscle of coronary artery disease patients after concentric and eccentric endurance training

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Abstract Low-intensity concentric (CET) and eccentric (EET) endurance-type training induce specific structural adaptations in skeletal muscle. We evaluated to which extent steady-state adaptations in transcript levels are involved in the compensatory alterations of muscle mitochondria and myofibrils with CET versus EET at a matched metabolic exercise intensity of medicated, stable coronary patients (CAD). Biopsies were obtained from *vastus lateralis* muscle before and after 8 weeks of CET ($n=6$) or EET ($n=6$). Transcript levels for factors involved in mitochondrial biogenesis (PGC-1α, Tfam), mitochondrial function (COX-1, COX-4), control of contractile phenotype (MyHC I, IIa, IIx) as well as mechanical stress marker (IGF-I) were quantified using an reverse-transcriptase polymerase chain reaction approach. After 8 weeks of EET, a reduction of the COX-4 mRNA level by 41% and a tendency for a drop in Tfam transcript concentration (−33%, $P=0.06$) was noted. This down-regulation corresponded to a drop in total mitochondrial volume density. MyHC-IIa transcript levels were specifically decreased after EET, and MyHC-I mRNA showed a trend towards a reduction ($P=0.08$). Total fiber cross-sectional area was not altered. After CET and EET, the IGF-I mRNA level was significantly increased. The PGC-1α significantly correlated with Tfam, and both PGC-1α and Tfam significantly correlated with COX-1 and COX-4 mRNAs. Post-hoc analysis identified significant interactions between the concurrent medication and muscular transcript levels as well as fiber size. Our findings support the concept that specific transcriptional adaptations mediate the divergent mitochondrial response of muscle cells to endurance training under different load condition and indicate a mismatch of processes related to muscle hypertrophy in medicated CAD patients.

Keywords Muscle plasticity · Mechanical stress · Metabolic stress · mRNA · Eccentric exercise

Abbreviations CET: Concentric endurance-type training · EET: Eccentric endurance-type training · RT-PCR: Reverse-transcriptase polymerase chain reaction

J. Zoll · 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

R. Steiner · K. Meyer
Cardiovascular Prevention and Rehabilitation, Department of Cardiology, University Hospital, 3010 Bern, Switzerland

Introduction

Skeletal muscle is a highly adaptive tissue, capable of altering its contractile and metabolic properties in response to repeated bouts of contractile activity. Muscle responds to mechanical or metabolic signals by adjusting protein synthesis, leading to structural, metabolic and physiological adaptations (Booth and Thomason 1991). Contractile activity-induced structural adaptations in muscle are highly specific and depend on the frequency, intensity, duration and the type of exercise (see Hoppeler 1986). The magnitude of mechanical, metabolic and neuronal stimuli in exercise sessions varies according to the type of muscle contraction, as dictated by the force–velocity relationship (Komi 1986). When a skeletal muscle actively shortens (concentric contractions), the force produced, and hence the mechanical stimulus is less than during lengthening (eccentric) contractions at the same metabolic load. Indeed, eccentric exercise allows an over fourfold higher muscle loading than conventional concentric exercise for the same metabolic demand (Lindstedt et al. 2001). The eccentric training paradigm hence represents an option to perform high load muscular exercise training with small cardiovascular demand (Meyer et al. 2003). Eccentric training interventions therefore may be of

benefit for cardiac rehabilitation where hemodynamic load needs to be tightly controlled.

It was demonstrated that eight weeks of eccentric endurance-type training (EET) of healthy subjects resulted in a 40% increase in both muscle strength and cross-sectional area of muscle fibers (LaStayo et al. 2000). Eccentric exercise has been shown to enhance the gain in muscle strength more than concentric training alone (Komi and Buskirk 1972; Friden et al. 1983; Colliander and Tesch 1990; Hortobagyi et al. 1996; LaStayo et al. 2000). Recently we have analyzed the effect of EET in stable coronary artery disease (CAD) patients with essentially normal central and peripheral performances (Steiner et al. 2004). The study demonstrated a significant increase in the subsarcolemmal mitochondrial volume density of muscle fiber after concentric endurance-type training (CET) which was associated with a decrease of myofibril volume density (Steiner et al. 2004). By contrast, EET produced a reduction of total mitochondrial volume density with a concomitant increase of myofibril volume density. Subsarcolemmal mitochondria form the smaller portion of the mitochondrial population, and are known to be preferentially increased after CET (Hoppeler et al. 1985; Hoppeler 1986). These observations support the concept of a differential muscular adaptation to CET and EET (LaStayo et al. 2000).

To date, the molecular mechanisms conferring specificity for structural and functional modifications after concentric and eccentric training are not established. In particular, the critical links between physical activity, involving mechanical, metabolic, hormonal and neuronal stress, and changes in muscle cell phenotype are still unclear (Fluck and Hoppeler 2003). The cumulative effects of transient increases in transcription during recovery from consecutive bouts of exercise have been shown to be the basis for the cellular adaptations associated with exercise training (Pilegaard et al. 2000; Fluck and Hoppeler 2003; Hildebrandt et al. 2003). In fact, in vitro and in vivo studies have identified a number of regulatory and/or signaling pathways with the potential to modify these fundamental cellular and molecular processes in skeletal muscles (Booth et al. 1998; Musaro et al. 1999; Haddad and Adams 2002; Fluck and Hoppeler 2003; Hoppeler and Fluck 2003). For this study, it was hypothesized that CET and EET would provoke divergent adjustments of the transcript level of markers of mitochondrial metabolism and contractile phenotype commensurate with the modifications of muscle ultrastructure and function observed with these interventions (Steiner et al. 2004).

To this end we selected mRNAs coding for transcriptional co-activators and transcription factors implicated in mitochondrial biogenesis (PGC-1α and Tfam; Hood 2001), for proteins implicated in the definition of mitochondrial phenotype (mitochondrial- and nuclear-encoded subunits of respiratory chain complex IV [cytochrome oxidase subunit 1 and 4 (COX-1, COX-4); Hood 2001], or related to contractile phenotype (MyHC I, IIa, IIx) and hypertrophy [Insulin-like growth factor-I (IGF-I); Bamman et al. 2001]. In view of the findings of specific structural modifications with CET and EET-training we hypothesized that the mRNA levels of mitochondrial factors would be increased after CET while being reduced with EET and analyzed whether MyHC type I and IIa transcript species would be differently affected by CET and EET. Furthermore, it was hypothesized that mRNA expression of factors related to mechanical stress would be selectively increased after training in EET. Since subjects were under prophylactic medication with statins and angiotensin-converting enzyme (ACE) inhibitors known to interfere with mitochondrial function (statin; Paiva et al. 2005; Phillips et al. 2002) and muscle hypertrophy (ACE; Gordon et al. 2001) we tested post-hoc whether this had interfered with the suspected muscular adjustments.

Methods

Subjects and conditioning program

Twelve male patients with stable CAD who were participating in the out-patient cardiac rehabilitation program at the University Hospital of Bern were included in the study. The study protocol was approved by the local Ethical Committee on Human Research in accordance with the terms defined in the Helsinki convention. All patients gave informed written consent before participating. They were randomly assigned to the CET or EET groups. The concentric training was done on a conventional cycle ergometer and EET was performed on a custom-built motor-driven ergometer as described (Steiner et al. 2004). The entire training period lasted 8 weeks, CET and EET was carried out three times per week for half an hour. Training was carried out at the same metabolic exercise intensity for the EET and the CET group. Exercise intensity was increased gradually over the first 5 weeks of the rehabilitation program to a level corresponding to 60% of peak oxygen uptake achieved in the ramp test performed at the entry into the rehabilitation program (Meyer et al. 2003). Based upon measures of central and peripheral performance, as assessed by left ventricular ejection fraction and maximal oxygen uptake (Meyer et al. 2003), the stable CD patients of our study matched normal active individuals as defined by clinical and physical definitions.

Muscle biopsy samples

Using the Bergstrom technique (Hultman and Bergstrom 1967), biopsies were taken at mid-thigh level from *vastus lateralis* muscle before and after the 8-week training period. Before the biopsies, subjects were minimally 48 h without any exercise activity [interval of

5.5 ± 1.5 (mean ± SEM) days post-training]. The data presented in this study thus represent steady state adaptations of concentrations for the RNA species analyzed. For mRNA analysis, the major part of the muscle tissue was immediately frozen in isopentane cooled by liquid nitrogen, and then stored in the latter until required for analyses. The other part was processed for electron microscopy and morphometry as described previously (Steiner et al. 2004).

Morphometry

Ultrastructural alterations of *m. vastus lateralis* were determined by morphometric analysis of biopsies as previously described (Steiner et al. 2004).

Histochemistry

Twelve micrometer cryostat cross-sections were processed for myofibrillar ATPase (alkaline or acid pre-incubation at pH 10.4 and pH 4.5) as described (Billeter et al. 1980). Consistently with the proposition of Berchtold et al. (2000), the muscle fibers were classified into type I, IIA and IIX fibers. The percentage of each fiber type was obtained from stained sections. One to three sections from different areas of each muscle biopsy were analyzed, depending on the size of the specimens. All the fibers that appeared reasonably cross-sectioned (minor to major fiber axis >0.5) were counted. On average, 178 fibers were counted per biopsy.

RNA extraction and reverse transcription

Total RNA was extracted from the human *vastus lateralis* muscle samples using the RNeasy minikit (Qiagen AG, Basel, Switzerland) as described (Fluck et al. 2003). Formaldehyde-Agarose gel analysis demonstrated the integrity of all RNA samples. RNA aliquots (600 ng) of these reactions were reverse transcribed in 20 µl into cDNA, with 4 U of Reverse Transcriptase using random hexamer primers (1 µM) and 0.5 mM dNTPs (= RT-reaction), following the manufacturer's instructions (Omniscript Reverse Transcriptase kit, Qiagen, Basel, Switzerland).

Real-time polymerase chain reaction (PCR) amplification reactions were carried out in triplicates on 30 µl aliquots in a 96-well plate with an ABI Prism 5700 Sequence Detection System using cDNA signal detection via SYBRGreen (PE Biosystem, Rotkreuz, Switzerland). Primers were designed with the Primer Express software (PE Biosystems, Rotkreuz, Switzerland). Emphasis was put to detect all known splice forms of these genes in toto. Sequences of the primers used are given in Table 1. Real-time PCR reactions with one specific primer set were conducted on the same 96-well plate for all samples in parallel. PCR reactions for level determination of cDNAs encoding an mRNA (PGC-1α, Tfam, COX-1, COX-4, IGF-I, MyHC I, IIa, IIx) were carried out with 2 µl aliquots of a 1:10 dilution of the RT-reaction, whereas the PCR reactions for the cDNA corresponding to 28S rRNA were performed with a 1:100 dilution of the RT-reaction. Care was taken to assay only samples with the same number of freeze-thaw cycles in the simultaneous PCR reaction for a transcript. The amount of target cDNA encoding mRNA relative to the reference (28S) was calculated using the C_T (comparative threshold cycle for target amplification) method as described (Fluck et al. 2003).

Specificity of amplified cDNA was verified from the dissociation curve as determined on the ABI Prism 5700 and by checking the amplified fragment for correct size after separation of the PCR reaction on a 1% Agarose gel. The mean C_T values for 28S were not different before and after both training modalities. 28S-related mRNA levels were normalized to the mean values of the respective training group before the 8 weeks of training.

Statistical analysis

Grubbs test for outliers was applied to test for the possible qualification of extreme data points as outliers (Grubbs 1969). This revealed that one data point (COX-1, post EET) was an outlier at a significance level of <0.01. Hence this point was removed from the calculation of inter-gene correlations of mRNA levels.

Differences between pre- and post-training values for absolute 28S-related RNA concentrations, estimates of muscle ultrastructure and muscle fiber types were analyzed using a paired Wilcoxon test. The particular conditions of the test were chosen dependent on the hypothesis. With a distinct hypothesis on the direction of the expressional change a one-tailed test was applied. In absence of a distinct prognosis a two-tailed test was used. Interaction of training modality (CET, EET) or training (pre, post) on transcript levels was verified with a two-way analysis of variance (ANOVA) for repeated measures with Fisher's least significance difference post-hoc test. Coefficients of correlation were calculated using Pearson Product Moment Correlation for pre- and post-training values separately. The probability level for statistical significance was set at $P \leq 0.05$. An alteration was considered as a trend at $P \leq 0.10$ and $P > 0.05$. Interaction effects of each medication with training modality (CET, EET) and/or training (pre, post) was tested for each muscular measurement with a 3-way ANOVA. All statistical analyses were carried out with the Statistica software package 6.1 [StatSoft (Europe) GmbH, 20253 Hamburg, Germany]. For the preparation of graphical presentations Sigmaplot 2002 vs. 8.0 (SPSS Inc.) was used.

Table 1 mRNA primer sequences used for real-time PCR

Transcript	Transcript name	Genebank	Forward primer	Reverse primer
Tfam	Mitochondrial transcription factor A	NM003201	5′ CCAAAAAGACCTCGTTCAGCTTA 3′	5′ TGCGGTGAATCACCCTTAGC 3′
PGC-1α	Peroxisome proliferator activated receptor gamma co-activator 1α	NM_013261	5′ GTAAATCTGCGGGATGATGGA 3′	5′ GCAGCAAAAGCATCACAGGTAT 3′
COX-1	Cytochrome oxidase subunit 1	M10546	5′ CTATACCTATTATTCGGCGCATGA 3′	5′ CAGCTCGGCTCGAATAAGGA 3′
COX-4	Cytochrome oxidase subunit 4	X54802	5′ GCCATGTTCTTCATCGGTTTC 3′	5′ GGCCGTACACATAGTGCTTCTG 3′
IGF-I	Insulin-like growth factor-I	M27544	5′ TGTGATTTCTTGAAGGTGAAGATGC 3′	5′ AGATCACAGCTCCGGAAGCAGCAC 3′
MyHC-I	Myosin heavy chain I	M21665	5′ AAGGTCAAGGCCTACAAGC 3′	5′ CGGAACTTGGACAGGTTGGT 3′
MyHC-IIa	Myosin heavy chain Iia	AF111784	5′ CAATCTAGCTAAATTCCGCAAGC 3′	5′ TCACTTATGACTTTTGTGTGAACCT 3′
MyHC-IIx	Myosin heavy chain IIx	AF111785	5′ GGAGGAACAATCCAACGTCAA 3′	5′ TGACCTGGGACTCAGCAATG 3′
28S	Ribosomal 28S RNA	M11167	5′ ATATCCGCAGCAGGTCTCCAA 3′	5′ GAGCCAATCCTTATCCCGAAG 3′

Results

Muscle morphometry

Muscle morphological characteristics are reported in Table 2. With CET, the volume density of total mitochondria was unchanged but after EET it was decreased (−20%). The volume density of subsarcolemmal mitochondria, increased after CET (+42%) whereas the volume density of central, but not subsarcolemmal mitochondria, showed a trend towards a lower level after EET (−17% respectively, $P = 0.07$). As a consequence of these mitochondrial alterations, the volume densities of myofibrils for both training modalities changed in opposite direction (−4.6 and +2.8% after CET and EET, respectively). Muscle fiber cross-sectional area showed a trend towards an increase in CET only (+19%, $P = 0.08$).

Fiber type distribution

The data on fiber type distribution is reported in Table 3. The initial distribution of fiber types in coronary patients was not different between the CET and EET group. After 8 weeks of EET, the percentage of type IIA fiber was increased (+32%) with no significant alterations neither in type I nor IIX fibers. There was no indication for a change in the fiber type composition after 8 weeks of CET.

mRNA expression analysis

Normalized values obtained for each mRNA level as determined by reverse-transcriptase polymerase chain reaction (RT-PCR), before and after 8 weeks of training are given in Fig. 1.

Mitochondrial biogenesis

PCR quantification showed no significant changes of the mRNA level of the transcriptional co-activator PGC-1α after both training modalities. Tfam, which was not altered after CET, showed a tendency towards a decrease of 33% after EET ($P = 0.06$).

Oxidative enzymes

The hypothesis of an increase of the COX-1 and COX-4 mRNA levels after CET was rejected. On the other hand, after EET, COX-4 was significantly decreased by 41% whereas COX-1 remained unchanged. PGC-1α significantly correlated with Tfam, and both Tfam and PGC-1α correlated significantly with COX-1 and COX-4 mRNA levels (Fig. 2).

Mechanical stress pathway

Expression of IGF-I mRNA was significantly increased after CET and EET (+31 and +78%, respectively). When pre-training values from both groups were combined, the transcript level of IGF-I, was significantly and positively correlated with those involved in mitochondrial biogenesis, Tfam ($r = 0.92$), and mitochondrial respiration COX-1 ($r = 0.83$) and COX-4 ($r = 0.85$). These associations were lost after both training interventions (not shown).

MyHC isoforms

The hypothesis of increased mRNA levels for either of the MyHC isoforms was rejected implying that MyHC

Table 2 Muscle structure before and after 8 weeks of concentric (CET) and eccentric (EET) cycle ergometer training [adapted from (Steiner et al. 2004)]

	CET		EET	
	Pre	Post	Pre	Post
Mitochondria total (%)	5.2 ± 0.7	6.0 ± 0.5	5.3 ± 0.6	4.2 ± 0.3*
Central (%)	4.4 ± 0.5	4.6 ± 0.4	4.4 ± 0.5	3.7 ± 0.2**
Subsarcolemmal (%)	0.8 ± 0.2	1.2 ± 0.2*	0.9 ± 0.2	0.5 ± 0.1
Myofibrils (%)	81.3 ± 1.0	77.5 ± 0.9*	79.6 ± 1.1	81.8 ± 0.7*
Fiber cross-sectional area (μm^2)	3,524 ± 216	4,209 ± 417**	4,523 ± 460	4,402 ± 282

Values are means ± SEM
Significant difference to pre-training values, *$P < 0.05$; **$P < 0.10$

Table 3 Fiber type distribution

	CET		EET	
	Pre	Post	Pre	Post
% Type I fibers	47.3 ± 4.3	54.5 ± 2.8	48.0 ± 5.3	45.9 ± 3.5
% Type IIA fibers	29.0 ± 3.9	25.9 ± 3.6	24.5 ± 2.0	32.3 ± 1.9*
% Type IIX fibers	23.7 ± 4.0	19.6 ± 1.8	27.6 ± 6.4	21.8 ± 3.3

Values are means ± SEM
Significant differences between pre- and post-training values, *$P < 0.05$

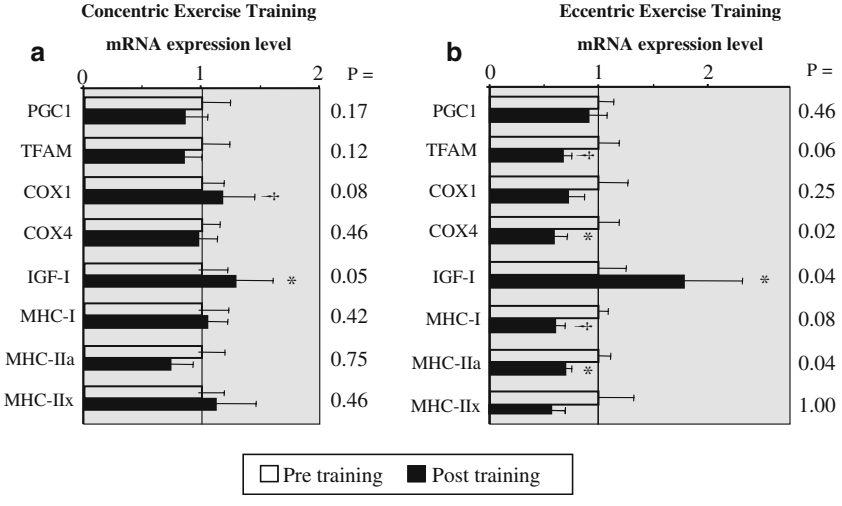

Fig. 1 Relative mRNA concentrations for gene products involved in muscular phenotype definition before and after concentric (**a**) and eccentric (**b**) endurance-type training. *Open bars* represent pre training values and *solid bars* are post training values of 28S-related mRNA concentrations in *vastus lateralis* muscle of coronary patients. Post-training values were related to the pre-values, which are set to 1. Data are means ± SEM. *$P < 0.05$ versus before training. †$p < 0.10$ versus before training. *Underlined P* values refer to those changes verified with a one-tailed Wilcoxon test. For gene names see list of abbreviations

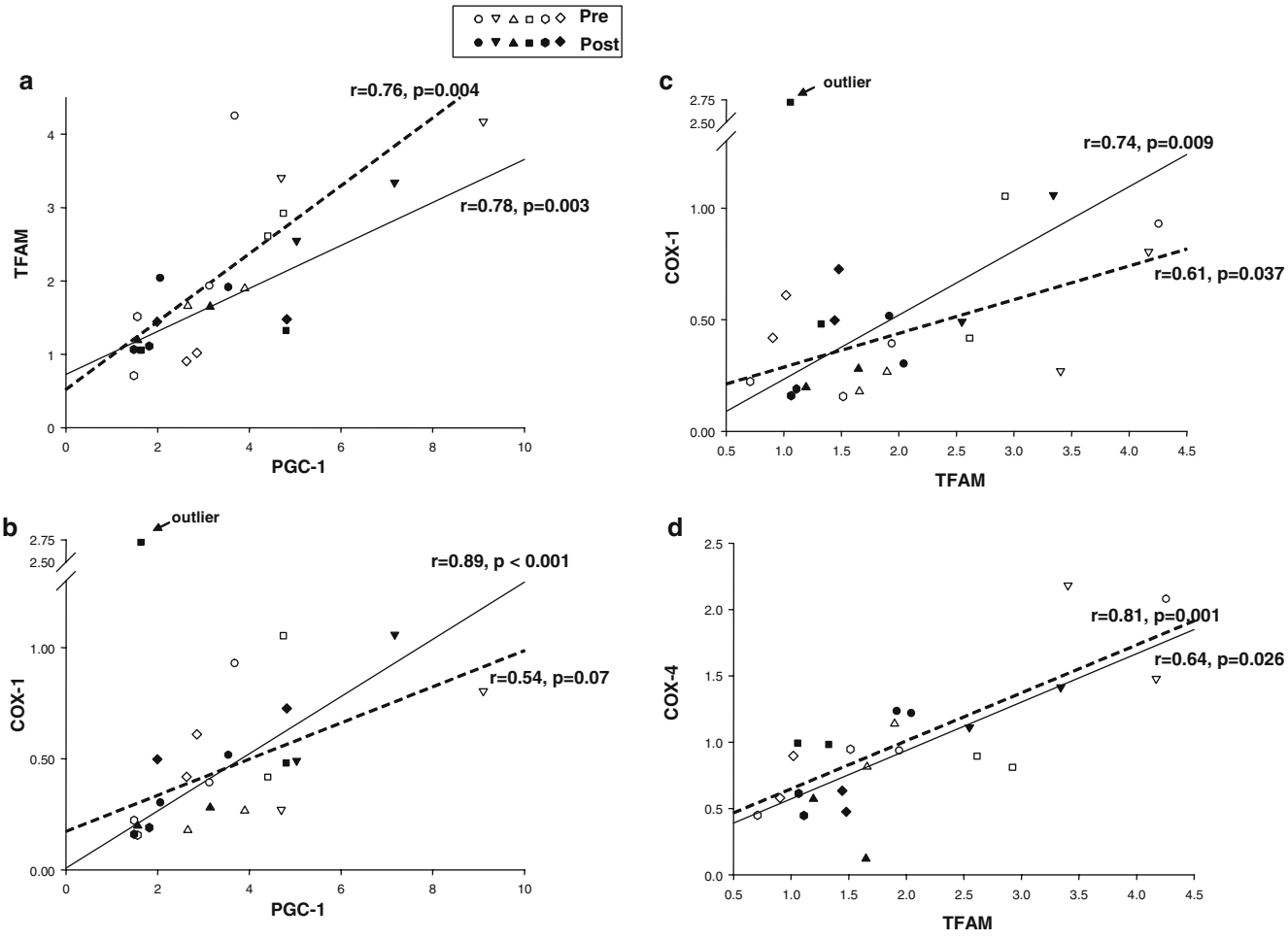

Fig. 2 Correlations analysis of mRNA levels for factors of mitochondrial biogenesis. Pearson correlation analysis between 28S-related mRNA concentrations for **a** PGC-1α and Tfam; **b** PGC-1α and mitochondrial DNA-encoded COX-1; **c** Tfam and COX-1 and **d** Tfam and nuclear DNA-encoded COX-4 in *vastus lateralis* muscle of coronary patients. Dotted and straight lines indicate the regression lines for pre- and post-training values. Correlation coefficient (r) and the level of statistical significance (P) are given beneath each regression line. The data point indicated by the *arrow* qualified as an outlier and was excluded from the calculated correlation. For gene names see list of abbreviations

expression remained stable after CET. In contrast, after EET the MyHC-IIa mRNA level was significantly decreased (−40%) and MyHC-I showed a trend towards a reduction (−31%, $P = 0.08$). MyHC-IIx mRNA levels were not altered with either type of training.

Table 4 Interactions of medicaments with muscular parameters

Pre	ACE	Statin
MHC-I mRNA	0.06	NS
MHC-IIA mRNA	0.06	NS
Fiber size	0.06	NS
COX-4 mRNA	NS	0.10

Pre/post	ACE	ACE × training	Statin
IGF-I mRNA	0.07	NS	NS
MHC-I mRNA	NS	0.06	NS
MHC-IIA mRNA	NS	0.04	NS
Fiber size	0.08	NS	0.05
COX-4 mRNA	NS	0.04	0.03

Data were statistically evaluated with a 3-way ANOVA (medicament × training modality × training). Numbers denote P-values
NS not significant ($P > 0.20$)

Effect of medication

There was a significant interaction between statin intake and training-induced alterations of COX-4 mRNA levels as well as fiber size (Table 4). Additionally, there was an interaction effect of ACE–intake × training on COX-4 with MHC-IIA mRNA as well as there was a trend for MHC-I mRNA ($P = 0.06$). With regard to the pre-training state, trends for an effect of ACE-intake were given for MHC-I, MHC-IIA mRNA as well as for fiber size.

Discussion

Major findings

This is the first study exploring the transcriptional mechanisms involved in the discrete contractile and metabolic muscular adaptations of stable coronary patients to 8 weeks of CET and EET. We assessed the expression levels of a set of selected mRNAs. RT-PCR experiments indicate that specifically after EET there is a reduction in the expression of the mitochondrial respiratory chain component COX-4, and suggest a decreased transcript level of the factor of mitochondrial biogenesis, Tfam. Both of these transcript alterations are in accordance with the decrease of the mitochondrial volume density. Surprisingly, we also found a trend for a reduction in the expression of genes coding for proteins implicated in contractile properties (MyHC-I, MyHC-IIa) after EET. CET and EET increased the mRNA level of the growth factor IGF-I.

Potential limitations of this pilot study

These are the first data on the functional (Meyer et al. 2003), structural (Steiner et al. 2004) and molecular response (this report) to chronic eccentric exercise during cardiovascular rehabilitation of CAD patients. In the design of the study we were therefore limited by constraints of the experimental protocol imposed by the ethical committee. This has lead to a number of limitations inherent in our experimental design, which we were unable to change a priori. We had constraints on patient selection as well as the final workload these patients were allowed to perform. We were further unable to influence medication; hence we are faced with possible interferences from this pharmacological treatment. Despite these confounding factors we believe that important biological information on the adaptive transcriptional processes activated by eccentric versus concentric training can be derived from our investigation (see below). This is of particular importance for patients when considering recovering from CADs following exercise rehabilitation. The current set of novel molecular data thus reflects an important complement to the functional and structural observations published previously.

Increasing the workload more than we were allowed could amplify the molecular adaptations described to occur in skeletal muscle after training in this study. Especially after CET, it is expected that markers of oxidative metabolism are over-expressed (Puntschart et al. 1995; Bengtsson et al. 2001; Vogt et al. 2001). The contention that the unchanged fiber size after EET is due to insufficient hypertrophic stimuli provided with the eccentric training is supported by the results on fiber cross-sectional area of the CET group (Table 2). With regard to the measure of mRNA levels in a post-training steady-state, care was taken to avoid heavy exercise 48 h prior to the biopsy sampling. However, the extended time period between the last exercise training and some biopsies may have led to an underestimation of the pre-post training differences in some transcripts. Despite this, it has been noted that transcripts can be remarkably stable within the post-exercise period (Simsolo et al. 1993; Pilegaard et al. 2000). In support of this contention we find no effect of time on transcript levels.

Specific molecular alterations after exercise training

Mitochondrial alterations.

As EET was carried out at a similar systemic metabolic load as CET but with a nearly fourfold higher mechanical workload (Meyer et al. 2003), the comparison of both training modalities potentially allows to separate the muscular adaptations resulting from mechanical and metabolic stress during exercise. After EET, we showed a specific drop in COX-4 mRNA level (i.e., the nuclear-encoded subunit 4 of respiratory chain complex IV) and a trend ($P = 0.06$) towards a reduced Tfam transcript concentration. Tfam is crucial for mitochondrial biogenesis and abundant enough to activate mitochondrial transcription in a normal state (Alam et al. 2003; Montoya et al. 1997; Hood 2001; Adhihetty et al. 2003). The involvement of Tfam in control of mitochondrial volume and COX-4 mRNA expression is supported by the observation on a linear relationship between Tfam mRNA levels and the transcript concentration of COX-1 and COX-4 (Fig. 2). This down-regulation of nuclear-encoded Tfam and COX-4 mRNA levels after EET implicates that the observed reduction of volume densities of mitochondria after EET (Table 2) involves a specific transcriptional mechanisms. The drop of mitochondrial volume density does therefore not simply result from dilution of a constant mitochondrial volume in larger muscle fibers (MacDougall et al. 1979; Luthi et al. 1986). The absence of significant alterations in mitochondria-associated transcript levels after CET is explained by the fact that only the small subpopulation of subsarcolemmal mitochondria but not total or central mitochondrial content was increased after the concentric training conditions (Table 2). Because the systemic metabolic load was similar in CET and EET, these findings further suggest

that different, i.e., mechano-dependent transcriptional adaptations mediate the divergent influence of lengthening as opposite to shortening contractions on the metabolic muscular phenotype.

Concerning the involvement of the major positive factor of muscle mitochondrial biogenesis, PGC-1α (Wu et al. 1999; Lehman et al. 2000), the results showed a stable PGC-1α mRNA level after both trainings (Fig. 1). These results relate to the observation on a similar steady-state PGC-1α mRNA level in *m. vastus lateralis* muscle of untrained and trained legs (Pilegaard et al. 2003). These data do not contradict the suggested role of PGC-1α in control of the skeletal muscle oxidative capacity in mammals (Garnier et al. 2003) in as much as the PGC-1α mRNA level was correlated with expression of Tfam when compared over all subjects and the training interventions (Fig. 2). Moreover, in response to exercise, PGC-1α mRNA increase was transient in both legs, returning to resting levels 24 h after exercise (Pilegaard et al. 2003). Since our biopsies were realized minimally 48 hours after the last exercise, this could be the reason why the PGC-1α mRNA level was not altered.

Contractile alterations

EET leads to a decrease of MyHC-I ($P=0.08$, trend) and a drop of MyHC-IIa ($P=0.04$) mRNA expression levels whereas their levels remained unchanged after CET. With regard to the changes in fiber size this supports the notion of translational modifications or myofibrillar protein turnover changes. Previous work reported enhanced abundance of ribosomes after 8 weeks of eccentric ergometer training (Friden 1984), a more efficient mRNA translation after a single bout of heavy resistance exercise (Chesley et al. 1992) or after a short resistance exercise training (Welle et al. 1999).

It has been suggested that during periods of increased loading, myofibers upregulate the expression and secretion of IGF-I (Adams 1998). IGF-I stimulates myofibrillar protein synthesis, satellite cell activation and consequent myofiber hypertrophy in an autocrine/paracrine fashion (Adams 1998; Musaro et al. 2001). Hence IGF-I represents a potential hypertrophic agent which is active during the recovery phase from exercise. In our study we demonstrated that IGF-I mRNA expression was significantly increased after both training conditions (Fig. 1). The higher IGF-I mRNA level after CET suggests that IGF-I expression is not only controlled by acute load-induced damage (Bamman et al. 2001), but also linked to the augmentation of mechanical and metabolic activities in untrained individuals. The finding of a tendency for an increase of fiber cross-sectional area and IGF-I mRNA expression after the exercise training protocol with shortening contractions supports the implication of this growth factor in the muscular remodeling response following an endurance training in untrained subjects (McKoy et al. 1999; Hameed et al. 2003). This increase of IGF-I expression could be one of the principal transcriptional events with endurance exercise.

Pathophysiology

The coincident downregulation of gene expressional as well as structural indicators of the mitochondrial phenotype in conjunction with the drop in MHC mRNA levels and the stagnant mean fiber size with eccentric training was a remarkable observation of our investigation. These adaptations invoke possible negative effects of the mechanical protocol for the recruited muscle groups of the studied CAD patients. This is in contrast to the improvement of volume density of subsarcolemmal mitochondria in the matched concentric group (see Table 2). In this respect, the CAD patients under study did from a haemodynamic point of view not show any signs of central or peripheral abnormalities under the imposed training conditions (Meyer et al. 2003). However, recent studies indicate that the prophylactic treatment against various cardiovascular risk factors may interfere with muscle function. For instance, ACE inhibitors and statins can exert adverse effects on muscle growth (Gordon et al. 2001) and mitochondrial function (Paiva et al. 2005; Phillips et al. 2002; Thompson et al. 2003). This contention is supported by several significant interactions between statin or ACE use transcript levels as well as interactions with fiber size (Table 4). This suggests that the concurrent prescription of medicaments (see Meyer et al. 2003) may have interfered with the expected gain of fiber cross-sectional area post EET. This finding is supported by evidence that exercise can unmask negative effects of statins on muscle in some patients (Thompson et al. 2003). Collectively, the interactions indicate that possibly a number of treatments will interfere with exercise when both are prescribed during rehabilitation. This constitutes an area worth of further study.

Conclusions

Our findings indicate that muscle tissue reacts specifically and differently to the combination of mechanical and metabolic stress induced by CET and EET in a population of stable CAD. In particular we observed a tendency towards a decrease in mRNA levels of TFAM and COX-4 as well as MyHC-I and -IIa in EET muscles with a concomitant increased myofibrillar volume density when the mRNA level of the hypertrophy factor IGF-I was enhanced. This suggests a transcriptional regulation for mitochondria and a mismatched hypertrophy process in the muscles of the medicated CAD patients under the mechanical stimulation applied with the eccentric protocol. This first pilot study suggests important biological information on the adaptive transcriptional processes activated by eccentric vs. concen-

tric training, which could be of particular importance for patients when considering their recovering from CADs.

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