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Different Molecular and Structural Adaptations with Eccentric and Conventional Strength Training in Elderly Men and Women

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Key Words
Eccentric strength training · Gene expression · Physical performance · Exercise training · Mitochondria · Sarcopenia

Abstract
Reprogramming of gene expression contributes to structural and functional adaptation of muscle tissue in response to altered use. The aim of this study was to investigate mechanisms for observed improvements in leg extension strength, gain in relative thigh muscle mass and loss of body and thigh fat content in response to eccentric and conventional strength training in elderly men (n = 14) and women (n = 14; average age of the men and women: 80.1 ± 3.7 years) by means of structural and molecular analyses. Biopsies were collected from m. vastus lateralis in the resting state before and after 12 weeks of training with two weekly resistance exercise sessions (RET) or eccentric ergometer sessions (EET). Gene expression was analyzed using custom-designed low-density PCR arrays. Muscle ultrastructure was evaluated using EM morphometry. Gain in thigh muscle mass was paralleled by an increase in muscle fiber cross-sectional area (hypertrophy) with RET but not with EET, where muscle growth is likely occurring by the addition of sarcomeres in series or by hyperplasia. The expression of transcripts encoding factors involved in muscle growth, repair and remodeling (e.g. IGF-1, HGF, MYOG, MYH3) was increased to a larger extent after EET than RET. MicroRNA 1 expression was decreased independent of the training modality, and was paralleled by an increased expression of IGF-1 representing a potential target. IGF-1 is a potent promoter of muscle growth, and its regulation by microRNA 1 may have contributed to the gain of muscle mass observed in our subjects. EET depressed genes encoding mitochondrial and metabolic transcripts. The changes of several metabolic and mitochondrial transcripts correlated significantly with changes in mitochondrial volume density. Intramyocellular lipid content was decreased after EET concomitantly with total body fat. Changes in intramyocellular lipid content correlated with changes in body fat content with both RET and EET. In the elderly, RET and EET lead to distinct molecular and structural adaptations which might contribute to the observed small quantitative differences in functional tests and body composition parameters. EET seems to be particularly convenient for the elderly with regard to improvements in body composition and strength but at the expense of reducing muscular oxidative capacity.

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Introduction

Physical exercise always invokes a mixture of metabolic, hormonal, neural and mechanical stimuli. It is a difficult challenge to distinguish these contributions. Endurance exercise has a pronounced metabolic component, while in pure eccentric exercise mechanical stress is predominant and the metabolic component is thought to be small. Given that sensors/integrators of metabolic (AMPK) and mechanical stress (mTOR) are thought to signal in an antagonistic manner, a continuum of adaptations can be expected, depending on the relative contributions of the stimuli which are dependent on training intensity, duration, frequency, type of exercise (strength vs. endurance) and contraction mode (eccentric vs. concentric) [1].

Exercise training, by integration and accumulation of these stimuli, leads to specific adjustments of gene and protein expression, partly by nuclear reprogramming of gene transcription, which eventually results in the phenotypic adaptation seen after prolonged periods of strength or endurance training [2]. Athletes with a several-year training history were found to differ in the expression of key regulators compared to sedentary individuals. This is thought to be a key part in the adaptation to the trained muscle phenotype [3]. Some of these orchestrated adaptations are thought to be driven by so called 'master' genes which mostly encode transcription factors or transcriptional coactivators that regulate transcription of clusters of genes responsible for specific functions and pathways, respectively. Stepto et al. [4] investigated the transcriptome adaptation in response to long-term strength and endurance training, and observed contrasting regulation of transcripts related to oxidative metabolism, which were increased after endurance and decreased after strength training. Accordingly, good correlations between mRNA and protein levels as well as structural and functional parameters, e.g. mitochondrial density and maximal oxygen consumption have been described by Hoppeler and Fluck [5]. In the muscles of highly endurance-trained athletes, mitochondrial density and capillarity are double when compared to sedentary individuals as are the levels of transcripts coding for proteins involved in mitochondrial biogenesis and angiogenesis [6, 7]. These observations point to a dominant long-term adaptation at the mRNA level for these systems [8–10].

MicroRNAs are thought to be another group of 'master modulators' since these small noncoding RNAs can affect a whole array of targets by binding to 3′-untranslated regions of specific mRNAs, thereby inhibiting translation and/or initiating degradation. McCarthy and Esser [11] have observed decreased expression of microRNA 1 (miR-1) and miR-133a during muscle hypertrophy. Proposed downstream effects include regulation of chromatin remodeling by targeting expression of histone deacetylases as well as modulations in the expression of transcription factors, growth factors and growth factor receptors such as serum response factor, hepatocyte growth factor (HGF), HGF-receptor (c-MET) and insulin-like growth factor 1 (IGF-1) [11, 12].

Eccentric strength training is proposed to be most effective in increasing strength, due to the higher mechanical forces that can be exerted during eccentric contractions compared to concentric contractions [13]. A recent meta-analysis confirmed the greater extent of strength gain and muscle hypertrophy upon eccentric training [14] (for further discussion see also [15]). Most of these studies have used high loads. Using a moderate load 'endurance-like' chronic eccentric exercise training program, Zoll et al. [16] compared the effects of eccentric and concentric cycling in a population of coronary disease patients. The low-energy demand of such a regimen allowed a high mechanical stimulus at comparatively low oxygen demand, thus avoiding a high cardiopulmonary load. Comparing eccentric with concentric cycling at the same relative metabolic load, Meyer et al. [17] showed that the eccentrically exercising subjects trained at approximately four times higher workloads than those training concentrically. On the level of gene expression, these authors reported differential adaptations of gene transcripts involved in mitochondrial biogenesis. The mRNAs of transcription factor A (TFAM) and cytochrome C oxidase (COX4) were both downregulated after eccentric but not concentric training, which supports the hypothesis of a dominant mechanical stimulus with eccentric training [18].

Using a systems biology approach, we compared long-term molecular, structural and functional adaptations in a steady-state condition in response to eccentric ergometer training (EET) or conventional resistance training (RET) in elderly subjects. These two training modes were chosen because they have been shown to induce similar functional benefits. RET is recommended to prevent age-related muscle atrophy and the functional readout of EET resembles more the effects of conventional strength training than endurance training [15]. We have previously reported similar and differential functional and structural adaptations with RET and EET in a population of elderly [15]. Most strikingly, gain in leg extension strength and loss of body fat content were exclusively observed with EET but not with RET, despite similar relative gains in

Table 1. Parameters of RET (n = 13) and EET (n = 14) subjects (mean ± SE)

	Women	Men	All	RET (6♀; 7♂)	EET (7♀; 7♂)
Age, years	80.1 ± 1.2	80.2 ± 0.8	80.1 ± 0.7	79.9 ± 1	80.3 ± 1
Height, cm	162.1 ± 1.5*	175.6 ± 1.6*	168.9 ± 1.7	169.8 ± 2.4	168 ± 2.4
Body mass, kg	69.3 ± 3.7	73.6 ± 2.4	71.5 ± 2.2	74.9 ± 3.4	68.5 ± 2.7
Body fat before, kg	21.9 ± 2.4*	15.8 ± 1.7*	19 ± 1.6**	20.2 ± 2.9	17.8 ± 1.3
Body fat after, kg	22.1 ± 2.6*	15.3 ± 1.6*	18.7 ± 1.6**	20.1 ± 3.1	17.3 ± 1.2
Thigh fat before, kg	5.4 ± 0.7*	3.1 ± 0.3*	4.2 ± 0.1**	4.6 ± 0.8	3.9 ± 0.3
Thigh fat after, kg	5.2 ± 0.7*	3 ± 0.3*	4.1 ± 0.1**	4.5 ± 0.8	3.7 ± 0.3
Thigh muscle before, kg	9.1 ± 0.4	9.6 ± 0.3	9.3 ± 0.3**	9.4 ± 0.4	9.2 ± 0.3
Thigh muscle after, kg	9.3 ± 0.4	9.8 ± 0.3	9.6 ± 0.3**	9.7 ± 0.4	9.4 ± 0.3
Relative MEL before, N/kg	14.1 ± 1.1	14.7 ± 1.4	14.4 ± 0.9**	14.9 ± 1.4	14 ± 1.1
Relative MEL after, N/kg	15.3 ± 1.1	15.9 ± 1.1	15.6 ± 0.7**	15.9 ± 1	15.3 ± 1.1

Relative MEL = Maximal isometric leg extension strength relative to body weight. The changes from before to after training were significant for pooled subjects (EET + RET, n = 27) for body fat, thigh fat, thigh muscle and relative MEL.

* $p < 0.05$ (ANOVA), significant difference between women and men; ** $p < 0.05$, ANOVA with repeated measures, Tukey's post-hoc analysis.

relative thigh muscle mass. The aim of the current analysis was to investigate the molecular and ultrastructural long-term adaptive mechanisms contributing to the observed phenotypic changes. We expected to find differences in gene expression profiles and distinct muscle ultrastructural adaptations [16, 19].

Subjects and Methods

Subjects and Study Design

The subjects described in this study were participating in a larger trial whose training programs and subject characteristics have been described in detail previously [15]. In brief, 62 elderly untrained subjects (80.2 years on average) with stable medication and health conditions were randomly assigned to one of three training groups: cognitive training, RET and EET. Cognitive training consisted of nonphysical computer-guided cognitive training (10 women, 6 men), and served to account for the positive social aspects of training sessions [for more details, see Buschkuehl et al. 20]. RET was carried out by 23 subjects (13 women, 10 men) in a gym, and comprised four exercises for the lower extremity (leg press, knee extension, leg curl, hip extension). The sessions consisted of a 10-min warm-up, 20-min specific training and 10-min cool-down with stretching. Exercises consisted of three sets with 8–10 repetitions. EET was carried out by 23 subjects (13 women, 10 men) on a custom-built motor-driven ergometer [17]. The training session started with a 10-min warm-up and ended with a 10-min cool-down, while the actual EET lasted 20 min. To avoid severe delayed onset of muscle soreness, the initial load on the eccentric bike was set low (women 30 W, men 50 W), and lasted only 5 min. In the following sessions, the duration was gradually increased in 5-min steps until it reached 20 min. Thereafter, the load was ramped in consecutive sessions by 20% of the individual maximal power output achieved in the initial incremental exercise test to exhaustion on a (concentric) cycling ergometer [15, 21]. All groups trained for 12 weeks with two sessions per week. Muscle biopsies were collected from m. vastus lateralis from a subgroup of 13 RET and 14 EET subjects in a nonfasted state prior to the start of the training intervention and between 48 to 72 h after the last training bout. Time of day for the biopsy was variable between subjects, but the same for the pre- and post-biopsies of a given individual. The anthropometric and morphological data of this subgroup are reported in table 1. The biopsies were divided into portions used for RNA isolation, which was immediately frozen in liquid nitrogen-cooled isopentane and stored in liquid nitrogen, and a portion processed for morphometric analysis which was fixed in 6.25% glutaraldehyde as described previously [22].

RNA Isolation and Reverse Transcription

Total RNA was isolated with the RNeasy All Prep Kit from Qiagen (Hombrechtikon, Switzerland) in the protocol adapted for the simultaneous extraction of small RNAs (such as microRNAs) and mRNA. (See QIAGEN supplementary protocol at http://www1.qiagen.com/literature/protocols/pdf/ry26.pdf.) In order to relate the total RNA content to the amount of tissue used, we determined the amino acid and the DNA content of the whole proteinase K-digested lysate. Amino acid analysis was performed by the Department of Chemistry and Biochemistry (University of Bern, Switzerland) using total acid hydrolysis followed by high-performance liquid chromatography on a silica column. DNA was collected on a silica membrane column. The concentration of the eluate was measured from the absorbance at 260 and 280 nm on a Nanodrop photospectrometer (Wilmington, N.C., USA). RNA was collected according to the manufacturer's protocol for total RNA isolation from fibrous tissue with a proteinase K digestion for muscle tissue. Total RNA yield was between 4.3 and 20.6 μg. For reverse transcription of mRNAs, the High Capacity RNA reverse transcription kit from Applied Biosystems (Foster City,

Table 2. Morphological parameters as estimated from m. vastus lateralis biopsies by electron microscopy of RET (n = 13) and EET (n = 14) subjects (mean ± SE)

	Eccentric (EET)		Resistance (RET)	
	after	before	after	before
Vv (mc, f), %	4.6 ± 0.9	4.32 ± 0.65	4.86 ± 0.24	5.17 ± 0.22
Vv (ms, f), %	0.6 ± 0.99	0.54 ± 0.06	0.72 ± 0.4	0.65 ± 0
Vv (mt, f), %	5.19 ± 1.88	4.85 ± 0.6	5.59 ± 0.64	5.82 ± 0.23
Vv (li, f), %	0.54 ± 0.32	0.3 ± 0.12*	0.44 ± 0.03	0.49 ± 0.17
Vv (fi, f), %	82.94 ± 2.72	84.29 ± 2.04	82.6 ± 2.96	82.3 ± 1.93
Vv (re, f), %	11.33 ± 0.52	10.55 ± 2.52	11.38 ± 3.57	11.39 ± 1.98
NN (c, f)	1.05 ± 0.08	1.14 ± 0.08	1.26 ± 0.15	1.24 ± 0.12
a (f), μm^2	3,125.48 ± 182.99	3,266.06 ± 210.89	3,489.13 ± 388.71	3,714.1 ± 329.81

Vv (mc, f) = Central mitochondrial volume per fiber volume; Vv (ms, f) = subsarcolemmal mitochondrial volume per fiber volume; Vv (mt, f) = total mitochondrial volume per fiber volume; Vv (li, f) = volume of intramyocellular lipid per fiber volume; Vv (fi, f) = volume of myofibers per fiber volume; Vv (re, f) = 'Residual' volume per fiber volume; NN (c, f) = number of capillaries per fiber; a (f) = mean cross-sectional area per fiber.

A significant decrease in Vv (li, f) could be recorded with EET (two-tailed paired Student's t test; * p = 0.02).

Calif., USA) was used, with 700 ng RNA input per sample (download protocol at http://www3.appliedbiosystems. com/cms/groups/mcb_support/documents/generaldocuments/cms_042557.pdf). Reverse transcription for microRNA analysis was carried out with the miScript reverse transcription kit from Qiagen according to the manufacturer's protocol, as were the other procedures (http://www1.qiagen.com/literature/render.aspx?id=104793). cDNA was aliquoted and stored at –20°C, and only thawed immediately prior to use. Repeated freeze-thaw cycles were avoided.

Gene Expression and MicroRNA Analysis

For PCR quantitation, TaqMan arrays (microfluidic cards) from Applied Biosystems were used. Two different 384-well microfluidics cards in format 96a were custom-designed, allowing the relative amount of 187 muscle-specific mRNA transcripts to be determined for each biopsy, using cDNA from 400 ng reverse-transcribed RNA as input. A complete list of transcripts can be seen in online supplementary table 4 (for all online suppl. material, see www.karger.com/doi/10.1159/000323267). Cycling conditions and procedure were followed as recommended by the manufacturer. TaqMan arrays measure the content of each cDNA relative to the amount of this cDNA in a calibrator sample of which an identical aliquot is run on each array. For this study, the calibrator cDNA was a mixture of cDNA from each biopsy in order to ensure coverage of all expressed transcripts. Relative expression was estimated by the ΔΔCt (cycle threshold) method using 18S cDNA normalization. We have chosen 18S as a standard because we can exclude significant fluctuations in the yield of total RNA, based on our measurements of amino acid as well as DNA content. Ribosomal RNA content makes up 80% or more of total RNA and can be assumed to be stable if the content of total RNA remains unchanged. Transcripts yielding a Ct ≤35 were included in the analysis. The miScript primer assay detection system from Qiagen was used to analyze microRNA expression. miR-1 expression was normalized to U6 small nuclear RNA [23].

Biopsy Sampling, Morphometry, Histochemistry and Estimation of Body Composition

Biopsy sampling of m. vastus lateralis and subsequent histochemical analysis were performed as described previously [15]. Ultrastructural parameters were determined by morphometric analysis as described by Steiner et al. [22]. Body composition parameters were assessed by dual energy X-ray absorptiometry as described previously [15].

Statistical Analysis

Differences in muscle ultrastructure and muscle fiber type composition were verified using a two-tailed Student's t test. Interactions of training modality (RET, EET) with transcript levels were checked using analysis of variance (ANOVA) for repeated measures with Tukey's honest significant differences post-hoc tests. Coefficients of correlation were calculated using Pearson product moment correlation. The probability level for statistical significance was set at $p < 0.05$. All statistical analyses were carried out with the Statistica software package 6.1 [StatSoft (Europe) GmbH, Hamburg, Germany]. The analysis of some of the functional data has been described previously by Mueller et al. [15].

Results

Anthropometry and Muscle Structure

The anthropometric data of the study subjects are reported in table 1, the morphology data of their muscle biopsies in table 2. Thigh muscle mass was increased by 2.8% with RET and 2.1% with EET along with a reduction in thigh fat content (RET: –2.8%; EET: –5.4%). The

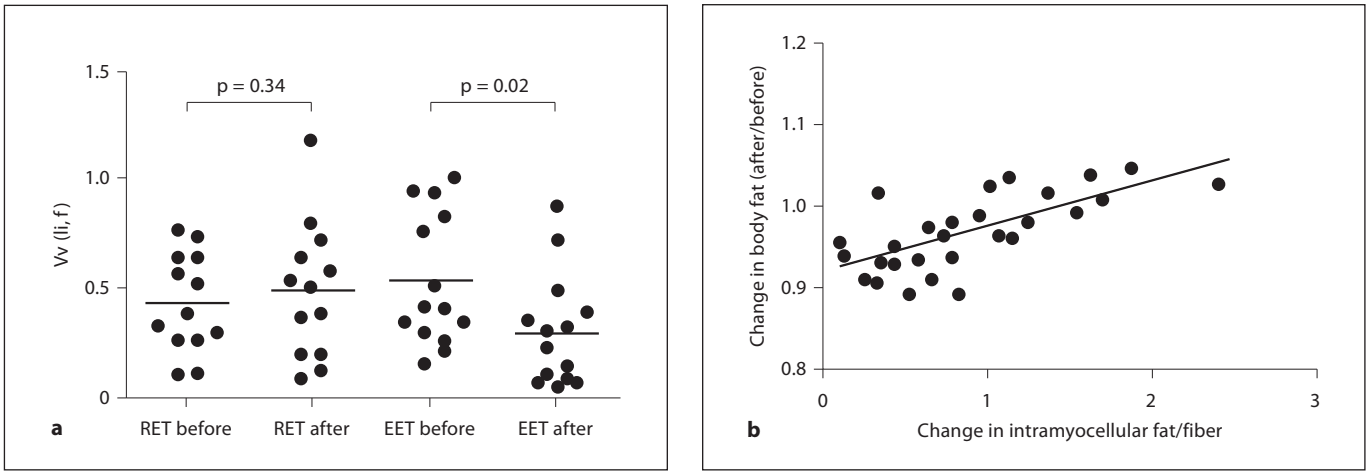

Fig. 1. a IMCL volume per fiber volume [Vv (li, f)] in RET (n = 13) and EET (n = 14) subjects, before and after training, respectively. A statistically significant change was found in the biopsies of EET subjects who lost 43% of IMCL with training. Indicated p values were from a two-tailed paired Student's t test. **b** This figure illustrates the highly significant ($p < 0.01$) positive correlation of change in body fat content with change in IMCL content in all subjects (n = 27). The Pearson coefficient for the correlation is 0.7.

means of body fat content were lower by –0.2% (RET) and –3.1% (EET), respectively. When pooled (n = 27), all changes were statistically significant. Most muscle ultrastructural parameters did not change significantly over time, only intramuscular lipid (IMCL) content per muscle fiber was decreased in eccentrically trained subjects after the training period (table 2; fig. 1a). The changes (post-/pre-ratio) in IMCL content correlated with the changes in body fat content (fig. 1b). Fiber cross-sectional area did not significantly change with training, nor did the volume density of mitochondria or capillary density. In agreement with the classic concept of muscle hypertrophy, the individual changes in fiber cross-sectional area correlated well with the changes in thigh muscle content with RET. However, this was not the case with EET, where changes in thigh muscle mass were not related to changes in fiber cross-sectional area (fig. 2).

Gene and Micro-RNA Expression Analysis

Our custom-designed low-density arrays contained primer and probe pairs for 187 different gene transcripts including the 18S ribosomal RNA used for normalization (transcripts are listed in online suppl. table 4). 178 transcripts yielded an average Ct of ≤35 over all subjects and were included in the analysis. The mean Ct of all gene transcripts was 27. As already indicated, our normalization to 18S expression was based on the observation that we obtained similar yields of total RNA from all biopsy portions when related to their protein content measured by amino acid analysis. The amount of total RNA did not significantly change with training (EET: +9%; RET: –6%).

Among the 178 analyzed transcripts, 60 were significantly changed with training. Four transcripts were exclusively changed with RET, 29 with EET and 27 reached the level of significance only when the subjects were pooled. Those transcripts that significantly changed in the same direction in response to strength training (both groups taken together) are indicated as 'all' in table 3. The transcripts are grouped according to their function. Those coding for metabolic enzymes and mitochondrial proteins were consistently downregulated with EET, in contrast to transcripts coding for factors involved in repair and remodeling, which were upregulated after EET. The individual values for the expression of metabolic and mitochondrial genes correlated highly significantly with each other (online suppl. fig. 6). In addition, the levels of transcripts involved in mitochondrial function also correlated strongly ($p < 0.01$) with mitochondrial density (see fig. 4 and online suppl. fig. 5).

The expression of miR-1 was decreased after training, with no significant differences between the changes in the two groups (fig. 3a). An analysis of potential mRNA targets (miRanda, www.microrna.org) of miR-1 revealed 3,343 potential targets, among them IGF-1, a potent mod-

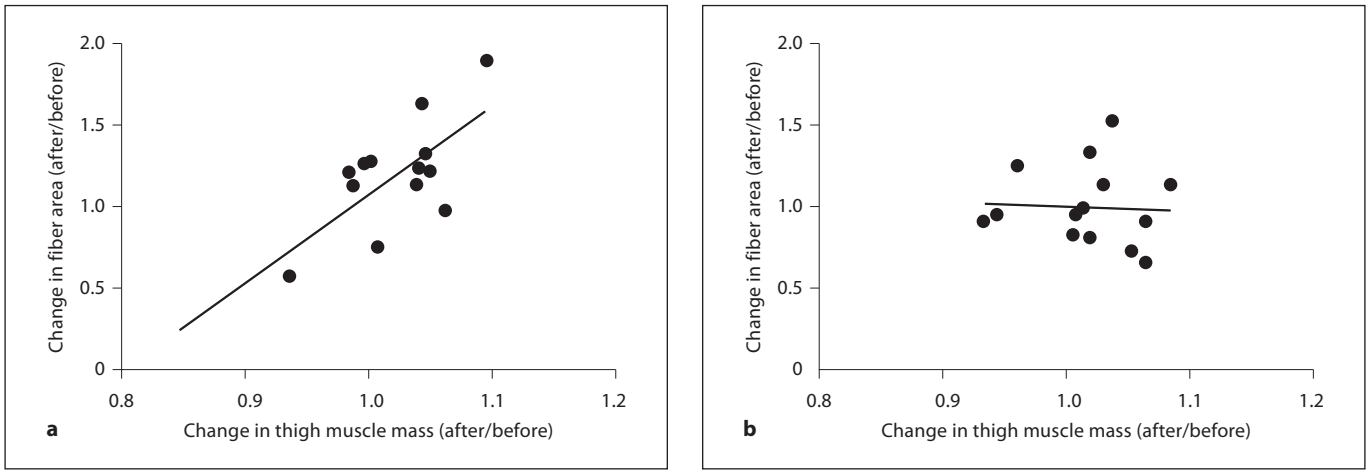

Fig. 2. A highly significant correlation between changes in thigh lean mass content and mean fiber cross-sectional area could be observed exclusively in the RET subjects (n = 13; **a**) but not in EET subjects (n = 14; **b**). Pearson coefficients were 0.7 (**a**) and 0.1 (**b**).

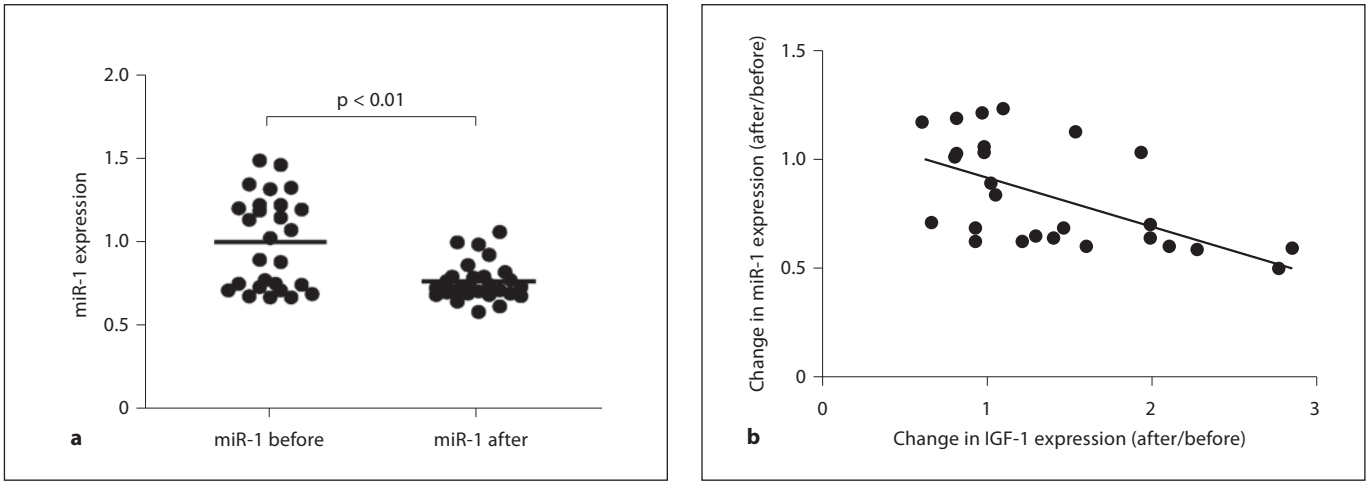

Fig. 3. a Expression of miR-1 before and after training in the biopsies of all subjects (RET and EET group, n = 27). A significant drop in response to training is indicated (paired two-tailed Student's t test). **b** Significant negative correlation ($p < 0.01$) between the changes in miR-1 and IGF-1 mRNA of pooled RET and EET subjects (n = 27). The Pearson coefficient was –0.6.

ulator of muscle hypertrophy [24, 25]. We observed a significant correlation between changes in miR-1 and IGF-1 expression (not distinguishing between the IEa and IEc isoforms; for further discussion, see fig. 3b). Among other potential targets, we could detect significant correlations for IGF-1 with fibronectin 1 (R = –0.6), chemokine (C-C motif) ligand 2 (R = –0.7) and platelet-derived growth factor-α (R = –0.6).

Discussion

This study reports a quantitative structural and molecular analyses of data obtained from muscle biopsies of aged men (n = 14) and women (n = 13; 80.1 ± 3.8 years) before and after eccentric (EET) and RET for 12 weeks. The functional and body composition data from our larger group of training subjects have been reported previ-

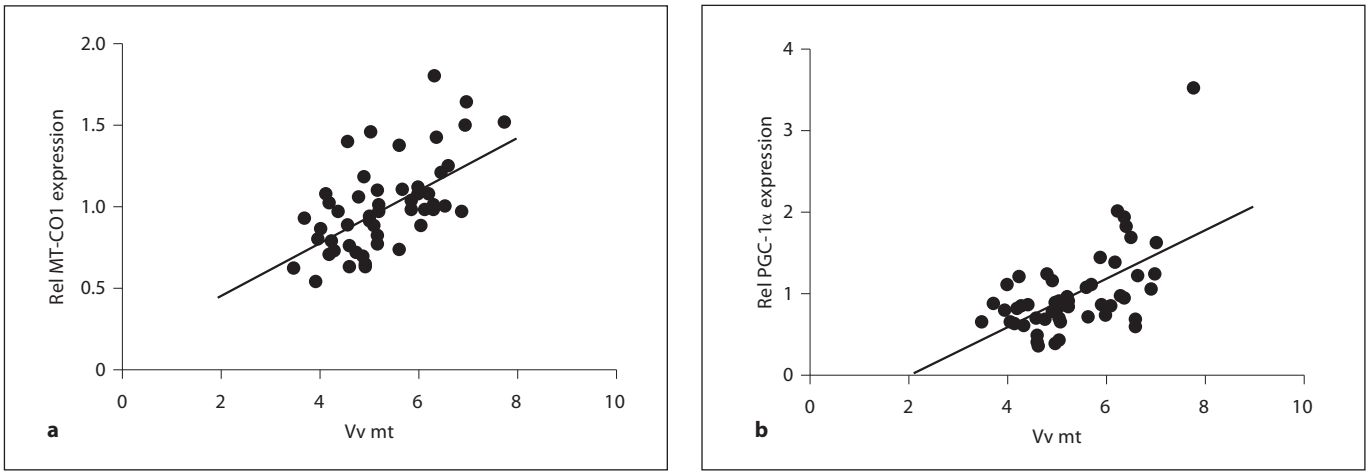

Fig. 4. Correlations ($p < 0.01$) between mitochondrial volume density (Vv mt) and the mitochondrially encoded cytochrome c oxidase 1 mRNA (MT-CO1; **a**), and between mitochondrial volume density and the nuclear-encoded transcriptional coactivator promoting mitochondrial biogenesis (PGC-1α; **b**). Both Pearson coefficients were 0.6; RET and EET subjects were pooled (n = 27, 54 data points).

ously [15]. In this larger group, we found significant but small increases in relative thigh muscle mass after either mode of training as well as significant losses in body and thigh fat content in EET subjects only. The strength gains in an isometric test were +8.9% for EET and +2.3% for RET, significant only for EET. Compared to conventional strength training studies with younger subjects, such changes are relatively small, but nevertheless important, given that the testing procedures were exercise independent and our subjects performed only two training sessions per week and were of very good physical condition for their age [15]. The data from the subset of subjects that were biopsied did not reach significant group-specific functional or body composition improvements, although there is a tendency in that direction. The pooled data from the subjects in both training groups showed significantly increased thigh muscle mass and strength (table 1). Thigh fat mass was reduced with both training modalities (significant for pooled data), but this was more pronounced with EET. We therefore assume the subgroup of subjects discussed in this analysis to have adapted in the same manner as the larger cohort, but with individually large variations that prevented a statistically significant demonstration of functional improvements on the basis of our conventional functional tests.

Although the actual calculations were performed by normalizing the PCR data to 18S cDNA, we interpret our gene expression data as relative measures of tissue content for a given mRNA. By estimating the RNA, DNA and amino acid contents from the same muscle biopsy specimens, we were able to control eventual fluctuations of total RNA (rRNA, mRNA , tRNA, microRNA) as well as DNA content (nuclear domains, infiltration of non-muscle cells) by standardizing to protein content. Changes in RNA and DNA levels were not significant. Our normalization procedure is therefore independent of housekeeping genes [10]. In addition, we used the ultrastructure morphometry of mitochondria as a measure of specific protein content to reflect bulk structural consequence of transcriptomic changes observed. The significant correlations of mitochondrially (MT-ATP5, MT-CO1, MT-ND1) as well as nuclear-coded transcripts (ATP5B, COX5B, PGC-1α) with mitochondrial volume (fig. 4 and online suppl. fig. 5) further validate our normalization strategy as well as the time point of sampling.

The gains in relative thigh muscle mass (as determined by dual energy X-ray absorptiometry) were similar after RET (2.8%) and EET (2.1%). The increases were small and reached the level of significance only for RET and EET combined. A more in-depth analysis of the individual data suggests that the muscle mass may have increased via different mechanisms in the two groups. In RET but not EET, we found a significant correlation between the change in thigh muscle mass and the increase in fiber cross-sectional area (fig. 2). This is compatible with the hypothesis that RET leads to fiber hypertrophy [26]. Upon eccentric training, muscle growth seems to occur without significant changes in fiber CSA, possibly driven

Table 3. Significantly changed expression of gene transcripts with training

Gene	Function	RET	EET	All
COL1A1	extracellular matrix	0.81	**3.78**	**1.76**
COL3A1	extracellular matrix	1.15	**3.50**	**2.03**
COL4A1	extracellular matrix	1.48	1.36	**1.42**
COL6A1	extracellular matrix	1.20	1.52	**1.33**
LAMA2	extracellular matrix	1.23	1.29	**1.25**
CDH15	cell adhesion	1.20	1.13	**1.16**
ITGB1	cell adhesion	1.22	1.23	**1.23**
VCL	cell adhesion	1.34	1.27	**1.31**
ACTA1	cytoskeleton	**1.18**	0.86	1.01
ACTN1	cytoskeleton	1.83	1.43	**1.63**
DAG1	cytoskeleton	1.36	1.16	**1.26**
DES	cytoskeleton	1.06	*0.75*	0.89
DMD	cytoskeleton	1.07	*0.74*	0.91
MYH1	cytoskeleton	0.85	0.46	**0.73**
MYH3	cytoskeleton	1.31	**2.55**	2.06
MYH6	cytoskeleton	1.43	1.92	**1.68**
TNNC2	cytoskeleton	0.96	*0.62*	0.79
ANGPT1	angiogenesis	1.37	1.22	**1.29**
ANGPT2	angiogenesis	1.37	1.25	**1.32**
VEGFB	angiogenesis	**1.16**	0.94	1.05
CDKN2D	cell cycle	1.37	1.10	**1.23**
CCND1	cell cycle	1.29	1.22	**1.26**
GADD45	DNA repair	1.74	2.38	**2.09**
FGFR4	cell growth	0.98	**1.82**	1.38
HGF	cell growth	1.36	1.79	**1.56**
IGF1	cell growth	1.22	1.71	**1.43**
IGFBP5	cell growth	0.84	0.75	*0.80*
RPSA	translation	0.97	0.81	*0.89*
MEF2C	transcription factor	1.23	1.10	**1.16**
MYOD1	transcription factor	1.49	1.18	**1.32**
MYOG	transcription factor	1.40	1.73	**1.58**
Pax7	transcription factor	1.21	1.30	**1.26**
SRF	transcription factor	**1.18**	0.90	1.05
CAMK2B	kinase	1.02	*0.76*	0.88
DMPK	kinase	1.09	1.57	**1.34**
CKM	metabolism	1.05	*0.66*	0.85
CS	metabolism	1.12	*0.68*	0.89
GAPDH	metabolism	1.10	*0.73*	0.91
DCI	metabolism	0.95	*0.76*	0.86
MDH2	metabolism	0.96	*0.76*	0.87
ALDOA	metabolism	1.04	*0.65*	0.86
HADH	metabolism	0.97	*0.72*	0.85
ACADVL	metabolism	1.05	*0.80*	0.92
HADHB	metabolism	1.06	*0.78*	0.92
ACAT1	metabolism	1.06	*0.73*	0.90
ACADL	metabolism	0.94	0.79	*0.87*
COX5B	mitochondria	1.03	*0.81*	0.92
MT-ND1	mitochondria	1.06	*0.71*	0.88
MT-CO1	mitochondria	1.09	*0.75*	0.91
ATP6	mitochondria	1.08	*0.82*	0.95
ATP5B	mitochondria	0.89	0.76	*0.83*
CYC1	mitochondria	0.98	0.83	*0.90*
PPARGC1A	transcription factor mt	1.26	*0.68*	0.95
TFAM	transcription factor mt	1.17	*0.90*	1.04
SLC27A1	fatty acid transporter	0.88	*0.82*	0.85
CD36	FAT	1.13	1.33	**1.23**
CAPN3	protease	1.09	*0.70*	0.90
SOD2	redox/pH	**1.22**	0.91	1.06
NOS1	signaling	1.09	*0.62*	0.86
MB	oxygen carrier	0.86	0.77	*0.82*

Figures represent after/before ratio of RET (n = 13), EET (n = 14) and all trained subjects (n = 27), respectively.

Figures in bold indicate significant upregulation, figures in italics significant downregulation.

Before/after differences were verified with ANOVA with repeated measures and Tukey's HSD post-hoc analysis with 5% level of significance.

Transcript names are according to US National Library of Medicine.

by hyperplasia [27, 28] or by addition of sarcomeres in series, i.e. by lengthening of muscle fibers [29, 30]. Reeves et al. [31] have shown that eccentric exercise increases fascicle length in pennate muscles, leading to increased CSA. They used a protocol of three training sessions per week for 14 weeks in subjects aged 67 years on average. Using a training mode-independent isometric testing procedure, they also found strength gains of comparable size to our study with eccentric and concentric training regimes. Given the remarkably different correlations between gains in fiber area and thigh muscle mass between RET and EET, we suggest that in our study, EET may have led to muscle gain via increased fascicle, i.e. fiber length.

Zoll et al. [16] reported a significantly decreased skeletal muscle mitochondrial content paralleled by a decrease in IMCL content (–24%, not significant, unpubl.) and increased fiber/muscle area (+2.76%, p = 0.01, unpubl.) in response to eccentric exercise training. The subjects in this study were slightly younger (mean age 55 years) and performed a more aggressive but otherwise

similar training protocol [22]. The data in our current study point in the same direction. Intramuscular lipid content was found to be significantly reduced in EET subjects (fig. 1a) while mitochondrial content was not significantly changed. Among individuals, the decrease in IMCL was significantly correlated with the decrease in body fat content (fig. 1b). Using concentric exercise training protocols, it has been consistently found that intracellular lipids are increased concurrent with the increases in mitochondrial volumes [32]. Eccentric exercise has been associated with lowered blood triacylglycerol after a high-fat meal in the post-exercise period [33]. When older subjects (average age 66 years) were subjected to a hyperglycemic clamp 48 h after a bout of eccentric exercise, their lipid oxidation increased at the expense of carbohydrate oxidation compared to the rested control state. In contrast, a cohort of younger subjects (average 23 years) showed the expected increased carbohydrate oxidation at the expense of lipid oxidation [34]. Eccentric exercise is known to induce transient insulin resistance, possibly due to the muscle's inflammatory state during the repair phase [35]. It is possible that in elderly, the reduced insulin sensitivity could be compensated sufficiently via increased insulin secretion to the glucose challenge. The subjects in our study are distinctly older (average 80 years); thus, it is conceivable that even a balanced meal could have been sufficient to elicit enhanced lipid oxidation.

An increase in steady state gene expression is thought to be the main mechanism of muscle plasticity in endurance type training situations [10]. As such, we expected significant time (before vs. after) and group (RET vs. EET) differences. Most strikingly, the biopsies of the EET subjects showed a significantly decreased expression of transcripts coding for mitochondrial proteins and metabolic enzymes (table 3) which was not observed in RET subjects. This finding is in accordance with a previous eccentric training study in which Zoll et al. [36] observed a decrease in the steady-state expression of mitochondrial transcripts with EET (TFAM, COX4). Monitoring the immediate transcriptome response after a single bout of eccentric ergometry, Klossner et al. [19] observed a significant depression of a large number of metabolic gene transcripts over a 24-hour period. In contrast, using a single bout of concentric, endurance type exercise, Schmutz et al. [3] observed a significant upregulation of a range of metabolic and mitochondrial gene transcripts after an initial drop in mRNA abundance in the first hour after exercise. The short-term response of metabolic transcript expression therefore corresponds to the long-term (steady-state) adaptation seen after both prolonged concentric and eccentric exercise training [37]. The overall abundance of most of mitochondrial transcripts correlated significantly with mitochondrial volume density (fig. 4 and online suppl. fig. 5) which supports the idea that EET tends to decrease mitochondrial density.

Our data show a trend in the reduction of fatty acid synthase mRNA by approximately 60% in the EET subjects. We did not find increased expression of uncoupling proteins which have in other studies been linked to enhanced mitochondrial fat oxidation [38, 39]. In light of the decreased mitochondrial protein transcripts and the tendency to decreased mitochondrial volumes, we would suggest increased basal but not maximal fatty acid breakdown in response to the eccentric training. Further studies will be necessary to confirm this.

In accordance with the higher mechanical stress experienced by the muscles of the EET subjects, upregulation of transcripts coding for ECM components was more pronounced after EET than after RET. While expression of COL4A1 and COL6A1 mRNAs increased independent of training regime, COL1A1 and COL3A1 mRNAs were elevated significantly, by almost 4-fold after EET. The finding of an increase in interstitial tissue components upon EET is in line with the observation that EET generates a stiffer muscle phenotype [40, 41]. The expression of embryonic myosin heavy chain (MYH3) and α-cardiac myosin heavy chain (MYH6), both markers of repair, as well as transcripts encoding proteins involved in cell cycle and DNA repair (CCND1, CDKN2D, GADD45) were all elevated independent of training mode. GADD45 (growth arrest and DNA damage inducible) encodes a DNA repair enzyme which is elevated after general stress. The stronger induction of GADD45 mRNA after EET (+140%) supports the hypothesis that EET has a more distinct molecular signature due to larger mechanical stress. Remodeling of muscle tissue both as neoformation or hypertrophy of muscle fibers requires activation of quiescent satellite cells. Activated satellite cells express a variety of unique markers such as CDH15 (M-cadherin), HGF and Pax7. As expected, mRNA expression of all these markers was increased after training with both training regimes, suggesting an increased remodeling of muscle tissue in strength-trained subjects. Expression of MYH1, the isoform almost exclusively expressed in type IIX muscle fibers, was decreased with training. This is in line with the more pronounced observed loss of type IIX fiber after eccentric training [15].

Marker transcripts involved in muscle growth such as myoD, myogenin, IGF-1 and HGF are significantly up-

regulated at the mRNA level after training in pooled RET and EET subjects (table 3). The mRNAs for myogenin, IGF-1 and HGF were induced by more than 70% in EET, but only by 20–40% in RET subjects (statistically not significant). IGF-1 was detected using primer pairs spanning the exon 3–4 boundary. As a consequence, we did not distinguish between the IGF-IEa and the mechano-growth factor (MGF; IGF-IEc) isoforms which differ in exon 5 and were shown to be differentially spliced upon mechanical stress [42]. Of the micro-RNAs, we found miR-1 to be downregulated, which correlated inversely with the upregulation of IGF-1 mRNA, a potential inhibitory target of miR-1 (fig. 3). The downregulation of miR-1 is an expected result [11]; however, the mechanism by which miR-1 could influence protein abundance of IGF-1 is not clear and could include translational repression as well as mRNA degradation. Since the potential priming site for miR-1 is present in the 3′-UTR of IGF-1, both translational inhibition and effects on IGF-1 mRNA abundance are possible mechanisms. The expression of other potential targets such as serum response factor and HGF did not correlate with miR-1 expression in our analysis. This suggests that the translation of these mRNAs could be inhibited or they may not represent bona fide targets of miR-1 [11]. Work from Rao et al. [43] has shown that myoD and myogenin bind to the promoter region of miR-1 and can promote its transcription. In our study, we found both myogenin and myoD mRNA expression increased, while miR-1 abundance was decreased in response to training. This apparent discrepancy might be explained by a differential availability other of cofactors at the two time points in this study.

Conclusions

Both EET and RET training regimes showed similar functional improvements, but distinct patterns of marker transcript accumulations. Pronounced mechanical stress combined with the smaller metabolic load of EET resulted in depressed expression of mitochondrial transcripts along with increased transcripts involved in remodeling and repair. RET led to a lesser disturbance of the muscle gene expression profile, with smaller increases in expression of remodeling and repair genes and unchanged expression of mitochondrial and metabolic transcripts. While EET seems to be at least as effective as RET with regard to the functional outcome of the strength training intervention, the depression of metabolic genes is at first sight undesirable. It also contradicts the decrease in IMCL content in response to EET (which remarkably correlated with body fat), which may well be a consequence of reduced insulin sensitivity in the elderly. Given the unanimously positive response we had in terms of improved life quality in response to the training from the participants, it looks as if for a cohort of this age, fitness is more limited by muscle mass than endurance capacity.

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