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ORIGINAL ARTICLE

Effects of strength training with eccentric overload on muscle adaptation in male athletes

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Abstract In classic concentric/eccentric exercise, the same absolute load is applied in concentric and eccentric actions, which infers a smaller relative eccentric load. We compared the effects of 6 weeks of classic concentric/eccentric quadriceps strength training (CON/ECC, 11 subjects) to eccentric overload training (CON/ECC+, 14 subjects) in athletes accustomed to regular strength training. The parameters determined included functional tests, quadriceps and fibre cross-sectional area (CSA), fibre type distribution by ATPase staining, localisation of myosin heavy chain (MHC) isoform mRNAs by situ hybridization and the steady-state levels of 48 marker mRNAs (RT-PCR) in vastus lateralis biopsies taken before and after training. Both training forms had anabolic effects with significant increases in quadriceps CSA, maximal strength, ribosomal RNA content and the levels of mRNAs involved in growth and regeneration. Only the CON/ECC+ training led to significantly increased height in a squat jump test. This was accompanied by significant increases in IIX fibre CSA, in the percentage of type IIA fibres expressing MHC IIx mRNA, in the level of mRNAs preferentially expressed in fast, glycolytic fibres, and in post-exercise capillary lactate. The enhanced eccentric load apparently led to a subtly faster gene expression pattern and induced a shift towards a faster muscle phenotype plus associated adaptations that make a muscle better suited for fast, explosive movements.

Keywords Skeletal muscle · Muscle adaptation · Muscle fibre · Training · Gene expression

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Introduction

Systematic strength training is of increasing importance as a preventative measure, in physiotherapy and in a wide variety of sports at all levels of performance. Heavy resistance training, used to enhance performance in many different sports, leads to muscular adaptations that result in muscle hypertrophy as well as changes in contractile characteristics. A vast majority of exercises used in strength training involve resistance against concentric as well as eccentric loads. Combined concentric/eccentric leg extension exercise has been shown to effectively increase the cross-sectional area (CSA) of the quadriceps femoris muscle (Narici et al. 1996; Ahtiainen et al. 2003), as well as fibre CSAs, especially of type II (fast) fibres (Hortobágyi

et al. 1996; McCall et al. 1996; Andersen and Aagaard 2000; Hortobágyi et al. 2000; Aagaard et al. 2001; Campos et al. 2002; Kosek et al. 2006).

Recent investigations into heavy resistance training have provided evidence that the resulting skeletal muscle hypertrophy is due to increased protein translation (Bodine 2006; Coffey and Hawley 2007), enhanced expression of genes involved in anabolic mechanisms (Bickel et al. 2005; Kosek et al. 2006; Kvorning et al. 2007) as well as satellite cell activation and proliferation to provide additional nuclei to the enlarging myofibres (Kadi and Thornell 2000; Kadi et al. 2004; Olsen et al. 2006; Petrella et al. 2006). Anabolic effects are mediated via the interaction of testosterone with the androgen receptor (AR) that leads to upregulation of several muscle-specific transcripts and probably also increased muscle availability of insulin-like growth factor (IGF)-1 (Bamman et al. 2001; Kraemer and Ratamess 2005). Although it is well known that the serum levels of testosterone can be acutely and chronically elevated after resistance training, there are few studies which have investigated AR expression in human skeletal muscle. IGFs, which are secreted by liver and skeletal muscle, play a prominent role in mediating muscle hypertrophy in response to strength training. Significant acute increases in the mRNAs of IGF-1 have been reported in human muscle after concentric/eccentric strength training (Hameed et al. 2003; Kim et al. 2005a, b; Petrella et al. 2006) and IGF-1 mRNAs were also found to be significantly increased after several weeks of heavy quadriceps strength training (Haddad et al. 2005; Kvorning et al. 2007; Petrella et al. 2006).

In addition to the development of muscle hypertrophy, a change in fibre type distribution towards increased percentage area of type IIA fibres has been reported in untrained subjects after heavy resistance training. Human type IIA fibres have somewhat slower contraction times than IIX fibres (Bottinelli et al. 1996) and it is thought that type IIX to IIA transformations render a trained muscle somewhat slower. Myosin heavy chain (MHC) isoforms are the principle (but not the only) determinants of a fibre's twitch characteristics (Spangenburg and Booth 2003). Human muscle fibre types are also distinguished by their metabolic properties, but to our knowledge, effects of strength training on metabolic gene expression have been under investigated.

In conventional concentric/eccentric quadriceps training, the same absolute load is used for the concentric as well as the eccentric actions, i.e. the relative workload is smaller for the eccentric contractions, because the maximal voluntary force is greater in eccentric than in concentric muscle actions (Komi and Vitasalo 1977). It has repeatedly been shown that muscle eccentric loading is beneficial for the development of muscle hypertrophy (Hather et al. 1991; Hortobágyi et al. 2000). Increased IGF-1 and myostatin mRNA responses to eccentric loading when compared with equivalent relative concentric loading have been reported after knee extension exercise in humans (Bamman et al. 2001) and after electrical stimulation of the medial gastrocnemius muscle in rats (Heinemeier et al. 2007); such findings plus the observation that in in vitro studies, mechanical stress (especially stretch caused by eccentric contractions) is converted into specific signalling (Coffey and Hawley 2007) suggest that concentric/eccentric strength training with increased eccentric load might be superior for force development compared with conventional concentric/eccentric training.

We have previously investigated concentric/eccentric overload training in untrained subjects with a low resistance, high repetition scheme (Friedmann et al. 2004). Although this training programme was not designed to induce hypertrophy, we observed a tendency towards increased muscle CSA. Enhanced hypertrophy of the quadriceps femoris muscle has recently been reported after 5 weeks of heavy knee extension exercise applying eccentric overload during training with a non-gravity dependent exercise system (Norrbrand et al. 2008). In addition, we found significantly increased mRNAs for MHC IIa and lactate dehydrogenase (LDH) A as well as high correlations between LDH A and MHC IIx mRNA content in biopsies obtained from m. vastus lateralis. Based on these findings, we suggested that the adaptations to concentric/eccentric-overload training are towards a faster and stronger muscle.

The aim of the current study was to compare the effects of high resistance concentric/eccentric quadriceps training with eccentric overload (CON/ECC+) to conventional high resistance concentric/eccentric quadriceps training (CON/ECC) in athletes accustomed to regular resistance training. Training loads were higher and the execution of the exercises faster than what can be managed by untrained subjects. The CON/ECC group trained with the same absolute load in the concentric as well as the eccentric phase, while the CON/ECC+ group exercised with similar relative loads in both the concentric as well as the eccentric phase, i.e. the eccentric loading was in average 1.9-fold higher than the concentric loading. Similar schemes are used in elite sports practice. CON/ECC+ is thought to provide a competitive edge in sports where explosive strength is crucial, such as jumps, sprints or throws. Before and after a 6-week training period, functional parameters as well as muscle CSA were determined. Biopsies from the vastus lateralis muscle were obtained to determine fibre type distribution, fibre CSA, analysis of MHC isoform RNAs in the different fibre types by in situ hybridization and the steady-state expression of 48 selected marker mRNAs which served as indicators for the adaptation of selected cellular processes, encoding myofibrillar proteins, muscle growth factors, energy metabolism enzymes, transporters, proteins involved in muscle fibre regeneration and/or signalling networks.

Materials and methods

Subjects

Thirty male athletes with a strength training background volunteered for the study. They had to be active in sports where fast, forceful movements (explosive strength) are important, e.g. track and field jumps or sprints, basketball, volleyball or judo. Most of them had performed 1–2 (average 1.26) dedicated strength training sessions per week during the last 1–15 (average 5) years. None of them had used anabolic steroids nor supplemented creatine. We did not recruit body builders or weight lifters, because their training aim is primarily hypertrophy. Written informed consent was obtained from each subject. The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg, Germany, and conformed to the standards set by the Declaration of Helsinki. The subjects were randomly assigned for 6 weeks of either conventional concentric/eccentric quadriceps strength training (CON/ECC) or concentric/eccentric-overload strength training (CON/ECC+). Two of the subjects dropped out due to muscle soreness or injury, another one injured his ankle during activities not related to the study, one succumbed to a viral infection and one subject left the local area for private reasons, leaving a total of 25 subjects to complete the study and whose muscle biopsies were analysed. (CON/ECC: $n = 11$, 24.5 ± 4.2 years, 184 ± 7 cm, 80.5 ± 7.8 kg; CON/ECC+: $n = 14$, 24.3 ± 3.7 years, 185 ± 7 cm, 79.8 ± 8.6 kg).

Training protocol

Resistance training was conducted three times per week (Monday, Wednesday, Friday) for 6 weeks under the supervision of a certified coach with several decades of experience in strength training of elite athletes as well as untrained subjects. Prior to each training session, the subjects completed a standardised warm-up programme. The subjects then performed heavy one-leg knee extension exercise in sitting position either on a conventional device (m3, Schnell, Peutenhausen, Germany, CON/ECC) or on a computer-driven device (Motronik, Schnell, Peutenhausen, Germany, CON/ECC+) which allows the eccentric load to be specifically adjusted (Friedmann et al. 2004). Both legs were trained at the eight repetition maximum (8RM), i.e. the load was chosen to cause exhaustion after eight repetitions that had to be performed very fast, within 10–12 s. Specific speeds, e.g. angular velocity, were not measured. The effort in each session was monitored by the coach and the load was continuously increased to 8RM throughout the training period. In the case of CON/ECC, the same absolute load was applied in the concentric as well as the eccentric phase, whereas in CON/ECC+, the subjects were instructed to develop maximal force onto the lever arm during the eight repetitions in the concentric as well as the eccentric phase, which resulted in an eccentric load that was approximately 1.9-fold higher than the concentric load. The movements on the CON/ECC+ machine were similar to the conventional leg extension device, i.e. the kinetics of movement was also constantly changing. The series of eight repetitions were separated by 4-min rest. In an attempt to reach the same amount of exertion in both groups, pre-tests were conducted before the beginning of the study, and the work performed during 8RM was measured for both training modes. Equivalent amounts of work were reached if the subjects of the CON/ECC group performed six sets of eight repetitions and the subjects of the CON/ECC+ group five sets of eight repetitions. This was adhered to throughout the training period. Throughout the study, the subjects continued with their usual activities, but refrained from additional strength training for their legs and from any intense exercise for at least 24 h prior to all testing.

At the end of each training session, the subjects were asked to rate their perceived exertion (RPE) according to the Borg Scale (Borg 1982). RPE was 14 ± 1 (mean value $\pm$ standard deviation, CON/ECC) and 15 ± 2 (CON/ECC+) after the second training session and had significantly increased to 17 ± 2 (CON/ECC) and 16 ± 3 (CON/ECC+) after the penultimate training session; there were no significant differences between the two training groups in rating the resistance training as heavy.

In the second and in the penultimate training session, the maximal training-induced capillary blood lactate concentration was determined. 20 µl of capillary blood was drawn from the earlobe after the warm-up programme and after 1, 3, 5, 10 and 15 min following the last set of eight repetitions. Lactate was measured with an automated system (EBIO plus, Eppendorf, Hamburg, Germany).

Testing procedures

Before and after the 6-week training period, quadriceps femoris muscle CSA was determined by magnetic resonance imaging (MRI). Before the start of the first and the last training session, one-leg concentric one repetition maximum (1RM) was measured and the performance in a squat jump test determined. Biopsies from the right vastus lateralis muscle were obtained before and 3 days after the end of the 6-week training period.

Strength tests

After a standardised warm-up procedure on a bicycle ergometer, concentric 1RM was determined on the conventional training device (m3, Schnell, Peutenhausen, Germany)

for both legs separately. Subjects were seated 5° reclined and firmly strapped in at their shoulders and hips. Sitting positions and knee angle (100°) were individually adjusted for each test. The subjects were asked to lift progressively heavier weights until they reached the weight they failed to raise to full leg extension. In all cases, 1RM was reached after no more than five trials with 4-min rest in between the attempts.

Squat jump test

After a 10-min break following the 1RM strength test, the subjects were required to perform three maximal squat jumps on a force platform (Leonardo-Mechanography™, Novotec, Pforzheim, Germany). The squat jump was performed as a maximal vertical jump from an initial static position of 90° knee flexion with the hands rested on the subjects' hips. Maximal performance was identified as maximal jump height as calculated on the basis of the time spent in the air between leaving the force platform and landing by a preinstalled software programme. The result of this jump test is thought to depend to a large extent on quadriceps explosive power.

Magnetic resonance imaging

Magnetic resonance imaging of both thighs was performed in the supine position on a 1.5-T clinical MR system (MAGNETOM Symphony, Siemens AG Medical Solutions, Erlangen, Germany) using the manufacturer's standard phased array coil for signal reception. The imaging protocol comprised an axial and coronal T1-weighted spin-echo (SE) sequence (repetition time (TR)/echo time (TE) in ms, 500/15), an axial T2-weighted turbo-spin-echo (TSE) sequence (4,000/119), an axial short tau inversion recovery sequence (4,000/119) and a fat-suppressed T1-weighted TSE sequence (645/11). CSA of both quadriceps femoris muscles was determined at 20 cm distance from the trochanter major on T1-weighted images before and after the training period. This image analysis was performed by two independent researchers. All MR images were displayed as softcopies in fully electronic monitored fashion using a picture archiving and communication system (PACS) with large screen high-resolution cathode ray tube displays, which enabled the review of eight images simultaneously. Each researcher also had the opportunity to individually select the different MR sequences using the PACS, to e.g. clarify the facial boundaries of the quadriceps muscle. All MR examinations were jointly randomized and presented to the researchers. Identifying parameters such as patient's name were omitted. Both researchers were blinded to the clinical and training data of the volunteers as well as the interpretations of the other researcher. CSA was recorded separately for the right and left thighs. CSA was measured by each researcher on T1-weighted images using a computerised digitiser that is part of the standard tool of the PACS and allows for precise and reproducible measurement of CSA. The mean CSA values obtained by the measurements of both researchers were used for statistical analysis. CSA of the whole quadriceps femoris muscles was determined, because the facial boundaries between the lateral and deep vastus muscles could not always be clearly identified, as described previously (Friedmann et al. 2004).

Muscle biopsy sampling

Before and after the 6 weeks of training, muscle biopsy samples were taken from the same region at mid-thigh level of the right vastus lateralis muscle under local anaesthesia, using the Bergström (1975) technique. The second biopsy was taken 3 days after the last training session to ensure that we were determining long-term steady-state changes in the mRNAs chosen, thus avoiding the influence of short-term transient changes after an exercise bout (Friedmann et al. 2003). The muscle tissue was immediately freed from blood and visible connective tissue, rapidly frozen in isopentane cooled by liquid nitrogen and subsequently stored at −80°C. To avoid residual effects from preceding biopsies, biopsy sites were spaced apart by about 1 cm from proximal to distal.

Histochemistry

Serial transverse sections (6 μm) were cut in a cryostat at −20°C and stained for myofibrillar ATPase after preincubations at pH 4.35 (5 min, room temperature), 4.8 (5 min, room temperature) and 10.5 (15 min, 37°C) (Brooke and Kaiser 1970). Using this method, four fibre types (I, IIA, IIAX, IIX) could be distinguished after preincubation at pH 4.8 and 3 fibre types (I, IIC, II) after preincubation at pH 10.5. On the average, 407 ± 185 fibres were classified in each sample. Microscopic images of the ATPase stained cross sections (pH 4.8) were recorded with a video camera (Olympus HCC-3600 P high gain) and digitized on a personal computer equipped with an image analysis system (VIBAM 0.0-VFG 1 frame grabber) as described earlier (Friedmann et al. 2003, 2004). The type IIC fibres were added to type I fibres and the type IIAX to the type IIA for the analysis of fibre type distribution.

RNA extraction and reverse transcription

For RNA extraction, 25 μm sections were cut on a cryostat. The area of the cutting surface was estimated by planimetry. The number of sections cut was adjusted to reach approximately 10 mm^3 (equivalent to 10 mg tissue,

assuming an approximate density of 1). From these sections, total RNA was isolated using a modification of the Quiagen mini-protocol for skeletal muscle as described previously (Quiagen, Hilden, Germany) (Friedmann et al. 2003, 2004). This procedure yields RNA with a 28S/18S ratio between 1.8 and 2 after formaldehyde gel electrophoresis that is virtually RNAse free (i.e. stable o/n at room temperature). The isolated RNA was quantified with RiboGreen (Quant-iT™ RiboGreen®RNA Reagent and Kit, Invitrogen, Paysley, UK). For RT, 380 ng of RNA from each sample was dissolved in water and reverse transcribed using the Superscript™ III First Strand Synthesis System for RT-PCR (Invitrogen, Paysley, UK) in a 10 µl reaction according to the manufacturer's specifications, with random hexamer priming. The resulting cDNA was stored at −80°C for subsequent PCR. As a negative control, 60 ng of each RNA was also processed under identical conditions, but without the reverse transcriptase.

Real-time PCR

PCR quantification was done using a real-time PCR system (ABI PRISM® 7900HT Detection System, Applied Biosystems, Foster City, USA) with 384 well TaqMan arrays (Micro Fluidic Cards), which were preloaded with an identical set of 48 predesigned probes and primers in each of the eight lanes; their Applied Biosystems assay IDs and the gene bank accession numbers corresponding to their sequences are shown in Table 1. For each target mRNA, 75 ng cDNA was combined with the TaqMan® Universal PCR Master Mix and loaded into a specific lane on the card. This system quantifies cDNA relative to a calibrator sample which is run on each card. Our calibrator consisted of cDNA generated from an mRNA pool containing portions from 12 of our samples that include pre- and post-training biopsies. Relative quantification was done with the machine's preinstalled software programme (SDS 2.2). The ratio relative to the calibration probe was determined based on the delta CTs and related to the average content of 5 cDNAs which were chosen as "housekeeping" transcripts. In the 10 runs performed, the average standard error of the mean of the calibrator sample for these 48 assays was 5.3%.

Originally, β2-microglobulin (β2M) and β-actin were selected as housekeeping transcripts, together with 18S RNA, the former two being described as the most stably expressed housekeeping transcripts after resistance exercise (Mahoney et al. 2004). These "housekeepers" turned out to be unsuitable for our study: As expected, the values for 18S cDNA in the PCR correlated strongly ($P < 0.0001$) with the yield of RNA from 10 mg tissue as determined by RiboGreen. However, the yield of total RNA had increased to an extent previously not described in response to CON as well as CON/ECC+ training (Fig. 1). The values for β2M and β-actin cDNA were highly correlated ($P < 0.0001$), but varied up to 20-fold or more between the samples. They also correlated strongly ($P < 0.001$ or less) with most of the mRNAs involved in muscle fibre regeneration in our selection (EIF2B5, HGF, IGF1 receptor, MYF5, MRF4, myogenin, embryonic myosin light chain, Cyclin D1, p21). We suggest that β2M and β-actin mRNAs are upregulated during muscle fibre regeneration and are, therefore, not suitable housekeeping RNAs for the biopsies described in here. Ideally, we could have used total RNA yield as correction factor to relate the PCR values to mg biopsy tissue, but these estimates were not precise enough to serve as a direct basis for individual PCR ratios. As a consequence, and also to compensate for the inherent variability introduced by the RT step (probably mainly during random priming), an aggregate of five housekeeping cDNAs was used: citrate synthase (CS), fatty acid-binding protein (FABP3), MyoD, monocarboxylate transporter 1 (MCT1) and GLUT4. The values for each of them had similar, statistically significant negative correlations with total RNA yield and no distinct fluctuations were obvious amongst the different biopsies or in the aggregate. The PCR values in this study, therefore, represent approximate relative values of tissue content.

In situ hybridization

In situ hybridizations were performed on a subset of biopsies that were sufficiently large for additional investigations after the preparation of RNA. This was the case for the biopsies from six subjects in the CON and for nine subjects in the CON/ECC+ group. For in situ hybridization, we used digoxigenin (DIG)-labelled cRNA probes for the mRNAs of human MHC I, IIa and IIx. The fragments used (MHC I: nucleotides (nt) 5871–6008 of gene bank sequence NM_000257; MHC IIa nt: 5916–6047 of NM_017534, MHC IIX: nt 5804–5925 of NM_005963) were very similar or equal to the ones used in a previous study (Andersen and Schiaffino 1997). They were subcloned pGEM-T Easy (Promega), from which antisense cRNA probes were synthesised by in vitro transcription from SP6 or T7 promoters using the Mega Script kit (Ambion), at a ratio of ~4 unlabelled UTPs (Ambion) to 1 DIG-UTP (Roche).

The protocol for in situ hybridization was adapted from Braissant and Wahli (1998). 10 µm cryostat sections were fixed in 4% paraformaldehyde/phosphate buffered saline for 10 min at room temperature and incubated twice for 10 min in acetic anhydride (0.25%) in 0.1 M triethanolamine (pH 8). The sections were then equilibrated in 5× saline-sodium citrate buffer (SSC) at room temperature for 15 min before a 2 h prehybridization at 58°C in 50% formamide, 5× SSC and 40 µg/ml herring sperm DNA. Hybridization with labelled cRNA anti-strand probes took place overnight at 58°C (MHC I probe) or 48°C (MHC IIa and

Table 1 Applied Biosystems assay IDs and gene-bank accession numbers of probes and primers used for real-time PCR

mRNA	Assay ID	Gene-bank accession no.
"Housekeeping" transcripts		
β2-Microglobulin (β2M)	Hs99999907_m1	NM_004048
β-Actin	Hs99999903_m1	NM_000476
18S rRNA	4342379	X03205.1
Myofibrillar proteins		
MHC I	Hs00165276_m1	NM_000257
MHC IIa	Hs00430042_m1	NM_017534
MHC IIx	Hs00428600_m1	NM_005963
MHC IIb	Hs00757977_m1	NM_017533
MLC 1f	Hs00365252_m1	NM_079420
MLC 1sb	Hs00365258_m1	NM_079422
MLC 3f	Hs00264820_m1	NM_000258
Enzymes of energy metabolism and transporters		
Creatine kinase (CK)	Hs00176490_m1	NM_001824
Adenylat kinase 1	Hs00176119_m1	NM_000476
Phophofructokinase (PFK)	Hs00175997_m1	NM_000289
Lactate dehydrogenase (LDH) A	Hs00855332_g1	NM_005566
LDH B	Hs00600794_mH	NM_002300
Citrate synthase (CS)	Hs00830726_sH	NM_004077| NM_198324
GLUT4	Hs00168966_m1	NM_001042
Monocarboxylic acid transporter (MCT)1	Hs00161826_m1	NM_003051
MCT4	Hs00190794_m1	NM_004696
Fatty acid-binding protein 3 (FABP3)	Hs00269758_m1	NM_004102.3
Anabolic signalling		
Androgen receptor (AR)	Hs00171172_m1	NM_000044
Insulin-like growth factor 1 (IGF1)	Hs00153126_m1	NM_000618
IGF-1 receptor	Hs00609566_m1	NM_000875
Insulin-like growth factor 2 (IGF2)	Hs00171254_m1	NM_000612
IGF-binding protein 3 (IGFBP3)	Hs00426287_m1	NM_000598
IGFBP4	Hs00181767_m1	NM_001552
IGFBP5	Hs00181213_m1	NM_000599
Muscle growth and regeneration		
MyoD	Hs00159528_m1	NM_002478
Myogenin	Hs00231167_m1	NM_002479
Myogenic factor 5 (MYF5)	Hs00271574_m1	NM_005593
Myogenic regulatory factor 4 (MRF4)	Hs00231165_m1	NM_002469
Myostatin	Hs00193363_m1	NM_005259
Cyclin D1	Hs00277039_m1	NM_053056
Cyclin-dependent kinase inhibitor (p21)	Hs00355782_m1	NM_000389| NM_078467
Hepatocyte growth factor (HGF)	Hs00300159_m1	NM_000601
Myosin heavy chain neonatal (MHCneo)	Hs00267293_m1	NM_002472
Myosin light chain embryonic (MLCemb)	Hs00267321_m1	NM_002476| NM_001002841
Signalling network proteins		
Calsarcin 1	Hs00222007_m1	NM_021245
Calsarcin 2	Hs00213216_m1	NM_016599
Calsarcin 3	Hs00370235_m1	NM_133371

Table 1 continued

mRNA	Assay ID	Gene-bank accession no.
Ankyrin repeat domain 2	Hs00220469_m1	NM_020349
Ankyrin repeat domain 23	Hs00171172_m1	NM_000044
LIM protein	Hs00185787_m1	NM_003476
mTOR	Hs00234522_m1	NM_004958
Eukaryotic translation initiation factor 2B, 5 epsilon (EIF2B5)	Hs00384949_m1	NM_003907
EIF4E-binding protein 1 (4EBP1)	Hs00607050_m1	NM_004095
Muscle atrophy F-box (MAFbx)	Hs00369714_m1	NM_148177| NM_058229
Forkhead box (FOXO1A)	Hs00231106_m1	NM_002015

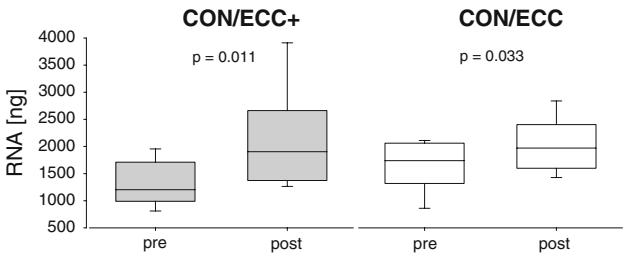

Fig. 1 RNA yield from approximately 10-mg biopsy tissue, determined using RiboGreen, before (*pre*) and after (*post*) 6 weeks of concentric/eccentric-overload (CON/ECC+) or conventional concentric/eccentric (CON/ECC) quadriceps strength training. Median values, the 5th, 25th, 75th and 95th percentiles are shown

IIx probes) with 0.3 ng/µl of DIG-labelled cDNA probes in 50% formamide, 5× SSC and 40 µl/ml herring sperm DNA. After hybridization, the sections were washed for 30 min in 2× SSC at room temperature, followed by two 1 h washes at 45°C (MHC IIx probe), 52°C (MHC IIa probe) or 62°C (MHC I probe), the first in 2× SSC, the second in 0.1× SSC. This was followed by RNAse treatment with 0.5 µg/ml RNAse A (Sigma) for 30 min to degrade unbound probe. The sections were then washed twice in 2× SSC for 5 min before another stringent wash in 0.5× SSC and 20% formamide at 45, 52 or 62°C for 10 min. The sections were washed again twice in 2× SSC for 5 min. They were then adjusted for 5 min in DIG buffer 1 (100 mM Tris, 150 mM NaCl, pH 7.5), followed by DIG buffer 2 (same as DIG buffer 1 but with 1% blocking reagent; Roche) for 1 h, before the application of anti-DIG alkaline phosphatase coupled Fab (Roche), in DIG buffer 2 at a dilution of 1:5,000 for 2 h. Unbound antibody was subsequently washed away by two 15 min washes in DIG buffer 1. Before the visualisation of the bound phosphatase, the sections were adjusted for 5 min in DIG buffer 3 (100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5). They were then incubated overnight in DIG buffer 3, supplemented with 10% polyvinyl alcohol (De Block and Debrouwer 1993) and 0.4 mM NBT (4-nitroblue-tetrazoliumchloride, Roche) and 0.4 mM X-phosphate (5-bromo-4-chloro-3-indolylphosphate, Roche). The staining reaction was stopped with three 15 min washes in TE buffer (0.1 M Tris, 1 mM EDTA, pH 8.0). The sections were embedded in Kaiser's gelatine. Some series included sense strand probes as negative control, which gave signals equivalent to non-reactive fibres.

The aim of the in situ hybridizations was to detect fibres "in transition", which in the context of this work are defined as being of one type (IIA or IIX) according to their ATPase stain, but expressing significant amounts of the mRNA of the other MHC isoform (IIx or IIa, respectively). This is not straightforward, since in human muscles, there is a continuum of hybrid fibres between types IIA and IIX fibres and the signals of in situ hybridizations are a semi-quantitative measure of RNA content at the best, which precludes a quantitative analysis of all fibres. Our analysis, therefore, primarily concentrated on fibres that were histochemically unambiguous IIA or IIX. All fibres that were a shade darker than unambiguous IIA or a shade lighter than unambiguous IIX were taken as IIAX fibres. Fibres "in transition" from IIA to IIX were defined as fibres with a clearly identifiable IIx mRNA signal and an ATPase stain that was as light as surrounding IIA fibres with no IIx mRNA expression (Fig. 5). IIX fibres in transition to IIA were defined as fibres with a clearly identifiable IIa mRNA signal and an ATPase stain as dark as surrounding IIX fibres without IIa mRNA signal and such fibres were rare.

Statistics

Most statistical procedures were performed using Sigmastat 3.0 and Sigmaplot 8.0 for Windows from Jandel Scientific (San Rafael, CA, USA). Statistical analysis of microarrays (SAM) and cluster analysis was done using J-Express Pro 2.7 (Molmine). Since most of the data were not normally distributed, we presented them as median, 5th, 25th, 75th

and 95th percentiles for the data obtained from tests of the right leg only, since the muscle biopsies were obtained from the right m. vastus lateralis. Differences between values obtained before and at the end of the training period in the strength and jump tests as well as the muscle morphometric data were assessed by Wilcoxon signed rank test, in case of normal distribution of the data by Student's paired *t* test. Differences between training groups were evaluated using the Mann–Whitney rank sum test. The level of significance was set at $P \leq 0.05$. With regard to the non-normal distribution of half of the PCR series and expected small differences between the gains of the two similar training schemes, we performed bootstrap analyses (statistical analysis of microarrays, SAM) with 400 iterations on these data. The sensitivity of this procedure is similar to that of a *t* test, but does not require normally distributed data. A false detection rate (FDR) of 0.0 was judged as statistically significant. Correlation of selected parameters were analysed with help of the Pearson product moment correlation. For cluster analysis, we used hierarchical clustering with Pearson distance measures.

Results

Functional testing (Fig. 2)

In both the groups, concentric one-leg 1RM was significantly increased by 11–15 kg after 6 weeks of training (Fig. 2a). No significant difference was found between the two groups. Jump height in the squat jump test was significantly increased ($P < 0.05$) after training in the CON/ECC+ group only, by about 2–3 cm. No significant changes were observed in the CON/ECC group (Fig. 2b). When comparing the relative improvements (percentage gain) in response to CON/ECC+ versus CON/ECC, we noted a clear tendency ($P = 0.089$, unpaired *t* test) towards increased performance after CON/ECC+. The maximal training-induced increase in capillary lactate concentration was significantly enhanced in the penultimate training cession of CON/ECC+ compared with the second training session. No such difference was found after CON/ECC (Fig. 2c).

Morphometric measurements (Fig. 3; Table 2)

Whole quadriceps CSA was significantly increased after the training period in both groups, by 5.8 ± 4.3 cm^2 after CON/ECC+ and by 8.0 ± 6.5 cm^2 after CON/ECC (Fig. 3a). In both the groups, the percentage fibre type distribution remained unchanged in response to the training (Table 2). However, in the CON/ECC+ group, the median FCSAs of all three fibre types tended to increase after the training period, but this was statistically significant for the type IIX fibres only. No significant changes of FCSA could be detected in the CON/ECC group (Fig. 3b).

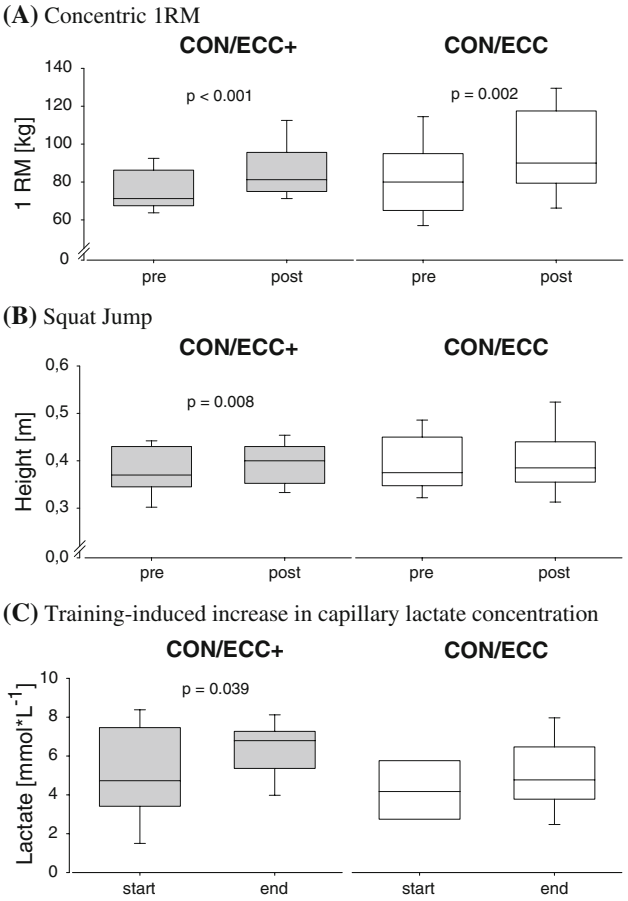

Fig. 2 Conventional one repetition maximum of the right leg (1RM **a**), jump height in the squat jump test (**b**) before (*pre*) and after (*post*) 6 weeks of concentric/eccentric-overload (CON/ECC+) or conventional concentric/eccentric (CON/ECC) quadriceps strength training and training-induced increase in capillary lactate concentration (**c**) at the beginning (start) and at the end of 6 weeks of CON/ECC+ or CON/ECC. Median values, the 5th, 25th, 75th and 95th percentiles are shown

Testing for significant correlations between morphometric measurements and the results of functional testing revealed that only after CON/ECC+, the changes (Δ) in 1RM were significantly correlated with Δ FCSA IIX ($R = 0.612$, $P = 0.020$) and with Δ FCSA IIA ($R = 0.600$, $P = 0.023$). There were no further significant correlations, especially none in the CON/ECC group, and Δ 1RM was not significantly correlated with Δ FCSA I.

RNA yield (Fig. 1)

In both the CON/ECC as well as the CON/ECC+ group, the amount of total RNA isolated from approximately 10-mg biopsy tissue was significantly increased after the training period, by an average of about 40 and 63%, respectively, as determined by RiboGreen. The difference in increase was

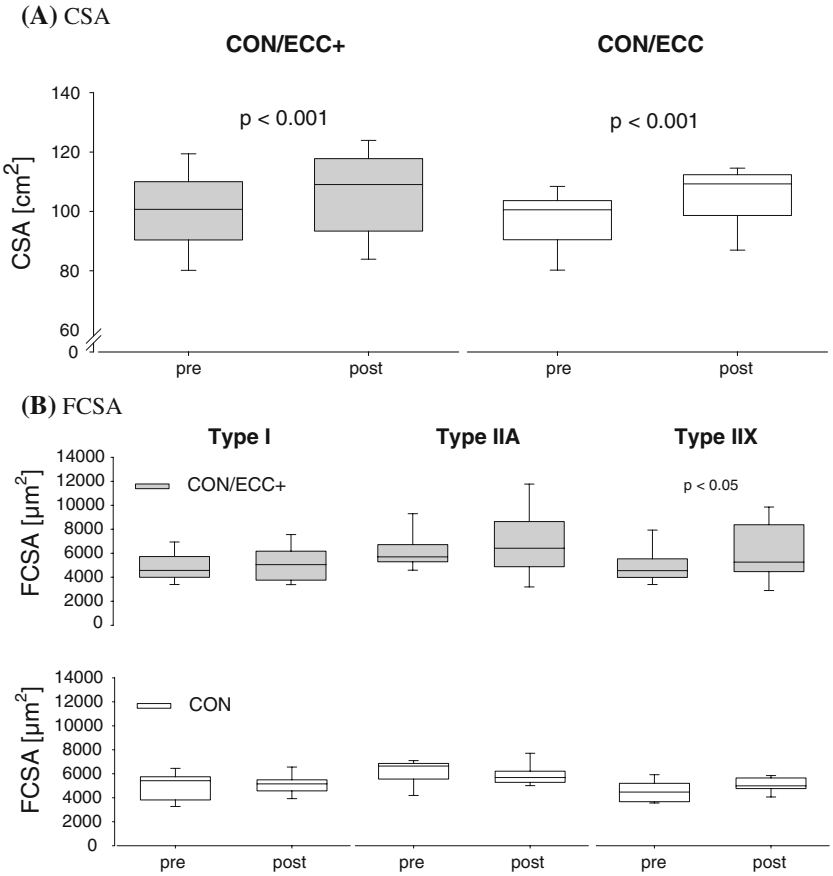

Fig. 3 Cross-sectional area of the right quadriceps femoris muscle as determined from magnetic resonance imaging (CSA **a**) and fibre cross-sectional areas (FCSA, ATPase staining **b**) before and after 6 weeks concentric/eccentric-overload (CON/ECC+) or conventional concentric/eccentric (CON/ECC) quadriceps strength training. Median values, the 5th, 25th, 75th and 95th percentiles are shown

Table 2 Fibre type distribution (%) before and after 6 weeks of heavy concentric/eccentric-overload (CON/ECC+) or concentric/eccentric (CON/ECC) knee extension exercise

Fibre type	CON/ECC+			CON/ECC		
	25%	Median	75%	25%	Median	75%
I						
Before	38.5	50.7	56.6	25.9	40.2	54.8
After	33.3	45.7	57.9	30.5	39.4	51.8
II A						
Before	30.5	34.7	37.2	31.6	47.1	51.0
After	28.0	37.7	44.5	37.2	50.7	58.3
II X						
Before	7.7	9.6	26.9	4.0	11.1	20.3
After	5.1	13.6	20.4	7.3	12.1	15.1

not significant between the training groups. Since about 90% of the total RNA is ribosomal, the observed increase is most likely due to increased ribosomal RNA.

Expression of selected marker transcripts (Fig. 4)

When analyzing the training-induced gains (post–pre training) of the selected 48 transcripts (listed in Table 1), two mRNAs were found to be differentially regulated in response to CON/ECC+ compared with CON/ECC, i.e. they had an FDR = 0.0 in the bootstrap method used for analysis. One was the mRNA of the fastest MHC isoform, MHC IIb, which was significantly increased after CON/ECC+, whilst after CON/ECC, it tended to decrease (Fig. 4a). MHC IIb mRNA was expressed at much lower level than the major MHC isoform mRNAs (MHC I, IIa and IIx), according to a rough estimate based on the CT values of the PCR by several hundred to a thousand fold. The other mRNA found to differ between the two training modes was androgen receptor (AR) mRNA, the training induced gain of which was significantly higher in the CON/ECC+ group compared with the CON/ECC group (Fig. 4b). In a comprehensive correlation analysis with the morphometric data, we found Δ AR mRNA only in the CON/ECC+ group to be significantly correlated: Δ AR mRNA was negatively correlated with Δ FCSAIIX ($R = -0.648$, $P = 0.012$) and with Δ FCSA IIA ($R = -0.586$, $P = 0.028$). Significant differences between post- and pre-training biopsies were found for 19 transcripts in the CON/ECC+ group; all of them were increased after training. In the CON/ECC group, only nine transcripts were significantly elevated after training. All were amongst the 19 transcripts which were also increased in the CON/ECC+ group.

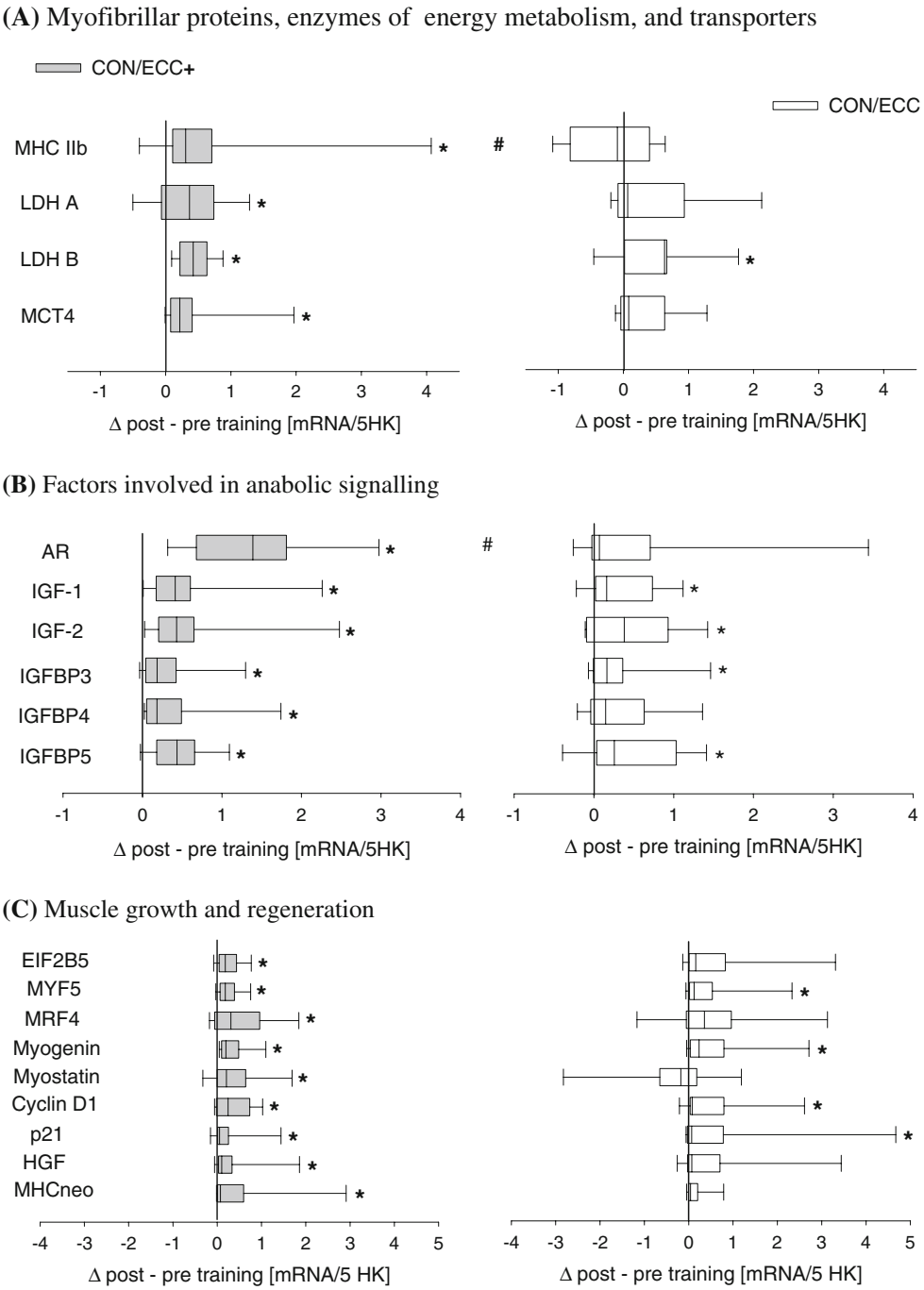

Fig. 4 Significantly changed marker mRNAs of myofibrillar proteins, enzymes of energy metabolism, transporters and factors involved in anabolic signalling, muscle growth and regeneration. These are relative values based on an aggregate of five housekeeping transcripts. Median values, the 5th, 25th, 75th and 95th percentiles are shown. *Significantly different from pre-training value (false discovery rate = 0 in SAM), #significantly different Δ post–pre training values between CON/ECC+ and CON/ECC (false discovery rate = 0 in SAM)

mRNAs of myofibrillar proteins, enzymes of energy metabolism and transporters (Fig. 4a)

With the exception of MHC IIb and MHCneo mRNA after CON/ECC+, no significant changes were found for the mRNAs of the MHC or MLC isoforms measured. After CON/ECC+, the mRNAs of both LDH isoforms (A and B) as well as the lactate transporter MCT4 were significantly increased (FDR = 0.0). In the CON/ECC group, we found only LDH B mRNA to be significantly increased. However, there were no significant differences in the gains of these mRNAs between the two training groups. The mRNAs of the other energy metabolism enzymes, of CK or of adenylate kinase 1 did not change significantly.

mRNAs involved in anabolic signalling (IGF axis) (Fig. 4b)

Most mRNAs coding for members of the IGF axis were significantly increased after the training period in both training groups, the exceptions being IGFBP4, for which mRNA levels did not significantly change in response to CON/ECC and IGF-1 receptor mRNA which was not different after both training protocols.

mRNAs involved in muscle growth and regeneration (Fig. 4c)

The mRNA of the translation initiation factor EIF2B5 was significantly increased in the CON/ECC+ group only. The mRNAs of three of the four known myogenic regulatory factors (MRF4, Myf 5 and myogenin) were increased after CON/ECC+. However, in the CON/ECC group, only MYF5 and myogenin were significantly upregulated, but not MRF4. There were concomitant significant increases in both training groups for the mRNAs of cyclin D1 and p21, both general markers of proliferation and differentiation. Myostatin mRNA content tended to decrease in response to CON/ECC, whilst it was significantly increased after CON/ECC+, although the difference between the training groups was not significant. Cluster analysis revealed a distinct cluster consisting of the mRNAs of MHC IIb, AR (the two transcripts that differed significantly between CON/ECC+ and CON/ECC) and myostatin. HGF and MHCneo mRNA expression increased significantly in the CON/ECC+ group only, whilst MLCemb mRNA did not change significantly in either group.

mRNAs of signalling network proteins

No significant changes were found for mRNAs of proteins known to be involved in anabolic signalling (mTOR, 4EBP1), atrophy signalling (MAFbx, FOXO) or calcium signalling (calsarcin 1, 2, 3).

Detection of fibres in transition by in situ hybridization (Fig. 5)

Although such fibres were relatively rare (in a total of 1,429 IIA fibres, 28 contained IIx mRNA), there was a significant increase in the percentage of type IIA fibres expressing MHC IIx mRNA after 6 weeks of CON/ECC+, but not after CON/ECC. The difference between the CON/ECC+ and the CON/ECC group was also statistically significant. In neither training group did we find significant changes in type IIX fibres expressing MHC IIa mRNA. There were too few clear type I fibres that expressed significant amounts of type II myosin mRNA (2 in total) for further analysis.

Discussion

The present study is special insofar as it uses athletes experienced in strength training to compare the effects of two modes of resistance training. Both, CON/ECC and CON/ECC+, were performed with fast movements, so fast that they could only be efficiently executed by athletes with significant training practice. Based on the information obtained from coaches and athletes, we hypothesised that both modes would lead to distinctive adaptations, with CON/ECC+ inducing muscular adaptations consistent with a shift towards a faster muscle phenotype. The athletes were well trained: on an average, they were able to lift 75 kg (CON/ECC+) and 81 kg (CON/ECC) with the right leg in the unilateral 1RM (knee extension) test performed before the start of the 6-week training period. Both training modes were effective, leading to significant increases in the 1RM (~18%) and in the quadriceps CSA (~7%) after 6 weeks of training. To our knowledge, responses of such magnitude have hitherto only been observed in studies with untrained subjects (Narici et al. 1996; Ahtiainen et al. 2003; Norrbrand et al. 2008). At a molecular level, the distinct hypertrophic response to both forms of heavy resistance training became evident by significant increases in the steady-state levels of nine marker mRNAs, all but one falling into the ontology categories of muscle hypertrophy and regeneration, in addition to an increase in ribosomal RNA content in the post-training biopsies to an extent hitherto not reported.

When comparing the adaptations to the two training regimens, a considerable number of the parameters determined showed subtle differences in their training response which all point in direction of a muscle better suited for fast, explosive movements after CON/ECC+ compared with CON/ECC: Functionally, there was a tendency towards larger training induced gains by the CON/ECC+ group versus CON/ECC in the squat test ($P = 0.089$). After CON/ECC+ but not after CON/ECC, FCSA of IIX fibres was significantly increased; IIA and mean fibre FCSA showed a tendency in the same direction. In the CON/ECC+ but not in the CON/ECC group, the increase in 1RM was significantly correlated with the increase in FCSA IIX and with the (non-significant) increase in FCSA IIA, indicating that in this group, training-induced hypertrophy of the fast fibre types contributed to the improvement in maximal strength. In addition, the proportion of type IIA fibres expressing MHC IIx mRNA was larger and significantly different in response to CON/ECC+ versus CON/ECC. Amongst the 48 marker transcripts, the gains in MHC IIb and AR mRNAs were significantly different between CON/ECC+ and CON/ECC. They were increased after CON/ECC+, but not after CON/ECC, as was the case for eight further transcripts. Six of these are thought to be involved in muscle growth and

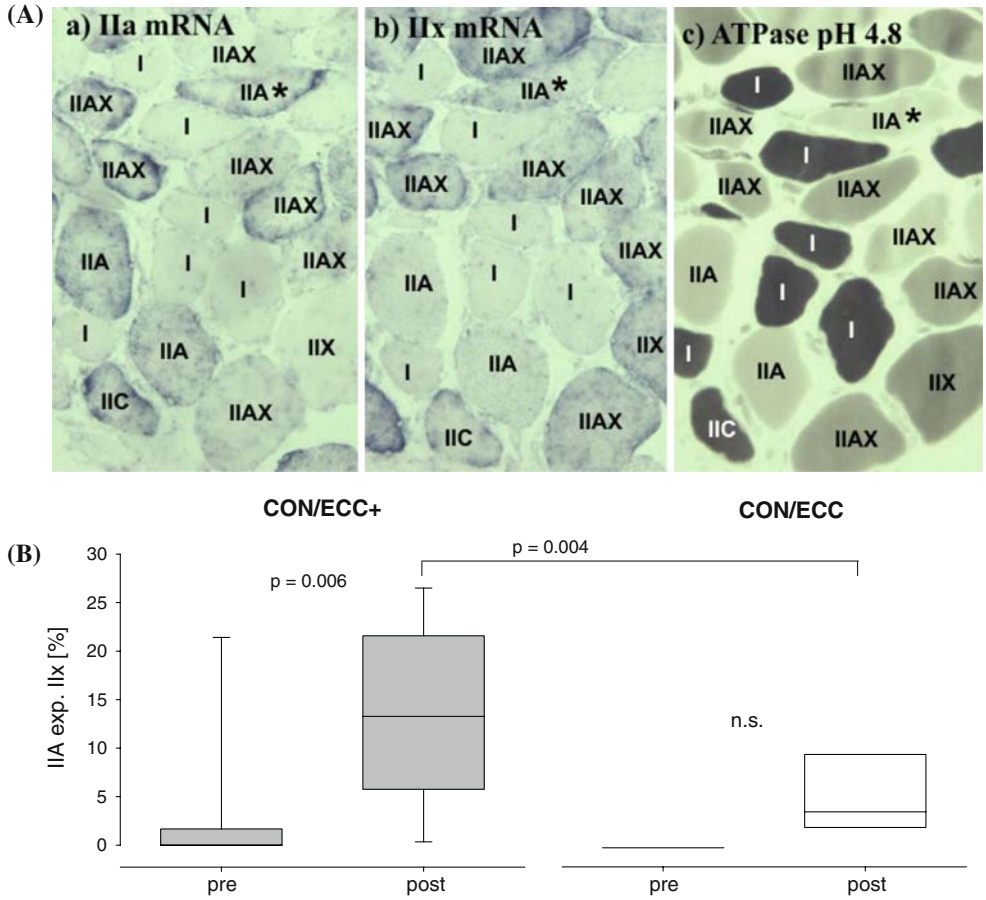

Fig. 5 **a** *a* In situ hybridisation for myosin heavy chain IIa mRNA, *b* in situ hybridisation for myosin heavy chain IIx mRNA, *c* ATPase staining after preincubation at pH 4.8. *A fibre "in transition". It is a histochemically unambiguous IIA fibre with significant amounts of IIx mRNA. This fibre is as light as the other IIA fibres that have no IIx mRNA. Because in situ hybridisations are at best semiquantitative only clear-cut type IIA or IIX fibres were investigated in the search for fibres "in transition". All fibres that were a shade darker than unambiguous IIA or a shade lighter than unambiguous IIX were not included in the analysis. **b** Percentage of type IIA fibres expressing primarily MHC IIX mRNA before and after 6 weeks concentric/eccentric-overload (CON/ECC+) or conventional concentric/eccentric (CON/ECC) quadriceps strength training. Median values, the 5th, 25th, 75th and 95th percentiles are shown

regeneration. The other two are preferentially expressed in fast, glycolytic fibres. This finding in turn corresponds to the enhanced training-induced increase in capillary lactate concentration observed after CON/ECC+. The fact that the changes of these parameters all point in the same direction suggests that they did not arise by chance. Rather, they probably represent small adaptations which together result in a subtly faster, stronger muscle after CON/ECC+, sufficient to give an athlete a competitive edge in a sport that depends on explosive force.

Hypertrophy

In light of the significant hypertrophy observed, and considering that muscle mass is primarily regulated at the level of protein turnover, it is not surprising to find an increased yield of total RNA, given that 90% of total RNA consists of ribosomal RNA and ribosomes are the key organelles in protein synthesis. From experiments in animals, it is known that mechanical overload induces increased in total RNA (Goldspink et al. 1995; Heinemeier et al. 2007), however, not much is known about the training-induced changes in the amount of ribosomal RNA per mg muscle tissue in humans. The changes that we found are in a range that could well have been missed without carefully controlling the amount of muscle tissue put into the RNA extraction, which is what we have tried to achieve in this study.

The results of several studies suggest that the IGF axis plays an important, however not an exclusive, role in the mediation of anabolic effects in response to several weeks of strength training (Haddad et al. 2005; Petrella et al. 2006; Kvorning et al. 2007; Spangenburg et al. 2008). Major players in the skeletal muscle IGF axis are IGF-1 and IGF-2, the IGF-1 receptor and several IGF-binding proteins, the modulations of which seem to be crucial for the development of hypertrophy. The assays used in the present

study tested for the cDNAs of IGF-1 (a region common to the known major splice variants), IGF-2 and IGFBPs, which were all significantly increased after 6 weeks of CON/ECC+ as well as of CON/ECC. These results correspond to the general increase in muscle CSA and the increased ribosomal RNA. Previous studies mainly focused on IGF-1, however, the regulation of IGF-1 mRNA and of its splice variants in response to strength training remains unclear. Studies on acute changes in human skeletal muscle provided equivocal results showing either significant increases in mRNAs of IGF-1 and its splice variant MGF (mechano-growth factor) (Bamman et al. 2001; Hameed et al. 2003; Kim et al. 2005a, b; Petrella et al. 2006), unchanged IGF-1 or downregulated IGF-1 gene expression (Psilander et al. 2003). To our knowledge, IGF steady-state mRNA levels were only shown to be increased after several weeks of classic strength training (Haddad et al. 2005; Petrella et al. 2006; Kvorning et al. 2007). In the present study, the mRNA of IGF-1 receptor remained unchanged, which is in accordance with the results reported by Haddad et al. (2005) who also observed increases in IGF-1 and IGFBP mRNA, but not in IGF-R mRNA, after 5 weeks of knee extension training.

IGFs are involved in the increase in protein synthesis by enhancing translation initiation via the PI3K (phosphatidylinositol 3,4,5-triphosphate)/Akt (protein kinase B)/mTOR pathway (Bodine 2006). In this study, we determined the mRNA for mTOR (FRAP1) and one of its substrates (EIF4EBP1), neither of which showed any changes, nor did the atrophy/hypertrophy markers MAFbx (FBXO32) and FOXO1A. It is possible that these transcripts only show a significant response during more extreme changes in muscle mass, as was the case in the immobilisation/recovery studies, where they have been found to change (Jones et al. 2004). The lack of measurable differences in their steady-state mRNA levels does not rule out the protein products of these transcripts from being important factors in generating the observed adaptations, through phosphorylation/dephosphorylation and subsequent association into regulatory complexes. The limited amount of tissue available from the biopsies did not allow such investigations in the present study.

Androgen receptor mRNA was one of the mRNAs with a significant increase after CON/ECC+ but not after CON/ECC, resulting in a significant difference between the two groups. AR is thought to be involved in anabolic regulation. In two previous studies, AR mRNA expression in vastus lateralis muscle was significantly increased 48 h after the first and the third session of heavy knee extension exercise, respectively (Bamman et al. 2001; Willoughby and Taylor 2004). Another investigation, however, showed unchanged steady-state AR mRNA levels in biopsies obtained from vastus lateralis muscle after 8 weeks of quadriceps strength training (Kvorning et al. 2007). In addition, no significant increase was observed 4 and 24 h after the last training bout of the 8-week training period (Kvorning et al. 2007). Training-induced changes in the AR expression might occur in a fibre-specific manner: In rats, only fast muscle fibres responded with a significant increase in AR protein expression after 11 weeks of resistance training (Deschenes et al. 1994). In the present study, the significant increase in the AR mRNA expression in the CON/ECC+ group was significantly correlated with the changes in the area of the fast myofibre types (FCSA IIX and FCSA IIA): Subjects with the greatest augmentation of FCSA IIX and FCSA IIA showed the smallest increase in the AR mRNA expression. This negative correlation might be due to a negative feedback mechanism that would also be in accordance with the above-mentioned results of previous studies. This explanation remains speculative as we did not measure AR protein expression.

Satellite cell activation and regeneration

The concomitant increases observed for the mRNAs of the myogenic regulatory factors MYF5, MRF4 and myogenin as well as the proliferation/differentiation markers cyclin D1 and p21 are indicative of satellite cell activation and proliferation, as has been described previously for muscles subjected to strength training (Psilander et al. 2003; Willoughby and Rosene 2003; Bickel et al. 2005; Kosek et al. 2006; Kvorning et al. 2007). Corresponding to the results of Kvorning et al. (2007), the steady-state levels of MyoD mRNA in this study remained unchanged. To our knowledge, the present study is the first in human muscle to additionally provide results for the mRNA expression of HGF, a growth factor that has been shown to activate satellite cells in vivo (Anderson and Wozniak 2004), and of MHCneo, which is expressed in myoblasts and myotubes. Both HGF and MHCneo are strong indicators of muscle fibre regeneration, and their mRNA expression was significantly increased after CON/ECC+ only. Enhanced satellite cell activation and differentiation in the CON/ECC+ group could be due to additional myotrauma induced by the increased eccentric load.

Myostatin steady-state mRNA levels in the present study were significantly increased after CON/ECC+ training, but showed a tendency towards a decrease in the CON/ECC group. Together with the gains in MHC IIb and AR mRNA, the gain in myostatin mRNA formed a distinct cluster in a cluster analysis of expression patterns of the 48 transcripts amongst the individuals of the two groups. This illustrates a tendency for these three transcripts to be co-regulated and therefore also myostatin to be differentially regulated in response to the two training modalities. The meaning of this result remains unclear, however. Myostatin is known to

reduce muscle growth by inhibiting myoblast proliferation and differentiation (Toigo and Boutellier 2006). Its mRNA has been shown to be acutely downregulated after one bout of knee extension exercise (Kim et al. 2005a, b). The results of long-term strength training studies have been conflicting. A significant reduction was found after 9 weeks of quadriceps strength training in one study (Roth et al. 2003), whereas two other investigations reported significantly elevated myostatin mRNA concentrations after 6, 12 and 21 weeks of heavy knee extension exercise (Willoughby 2004; Hulmi et al. 2007). In the study of Hulmi et al. (2007), a single exercise bout induced a significant acute decrease in myostatin mRNA, but only at the end of the 21-week training period, when the steady-state levels were elevated, indicating a complex regulation.

Fibre types

The analysis of muscle fibre diameters in our biopsies of the vastus lateralis muscle revealed a significant increase in the type IIX FCSA after CON/ECC+ only. Types I and IIA as well as mean FCSA also tended to increase in the CON/ECC+ group, but due to wide interindividual variation, the gains did not reach statistical significance. No such tendencies were observed in the CON/ECC group. These findings seem to be in apparent discrepancy to the significant increases we found in CSA for the quadriceps of both groups, but are in line with another study using eccentric-overload training. In this study (Norrbrand et al. 2008), the volumes of the four components of the quadriceps femoris muscle could be individually determined by MRI. After 5 weeks of conventional quadriceps strength training, the significant increase in total quadriceps femoris muscle volume was mainly due to a significant increase in the rectus femoris volume, whilst the vastus lateralis volume had not changed significantly. After 5 weeks of eccentric-overload training, however, significant increases were also found in the volumes of vastus intermedius, vastus medialis and vastus lateralis, with a strong tendency, for vastus lateralis only, towards a differential response to eccentric-overload training when compared with conventional strength training. Discrepancies between whole muscle and fibre CSAs can also arise from changes in muscle architecture (pennation angle), resulting in increased "physiological" but not necessarily "anatomical" muscle cross sections (Toigo and Boutellier 2006).

The significant increase in the FCSA of type IIX after CON/ECC+ only could be due to enhanced recruitment of IIX fibres in the combination of concentric with increased eccentric load applied in the CON/ECC+ group. Enhanced EMG activities during knee extension exercise with increased eccentric loading do suggest increased type-II fibre recruitment (Hortobágyi et al. 2001). The increased type IIX FCSA after CON/ECC+ only corresponds to the significantly increased percentage of type IIA fibres expressing significant amounts of IIx mRNA after CON/ECC+. These findings are in contrast to the ones usually found after conventional strength training in untrained subjects (Andersen and Schiaffino 1997), but fit our observation of a muscle better suited to explosive strength tasks after CON/ECC+. This observation is further corroborated by changes in muscle function, as indicated by the tendency towards better improvement in the squat jump test after CON/ECC+ and also by the significant correlation between Δ 1RM and Δ FCSA IIX in this training group. Overall, we found only a small number of fibres, which were obviously in the process of fibre type transition in the in situ hybridizations. These results are in accordance with the lack of change in the relative contents of the major MHC mRNAs (I, IIa, IIx) and also with the unchanged fibre type composition in response to the training, analysed by ATPase staining.

The mRNA of MHC IIb, however, was significantly increased in response to CON/ECC+ but not to CON/ECC. The difference in training-induced gain was statistically significant. MHC IIb mRNA was expressed at low level. Nevertheless, a clear PCR signal could be detected in each of the biopsies. Owing to the low abundance of its mRNA (and protein), MHC IIb seems not to be a major contributor to the fibre's contractile properties, rather, its transcription could be from a "leaky" promoter or it perhaps has an as yet undefined regulatory role. In animals, it is the fastest limb muscle MHC, indicative of the least oxidative, most glycolytic fibres. It is therefore conceivable that also in human muscle, its upregulation is indicative of a shift towards a faster muscle gene expression pattern.

It is widely accepted that genes involved in energy metabolism are expressed in a muscle fibre type specific fashion (Plomgaard et al. 2006). Changes in the metabolic profile of a muscle are thought to occur more rapidly than changes in its MHC content (Spangenburg and Booth 2003; Plomgaard et al. 2006). In the present study, the measured markers of energy metabolism were also compatible with a shift towards a slightly more glycolytic (faster) muscle after CON/ECC+. We found significant increases in the steady-state mRNAs of both LDH isoforms and the monocarboxylic acid transporter (lactate transporter, MCT 4) after CON/ECC+, whilst after CON/ECC, only LDH B mRNA was significantly upregulated. LDH A and MCT4 are predominantly found in fast muscle fibres (Pilegaard et al. 1999, Plomgaard et al. 2006): LDH A catalyses the conversion of pyruvate to lactate and MCT4 is thought to be regulated according to the need for lactate efflux from muscle (Bonen et al. 2000). LDH B catalyses the conversion of lactate to pyruvate. In line with these adaptations at the molecular level, the training-induced increase in capillary lactate

concentration was significantly enhanced only after CON/ECC+. As muscle lactate and protein levels of MCT4 were not determined we can only speculate that these findings indicate increased muscular lactate generation and diffusion during quadriceps strength training in the CON/ECC+ subjects at the end of the 6-week training period.

In summary, 6 weeks of supervised CON/ECC and CON/ECC+ induced a significant anabolic response in already strength-trained athletes. The significantly improved performance in the squat jump test after CON/ECC+ suggests adaptations towards better development of explosive strength. This is supported by changes in a further 15 of the 56 parameters determined, which point towards CON/ECC+ inducing adaptations in direction of a faster, stronger muscle. The changes were subtle and difficult to discern, but sufficient to provide an advantage in a competitive sport where explosive strength is important.

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Conflict of interest statement The authors have no conflict of interest.

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