# Muscular adaptations to computer-guided strength training with eccentric overload

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**Abstract**

**Aims:** In order to investigate the muscular adaptations to a novel form of strength training, 18 male untrained subjects performed 4 weeks of low resistance–high repetition knee extension exercise.

**Methods:** Nine of them trained on a conventional weight resistance device (Leg curler, CON/ECC group), with loads equivalent to 30% of the concentric one-repetition maximum (1RM) for both the concentric and eccentric phase of movement. The other nine trained on a newly developed computer-driven device (CON/ECC-OVERLOAD group) with the concentric load equivalent to 30% of the concentric 1RM and the eccentric load equivalent to 30% of the eccentric 1RM.

**Results:** Training resulted in significantly ($P \leq 0.05$) increased peak torque and a tendency ($P = 0.092$) to increased muscle cross-sectional area for the CON/ECC-OVERLOAD but not the CON/ECC group, while strength endurance capacity was significantly ($P \leq 0.05$) increased in the CON/ECC group only. RT-PCR revealed significantly increased myosin heavy chain (MHC) IIa and lactate dehydrogenase (LDH) A mRNAs, a tendency for increased MHC IIx mRNA ($P = 0.056$) and high correlations between the changes in MHC IIx and LDH A mRNAs ($r = 0.97$, $P = 0.001$) in the CON/ECC-OVERLOAD group.

**Conclusions:** These results indicate a shift towards a more type II dominated gene expression pattern in the vasti laterales muscles of the CON/ECC-OVERLOAD group in response to training. We suggest that the increased eccentric load in the CON/ECC-OVERLOAD training leads to distinct adaptations towards a stronger, faster muscle.

*Keywords* fibre types, gene expression, maximal strength, muscle cross-sectional area, resistance training, strength endurance capacity.

Resistance training increases muscular strength, primarily due to neuronal adaptations and adaptive changes in the trained skeletal muscles, such as hypertrophy and changes in the myosin heavy chain (MHC) composition of the muscle fibres. The extent and nature of the adaptations to resistance training depend on the specific mode of training. Combinations of concentric and eccentric exercise were found to be most successful for strength gain (Jones & Rutherford 1987, Colliander & Tesch 1990, Dudley *et al.* 1991a, Hather *et al.* 1991, O'Hagan *et al.* 1995, Hortobágyi *et al.* 2000). In some studies, detectable hypertrophy of muscle fibres, mainly

 

of type IIA, could only be realized if eccentric work was included in the strength training regimens (Hather *et al.* 1991, Hortobágyi *et al.* 2000). Metabolic stress was found to be lower in eccentric than in concentric exercise, thus the greater increase in strength after combined concentric/eccentric training is accomplished with reduced additional energy cost of the eccentric actions (Dudley *et al.* 1991b, Horstmann *et al.* 2001).

In most studies involving strength training in a concentric/eccentric mode, the same absolute load was used for concentric and eccentric actions, e.g. for lifting the weight during leg extension (concentric action) and for lowering it (eccentric action). In this procedure, the relative workload is smaller for the eccentric exercise, because the maximal voluntary force is greater in eccentric than concentric muscle actions (Komi & Vitasalo 1977). Recently, Hortobágyi *et al.* (2001) have shown that significant strength gain could be achieved after just seven consecutive days of low intensity strength training with a higher absolute load in the eccentric than in the concentric movements. The gain in maximal voluntary isometric and isokinetic eccentric strength was twofold higher than after standard low intensity strength training and could be attributed to neural adaptations.

In the present study, we focused on the muscular adaptations after a longer period of low resistance–high repetition strength training. Concentric/eccentric knee extension exercise was performed during 4 weeks, with one group (CON/ECC) exercising with the same absolute loads for concentric and eccentric quadriceps contractions and the other group (CON/ECC-OVERLOAD) with similar relative loads (i.e. higher absolute load during the eccentric contractions). The CON/ECC-OVERLOAD training was performed on a newly developed computer-driven resistance device (Motronic; Schnell, Peutenhausen, Germany), which allows a selective choice of concentric and eccentric loads for a muscle group in training. The kinetics of the movement on these machines are similar to the kinetics on a conventional leg curler, i.e. constantly changing. This is different to training on isokinetic dynamometers, which were designed to keep the velocity of movement constant.

Low intensity–high repetition strength training is frequently used in sports practice, because strength endurance capacity is an important determinant for performance in a variety of sports, for example, rowing, canoeing, and wrestling or for physical rehabilitation (Güllich & Schmidtbleicher 1999). However, only few studies have investigated the effects of low intensity–high repetition strength training on structural, cellular and molecular changes in skeletal muscle. Two systems are likely to adapt: glycolysis and fiber types (Cadefau *et al.* 1990, Sale *et al.* 1990). Some authors have described increased enzyme activities of the glycolytic pathway (Costill *et al.* 1979) but others, after combined concentric/eccentric strength training, found no differences in enzyme activities (Tesch *et al.* 1990).

Exercise-induced fibre transformations are well known. In human strength training, transformations of type IIX into type IIA fibres have been found in the majority of studies (Hather *et al.* 1991, Adams *et al.* 1993, Staron *et al.* 1994, Carroll *et al.* 1998, Andersen & Aagaard 2000, Hortobágyi *et al.* 2000, Williamson *et al.* 2001, Willoughby & Rosene 2001) and also in response to endurance training (Andersen & Henriksson 1977), where transformations to type I were also found (Howald *et al.* 1985). Transformations towards a fast muscle phenotype are difficult to achieve via training, all-out sprint exercises seem best suited to induce such transitions (Jacobs *et al.* 1987). Fibre-type transitions are linked to changes in the expression of MHC isoforms, which are the principle marker molecules of muscle fibre types. Changes of fibre types and MHC mRNAs do not happen in parallel: on the one hand, it is thought that a distinctly slower turnover of the MHC proteins in comparison with their mRNAs can lead to 'mismatches' between the MHC mRNAs and the accumulated protein isoforms in the myofibrils of a muscle fibre in response to altered muscle load (Andersen & Schiaffino 1997). On the other hand, there are indications of altered translation efficiencies in response to strength training (Welle *et al.* 1999). However, the results of other studies suggest that the long-term steady-state levels of the MHC mRNAs are reasonably correlated to fibre composition of a muscle biopsy (Hortobágyi *et al.* 2000, Friedmann *et al.* 2003).

We determined the relative levels of a number of mRNAs that code for the MHC isoforms and for enzymes of energy metabolism in muscle biopsies taken before and after a 4-week training period, using them as indicators of adaptive processes to low resistance–high repetition strength training with increased eccentric load. In particular, we hypothesized that training-induced shifts in fibre-type distribution and increased activities of glycolytic enzymes would lead to changes in the steady-state levels of the respective MHC isoform mRNAs and of the mRNAs of phosphofruktokinase (PFK), lactate dehydrogenase (LDH) A and B. In addition, strength endurance capacity, maximal strength, muscle cross-sectional area (MCSA), fibre-type distribution, and fibre cross-sectional areas (FCSA) were investigated. Based on results of recently published studies, which investigated the effects of eccentric overload training without providing biopsy data (Hortobágyi *et al.* 2001, Brandenburg & Docherty 2002), and on observations from coaches and athletes who have used concentric/eccentric training with the same relative loads, we expected this recently developed

 

mode of strength training to induce greater increases in strength and enhanced muscular adaptations, which could lead to better performance in sports that require explosive strength or very high power output.

## Methods

### *Subjects*

Eighteen male subjects volunteered for the study. They were untrained or recreationally active and had not participated in any systematic strength training for at least 1 year prior to the study. Written-informed consent was obtained in each case. The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg, Germany.

### *Training protocol*

The low resistance–high repetition training performed in this study was done in series of 25 leg extensions within 45 s, which is fast for subjects not used to such kinds of exercise. All the subjects were therefore familiarized with the movements during a 3-week lead-in-phase. During this period, they trained to flawlessly perform 25 repetitions of leg extension within 45 s on a conventional leg curler, using very low resistance [about 10% of concentric one-repetition maximum (1RM)] for three times per week. They were also accustomed to isokinetic testing during one of these lead-in days. The lead-in training likely induced some of the neural adaptations found in the early adaptation phase to strength training (Komi 1986) and ensured that the movements could be carried out with the correct technique despite higher fatigue in the ensuing 4-week resistance training phase (see below). It is possible that the endurance type activity of the lead-in period could have induced changes in the levels of the mRNAs determined, given these were untrained subjects. Considering the results of low intensity endurance training with untrained subjects presented by Vogt *et al.* (2001), we would expect such changes to be small, however. As the purpose of this study was to compare the adaptations with two levels of eccentric loads during concentric/eccentric training, it would have been ethically questionable to monitor also the lead-in phase with muscle biopsies.

After the lead-in phase, the subjects were randomly assigned to 4 weeks of low resistance–high repetition strength training either on a conventional leg curler (CON/ECC, $n = 9$, 24.3 ± 2.5 years, 179.3 ± 8.4 cm, 72.9 ± 9.0 kg) or on an corresponding computer-driven device (CON/ECC-OVERLOAD). Only seven subjects of the CON/ECC-OVERLOAD group (24.8 ± 4.2 years, 182.8 ± 6.7 cm, 78.3 ± 6.4 kg) finished the study. Two subjects dropped out because of muscle soreness or injury during the 4-week training period. The subjects maintained their habitual activity level during the experimental period.

Both groups performed one-leg knee extension exercise in sitting position three times per week (Monday, Wednesday, Friday) under continuous supervision of an experienced strength training coach. Before each training session, the subjects went through a standardized warm-up programme. For the training itself, the subjects of the CON/ECC group used a conventional leg curler (M3; Schnell, Peutenhausen, Germany). They completed six sets of 25 repetitions per leg and training session, taking 45 s per set. Their resistance was set at 30% of their individual 1RM as determined from concentric contraction. This load was raised (concentric action) and lowered (eccentric action) for each repetition. The subjects in the CON/ECC-OVERLOAD group trained on a computer-driven device (Motronic). They also raised a load equivalent to 30% of their concentric 1RM in each repetition, but the eccentric action was performed with a higher load. The lever arm of the machine pressed the leg downwards against the resisting extensors, subjecting them to an eccentric load equivalent to 30% of the individual eccentric 1RM. In average, the eccentric training load was 2.32-fold higher than the concentric training load (equivalent to 70% of the concentric 1RM). The movements on this machine are similar to the conventional leg curler, i.e. the kinetics of movement also constantly change. The subject's effort is adjusted through bio-feedback form an instant display of the force curve. In an attempt to achieve about the same amount of exertion for both groups, the CON/ECC-OVERLOAD group performed only three sets of 25 repetitions per training session. This is similar to the workloads chosen by Hather *et al.* (1991) in their comparison of concentric and combined concentric/eccentric training and also follows the recommendations of athletes and coaches who regularly use CON/ECC-OVERLOAD training. After 2 weeks of training, 1RM was again determined, in order to adjust the loads. In order to prevent muscular dysbalance, both legs were trained in each session, starting with the right side. The 25-repetition series were separated by 1-min rest periods, during which the other leg was exercised. All but one subjects of the CON/ECC group were able to perform the required six sets of 25 repetitions within 45 s in all training sessions (three sets in the CON/ECC-OVERLOAD group); this one subject was able to perform only 22 repetitions in sets 4–6 in three of the 12 training sessions. For the same reasons of preventing muscular dysbalance, the knee flexors were also trained after finishing the knee extension exercise, using a conventional device with 30% of the concentric 1RM for concentric and eccentric contractions.

 

### *Testing procedures*

The 3-week lead-in phase and the 4-week training period were separated by 1 week, during which strength tests, magnetic resonance imaging (MRI) and a muscle biopsy were performed. The biopsy was taken either 4 or 5 days after the last lead-in session. The same routine was followed in the week after the 4-week training period, with all the biopsies being taken 5 days after the last training session.

### *Strength tests*

The strength tests of the quadriceps muscles were conducted on an isokinetic device (System3Pro; Biodex Medical Systems, New York, NY, USA), in sitting position for both legs separately. The subjects were seated 5° reclined and firmly strapped in at shoulders, hips and thighs. Maximal concentric strength was determined as the peak torque in three maximal attempts at a movement velocity of $60° \, s^{-1}$. After a short rest, the subjects performed 50 repetitions of concentric quadriceps contractions, followed by concentric hamstring contractions in each cycle at a movement velocity of $180° \, s^{-1}$. Strength endurance capacity was measured as the sum of work performed during the concentric quadriceps contractions. During these tests, the subjects were verbally encouraged to exercise with maximal effort. Both tests were performed within a 90–180° range of limb excursion. The recorded torque–angle curves were not corrected for the effect of gravity of the lower leg.

Because all the muscle biopsies were taken from the right mm. vasti laterales, only the strength data obtained from the right legs were subjected to statistical analysis.

### *Magnetic resonance imaging*

MRI scans of both thighs were performed in the supine position using a 1.5 T system (Symphony; Siemens, Erlangen, Germany) with a T2-weighted sequence [TSE, repetition time (TR) = 3100 ms, echo time (TE) = 119 ms, turbo factor = 17]. The field of view was 45 cm. MCSA of the quadriceps femoris was determined in the proximal, middle and distal third of both thighs at 10, 15 and 20 cm from the very distal part of the os pubis. A computerized digitizer with a trackball was used to trace each area as displayed on the computer's monitor using software provided by the manufacturer. The measurements were done in randomized order by two investigators; the mean values of both were used for statistical analysis. Our original attempt to determine the MCSAs of the vasti laterales muscles had to be abandoned, because especially in the proximal axial MRI scans, the fascia boundaries between the lateral and deep vasti could often not be identified. Similar findings have recently been reported elsewhere (Aagaard *et al.* 2001). Therefore, in the present study, the MCSAs of the whole quadriceps muscles were determined.

### *Muscle biopsy sampling*

Muscle biopsy samples were taken from the same region of the vastus lateralis muscle at mid-thigh level under local anaesthesia, using the Bergström technique (Bergström 1975). The muscle pieces were immediately frozen in isopentane, cooled by liquid nitrogen, and then stored at −80 °C. The first biopsy was taken after the 3-week lead-in phase with training in the same rhythm but very low resistance loads compared with the actual training period. The second biopsy was obtained after 4 weeks of low resistance–high repetition strength training as described above. The first biopsy was taken 4–5 days after the last training session of the lead-in phase, all the second biopsies were obtained on day 5 after the last training session. This lag period was chosen to ensure that we would determine long-term changes in the steady state of the mRNAs [which correlate best with the structural and biochemical changes in response to altered muscle load (Booth & Baldwin 1996)] and transient regulatory phenomena would not interfere. Transient increases in transcription in the hours after a bout of exercise have been described for some genes (Pilegaard *et al.* 2000), which partially resulted in transient increases in mRNA levels. It is difficult to predict the extent and duration of such transient changes for the mRNAs under study, since the molecular events during recovery from exercise are not well known. We chose a period of 5 days between the last exercise session and the biopsy, in accordance with Vestergaard *et al.* (1994), who recommended a period of 4–5 days without exercise, based on their experience on post-exercise modulations of the glucose uptake system. This is the best-characterized muscular post-exercise response to date, according to our knowledge.

### *Histochemistry and morphometry*

Serial transverse sections (6 $\mu$m) were cut in a cryotome at −20 °C and stained for myofibrillar ATPase after preincubations at pH 4.35 (5 min, room temperature), 4.6 (5 min, room temperature) and 10.5 (15 min, 37 °C) (Brooke & Kaiser 1970). Based on their staining intensities, four fibre types (I, IIA, IIAX, IIX) could be distinguished after preincubation at pH 4.6 and three fibre types (I, IIC, II) after preincubation at pH 10.5. Biopsies with less than 100 fibres were not analysed, which led to subjects with too small biopsies being

 

excluded from the statistical analysis. On average, 375 ± 219 fibres were classified in each sample. Microscopic images of the ATPase stained cross-sections (pH 4.6) were recorded by a video camera (Olympus HCC-3600 P high gain; Hamburg, Germany) and digitized by a personal computer equipped with an image analysis system developed by our own group (VIBAM 0.0–VFG 1 frame grabber), as described earlier (Kinscherf *et al.* 1995). FCSAs were determined at a 200-fold magnification. As the number of the hybrid fibre types IIC and IIAX was very small, reliable statistical comparison of changes in their FCSA was not possible. The statistical analysis was therefore only performed for the major fibre types. For the analysis of fibre-type distribution, the (few) type IIC fibres were added to type I fibres and the type IIAX to the type IIA. The fibre-type specific cross-sectional areas could not be determined in all muscle samples, because in few of them, insufficient numbers of fibres with perpendicular cross-sections could be found.

### *RNA extraction*

For the extraction of total RNA, a modification of the Quiagen mini-protocol for heart, skeletal muscle and skin was used [Quiagen, Hilden, Germany (Wittwer *et al.* 2002)]. Briefly, about 10 mg of a biopsy (estimation by planimetry) were cut into 25 μm sections with a cryotome at −20 °C and stored at −80 °C. After adjusting to −20 °C, the cut tissue was homogenized in 333 μL of lysis buffer (RLT buffer; Quiagen). After threefold dilution with water, 30 mAU of Proteinase K were added and proteins digested for 1 : 45 h. Thereafter, one volume each of the RLT and ethanol were added and the RNA bound to a Quiagen mini column, washed twice with buffer RW 1 and subjected to DNAse I digestion on the column. This step, as well as the following washing and elution of the RNA, were done according to the manufacturer's instructions. Ten milligrams adult human skeletal muscle yield about 1 μg total RNA.

### *Reverse transcription*

The RNA was ethanol precipitated and the pellet dissolved in 11 μL water. 5 μL RNA were reverse transcribed with Superscript II reverse transcriptase (Invitrogen, Paysley, UK) in a 20 μL reaction according to the manufacturer's specifications, using random hexamer priming. After 50 min at 42 °C, the enzyme was inactivated by incubation at 70 °C for 15 min and the tubes subsequently cooled on ice for 2 min or longer. The resulting cDNA was then diluted to 200 μL in TE (10 mM Tris, 1 mM EDTA) and aliquoted for direct use in PCR. One microlitre of each RNA was also processed in a 5 μL reaction under identical conditions, but without the reverse transcriptase, as negative control.

### *PCR and primers*

PCR quantification was done on a real-time cycler (LightCycler; Roche, Mannheim, Germany), with SyBr green detection, with the exception of the 18S cDNA, where we used the TaqMan probe and the primers contained in the TaqMan® Ribosomal RNA Control Reagents obtained from Applied Biosystems (ABI, Foster City, CA, USA). For every PCR run, a master mix was prepared using the reagents of the LightCycler Fast Start DNA Master SYBR Green I according to the manufacturer's instructions. One microlitre aliquots of the cDNAs (diluted as given above) were combined with 9 μL of master mix in the LightCycler capillaries. For all transcripts, three independent measurements were performed for each cDNA sample and their values averaged and related to the values of 18S cDNA to correct for variations in input total cDNA. Primers and PCR conditions for each assay are given in Table 1. Ten of the experimental cDNA samples were

**Table 1** Primers and PCR conditions for RT-PCR quantification of mRNAs

| cDNA probed for | GenBank accession number | Primer location | | Annealing temperature (°C) | Number of cycles | From |
|---|---|---|---|---|---|---|
| | | Forward | Reverse | | | |
| MHC I | M58018 | 5895–5914 | 5972–5991 | 55 | 35 | Welle *et al.* (1999) |
| MHC IIA | AF111784 | 5829–5845 | 5926–5949 | 46 | 40 | Welle *et al.* (1999) |
| MHC IIX | AF111785 | 5819–5844 | 5882–5907 | 46 | 40 | Welle *et al.* (1999) |
| PFK | NM000289 | 587–613 | 768–794 | 58 | 35 | Own design |
| LDH A | X02152 | 1143–1164 | 1264–1286 | 58 | 37 | Own design |
| LDH B | BC015122 | 32–53 | 142–165 | 58 | 35 | Own design |

For 18S rRNA, we used the primers and the TaqMan probe from the TaqMan® Ribosomal RNA Control Reagents obtained from ABI. The 18S PCR was run for 35 cycles with an annealing temperature of 60 °C in the reagents of the Fast Start DNA Master Hybridization kit from Roche.

 

chosen as reference standards and were measured in each run. Relative quantification was performed with the help of the 'fit point' method using the preinstalled software program of the LightCycler. For each run, ratios relative to each of the reference standards were determined based on the respective delta CTs and an average efficiency (determined graphically, SD 2–3%). These ratios were then averaged over all three runs and related to the average content of 18S cDNA in each sample. The obtained 'mRNA values' are therefore relative values based on total RNA content.

### *Statistics*

Statistical procedures were performed with the software programs Sigmastat 2.0 and Sigmaplot 2001 for Windows from Jandel Scientific (San Rafael, CA, USA). Data are presented as mean values ± SD for the data obtained from tests of the right leg only, since the muscle biopsies were obtained from the right m. vastus lateralis. Statistical analyses were performed by utilizing a 2 × 2 repeated measures ANOVA [group (CON/ECC, CON/ECC-OVERLOAD) × test (pre-training, post-training)]. Significant between-test differences were determined involving the *post hoc* Tukey test. Additionally, differences between values obtained pre- and post-training for each group were analysed by using Student's paired *t*-test. Correlations between selected parameters were computed with the Pearson product–moment. The level of significance was set at $P \leq 0.05$.

## Results

### *Strength endurance capacity, maximal strength and muscle cross-sectional area*

As all muscle biopsies were taken from the right vastus lateralis muscle, only the results of the right leg are presented. Neither significant group × test interactions nor significant group effects were found for strength endurance capacity, maximal strength or MCSA. A significant test effect was observed for strength endurance capacity ($F = 5.137$, $P = 0.040$, power = 0.465). *Post hoc* testing revealed a statistically significant ($P = 0.028$) increase in strength endurance capacity (about 8%) after 4 weeks of CON/ECC training (Fig. 1). The strength endurance capacity after CON/ECC-OVERLOAD training was not significantly different from the value before training ($P = 0.546$). For maximal strength, there was a tendency towards a test effect ($F = 3.180$, $P = 0.096$, power = 0.267), (Fig. 1). The increase (5% in average) after CON/ECC-OVERLOAD training reached statistical significance ($P \leq 0.05$). No significant difference was found after CON/ECC training ($P = 0.482$). A significant test

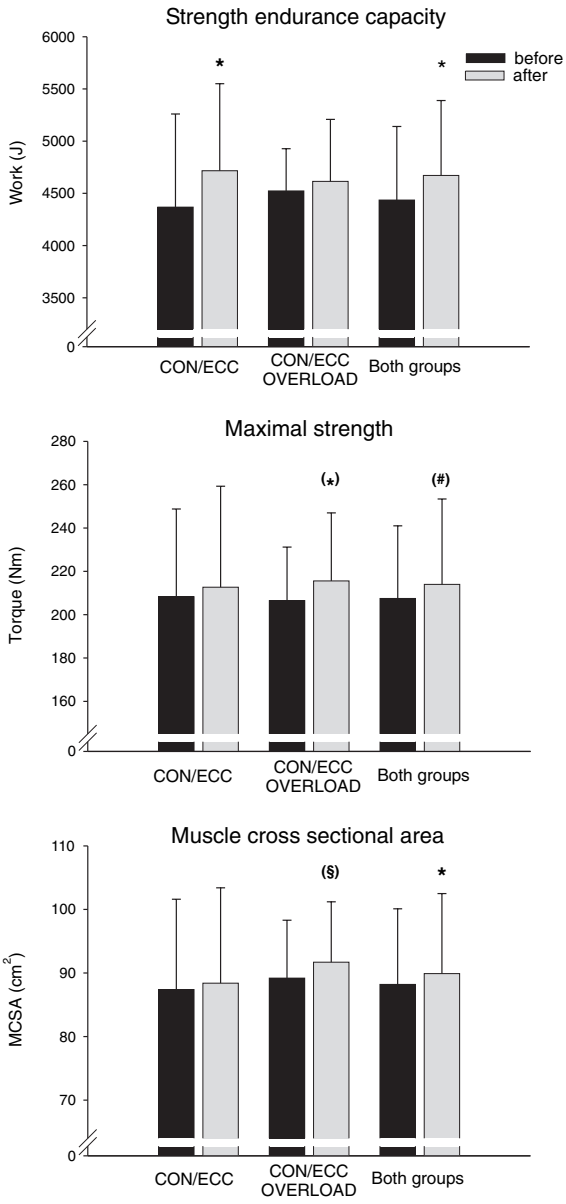


**Figure 1** Strength endurance capacity, maximal strength, and muscle cross-sectional area (MCSA): mean values ± SD for the data obtained from the right leg before and after 4 weeks of concentric/eccentric (CON/ECC) and concentric/eccentric-overload (CON/ECC-OVERLOAD) training are shown, as well as mean values ± SD for the aggregate of both groups. For MCSA, average values for the 10-, 15- and 20-cm axial sites are shown. *$P < 0.05$; (*): $P \leq 0.05$, (#): $P = 0.096$, (§): $P = 0.092$ compared with the values before training.

effect was seen for MCSA (average values for the 10-, 15- and 20-cm axial sites) which was increased by $1.7 \pm 2.6$ cm$^2$ for the collapsed groups (i.e. by an average of 2%, Fig. 1; $F = 7.573$, $P = 0.016$, power = 0.668). In the CON/ECC-OVERLOAD group, there was a tendency ($P = 0.092$) for MCSA to be increased after training (average increase of $2.5 \pm 3.3$ cm$^2$).

**Table 2** Fibre-type distribution. The data are shown as mean values ± SD before and after 4 weeks of concentric/eccentric or concentric/eccentric-overload training

| Fibre types (%) | Test | CON/ECC-OVERLOAD | CON/ECC | Both groups |
|---|---|---|---|---|
| Type I | Before | 56.3 ± 12.0 | 50.1 ± 12.4 | 53.4 ± 11.9 |
| | After | 48.3 ± 15.5 | 50.6 ± 9.4 | 49.4 ± 12.3 |
| Type IIC | Before | 0.2 ± 0.3 | 0.1 ± 0.2 | 0.1 ± 0.2 |
| | After | 0.8 ± 1.2 | 0.4 ± 2.9 | 0.4 ± 0.9 |
| Type IIA | Before | 30.4 ± 8.8 | 36.6 ± 6.5 | 33.5 ± 8.0 |
| | After | 38.2 ± 10.5* | 38.7 ± 6.6 | 38.4 ± 8.4** |
| Type IIAX | Before | 1.1 ± 1.9 | 2.9 ± 3.4 | 2.3 ± 2.9 |
| | After | 0.1 ± 0.4 | 1.3 ± 1.6 | 0.5 ± 1.1 |
| Type IIX | Before | 13.0 ± 8.3 | 13.4 ± 9.9 | 13.2 ± 8.7 |
| | After | 13.5 ± 9.7 | 10.8 ± 7.1 | 12.2 ± 8.2 |

*$P = 0.084$, **$P = 0.039$ compared with values before training.

**Table 3** Fibre cross-sectional areas (FCSA). The data are shown as mean values ± SD before and after 4 weeks of concentric/eccentric or concentric/eccentric-overload training

| FCSA ($\mu m^2$) | Test | CON/ECC-OVERLOAD | CON/ECC | Both groups |
|---|---|---|---|---|
| Type I | Before | 4182 ± 933 | 4298 ± 1710 | 4252 ± 1385 |
| | After | 4820 ± 637 | 5525 ± 1428 | 5243 ± 1184 |
| Type IIA | Before | 5118 ± 1541 | 5458 ± 2556 | 5322 ± 2096 |
| | After | 6475 ± 1718 | 6193 ± 1598 | 6306 ± 1557 |
| Type IIX | Before | 4190 ± 1364 | 4282 ± 2218 | 4241 ± 1778 |
| | After | 4720 ± 1125 | 4804 ± 720 | 4772 ± 812 |

### *Fibre-type distribution and fibre cross-sectional areas*

Neither significant group × test interactions, nor significant group effects could be detected for the different fibre types or for FCSA of the different fibre types or for mean FCSA. A significant test effect was seen for the percentage of type IIA fibres ($F = 5.668$, $P = 0.039$, power = 0.491). In the CON/ECC-OVERLOAD training group, the percentage of type IIA fibres was increased in five subjects and unchanged in one subject. The statistical treatment gave a tendency for an average increase (8%, $P = 0.084$). In the CON/ECC group, the proportion of type IIA fibres was found to be increased in four and decreased in two subjects after training ($P = 0.273$) (Table 2). The FCSA values were not significantly different between pre- and post-training, or between groups (Table 3). In the CON/ECC-OVERLOAD group, FCSA could only be determined in the biopsies of four subjects. Their type IIA fibre FCSAs correlated significantly with maximal strength after the training period ($r = 0.966$, $P = 0.03$).

### *mRNAs coding for myosin heavy chain isoforms I, IIa, IIx*

The relative contents of all the mRNAs determined showed considerable inter-individual variation. Substantial variability was also seen when the values obtained from the biopsies before and after the training period were compared. This is illustrated in Figures 2 and 3. Neither a significant group × test interaction, nor significant group or test effects could be detected for MHC I mRNA. For MHC IIa mRNA, a significant group × test interaction was observed ($F = 8.322$, $P = 0.016$, power = 0.689, Fig. 2). There were no significant group or test effects. MHC IIa mRNA was increased after CON/ECC-OVERLOAD training in each subject, between 4 and 84%. The resulting average increase (30%) was statistically significant ($P = 0.026$). In the CON/ECC group, MHC IIa mRNA decreased in five subjects between 22 and 37%, and increased by 54% in one subject. The average value after the training period was 25% lower, but this was not statistically significant ($P = 0.186$) (Fig. 2). The individual values for MHC IIx mRNA were increased in five subjects after CON/ECC-OVERLOAD training between 36 and 463%. In one subject, the value had decreased by 7%. The resulting average increase (320%) approached statistical significance ($P = 0.056$). In the CON/ECC group, the MHC IIx mRNA values had increased in four subjects, between 32 and 634% and decreased in the other two, between 78 and 98%. The after-training average was 24% lower, but this was statistically not significant (Fig. 2).

### *mRNAs coding for glycolytic enzymes (PFK, LDH A and LDH B)*

For PFK mRNA, neither a significant group × test interaction, nor significant group or test effects could be detected. For LDH A mRNA, a significant group ×

 

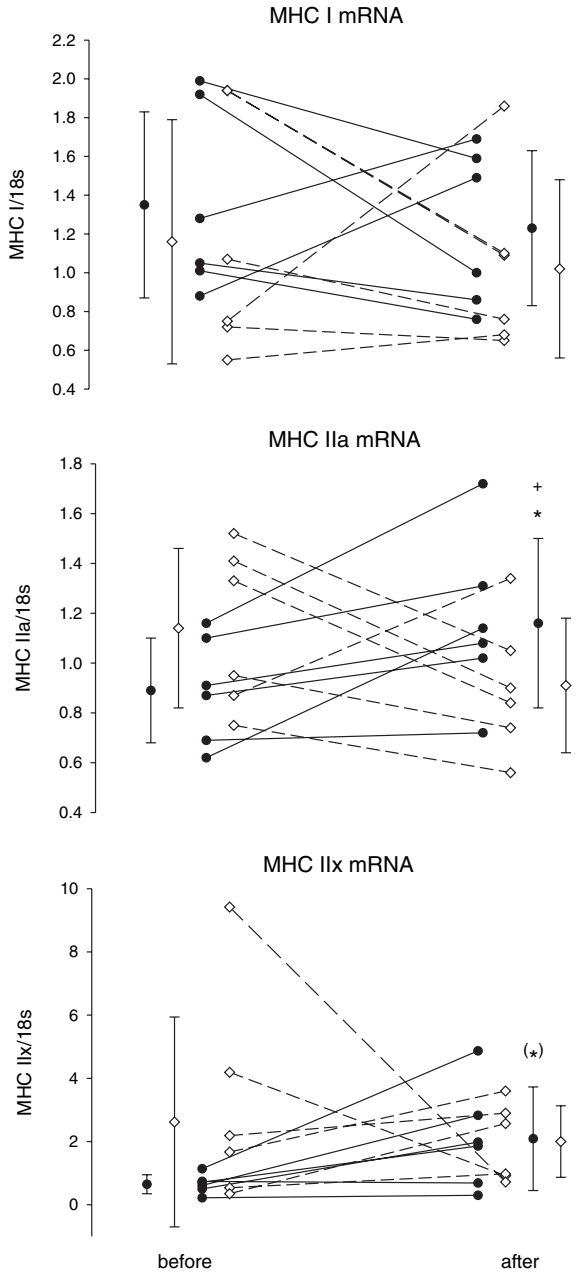


**Figure 2** mRNAs coding for myosin heavy chain isoforms (MHC) I, IIa, IIx: individual values and mean ± SD before and after 4 weeks of concentric/eccentric (◇) and concentric/eccentric-overload (•) training are shown to illustrate the inter-individual variability. *: $P = 0.026$, (*): $P = 0.056$ compared with value before training; +: $P = 0.016$ compared with CON/ECC (change in mRNA level).

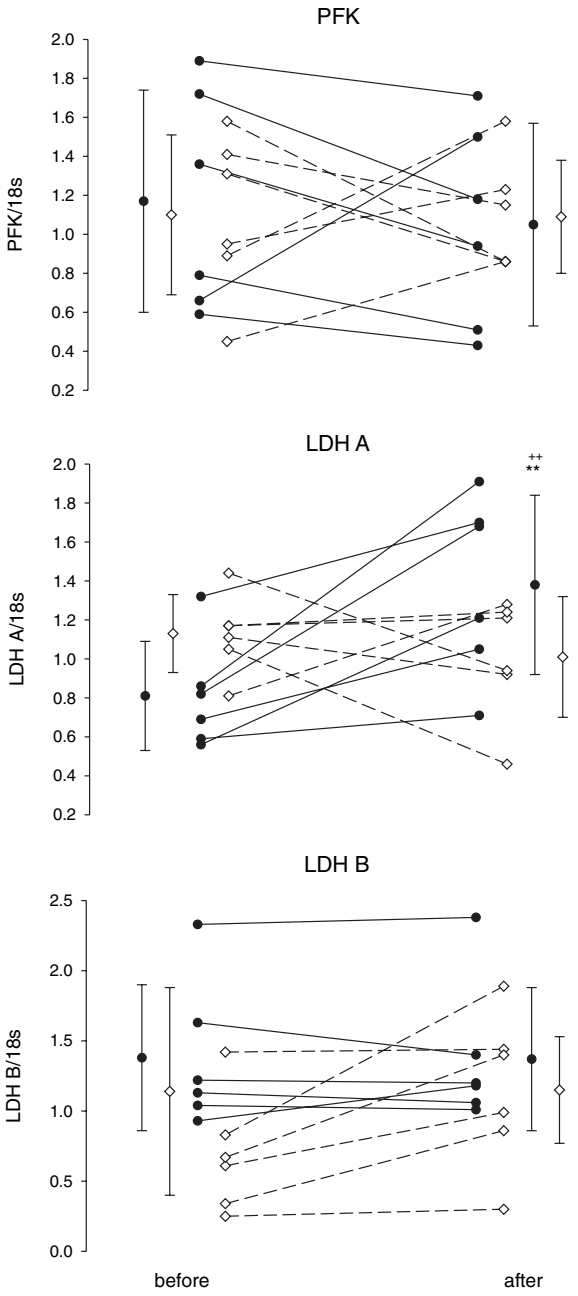


**Figure 3** mRNAs coding for the glycolytic enzymes phosphofruktokinase (PFK), lactate dehydrogenase (LDH) A and B: individual values and means ± SD before and after 4 weeks of concentric/eccentric (◇) and concentric/eccentric-overload (•) training are shown. **: $P = 0.01$ compared with value before training; ++: $P = 0.01$ compared with concentric/eccentric training group (change in mRNA level).

test interaction was obtained ($F = 10.224$, $P = 0.010$, power = 0.791). No group or test effects were found. LDH A mRNA was significantly increased ($P = 0.01$) after 4 weeks of CON/ECC-OVERLOAD training, by about 70%. Each subject of this group had increased LDH A mRNA values, between 20 and 122%. In the CON/ECC group, the individual LDH A mRNA values were decreased in half the subjects, between 17 and 66%, and increased in the other half, between 6 and 58% (Fig. 3). For LDH B mRNA, neither a significant group × test interaction nor significant group or test effects were observed (Fig. 3). A statistically significant

 

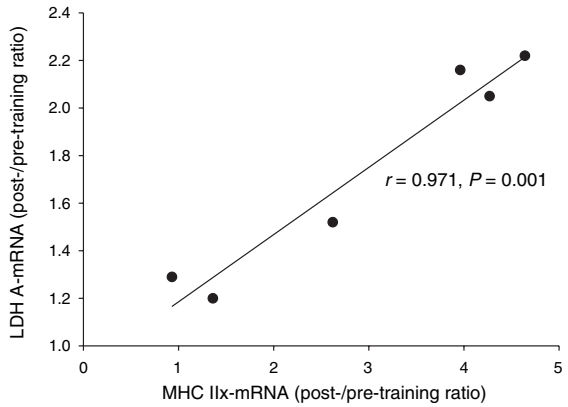


**Figure 4** Correlation between the post- to pre-training ratios of myosin heavy chain (MHC) IIx mRNA and lactate dehydrogenase (LDH) A mRNA in the concentric/eccentric-overload (CON/ECC-OVERLOAD) training group.

positive correlation was observed between the relative individual changes in MHC IIx mRNA and LDH A mRNA in the CON/ECC-OVERLOAD group (Fig. 4). In the CON/ECC group, the changes of these two mRNAs were not significantly correlated ($r = -0.405$, $P = 0.426$).

## Discussion

This study investigated structural and gene expression changes in response to two modes of concentric/eccentric strength training in human m. vastus lateralis: low resistance–high repetition knee extension exercise with equal absolute loads in the concentric and eccentric phases (CON/ECC) and low resistance–high repetition knee extension exercise with equal relative loads in the concentric and eccentric phases (CON/ECC-OVERLOAD).

Functional tests and structural analyses performed before and after a 4-week training period indicate that the two modes give rise to different adaptations: Strength endurance capacity increased significantly only in the CON/ECC group (by about 8%), while a significant increase in maximal strength (about 5%) was found in the CON/ECC OVERLOAD group only (Fig. 1). It cannot be ruled out that slightly different results might have been observed if the strength data had been gravity corrected. However, the functional differences were paralleled by differences in the expression of marker mRNAs: MHC IIa and LDH A mRNA were significantly increased in the biopsies after CON/ECC-OVERLOAD training, but not after CON/ECC training (Figs 2 and 3). For the mRNA of MHC IIx we found a tendency towards an increase ($P = 0.056$) in the CON/ECC-OVERLOAD group, but not in the CON/ECC group. In addition, the post- to pre-training ratios of MHC IIx mRNA were highly correlated with LDH A mRNA, but in the CON/ECC-OVERLOAD group only (Fig. 4).

These data indicate a shift in gene expression towards the RNA pattern of a more type II dominated muscle in response to CON/ECC-OVERLOAD, but not to CON/ECC training. Such a shift did not become manifest as a significant increase in the proportions of IIA and IIX fibre types measured by ATPase histochemistry, rather as tendency towards increased type IIA percentage ($P = 0.084$). It is possible that this is indicative of a transient state, with MHC isoform switches detectable at the level of mRNAs but not yet at the level of protein incorporated in the myofibrils. This would correspond to previous reports that show changes in MHC isoform mRNAs to precede changes in the histochemical fibre type, because the myosin proteins are thought to have much slower turnover rates than their mRNAs (Andersen & Schiaffino 1997). It should also be pointed out that ATPase histochemistry is a relatively crude indicator of the MHC composition of a given fibre. In fibres containing several isoforms, the minor ones are often not detected (Klitgaard *et al.* 1990, Staron 1991, Andersen *et al.* 1994). Thus this discrepancy could be the product of the additional fast MHC proteins being present but not detected by the histochemical stain because they are not the major isoform in all the adapting fibres. We cannot, however, exclude that this discrepancy arose from differential modulation of the translation efficiencies of the MHC mRNAs which has been described by Welle *et al.* (1999). They found an increase in myofibrillar synthesis, e.g. an increase in the synthesis rate of the aggregate of numerous myofibrillar proteins while relative mRNA levels of the MHC isoforms remained unchanged after three sessions of resistance training. At present, the relative importance of transcriptional and translational mechanisms for training-induced changes in the synthesis of muscle proteins still remains to be elucidated. At least, to our knowledge, there has been no study so far in which an adaptive shift in the levels of MHC isoform mRNAs was shown to be accompanied by an opposing translational modulation.

The increase in the mRNAs for the type II MHC isoforms as well as the LDH A isoform differs from the adaptations described in most strength training studies, which – considering mostly the levels of accumulated proteins – almost all point towards transformation of (more glycolytic) type IIX to (less glycolytic) IIA fibres (Colliander & Tesch 1990, Adams *et al.* 1993, Staron *et al.* 1994, Carroll *et al.* 1998, Andersen & Aagaard 2000, Hortobágyi *et al.* 2000, Williamson *et al.* 2001, Willoughby & Rosene 2001). In the present study, the lack of even slight a trend towards a decrease in the proportion of type IIX fibres in the

 

CON/ECC-OVERLOAD group gives additional support to the suggestion that this particular training mode induces unique adaptations.

There are few studies on strength training and MHC mRNA levels in humans. They either showed no significant changes in MHC mRNA levels (Welle *et al.* 1999, Hortobágyi *et al.* 2000), an increase in MHC I and MHC IIa mRNA, but no change in MHC IIx mRNA (Willoughby & Rosene 2001), or even an overall shift towards slow MHC expression (Balagopal *et al.* 2001) after high resistance–low repetition CON/ECC strength training. The only regimen leading to an increase in MHC IIx mRNA was high resistance–low repetition training with creatine loading, which led to relative increases in all MHC mRNA isoforms (Willoughby & Rosene 2001). No study so far has described a pattern matching the one found in our study (significant increase in MHC IIa mRNA with a tendency towards an increased MHC IIx mRNA in the CON/ECC-OVERLOAD group).

To our knowledge, mRNA levels of glycolytic enzymes have not been described in connection with strength training in human muscles. In addition, there are few data on enzyme activities from muscle homogenates. Tesch *et al.* (1990) did not find significant changes in the activities of glycolytic enzymes after concentric or combined concentric/eccentric strength training. Our LDH mRNA data, with the significant increases in relative LDH A mRNA levels after CON/ECC-OVERLOAD but not after CON/ECC training also suggest that the CON/ECC-OVERLOAD regimen leads to unique adaptations in the glycolytic pathway.

The mRNA estimates in the present study showed remarkable inter-individual variability (Figs 2 and 3). The figures overrepresent the variability to a small extent, because the scatter introduced by the RT reaction cannot be accounted for. The standard deviation of RT is 10–20% in our laboratory, as derived from cDNA array studies (Wittwer *et al.* 2002 and other unpublished results). The high variability in our mRNA data is in agreement with many studies on gene expression in human muscle biopsies, which found such variability, e.g. for MHC mRNAs (derived from estimates of the standard deviations in Welle *et al.* 1999) or for PFK mRNA (Vestergaard *et al.* 1994). Recent results from microarray studies confirm these observations: human muscle biopsy samples show remarkably variable patterns of gene expression, not only just between individuals, but also within a large biopsy (Bakay *et al.* 2002, Wittwer *et al.* 2004). Nevertheless, consistent differences between biopsies can be detected, provided they are large enough (Bakay *et al.* 2002).

In our CON/ECC training group, we found an increase in strength endurance capacity (Fig. 1), but no statistically significant changes in any of the mRNAs determined. It is possible that there were systematic changes in some of these mRNAs, but they were too small to be detected in the scatter of the individual results. However, it could also be that translational modulations and/or adaptations in systems other than those tested with the mRNA markers in this study were primarily responsible for the improved test results, either in the muscles themselves or in the nervous system.

The higher eccentric component of the CON/ECC-OVERLOAD training is likely responsible for the significant increase in maximal strength and the trend towards enhanced MCSA. Our results correspond well to the findings of Brandenburg & Docherty (2002) and Hortobágyi *et al.* (2001) who reported greater increases in maximal strength after comparable concentric/eccentric-overload strength training than after concentric/eccentric strength training. The higher intramuscular pressure due to the higher tension during the eccentric phase in the CON/ECC-OVERLOAD exercise probably impedes blood flow to a greater extent than during CON/ECC exercise, possibly leading to enhanced hypoxia, which could induce different adaptations. Hypoxia during endurance training has been shown to cause specific adaptations in muscle gene expression, such as increased mRNAs of vascular endothelial growth factor (VEGF) or myoglobin (Vogt *et al.* 2001). While myoglobin mRNA was not significantly changed after 4 weeks of low resistance–high repetition strength training in neither group, we even found a decrease in VEGF mRNA ($P \leq 0.05$) in the CON/ECC-OVERLOAD group (data not shown). It is, therefore, not likely that local hypoxia was a decisive factor. Another possibility is that these adaptations towards a faster, more glycolytic mRNA pattern were due to differential recruitment of fibre types. The combination of concentric with higher eccentric load in the CON/ECC-OVERLOAD strength training could have led to enhanced recruitment of IIA and IIX fibres, the enhanced glycolytic metabolism of which is more suited to deal with reduced blood flow. This is supported by the data of Hortobágyi *et al.* (2001) who used training modes similar to the ones of the present study and found increased EMG activities in the group following the concentric/eccentric-overload training regimen. It is possible that the high total eccentric workload of the CON/ECC-OVERLOAD training regimen stimulated overall MHC II synthesis (due to recruitment of additional units or other mechanisms) but the drop in MHC IIx mRNA, which usually occurs with concentric training, was prevented by the low proportion of concentric training to the total workout.

CON/ECC-OVERLOAD strength training is becoming an increasingly popular component in the preparation of athletes for sports involving explosive strength

 

and/or very high power output. The protocols used in this study are very similar to the regimens used in training practice in terms of loads and length of the training period. The changes in our marker mRNAs, which are compatible with a shift towards a faster, more glycolytic muscle after CON/ECC-OVERLOAD training are therefore in good agreement with the enhanced athletic performance observed.

It is possible that the RNA results of the current study were influenced by the muscle activity during the high repetition–very low resistance conventional strength training of the lead-in period, which would have set an endurance type stimulus, albeit a low one. Previously observed changes in mRNAs of energy metabolism enzymes after low intensity endurance training were small (Vogt *et al.* 2001), thus it is not likely that the results of this study were decidedly influenced by the lead-in phase. Nevertheless, the results of the present study deserve to be checked by further research on the effects of CON/ECC-OVERLOAD training, e.g. with athletes familiar with strength training, who do not need a lead-in phase, and also with additional strength tests on conventional devices besides isokinetic testing because isovelocity muscle actions are not natural to most activities.

In summary, we found different functional and cellular adaptations in the muscles of subjects training concentrically and eccentrically with the same absolute loads compared with subjects training with the same relative loads (eccentric-overload). Significant increases in the mRNAs coding for MHC IIa and LDH A and a strong tendency towards an increase in MHC IIx mRNA in the group following the concentric/eccentric-overload training regimen indicate a shift towards a more type II dominated mRNA pattern specific for this type of training.

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