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Effect of training with different mechanical loadings on MyHC and GLUT4 changes

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ABSTRACT

Purpose: There is an inverse relationship between insulin sensitivity and percentage of myosin heavy chain IIx isoform (MyHC IIx) in sedentary, obese and type 2 diabetic humans. How different exercise conditions may reduce the proportion of MyHC IIx, and in parallel elevate glucose transporter 4 (GLUT4) content is interesting in a therapeutic setting. This study investigates the nature of exercise signals regulating MyHC gene switching and whether it is accompanied by GLUT4 changes.

Methods: Thirty-two subjects performed high loading (60% of 1RM) or low loading (30% of 1RM) elbow extensions in a training apparatus and exercised 3 times week^{-1} for either 5 (low volume) or 8 weeks (high volume). MyHC and GLUT4 contents in the m. triceps brachii were measured by Western blotting pre- and post training and after 8 weeks of detraining.

Results: All training regimes resulted in MyHC IIx changes of similar magnitude, and differences in training volume had no effect on the outcome. The reduction in MyHC IIx content following high loading, high volume was similar to low loading, matching volume of training. Thus, there was no effect of training load on MyHC changes. GLUT4 increased more following high- than low loading ($P < 0.0.1$). In addition, the larger increases in the GLUT4 were associated with the larger reductions in MyHC IIx content ($r = - 0.56$, $P < 0.01$). Detraining returned GLUT4 to baseline, but MyHC IIx content was still higher than baseline ($P < 0.01$).

Conclusion: Magnitude of loading is not important for suppression of MyHC IIx, but for increases in GLUT4 content. The GLUT4 content responded, however, more rapidly to detraining than the MyHC IIx and IIa isoforms.

Keywords: EXERCISE, MUSCLE, CONTINUOUS, INTERMITTENT, MYOSIN HEAVY CHAINS, GLUCOSE TRANSPORTER 4

Paragraph 1

There is an ongoing discussion concerning the nature of exercise signals that regulate myosin heavy chain (MyHC) gene switching during long term training in humans. It has been suggested (1) that training volume is the sole determining factor for MyHC IIx suppression during repetitive, low force activity, but that the magnitude of mechanical loading is a governing factor for the suppression of MyHC IIx during training with higher loads (1). In contrast, results from other studies suggest that the magnitude of loading is not important for suppression of MyHC IIx in exercising muscle (6). Hence, there is no consensus regarding the magnitude of mechanical loading, whether it is important for fast MyHC isoform shifts (1) or not (6). If the training volume is important for fast MyHC shifts during repetitive training with low mechanical loading (e.g., continuous endurance training), one would expect that a large total volume of this type of training would suppress the MyHC IIx content more than a small total volume of training. In contrast, if the magnitude of mechanical loading is important, one might anticipate that training with a high mechanical load would suppress the MyHC IIx content more than training with less heavy loads, despite a matching total volume of training. If loading is of little importance, the implications are that training with low or higher mechanical loading will suppress the MyHC IIx content to similar levels. The response of the MyHC isoforms to different doses of exercise is, however, little investigated in human skeletal muscle, and we will analyse this relationship by comparing the effects of training with high or low relative loading combined with matching high or low volumes on MyHC isoform expression in the m. triceps brachii.

Paragraph 2

Training studies show that long term endurance training increases insulin sensitivity and glucose transporter 4 (GLUT4) content in humans (15) and it is suggested that the total amount of the GLUT4 protein available may be an important factor for governing insulin sensitivity (14). It is also reported that insulin sensitivity is inversely related to the percentage of MyHC

IIx muscle fibres in sedentary (12), obese (5, 21) and type 2 diabetic humans (5). Consequently, a reduction in proportion of MyHC IIx fibres and an increase in the protein content of GLUT4 would be associated with increased insulin sensitivity and responsiveness in type 2 diabetic individuals (for review, see 17). Decreased insulin stimulated glucose transport is one key aspect of insulin resistance, but glucose uptake into skeletal muscle cells by GLUT4 is also stimulated by contractile activity, independent of insulin (for review, see 18). Chronic training can elevate the GLUT4 content in human skeletal muscle (18) as well as reduce the percentage of MyHC IIx fibres (e.g., 6) and it is suggested that transformation of muscle fibre types from IIx to IIa should be considered when attempting to explain elevations in the GLUT4 content with training (14). In contrast, others have argued that the GLUT4 content may be more related to the activity level of the muscle fibre, than to its expression of contractile proteins (8, 9). Consequently, there are conflicting views on the association between GLUT4 and MyHC and on their regulation in response to training (e.g., 8, 14, 23, 24).

Based on this we will investigate how different combinations of exercise may change the proportion of MyHC in a favourable direction (MyHC IIx → MyHC IIa) and elevate the GLUT4 content. Thus, in a therapeutic setting the present study may provide valuable information regarding tailoring of physical activity programmes as part of a lifestyle intervention for type 2 diabetic persons. Specifically, we have tested the following hypotheses: 1) Will a large total volume of repetitive training with low mechanical loading (continuous training), suppress the MyHC IIx content more than a small total volume of this type of training? 2) Will a high mechanical load (intermittent training) suppress the MyHC IIx content more than training with less heavy loads, but with a matching total volume of training? 3) What type of training is the better to reduce the proportion of MyHC IIx and in parallel increase the GLUT4 content in the exercising muscles?

MATERIALS AND METHODS

Subjects. In the present study, we chose to include young, healthy and lean subjects since the subjects had to tolerate repeated biopsies and intensive training three times per week for maybe eight or more weeks. Prior to inclusion in the study, 77 subjects completed two questionnaires, one about self-reported health and another about habitual physical activity. Based on the results from a 1RM test of their elbow extensors and the questionnaires, we excluded 37 subjects with a history of regular exercise of the elbow extensors and those with the highest and the lowest 1RM.

The remaining subjects (n = 40), who were untrained in their m. triceps brachii, were included in the study and assigned to one of four different training regimes by a random number generator. This randomization resulted in three groups with nine subjects each (group HI/HV, HI/LV and LI/LV) and one group with 13 subjects (group LI/HV). The subjects were healthy and were not using any medication for chronic diseases. They had not been engaged in any type of regular exercise of the elbow extensors for at least six months prior to the start of this study. During the study, the subjects were instructed not to engage in physical activities that exercised their triceps muscle other than their prescribed training programme. The subjects' adherence to this regimen was controlled by their self-reported training diary. There was a total of eight dropouts from the study (three men and five women), resulting in the following group sizes: HL/HV; n = 6 (1 male and 5 females), HL/LV; n = 5 (2 males and 3 females), LL/HV; n = 13 (6 males and 7 females) and LL/LV; n = 8 (3 males and 5 females). The mean (SE) age, height, bodyweight and body mass index (BMI) of the 32 persons who completed this study was 22.0 (0.6) years, 172.8 (1.3) cm, 64.6 (1.2) kg and 21.6 (0.3). Written informed consent was obtained from all subjects, and the study was approved by the Regional Committees for Medical Research Ethics (REK) in Norway.

Paragraph 3

Study design (Fig. 1). All subjects performed elbow extensions 3 times week $^{-1}$ on alternating days with both arms simultaneously in a commercial training apparatus (Triceps Extension Machine, Cybex International Inc., USA). The training groups differed in terms of type of training, the relative load applied, the total number of repetitions per session and the total number of weeks in training.

Training load. The loading during training was calculated as a percentage of the subjects' 1RM of the elbow extensors. Relative to each other, the subjects performed either High Loading training (HL) or Low Loading (LL) training. 1RM was tested once every week, and the training load was adjusted progressively to keep the loading at the designated percentage of 1RM

<u>High Loading, intermittent training (60% of 1RM)</u> was: 20 repetitions during 30 seconds (paced by a metronome), followed by 30 sec. recovery. A total of 100 repetitions completed one set, and the subjects performed 4 sets session^{-1} with 5 min. recovery between sets. The subjects performed a total of 400 repetitions session.$^{-1}$

<u>Low Loading, continuous training (30 % of 1RM)</u> was: 40 repetitions minute^{-1} (i.e., the same pace as intermittent training) for a total of 800 repetitions session.$^{-1}$ There were no recovery periods during the training session.

Training volume and duration. The total volume of training is calculated as the total weight lifted per session times the total number of sessions. The total volume was matched between the two Low Volume groups and the two High Volume groups. The Low Volume subjects trained for an average (SE) of 5 (1) weeks, while the High Volume subjects trained for an average of 8 (0.8) weeks.

Detraining. After completion of the training period the subjects went through a period of detraining lasting eight weeks. During this period, the subjects were instructed to refrain from regular training of the elbow extensors.

Biopsy protocol. The subjects reported to the lab after an overnight fast. Needle biopsies were taken from the lateral head of m. triceps brachii of the non-dominant arm prior to training (Pre-T), after termination of the training period (Post-T) and eight weeks after the Post-T biopsy (De-T). Post-T biopsies were taken five days following the last exercise session.

Analysis of GLUT4. Muscle membrane preparations (red blood cells, contractile proteins and insoluble materials removed) were prepared as described previously (10). Total protein concentration of the samples was determined using the bicinchoninic acid (Micro-BCA, Pierce, USA) protein assay. The membrane preparations were diluted with sample buffer to give equal amounts of protein. The samples were then heated for 4 min. at 100 ℃ in a water bath. Proteins were separated using a 4% stacking and 12 % separating gel (Mini-Protean 3, BioRad Laboratories, USA). Electrophoresis was run at 150 volt (V) for ~60 min. Separated proteins were transferred from gels to 0.2 µm PVDF membranes (BioRad Laboratories, USA). Protein transfer was done at 100 V for 70-80 minutes (Mini Trans Blot system, BioRad Laboratories, USA). The PVDF membranes were subsequently blocked with skimmed milk powder in 0.1%TBS-Tween (TBS-T) on a shaker over night at 4℃. After blocking, PVDF membranes were incubated at room temperature with a GLUT4 antibody (1: 3000, # 4670-1704, Biogenesis Ltd., UK). After incubation with primary antibody, membranes were washed in TBS-T and incubated with an HRP conjugated secondary antibody (NA 934, Amersham) for 60 minutes. Following incubation with the secondary antibody, membranes were repeatedly washed with TBS-T before detection of protein bands. Membranes were subsequently incubated with an ECL+ reagent (RPN 2132, Amersham) and proteins bands visualized on X-ray films.

Paragraph 4

Separation of MyHC isoforms. Homogenisation and electrophoretic separation of myosin heavy chains (MyHC) were performed on biopsy samples (4). Total protein concentration of the samples was determined using the bicinchoninic acid (Micro-BCA, Pierce, USA) protein

assay. After electrophoretic separation of MyHC proteins, protein bands were visualized by Coomassie staining. Identification of MyHC isoforms was confirmed by Western blotting (3). Antibodies used were: Anti MyHC IIa + IIx or anti MyHC I + IIa (SC-71 and BF-35, respectively, kindly provided by prof. S. Schiaffino, University of Padua, Italy), and anti MyHC I antibody (NCL-MyHC, Novacastra Laboratories, UK). A typical response of GLUT4 and MyHC responses following training and subsequent detraining is shown in fig 2.

Quantification. Digitized scans of Coomassie stained SDS gels (MyHC) and X-ray films (GLUT4) were quantified by image analysis software (TotalLab, Nonlinear Dynamics, UK). All samples were run in duplicate and averaged. GLUT4 values are reported as mean integrated optical density (IOD) units. IOD is calculated as the sum of pixel gray level values within a defined area divided by the number of pixels within the same area. We did not use a control sample to correct for differences between gels, but before quantification of IOD levels, each digitized scan was calibrated by adjusting the OD levels of the scanned image to the expected values of a an optical density control scale (Kodak control scale T-14, N.Y, USA). MyHC isoforms were identified as MyHC I, IIa or IIx and each isoform was expressed as a percentage of the total MyHC content detected in the samples.

Statistical analyses. The variables were tested by two-way repeated measures ANOVA with *time* as within-subjects variables, *load* and *volume* as between-subjects factors and *gender* as a covariate. Significant main effects were subsequently analyzed by univariate analysis with a LSD post hoc test to examine differences between the training groups. Linear regression was used to investigate the relationships between the variables. All calculations were done in SPSS statistical programme (SPSS Inc., Chicago, USA). Statistical significance was set at $P < 0.05$.

RESULTS

Main results (Table 1). Long-term training increased the GLUT4 content by 64 % (Pre-T vs. Post-T; $P < 0.001$), while the reduced percentage of the MyHC IIx isoform was accompanied by a reciprocal increase of the MyHC IIa isoform (Post-T vs. Pre-T; $P < 0.001$ for both). Following detraining, the MyHC IIx and MyHC IIa isoform contents were reversed (Post-T vs. De-T; $P < 0.01$ for both), but the De-T contents of both isoforms were still different compared to their respective Pre-T values (both; $P < 0.01$). In contrast, GLUT4 levels had returned to Pre-T levels.

Group comparisons. The changes in GLUT4 content were clearly dependent on the magnitude of loading during training. In this respect, the GLUT4 content increased more following training with high loading (groups HL/HV and HL/LV) than following training with low loading (groups LL/HV and LL/LV), (128 % vs. 13 %, respectively; $P < 0.01$). In contrast to the GLUT4 changes, the type of training did not influence the outcome of MyHC IIx and MyHC IIa isoform changes. In this respect, no significant differences between the training groups were observed for training induced changes (Post-T values minus Pre-T values) in the MyHC IIa or MyHC IIx contents (Fig. 3A and B). Thus, all training regimens regardless of mechanical loading and total training volume resulted in changes of similar magnitudes with a general reduction of the MyHC IIx content and a reciprocal increase in the MyHC IIa content.

Correlations. There were no significant correlations between MyHC isoforms and GLUT4 contents neither Pre-T, Post-T nor De-T. The changes in MyHC IIa and IIx content following detraining were inversely related to the magnitude of their changes following training ($r = - 0.65$, and $r = - 0.56$, respectively, $P < 0.001$, for both). Thus, the larger changes in MyHC IIa and IIx following detraining were observed in the subjects with the larger changes following training.

The protein content of GLUT4 increased with an increasing Post-T percentage of MyHC IIa in the triceps muscle (Fig. 4), but only for the subjects that performed intermittent training with high loading ($r = 0.73$, $P < 0.01$). On the other hand, the GLUT4 protein content following continuous low loading training was similar over the whole range of Post-T MyHC IIa content. There was an inverse relationship between training induced reductions (Post-T minus Pre-T values) in MyHC IIx content and the increases (Post-T minus Pre-T values) in GLUT4 content following training ($r = - 0.56$, $P < 0.01$), meaning that the larger increases in GLUT4 content were observed in the subjects with the larger reductions in MyHC IIx content.

Total training volume. Total average (SE) training volume for the Low Volume groups (HL/LV and LL/LV) was 107 (2.9) tons, while the High Volume groups (HL/HV and LL/HV) lifted an average of 179 (1.9) tons (HV vs. LV; $P < 0.001$).

DISCUSSION

Effects of training. In the present study both high loading (HL) and low loading (LL) training induced transformation of muscle fibre types from MyHC IIx to MyHC IIa, but only HL training increased the GLUT4 expression in the m. triceps brachii. The relationship between GLUT4 and MyHC isoform expression is, however, not completely resolved, and it has been suggested that transformation of muscle fibre types from MyHC IIx to MyHC IIa should be considered when attempting to explain elevations in the GLUT4 content with training (14). Interestingly, we observed that the larger increases in the GLUT4 content occurred in the individuals with the larger reductions in the MyHC IIx content. It is, however, unlikely that the MyHC IIx *directly* determines the GLUT4 expression, and others have argued that the GLUT4 content may be more related to the activity level of the muscle fibre, than to its expression of contractile proteins (8, 9).

Paragraph 5

Exercise induced adaptation of contractile and metabolic properties in muscle may be regulated by different signalling pathways (e.g., 20). Previous research on signalling pathways have, however, suggested that metabolic properties and MyHC isoforms are independently regulated and that metabolic changes can occur without transformation of fast MyHC isoforms (23). In contrast, Russel et al. (24) argues that increased expression of the transcriptional coactivator PGC-1 (peroxisome proliferator-activated receptor-γ coactivator-1) might be linked to MyHC transformations and increases in GLUT4 content following endurance training. Thus, there are seemingly conflicting views on the association between GLUT4 and MyHC gene expression in muscle. If, however, multiple signalling pathways are activated during training, this may possibly integrate the metabolic changes with the MyHC transformations that are observed following training (29). This would ensure a timely match of contractile and metabolic properties to meet the altered workload imposed by chronic training (29). The exact signalling

mechanisms linking the different types of neuromuscular activity to altered gene expression, remains, however, to be elucidated.

Paragraph 6

The fact that most subjects in the present study experienced a reduction in the percentage of MyHC IIx fibres and a reciprocal increase in the percentage of MyHC IIa fibres and GLUT4 content (Table 1) is interesting also of therapeutic reasons. Although we trained young, healthy and lean subjects, our findings may have implications for a diseased population. In this regard it is reported that type 2 diabetes and insulin resistance in humans are associated with a greater percentage of MyHC IIx muscle fibres and it is suggested that a reduction in proportion of MyHC IIx fibres and an increase in the proportion of MyHC IIa fibres would increase GLUT4 levels and increase muscle insulin sensitivity and responsiveness in type 2 diabetic individuals (17). Thus, to look at a possible association between the transformation of the MyHC IIx isoform to the MyHC IIa isoform and GLUT4 expression, we plotted the training induced changes in GLUT4 levels in relation to the Post-T content of MyHC IIa (Fig. 4). Interestingly, there were only minor and quite similar increases in individual GLUT4 protein content following training with low mechanical loading, regardless of the individual Post-T MyHC IIa content (Fig 4; open circles). Evidently, this type of training is unable to stimulate to increases in the GLUT4 protein content, and given this scenario, there seems to be a relatively uniform expression of GLUT4 regardless of the percentage of muscle fibres expressing MyHC IIa. Previous investigations have argued that continuous training provides the highest stimulus for inducing the GLUT4 expression in skeletal muscle (26), but the present study show much larger increases in the individual GLUT4 content following intermittent training. In the present study, a high mechanical loading is necessary to stimulate to increases in the GLUT4 protein content, thus the activity level of the muscle fibres is clearly important (8). During high loading training, however, there seems to be an increase in GLUT protein content which is related to an increasing percentage of MyHC IIa muscle

fibres. Thus, there are larger increases of GLUT4 in the individuals with a high Post-T percentage of MyHC IIa (Fig. 4; closed circles). It is argued that it is difficult for many patients with type 2 diabetes to engage in endurance type training programmes (13), and studies on a disease population using a resistance type of exercise, are few. Holten et al. (13), however, found that resistance training for 30 minutes, three times per week, increased insulin action and increased the protein contents of GLUT4 in patients with type 2 diabetes. Since they did not determine the percentage of MyHC IIa and MyHC IIx in their biopsies, it is not possible to tell whether these changes were accompanied by transformation of fast MyHC isoforms. In our study, both training regimes reduced the MyHC IIx content in exercising muscles to the same extent (Fig. 3B), but HL training increased the GLUT4 content more than LL training. Thus, the present results support the view that a resistance type of training with high loading may represent an attractive alternative for patients with type 2 diabetes (13). Sedentary and obese persons may, however, have less tolerance to high training loads, thus the work/rest protocol during exercise needs to be carefully designed.

Paragraph 7

Given that the proportion of MyHC IIx muscle fibres in many muscles may be small, it is relevant to discuss if a reduced proportion of MyHC IIx have a physiological role for individuals with insulin resistance and impaired glucose metabolism. In this regard, Bandyopadhyay et al. (5) showed that the percent composition of MyHC IIx fibres in insulin resistant subjects (~16% MyHC IIx) was higher than in lean, healthy subjects (~5 % MyHC IIx). Furthermore, Venojärvi et al. (27) investigated the role of muscle fibre type composition in regulation of the glucose metabolism in subjects with impaired glucose tolerance (IGT). The subjects were divided in two groups with either a high proportion of MyHC I (IGT_slow) or a high proportion of MyHC II isoforms (IGT_fast). The actual MyHC IIx content in the m. vastus lateralis was ~17% (IGT_slow) and ~23% (IGT_fast), respectively, i.e. a difference of 6% in the MyHC IIx content. Despite this small difference, Venojärvi et

al. (27) argued that the difference between the proportions of MHC I and MHC II isoforms could partially explain why the IGT_fast subjects were more insulin-resistant than the IGT_slow subjects. Correspondingly, Hedman et al. (11) observed that muscle morphology explained 16% of the variation in insulin sensitivity in older men. Interestingly, half of this variation was attributed exclusively to the proportion of MyHC IIx fibres in the muscle. In summary, these data underscore the importance of further investigations on the association between GLUT4 expression and the content of fast MyHC isoforms.

Paragraph 8

Given that a transformation of MyHC IIx to MyHC IIa fibres may be interesting also in a therapeutic setting, it is interesting to investigate the nature of exercise signals regulating MyHC gene switching. There is, however, limited information on MyHC changes following different training loadings and concerning a putative role of the training volume *per se* on MyHC adaptations. In the present study, we find that training with a high mechanical load does not suppress the MyHC IIx expression more than training with less heavy loads, but with a matching total volume of training (Fig. 3B) and we believe our findings support the view that the mechanical load is of little importance in this matter (6). Admittedly, our subjects have not lifted as heavy loads as the subjects in other resistance training studies (e.g., 1, 19, 25) and consequently one may ask if those results are comparable to ours. In a resistance training study with three loading regimes (3-5RM, 9-11RM and 20-28RM), and similar volumes of training, the magnitude of reductions in MyHC IIx content was the same for all loading conditions (6). In the present study, the two high loading groups (HL) performed repeated series of 20 repetitions, and most of the subjects were close to fatigue at the end of each of the series. Thus, the HL type of training seems comparable to the 20-28RM loading regime previously mentioned (6). In addition, the two low loading groups (LL) in the present study performed elbow extensions at 30 % of the subjects' 1RM, but the type of changes in MyHC content following LL training was similar to the type of changes

observed during training with much higher mechanical loadings (e.g., 1). Consequently, the combined evidence from the present and other studies (6) does not support the view (1) that the magnitude of mechanical loading governs the suppression of the MyHC IIx content in exercising human muscles. Interestingly, we also find that that during repetitive, low force training (LL groups), the magnitude of reductions in MyHC IIx content was similar following either a high volume (HV) or a low volume (LV) of training (Fig. 3B). The total volume during HV training was on average ~70 % higher than the volume during LV training, hence we find no support for the view of that the training volume is the sole determining factor for MyHC IIx suppression during repetitive, low force activity (1). Since the magnitude of reductions in MyHC IIx content following low force, low volume (LL/LV) training was similar to the changes following a much larger total volume (LL/HV), our results may indicate that the downregulation of MyHC IIx is not related to the *total* volume of training, but perhaps to whether MyHC IIx fibres are recruited or not during training.

Paragraph 9

Effects of detraining. In the present study, detraining for eight weeks only partly reversed the training induced changes in MyHC IIx and IIa content in the m. triceps brachii. The De-T MyHC IIx and IIa contents were not fully restored to their respective Pre-T levels, hence there was no indication of a MyHC IIx overshoot, as reported in previous studies (1, 2). Since we detrained our subjects for a shorter period of time than the previously mentioned studies (8 vs. 12 weeks, respectively), we may have missed an overshoot because it may need more than eight weeks to develop. In this respect, serial biopsies of well trained endurance athletes have been performed one, two, three, eight and twelve weeks after cessation of an endurance training programme (7). Endurance training reduced the proportion of MyHC IIx, while detraining progressively increased the MyHC IIx content. Twelve weeks of detraining did, however, not elevate the IIx content any further than at eight weeks. In addition, a MyHC IIx overshoot has not been confirmed by other resistance studies (16, 25) exercising

the m. vastus lateralis and with similar or longer training and detraining periods as the previously mentioned studies (1, 2). Consequently, a possible induction of an overshoot in MyHC IIx expression after shorter or longer periods (8 – 32 weeks) of detraining is still an open question. The GLUT4 content responded, however, more rapidly to detraining than MyHC IIx and IIa isoforms, and after eight weeks of detraining, GLUT4 levels had returned to baseline (Table 1). Other studies of endurance trained athletes confirm our findings and show a significant reduction in the GLUT4 content following 6 (28) or 10 days (22) of detraining. Thus, muscle GLUT4 content starts to decline rapidly after cessation of contractile activity, and has returned to baseline after several weeks of detraining. Consequently, to maintain a favourable adaptation in type 2 diabetic persons, a break from training should not be too long

Paragraph 10

To summarize: Within the range of mechanical loading used in this study, the magnitude of mechanical loading is not important for suppression of MyHC IIx synthesis, and a large training volume do not suppress the MyHC IIx content more than a lesser volume of training. A high loading is, however, more effective than a lower loading in increasing the GLUT4 content. In addition, GLUT4 increases correlates with the MyHC IIa content in the trained triceps brachii, thus the suppression of MyHC IIx and the concomitant increase in MyHC IIa and GLUT4 following intermittent training may represent a favourable adaptation in a therapeutic setting. Finally, eight weeks of detraining is sufficient to return training induced changes in GLUT4 content to baseline, while the changes in MyHC IIx and IIa contents are not fully reversed.

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LEGEND TABLE 1 The content of MyHC isoforms and GLUT4 in the m. triceps brachii following training and detraining.

Values are means and SE (n = 32, all groups together). [a] = $P < 0.001$; Pre-T vs. Post-T, [b] = $P < 0.01$; Post-T vs. De-T, [c] = $P < 0.01$; Pre-T vs. De-T. IOD = integrated optical density, MyHC = Myosin heavy chain, GLUT4 = Glucose transporter 4.

LEGEND FIGURE 1 The study design.

The training mode (elbow extensions), velocity of movement (paced by a metronome), range of motion (defined by the training apparatus), frequency of training, and number of recovery days between training sessions were similar for all subjects. HL = High Loading, LL = Low Loading, HV = High Volume, LV = Low Volume.

LEGEND FIGURE 2 Electrophoretic separation of MyHC isoforms and immunoblot of the GLUT4 expression in the m. triceps brachii.

Typical response of Myosin Heavy Chain (MyHC) isoforms and Glucose transporter 4 (GLUT4) after High Loading training and subsequent detraining in one subject. kDa = kilo Dalton.

LEGEND FIGURE 3 Changes in MyHC IIa (A) and MyHC IIx (B) content following long term training and detraining in relation to different training groups.

Filled bars = Training changes (Post-T minus Pre-T values). Open bars = Detraining changes (De-T minus Post-T values). Values are means (SE). [a] = $P < 0.05$; Low Loading/High Volume vs. Low Loading/Low Volume.

LEGEND FIGURE 4 Changes in GLUT4 content in relation to Post-T MyHC IIa content.

Closed circles = High Loading, open circles = Low Loading. IOD = Integrated Optical Density (the integral of the mean optical density over the area of each band). Correlation is for High Loading, only.

TABLE 1 The contents of MyHC isoforms and GLUT4 in the m. triceps brachii following training and detraining.

	MyHC I (%)	**MyHC IIa (%)**	**MyHC IIx (%)**	**GLUT4 (IOD)**
Pre-T	33.6 (1.1)	49.3 (2.1)	17.1 (2.3)	836 (128)
Post-T	34.4 (1.3)	58.7 (1.4)[a]	6.9 (1.6)[a]	1375 (254)[a]
De-T	32.9 (1.3)	54.6 (1.7)[b, c]	12.5 (1.8)[b, c]	834 (146)[b]

FIG 1. The study design

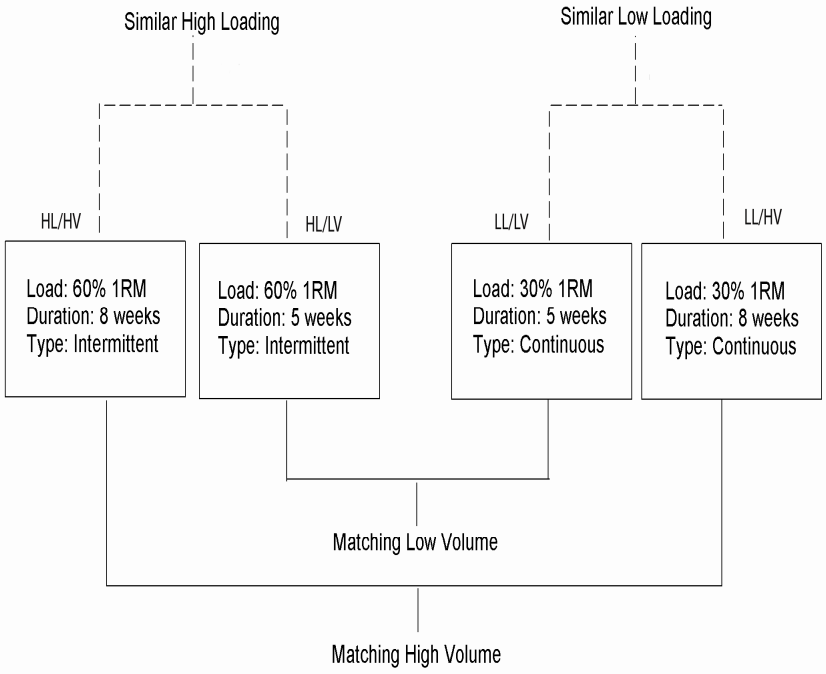

FIG 2. MyHC and GLUT4 adaptations following training and detraining

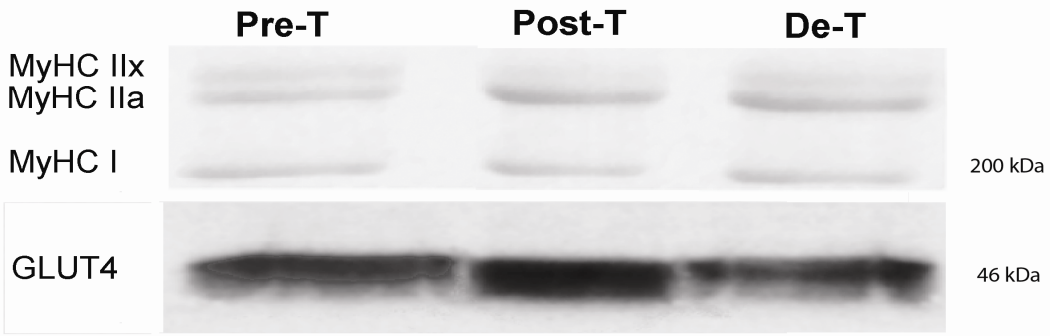

FIG 3. Changes in MyHC IIa content following training and detraining

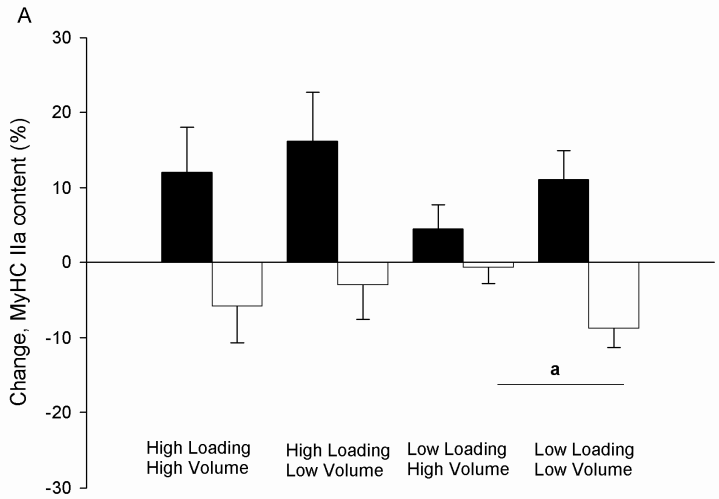

FIG 3. Changes in MyHC IIx content following training and detraining

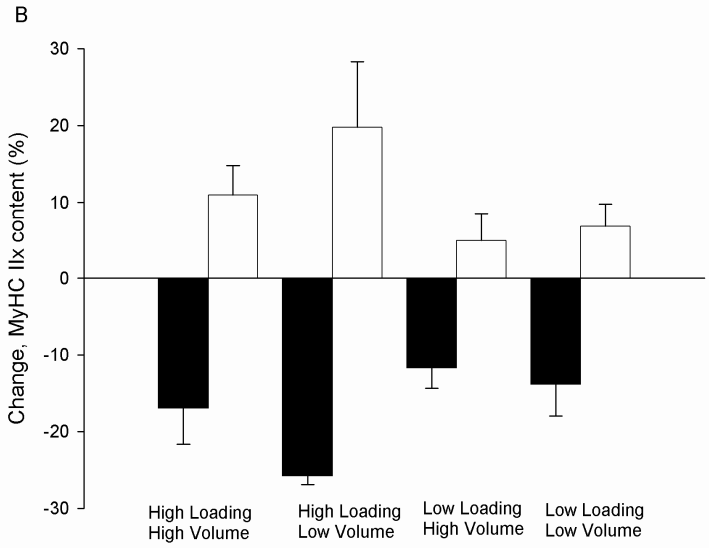

FIG 4. Changes in GLUT4 content in relation to Post-T MyHC IIa content