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Effects Of High-Intensity Strength Training On Steady-State Myosin Heavy Chain Isoform Mrna Expression

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DARRYN S. WILLOUGHBY AND STEPHEN PELSUE. Effects Of High-Intensity Strength Training On Steady-State Myosin Heavy Chain Isoform Mrna Expression. JEPonline, 3(4):13-25, 2000. The purpose of this study was to determine steady-state myosin heavy chain (MHC) isoform (Types I, IIa, and IIx) mRNA abundance in skeletal muscle after 8 wks of high-intensity strength training. Twelve untrained males were randomly assigned to either a control (CON) or strength training (STR) group. During the study, STR trained at 85%-90% 1-RM for 3 sets of 6-8 repetitions thrice weekly employing the bilateral leg press exercise while CON engaged in no strength training. Approximately 20-30 mg of muscle from vastus lateralis biopsies were obtained before and after the study and a competitive method of quantitative RT-PCR was performed to determine MHC isoform mRNA abundance. A synthetic DNA fragment (555 base pairs) was designed as a competitive internal control standard and a known amount co-amplified with the template DNA. As a result of training, STR underwent significant increases in muscle strength, thigh volume, and myofibrillar protein that were significantly different from CON (p < 0.05). STR was also shown to be expressing significantly more of the Type I and IIa isoform and less of the IIx isoform that was also significant from CON (p < 0.05). This study indicates that high-intensity strength training increases myofibrillar protein content and results in up-regulation of the expression of the Type I and IIa MHC genes with concomitant down-regulation of the IIx gene.

Key Words: strength, pre-translational, translational, RT-PCR

INTRODUCTION

Human skeletal muscle is a highly-adaptable tissue that commonly demonstrates significant plasticity in response to various types of exercise. This plasticity is well illustrated by the isoform diversity (Type I, IIa, and IIx) of the sarcomeric myosin heavy chain (MHC) genes (1). All the MHC isoforms are encoded by a closely conserved multigene family (2) of distinct genes that are expressed in a tissue-specific and developmentally regulated manner (1,3). The diversity of these isoforms is augmented in some instances by alternative RNA splicing of individual MHC genes; therefore, changes in muscle activity from weight training alter MHC gene expression and phenotype and affect muscle fiber types in characteristic ways (4). The adaptability of skeletal muscle appears to reside in the ability of the muscle fibers to transcribe different isoforms of MHC protein, each which have specific functional characteristics (5) relative to muscle contraction. Consequently, the polymorphism of the MHC plays a major role in the adaptability and contractile efforts of muscle fibers necessary for various types of exercise.

High-intensity strength training in humans is characterized by increases in muscle strength and hypertrophy (6,7,8). These training-induced adaptations are often thought to be a result of increases in the content of

myofibrillar proteins (9,10) and MHC isoform proteins (6,7,8) which is presumed to occur from increased expression of MHC isoform mRNA. However, little is known at the present time about the MHC gene expression characteristics in response to high-intensity strength training in humans.

Increases in the abundance of MHC isoform protein content contributes to increases in the content of myofibrillar protein and could be a result of: 1) enhanced transcription of MHC isoform mRNA thereby resulting in more mRNA molecules being translated or 2) increased rate of translation of each molecule of mRNA (11). It has also been concluded that increased muscle protein synthesis may also be caused by molecular events occurring post-transcriptionally (9). We categorize the term pre-translational as the events altering the abundance of mRNA. Incidentally, abundance is the algebraic sum of transcription, mRNA processing, and mRNA stability. The term translational indicates changes in the synthesis of protein in relation to mRNA activity while post-translational is indicative of the modification of protein (i.e., phosphorylation, proteolysis, etc.).

At the present time, few studies exist investigating MHC isoform mRNA expression in response to high-intensity strength training in humans. Most strength training studies investigating the response of MHC to training in humans have focused on the protein isoforms. As a result, it is well established that the MHC protein isoforms undergo training-induced transitions to a slower phenotype (IIx \rightarrow IIa \rightarrow I). In humans, it has been shown that after 3 weeks of immobilization 12 weeks of weight training of the knee extensors produced no significant changes in the mRNA of the three MHC isoforms (12). Similarly, it has also been shown in elderly humans that 7 days of weight training of the knee extensors at 80% of maximum had no significant change on MHC isoform mRNA levels (11). Using a qualitative method of the polymerase chain reaction (PCR) technique, we have recently shown (13) that the relative expression of Type I and IIa MHC mRNA in the elderly may increase after high-intensity weight training such that it is similar to the respective changes in MHC protein expression observed in other studies (6,7,8). However, the major weakness associated with the qualitative PCR technique is that it only provides the ability to detect the presence or absence of the target mRNA and, at best, can only be pseudo-quantified based on normalization to an external control standard. Therefore, in order to better determine MHC isoform mRNA expression using PCR, a quantitative method should be used.

Regardless of the PCR procedure used, it should be noted that in steady-state, mRNA expression usually parallels the pattern of MHC protein expression; therefore, it is assumed that MHC expression is primarily regulated at the pre-translational level (14). However, mechanical overload such as with strength training may actually create a mismatch between the relative expression of MHC mRNA and protein suggesting that upregulation of the MHC isoform genes as a result of exercise may not be directly correlated to the synthesis of the respective MHC protein. Presently, however, there is a limited amount of data available on MHC isoform mRNA expression after high-intensity strength training. As a result, the purpose of this study was to two-fold and sought to: 1) determine the steady-state level of MHC isoform mRNA abundance and MHC gene expression characteristics in response to high-intensity strength training and 2) compare the MHC mRNA expression after training using a quantitative and qualitative method of PCR.

METHODS

Subjects

Twelve untrained males with an mean \pm SD age of 19.88 \pm 0.53 yrs, height of 180.13 \pm 3.00 cm, and body weight of 74.63 \pm 7.92 kg volunteered to participate in the study and were randomly assigned to either a control group [CON, (n = 6)] which involved no strength training or a strength training group [STR, (n = 6)]. Subjects with contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) and/or who had engaged in consistent weight training 6 months prior to the study were not allowed to participate. All eligible subjects signed university-approved informed consent documents, and approval was given by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed

to the ethical consideration of the Helsinki Code. The subjects were explained the purpose of the training program, the protocol to be followed, and the experimental procedures to be used. Subjects were also instructed to maintain their normal dietary regimen and to not consume any type of sport supplements (e.g., creatine monohydrate, protein powder, etc.) during the course of the study.

Muscle Biopsies

Percutaneous muscle biopsies (20-30 mg) were obtained both before and after the 8-wk training period for each subject. Initial biopsies were obtained 1 wk before the initiation of exercise (to allow for adequate healing), and the follow-up biopsies were completed within one hour following the final exercise session. Muscle samples, extracted under local anesthesia (2% Xylocaine), were taken from the middle portion of the right vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. For the post-training biopsy, attempts were made to extract tissue from approximately the same location by using the pre-biopsy scar and depth markings on the needle. A successive incision was made approximately 0.5 cm to the former from medial to lateral (13). Muscle specimens were frozen in liquid nitrogen for later analysis.

Strength Testing and Thigh Volume Determination

Before and after the 8-wk training period, both groups were subjected to a testing session in which each subject's lower body maximum strength [one repetition maximum (1-RM)] was determined using a bilateral leg press machine (Cybex, Owatonna, MN). Due to differences in absolute muscular strength and body weight between groups at the onset of the study, each subject's absolute strength 1-RM was divided by their body weight to ascertain a relative measure of strength. Relative strength was used as a criterion variable because it corrects for variations in body weight among subjects, thereby providing a more accurate estimate of strength (15,16).

Thigh volume (m³) was estimated before and after the study from an equation and guidelines outlined previously (17) taking into account surface measurements of the length, circumference, and skin fold thickness of each subject's right thigh. The measurement was performed in the supine position and always prior to exercise to avoid the influence of possible exercise-induced muscle swelling.

Training Protocol

The training principles of overload and progressive resistance were incorporated in the weight training program based on previously established guidelines (13,15,16). In addition to the pre-test 1-RM, the 1-RM was assessed every two weeks to continually evaluate muscular strength so that adjustments could be made to accommodate for increases in strength and ensure that subjects continued to train at 85%-90% of their 1-RM based on the repetition continuum and guidelines previously established (18,19).

Training sessions occurred 3 days/wk on a Monday-Wednesday-Friday format for approximately 30 min/session (excluding warm-up and cool-down). The format and intensity for the training protocol involved 3 sets of 6-8 repetitions at 85%-90% 1-RM (8). A 90 sec rest period was required between each set and each exercise to help counteract fatigue (15). For warm-up and cool-down, workouts began and ended with 10 min of flexibility exercises combined with calisthenics. Members of CON did not participate in any weight training exercise during the course of the study (other than the pre- and post-training strength evaluations). Missed training sessions were made up on either Tuesdays or Thursdays and subjects were informed that missing three training sessions would result in disqualification from the study.

Total RNA Isolation

Total cellular RNA was extracted from the homogenate of biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate (13,20,21). Specifically, the samples were homogenized and incubated with the TRI-reagent (Sigma Chemical Co., St. Louis, MO). The RNA was precipitated with isopropanol, washed with 70% ethanol, and re-suspended in dH_2O . The RNA concentration was determined by optical density (OD) at

260 nm (by using an OD_{260} equivalent to 40 µg/µL), and the final concentration was adjusted to 1 µg/µL (5,12,20). Aliquots (5 µL) of total RNA samples were then separated with 1% agarose gel electrophoresis, ethidium bromide stained, and monitored under an ultraviolet light to verify RNA integrity and absence of RNA degradation. This procedure yielded undegraded RNA, free of DNA and proteins, as indicated by prominent 28s and 18s ribosomal RNA bands (Figure 1), as well as an OD_{260}/OD_{280} ratio of approximately 2.0 (5,13,21). The RNA samples were stored at -70 °C until later analyses.

Reverse Transcription and cDNA Synthesis

Two μ g of total skeletal muscle RNA were reverse transcribed to synthesize cDNA (21). A reverse transcription (RT) reaction mixture [2 μ g of cellular RNA, 10x reverse transcription buffer (20 mM Tris-HCL, pH 8.3;50 mM KCl;2.5 mM MgCl₂; 100 μ g of bovine serum albumin/ml), a dNTP mixture containing 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.8 μ M MgCl₂, 1.0 U/ μ L of rRNasin (ribonuclease inhibitor), 0.5 μ g/ μ L of oligo(dT)₁₅ primer, and 25 U/ μ g of AMV reverse transcriptase enzyme (Promega, Madison, WI)] was incubated at 42 °C for 60 min, heated to 95 °C for 10 min, and then quick-chilled on ice. Starting template concentration was standardized by adjusting the RT reactions for all samples to 200 ng prior to PCR amplification (5).

Figure 1. Electrophoresis of total RNA from the homogenate of a pre- and post-test muscle biopsy from a CON and STR subject used for the quantitative PCR procedure. Abbreviations are as follows: MW = EcoR-1 molecular weight marker; 28S rRNA = 28S ribosomal RNA; 18S rRNA = 18S ribosomal RNA



Oligonucleotide Primers for Qualitative and Quantitative PCR

The 5'-oligonucleotide for each polymerase chain reaction (PCR) amplification was designed from a highly conserved region in all known human MHC genes approximately 600 base pairs (bp) upstream of the stop codon. The three adult MHC isoforms (Type I, IIa, and IIx) are identical in this region, which enabled us to use the same "common sense" upstream primer with the following sequence: 5'-GCCAAGAAGGCCATCAC-3'(13,21,22). The 3'-oligonucleotide antisense (downstream) primers used in the PCR reactions were designed from the 3'-untranslated regions of each of the different MHC genes, where the sequences are highly specific for each MHC gene (Table 1) (13,21,22). We have previously shown these primers to amplify PCR products of 623, 655, and 609 bp, respectively, for Type I, IIa, and IIx MHC mRNA (13,21).

MHC mRNA	Antisense Primer	*Sample CDNA	*Control Fragment
Ι	5'CAAGAAGCTGTTACACAGGCTCCAGCATGGGGCTTTGCTGGCACC3'	623 bp	462 bp
IIa	5'GCTTTATTTCCTTTGCAACAGGGTAGAATACACAATAATTACAGAGGG3'	655 bp	510 bp
IIx	5'TGGAGTGACAAAGATTTTCACATTTTGTGCATTTCTTTGGTCACC3'	609 bp	555 bp

Table 1: 3'-Oligonucleotide RNA Primers Used for PCR Amplification

*Sample cDNA is the size of MHC mRNA product from PCR amplification in base pairs (bp); *Control fragment is the size of the internal DNA control standard (bp) co-amplified with the sample cDNA during PCR amplification using the same sense (upstream) and antisense (downstream) primers.

Design of Internal DNA Control Standard for Quantitative PCR

An internal DNA control standard was constructed by a technique of oligonucleotide overlap extension and amplification by PCR with a primer annealing temperature of $55^{\circ}C$ (5,23). The control standard uses the common primer as its 5'-oligonucleotide and contains the three MHC-specific isoform sequences as its 3'-oligonucleotide. The particular, unrelated, "uncommon sequence" originated from a stretch of coding region of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, which has little or no sequence homology to any of the relevant MHC genes.

Cloning of Internal DNA Control Standard for Quantitative PCR

The internal DNA control standard generated from PCR was cloned into a modified transcription vector. Prior to cloning, a T vector was constructed by cutting the pGEM-5Zf(+) vector (Promega, Madison, WI) with the EcoR-V restriction endonuclease and adding a 3'-terminal thymidine to both ends. The control fragment was ligated into the vector with T4 DNA ligase and then cloned into the pGEM-T Vector (Promega, Madison, WI); a vector in which the T7 and SP6 RNA polymerase promoters flank a multiple cloning region within the alphapeptide coding region of the β -galactosidase gene.

At this point, the vector was transformed into 1×10^8 cfu/µg DNA of competent JM109 *E. coli* bacterial cells (Promega, Madison, WI) by spreading aliquots of the ligation reaction onto agar plates containing Luria Broth (LB) and ampicillin [(AMP) Sigma Chemical Co., St. Louis, MO] and the cells grown overnight at 37°C. Random cell colonies were chosen, cultured using LB and AMP, and the PCR product of the internal control standard was then purified from possible contaminants such as primer-dimers and amplification primers with the Wizard Plus Midipreps DNA Purification System (Promega, Madison, WI).

Internal Control Standard Identification and Quantitation

The identity of the PCR products for the internal DNA control standard were verified by their specific lengths in an ethidium-bromide stained agarose gel as a result of restriction digestion. The internal DNA control standard sequence was cut away from plasmid DNA with the EcoR-I restriction endonuclease enzyme (Promega, Madison, WI) and PCR amplified with the respective MHC mRNA oligonucleotides (Table 1) which yielded fragments of 462, 510, and 555 bp, respectively for MHC Type I, IIa, and IIx mRNA (Figure 2). Further verification of the MHC control standard was determined by DNA sequencing using the Silver Sequence DNA Sequencing System (Promega, Madison, WI) according to manufacturer's guidelines.

The amount of restricted internal DNA control standard was quantitated by determining the absorbance at OD_{260} . The purity of the fragment was further determined by determining the ratio of the absorbance between OD_{260}/OD_{280} (5,13,21).

PCR Amplification for Quantitative PCR

One pg of the internal control fragment and 200 ng of cDNA were added to each 25 μ L PCR reaction. For an absolute negative control, a separate PCR reaction was amplified containing no cDNA. Specifically, each PCR reaction contained the following mixtures: [10x PCR buffer, 0.2 μ M dNTP mixture, 1.0 μ M of a cocktail containing both the sense and antisense RNA oligonucleotide primers for MHC and either of the three primers specific for I, IIa, or IIx isoforms (purchased from Ransom Hill Biosciences, Ramona, CA) (Table 1), 2 mM MgCl₂, 1.0 U/ μ L of Taq DNA polymerase (Sigma, St. Louis, MO), and nuclease-free dH₂O]. Each PCR reaction was amplified with a thermal cycler (Bio Rad, Hercules, CA). The amplification profile involved an initial denaturation step at 94°C for 60 sec, followed by 25 cycles, with each cycle consisting of denaturation at 94°C for 45 sec, primer annealing at 50°C for 60 sec, and extension at 72°C for 90 sec, and a final step of 72°C for 180 sec (5). The number of cycles was optimized so that the amplified signal was still on the linear portion of a plot with the yield expressed as a function of the absorbance at OD₂₆₀ and the number of cycles (Figure 3). To assess reliability between amplifications, two separate PCR amplifications were performed for each sample.



Figure 2. Electrophoresis of the internal DNA control standard after digestion with the EcoR-1 restriction endonuclease enzyme and PCR amplified with the respective MHC Type I, IIa, and IIx oligonucleotides. Lane 1 = 1 kb DNA MW marker; Lane 2 = Type IIx internal control standard (555 bp); Lane 3 = Type IIa internal control standard (510 bp); Lane 4 = Type I internal control standard (462 bp).



Figure 3. Evaluation of amplification efficiencies with the quantitative PCR technique. Amplification curves show the number of PCR cycles plotted against the absorbance at OD_{260} for Type I, IIa, and IIx MHC mRNA co-amplified with the internal DNA control standard to ensure that measurements were performed during the linear phase of PCR amplification.

PCR Amplification for Qualitative PCR

Four separate 25 μ L PCR reaction mixtures [1x PCR buffer, 0.2 μ M dNTP mixture, 1.0 μ M of a cocktail containing both the sense and antisense RNA oligonucleotide primers for MHC and either of the three primers specific for I, IIa, or IIx MHC isoforms and 0.5 μ M of a cocktail containing both sense and antisense RNA oligonucleotide primers for a-actin (purchased from Ransom Hill Biosciences, Sonoma, CA), 2.5 U/ μ L of Taq

DNA polymerase (Sigma, St. Louis, MO), 5 μ L of cDNA, and nuclease-free dH₂O] was then amplified with a thermal cycler (BioRad, Hercules, CA). The amplification profile involved denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute. This sequence was repeated for 35 cycles.

Gel Electrophoresis for Quantitative and Qualitative PCR

Aliquots (20 μ L) of the quantitative and qualitative PCR reaction mixtures were electrophoresed in 1.5% agarose gels in 1X Tris-Acetate-EDTA (TAE) buffer (5,13,21). The oligonucleotides within the gels were stained with ethidium bromide (present in the TAE buffer at 1 μ g/mL), illuminated with a UV transilluminator, and photographed with a Polaroid DS 34 camera (Polaroid, Cambridge, MA). Photographs were scanned (Hewlett Packard 5200C, Palo Alto, CA) and the density of bands were determined using the Scion Imaging software package, expressed in arbitrary density units (20).

According to previous guidelines (5), for quantitative PCR only the volume of the OD of a DNA band was corrected to the local background such that it was directly proportional to the amount of DNA over a wide range. Intensity (expressed in arbitrary density units) of each MHC band was divided by the intensity of the internal DNA control standard. The percent content of the mRNA of each MHC isoform was then calculated based on the fraction of specific MHC mRNA-corrected value relative to the total sum of expressed MHC mRNA isoforms in a given sample. For example, %Type IIa MHC mRNA = 100 x (Type IIa band intensity/corresponding control band intensity)/[sum (MHC band/control band) for all three MHC mRNA isoforms](5).



Figure 4. Electrophoresis of quantitative and qualitative PCR products from pre- and post-test muscle biopsy samples for a CON and STR subject. A = Quantitative PCR: Lane numbers indicate the following: 1,5 = Type I MHC mRNA, 623 bp; 2,6 = Type IIa MHC mRNA, 655 bp; 3,7=Type IIx MHC mRNA, 609 bp; 4,8 = absolute negative control (no cDNA); 9=molecular weight PCR marker . In lanes 1,2,3,5,6,7 the lower band corresponds to the respective internal DNA control standard (Type I = 462 bp, IIa = 510 bp, IIx = 555 bp). Also, Lanes 1-4 indicate pre-test and 5-8 post test. B = Qualitative PCR: Lane numbers indicate the following: 1=molecular weight PCR marker, 2,6=alpha-actin [external control Standard (313 bp)], 3,7 = Type I MHC mRNA (623 bp), 4,8 = Type IIa MHC mRNA (655 bp), 5,9 = Type IIx MHC mRNA (609 bp). Lanes 2-5 indicate pre-test and 6-9 post-test.

Myofibrillar Protein Isolation and Quantitation

Myofibrillar protein, still remaining in the phenol phase left over from the total RNA isolation procedure, was isolated with isopropanol, washed with 0.3 M guanidine hydrochloride, and dissolved in 1% SDS (24). Protein content was then determined spectrophotometrically based on the Bradford method at a wavelength of 595 nm and using bovine serum albumin as the standard (25).

Statistical Analysis

Separate two-way (Group x Test) analysis of variance (ANOVA) procedures were employed to determine significant differences for the following criterion variables: 1) body weight, 2) thigh volume, 3) strength, 4) percent mRNA of the three MHC isoforms, and 5) myofibrillar protein content. Separate independent-groups t-tests were performed to determine significant differences for the percent change from pre- to post-testing for the above-mentioned criterion variables. Additionally, to assess reliability between PCR reactions, paired t-tests were performed for each subject within both groups for the pre- and post-training biopsy samples. A probability level of 0.05 was adopted throughout for all statistical analyses.

RESULTS

Body Weight, Muscle Strength, and Thigh Volume

During the course of the study, there was no significant Group x Test interaction ($\underline{F}(2,15)=0.14$, p>0.05) for body weight (Table 2). Also, in regard to percent changes in body weight from pre- to post-test, there were no significant differences (p>0.05) among the two groups.

As a result of the strength training program, significant Group x Test interactions were found for muscle strength (F(2,15)=10.44, p=0.007) and thigh volume (F(2,15)=5.50, p=0.02). Analysis of the main effects revealed no significant differences between CON and STR at pre-test for either strength (F(2,15)=1.56, p=0.85) or thigh volume (F(2,15)=2.13, p=0.23). At the end of the study for CON, there were still no significant differences noted for strength or volume; however, STR was significantly different from CON for both strength (F(2,15)=8.33, p=0.005) and volume (F(2,15)=10.65, p=0.007).

In regard to the percent changes from pre- to post-training for muscle strength, results showed average increases of 2.72 ± 1.56 and $48.53\pm8.10\%$, respectively, for CON and STR. For thigh volume, increases of 0.60 ± 2.44 and $11.86\pm8.67\%$ were seen for CON and STR, respectively. For strength and volume, the changes observed for STR were significantly different (p<0.05) from CON.

MHC mRNA Isoform Abundance for Quantitative PCR

RT-PCR analysis yielded amplification products of 623, 655, and 609 bp, respectively, for the Type I, IIa, and IIx MHC mRNA isoforms (upper bands, Figure 4). Also, co-amplification of the internal DNA control standard yielded amplification products of 462, 510, and 555 bp, respectively for Types I, IIa, and IIx MHC (lower bands, Figure 4A).

For the abundance of MHC isoform RNA present in each of the samples for both groups, significant Group x Test interactions were located for Type I (F(2,15)=13.81, p=0.002), IIa (F(2,15)=11.61, p=0.001), and IIx (F(2,15)=13.81, p=0.002). Analysis of the main effects showed no significant differences between CON and STR at pre-test for the expression of the Type I (F(2,15)=0.614, p=0.55), IIa (F(2,15)=0.81, p=0.46), and IIx (F(2,15)=0.21, p=0.89) MHC mRNA. At the end of the study for CON, there were no significant differences noted in abundance for any of the three MHC isoforms. However, after training for STR, there were significant differences detected in abundance for MHC I MHC mRNA that were significantly different from CON.

Strength Training and Myosin Isoform Expression

In regard to the percent change in mRNA abundance from pre- to post-test for the two groups, results for CON revealed mean \pm SD increases of 1.81 \pm 3.22 and 2.49 \pm 5.82%, and a decrease of 1.88 \pm 8.68%, respectively, for the Type I, IIa, and IIx MHC isoforms (Table 2). In addition, for STR there were average increases of 17.08 \pm 5.35 and 15.89 \pm 8.32%, and a decrease of 49.69 \pm 22.38%, respectively, for Type I, IIa, and IIx that were all significantly different from CON (p<0.05).

Table 2: Group Means (±SD) for the Criterion Variables					
	Pre-Test	Post-Test	% Change		
		Group CON			
Body Weight (kg)	75.03±3.21	75.78±3.45	1.06±0.54)		
Rel. Strength	12.70±0.41	2.74±0.41	1.50±1.49)		
Thigh Volume (m ³)	5.58±1.11	5.65±1.09	0.06±2.44)		
Protein (mg /ml)	21.94±2.08	23.96±0.97	9.49±5.99)		
% MHC I mRNA [#]	44.03±7.86	44.65±6.92	1.41±4.63)		
% MHC IIa mRNA [#]	41.07±6.43	41.87±4.94	2.49±5.82)		
% MHC IIx mRNA [#]	13.90±4.63	13.48±3.75	1.86±8.68)		
MHC I mRNA [@]	984.56±56.97	992.65±82.76	0.82±1.47)		
MHC IIa mRNA [@]	756.26±46.83	766.45±58.36	1.34±2.67)		
MHC IIx mRNA [@]	328.58±36.87	340.17±38.95	3.52±2.79)		
		Group STR			
Body Weight (kg)	80.23±10.01	85.29±10.77	6.31±2.45		
Rel. Strength	2.69±0.25	3.88±0.51*	44.09±6.36**		
Thigh Volume (m ³)	7.46±1.36	8.39±1.97*	10.94±7.17**		
Protein (m /ml)	24.39±2.99	47.35±12.77*	92.09±24.68**		
% MHC I mRNA [#]	42.50±8.32	49.51±9.77*	17.08±5.35**		
% MHC IIa mRNA [#]	37.28±7.89	42.81±6.95*	15.89±8.32**		
% MHC IIx mRNA [#]	20.17±2.44	7.67±3.63*	-49.69±22.38**		
MHC I mRNA [@]	976.47±66.37	1,166.95±98.82*	19.51±8.34**		
MHC IIa mRNA [@]	781.58±56.28	986.84±65.60*	26.26±12.84**		
MHC IIx mRNA [@]	404.14±47.03	324.71±23.93*	-19.65±9.27**		

*Significant from CON at the post-test (p < 0.05); **Significant change from pre- to post-test (p < 0.05); Results for % MHC mRNA are based on the arbitrary densities of PCR amplified MHC products relative to the co-amplified internal standard as illustrated in Figure 4. The equation for calculating % MHC mRNA is described in the Methods section and elsewhere (5). #quantitative PCR; @qualitative PCR (numbers expressed in arbitrary density units).

Considering intra-assay reliability related to the consistency in mRNA abundance between the two PCR amplifications within each group, results showed no significant differences (p>0.05) for the three MHC isoforms. Additionally, the mean intra-assay coefficient of variations for the two PCR amplifications for CON were 0.03±0.04, 0.02±0.04, and 0.03±0.03 for Type I, IIa, and IIx, respectively, while the respective coefficients for STR were 0.025±0.005, 0.024±0.01, and 0.014±0.14.

Qualitative Comparison of MHC mRNA Isoform Expression

Myosin heavy chain isoform mRNA expression patterns were compared between all subjects within the two groups. Expression was determined by pseudo-quantification from the arbitrary density units of stained gels. No significant differences were detected at the pre-test for either CON or STR. However, at the post-test for STR significant differences were found for Type 1 (F(2,15)=26.43, p=0.002), IIa (F(2,15)=6.38, p=0.002), and IIx (F(2,15)=9.60, p=0.001) MHC mRNA. In regard to the percent change in expression between the pre- and post-test for MHC mRNA, CON showed no apparent differences in the Type I, IIa, and IIx isoforms. However, STR

was expressing significantly more of the Type I ($19.51\pm8.34\%$) and Type IIa ($26.26\pm12.84\%$) MHC isoforms with a concomitant decrease in the Type IIx ($19.65\pm9.27\%$) mRNA isoform (Table 2 & Figure 4B).

Myofibrillar Protein Content

For myofibrillar protein content, a significant Group x Test interaction (F(2,15)=9.74, p=0.006) was located. Analysis of the main effects revealed no significant difference between CON and STR at pre-test (F(2,15)=2.31, p=0.63). However, at the end of the study protein content for CON was not significant while STR was significantly different from CON (F(2,15)=11.44, p=0.008).

In regard to the percent changes from pre- to post-training for myofibrillar protein content (Table 2), results showed average increases of 9.49 ± 5.99 and $92.09\pm24.68\%$, respectively, for CON and STR. The changes observed for STR were significantly different (p<0.05) from CON.

DISCUSSION

In terms of the physiology of muscle adaptation to strength training exercise, it is important for exercise physiologists to understand the molecular mechanisms in which MHC genes may be sensitive to the intensity of the external training load. As a result, we may become more strategic at designing and implementing strength training programs that effectively manipulate the expression of the MHC genes which play a significant role in increasing muscle strength and hypertrophy. Studies investigating the effects of weight training on MHC gene expression in humans is limited, and most of the available data involves rodent models.

As can be seen from Figure 4B, qualitative PCR only provides the ability to pseudo-quantify MHC isoform mRNA expression from the intensity of ethidium bromide staining and normalized to alpha-actin as an external, positive control standard. As a result, qualitative PCR is only able to detect the presence or absence of MHC mRNA in a given sample and does not provide the ability for accurate quantitation. Ethidium bromide staining with agarose gel electrohyporesis and comparison of the target mRNA to the control mRNA only highlights differences in staining intensities which can possibly illustrate differences in mRNA content (Figure 4B). However, without knowing the concentration of the starting template, our attempts to normalize expression compared to alpha-actin is problematic for two reasons: 1) there is no quantification and standardization of the initial starting concentration of cDNA, and 2) since different primer pairs were used to amplify the MHC compared to the alpha-actin, fully correcting for efficiency of the PCR amplification is not possible. Therefore, this method of qualitative PCR is strictly descriptive and does not lend itself well to the determining of the abundance of MHC isoform mRNA as does our quantitative technique (Figure 4A).

Based on our modifications from previously established guidelines (5), in this study we employed a method of competitive PCR specific for quantitating MHC isoform mRNA expression in humans. The PCR method described here involves a constitutively expressed gene that is amplified simultaneously in the same reaction as the target MHC mRNA sequence, and corrected relative to the constitutive gene. Also, to correct for variability in PCR reaction efficiency, a known amount (1 pg) of an internal DNA control standard was added to each reaction and amplified simultaneously with the MHC mRNA by using the same set of primers as the MHC mRNA but yielding a product of different size. As can be seen in Figure 4A, each PCR reaction resulted in co-amplification of the MHC mRNA and the internal DNA control standard. The upper band corresponds to the higher molecular weight MHC mRNA while the lower band corresponds to the lower molecular weight internal DNA control fragment. The intensity of the upper bands is indicative of the abundance of the specific MHC mRNA in the total RNA sample relative to the internal DNA control standard. This method of competitive RT-PCR can accurately estimate the abundance of MHC mRNA in a given sample (5); although, this method only provides the accurate comparison of the relative amount of mRNA expression of each MHC isoform in a given sample.

The results of this study are in agreement with other studies which have shown that periods of 8-19 wks of highintensity strength training were effective in significantly increasing strength (6,7,8). In regard to thigh volume, however, there were no significant changes (p>0.05) in thigh skin fold thickness noted for CON ($1.56\pm0.04\%$) and STR ($1.12\pm0.02\%$) (data not shown). Therefore, the primary increase in volume measurements most likely came from thigh girth. It is possible that increases in thigh volume could have been due to increases in intramuscular and/or interstitial fluid levels; however, based on the observed increases in muscle strength and myofibrillar protein content it is more reasonable to assume that the increased volume occurred as a result of hypertrophy of the thigh musculature.

The present results illustrate significant training-induced increases in myofibrillar protein that are in agreement with previous studies (9,10). During weight training-induced hypertrophy, there is an increased accumulation of non-contractile and contractile proteins. The net quantity of these proteins is a result of synthesis vs. degradation rates and it appears that myofibrillar protein synthesis seems to be regulated at the translational or post-translational level (26). The present results also show that pre-exercise steady- state MHC mRNA levels are similar to other research involving humans (11) in that non-exercised muscle expresses a greater percentage of the Type I isoform with IIa also expressing more than IIx. Our results also illustrate that high-intensity strength training seems to cause fast to slow transitions in MHC isoform mRNA expression that seem to parallel the transitions in MHC protein isoforms seen in other studies (6,7,8). The fact that we showed increased in MHC isoform mRNA expression compared to other studies which did not (11,12) could be due to the higher training intensity and/or length of the training period of the present study.

The results of the present study also indicate a significant increase in the abundance of Type I and IIa MHC mRNA after training coupled with a concomitant decrease in the abundance of Type IIx. The decreased abundance in the Type IIx isoform could be due to the premise that the IIx gene is known as the "default gene" (27) and is very sensitive to high intensity resistance training and altered mechanical loading and differing muscle contraction velocities. A study with rodents has shown increased Type IIx MHC mRNA expression after high-resistance treadmill training (28). Therefore, based on the mode and intensity of the training stimulus, and possibly the animal model used, the Type IIx gene may have the ability to either preferentially increase or decrease its expression based on the recruitment profiles of the muscle fibers during the activity. Since high-intensity strength training involves large contractile efforts but slow velocity of shortening of the muscle fibers, the Type IIb muscle fibers may not be as heavily recruited as the Type I and IIa fibers (29). As a result, muscle fiber recruitment profiles related to training intensity (load) and speed of contraction may have played a role in dictating down-regulation in the expression of the Type IIx gene. As a result, this could imply that, in regard to the principle of specificity, the weight training intensity may actually play a key role in the differential expression of the Type I, IIa, and IIx MHC genes. Therefore, training regimens should be specific based on the type of exercsie and/or sport of participation.

Increasing the mRNA template available for translation and protein synthesis in muscle during hypertrophy can be a reflection of alterations in transcriptional efficiency, transcriptional capacity, and/or mRNA stability. The results of this study indicate that increases in Type I and IIa MHC mRNA abundance occur concomitant with increases in myofibrillar protein content. Therefore, a possible relationship may exist between the up-regulation of MHC Type I and IIa gene expression and high-intensity strength training that is necessary to support the training-induced increases in muscle strength and thigh volume observed in this study.

It should be noted, however, that the method of mRNA quantitation employed in this study is only reflective of steady- state levels of mRNA at the time of biopsy. When interpreting the MHC isoform mRNA expression during periods of physical inactivity, MHC mRNA and protein expression is often shown to occur in the same direction (26). However, during periods of training and muscle hypertrophy, a mismatch seems to occur

between the relative content of MHC mRNA and protein levels which may be reflective of differential timing between up- and down-regulation of MHC transcripts and their protein isoforms (29). This mismatch between pre-translational and translational activity may be due to the transient (0-4 hours post-exercise) nature of MHC gene expression compared to MHC protein expression (3-36 hours post-exercise) (26). As a result of the transient nature of mRNA expression, more than a single time point of mRNA determination may be required to confirm absolute interpretations of regulatory sites in MHC gene expression.

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