Stimulation of myofibrillar synthesis by exercise is mediated by more efficient translation of mRNA

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Welle, Stephen, Kirti Bhatt, and Charles A. Thornton. Stimulation of myofibrillar synthesis by exercise is mediated by more efficient translation of mRNA. *J. Appl. Physiol.* 86(4): 1220–1225, 1999.—Resistance exercises stimulate protein synthesis in human muscle, but the roles of changes in mRNA concentrations and changes in the efficiency of mRNA translation have not been defined. The present study was done to determine whether resistance exercise affects concentrations of total RNA, total mRNA, actin mRNA, or myosin heavy-chain mRNA (total and isoform specific). Eight subjects, 62–75 yr old, performed unilateral knee extensions at 80% of their one-repetition-maximum capacity on *days 1*, *3*, and *6* of the study. On *day 7*, biopsies of exercised and nonexercised vastus lateralis muscles were obtained. Myofibrillar synthesis was determined by stable-isotope incorporation, and mRNA concentrations were determined by membrane hybridization and PCR-based methods. The exercise stimulated myofibrillar synthesis [30 ± 6 (SE)%] without affecting RNA or mRNA concentrations. The effect of exercise on protein synthesis in individual subjects did not correlate with the effect on total RNA and mRNA concentrations. These data suggest that the stimulation of myofibrillar synthesis by resistance exercise is mediated by more efficient translation of mRNA.

actin mRNA; myosin heavy-chain mRNA; polyadenylated RNA; resistance exercise; protein synthesis

RESISTANCE EXERCISES STIMULATE muscle protein synthesis in humans (5, 8, 29, 32, 33). This response could result from more mRNA molecules being translated or from an increased rate of translation of each molecule of mRNA. Based on the observation that muscle protein synthesis increased significantly after resistance exercises without a change in the muscle concentration of total RNA, Chesley et al. (8) concluded that the increased muscle protein synthesis is caused by posttranscriptional events. However, it is not clear that such a conclusion can be made based on measurements of only the total RNA concentration. The contribution of mRNA to the total RNA concentration is very small, so that there could be a large increase in mRNA concentration without a detectable change in total RNA. If the availability of ribosomes or other translation factors is rate limiting for protein synthesis in human muscle, then the total mRNA concentration should not be a significant determinant of the protein synthesis rate. However, if there are "spare" ribosomes, initiation factors, and elongation factors available to translate mRNA, then an increased mRNA concentration could lead to increased protein synthesis. The studies of Wernerman et al. (30, 31) indicated that about one-half of the RNA in human muscle is in free ribosomes or ribosomal subunits, suggesting that an increased mRNA concentration might facilitate protein synthesis if other translation factors are available. Thus we examined whether or not the concentration of mRNA in human muscle was affected by a resistance exercise protocol that consistently increased myofibrillar protein synthesis (29).

Regardless of the effect of resistance exercise on total mRNA concentrations, the effect on the relative abundances of particular mRNA species is an important factor in the adaptation to exercise. Animal studies have indicated that the abundance of α-actin mRNA is sensitive to muscle activity (6, 18, 19). The pattern of expression of the various isoforms of myosin heavy chain is also sensitive to muscle activity (7, 14, 20). In humans, there is evidence that resistance training increases expression of type 2a myosin heavy chain relative to type 2b myosin heavy chain, resulting in a reduced percentage of the faster contracting type 2b fibers and a greater percentage of the slower type 2a fibers (1, 12, 15–17, 25–27). The ratio of type 2a to type 1 myosin heavy chain was higher in the vastus lateralis muscle of old weight lifters than in old sedentary men (16), but resistance training for 19 wk did not reduce the percentage of the type 1 isoform in the vastus lateralis of younger men (1). A gene for a type 2b myosin heavy chain has not been identified in humans. Human muscle fibers that have been classified as type 2b express the type 2x isoform (which also has been termed type 2d) rather than type 2b (9, 24). Only three myosin heavy-chain isoforms, types 1, 2a, and 2x, appear to be expressed in most adult human muscles. Changes in the relative abundance or rate of translation of the mRNAs encoding these different isoforms must precede major changes in the protein isoform composition by several weeks because of the slow turnover of myosin heavy chains in human muscle (4). This delay between exercise-induced changes in myosin heavy-chain mRNA isoform expression and alterations in protein isoform distribution is the basis for mismatches between mRNA and protein expression in individual fibers during training (3). Thus the myosin heavy-chain protein isoform concentrations may not be representative of gene expression early in a training program. We, therefore, examined the effect of resistance exercise on concentrations of mRNAs encoding the major adult isoforms of myosin heavy chain, as well as the abundances of total myosin heavy-chain mRNA and actin mRNA.

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METHODS

Subjects. The subjects were five men and three women, 62–75 yr of age. None of the subjects had any medical conditions or disabilities that interfered with their ability to perform the exercise, and they were in good health according to medical history, physical examination, electrocardiogram, and laboratory tests (thyroid-stimulating hormone, fasting glucose and electrolytes, liver enzymes, creatinine, urea nitrogen, albumin and total protein, complete blood count). None was involved in any form of resistance training. Written consent was obtained from all participants, after procedures and risks were explained verbally and in a written consent form. The project was approved by the University of Rochester Research Subjects Review Board.

These subjects were part of a larger group (9 men and 9 women) participating in a study of the effect of protein intake on the stimulation of myofibrillar protein synthesis by resistance exercise (29). A set of both sedentary and exercised muscle samples was not available from the other 10 subjects because all of the muscle was used for determination of protein synthesis.

Exercise protocol. The subjects exercised the quadriceps muscles of the right leg on a Universal (Cedar Rapids, IA) knee-extension machine. Proper form for lifting and breathing was demonstrated before the test, and the same investigator (S. Welle) supervised all testing and exercise sessions. On *day 1*, the one repetition maximum (1RM) of the right knee extensor was determined by progressive addition of weight in 2.2-kg increments (with rest periods) until a subject could not perform full extension. The 1RM was defined as the highest weight that could be lifted once with full knee extension. Subjects performed 4 sets of 10 repetitions, with brief rests between sets, at ~80% of the 1RM on *days 1* and *4*. On *day 6*, at ~1500, they performed 5 sets of 10 repetitions. Subjects were encouraged to raise and lower the weights smoothly and slowly (2–3 s). No subject complained of any major muscle soreness, and all were able to complete the sets, although some were unable to attain full extension for all lifts. Subjects were asked not to perform any strenuous activities involving the left leg but to otherwise continue with their normal activities.

Subjects went to the University of Rochester General Clinical Research Center immediately after exercising on *day 6*. There they received a standard meal, fasted overnight, and did not perform any activities more strenuous than walking.

Muscle biopsies. Muscle biopsies of the vastus lateralis were obtained bilaterally, with the muscle under local anesthesia with lidocaine, ~23 h after the final exercise session. The samples were frozen in liquid nitrogen within 10–30 s of being removed and then stored at −70°C until analysis. During the 7-h period before the muscle biopsies, the subjects had received small liquid meals every 30 min and had received an infusion of [^{13}C]leucine to trace myofibrillar protein synthesis. Details of the meals and protein synthesis measurements are presented elsewhere (29). All subjects had the same energy intake relative to energy requirements, but protein intake was low (7% of energy, $n = 3$), normal (14% of energy intake, $n = 3$), or high (28% of energy intake, $n = 2$). The level of protein intake did not affect the stimulation of myofibrillar protein synthesis (29). Obtaining simultaneous biopsies from exercised and sedentary muscles of each subject controlled for individual differences in nutritional, hormonal, or other factors other than the exercise that might influence gene expression in muscle.

RNA extraction. The frozen tissue was homogenized with a Polytron in 1 ml of Tri-Reagent (Molecular Research Center, Cincinnati, OH). The homogenate was centrifuged at 12,000 *g* for 15 min, and the aqueous supernatant containing the RNA was transferred to a separate tube for ethanol precipitation. The precipitate was dissolved in RNase-free water (2 μl/mg tissue) and stored at −70°C. The amount of RNA extracted from the tissue was determined by absorbance of ultraviolet light at 260 nm, with background compensation for the absorbance at 320 nm, by using a GeneQuant RNA/DNA calculator (Pharmacia Biotech, Piscataway, NJ) equipped with a 7-μl microcell.

Total mRNA. Total RNA (0.4 μg) was applied to a positively charged nylon membrane with a slot-blotting apparatus, and the polyadenylated RNA bound to the membrane was hybridized with a ^{32}P-labeled oligo(dT)$_{18}$ probe by using procedures that were described previously (28). The relative amount of probe bound to each slot was quantified with a PhosphorImager, by using the ImageQuant software provided by the manufacturer (Molecular Dynamics, Sunnyvale, CA). Data are presented as a percentage of the mean value in sedentary muscle.

Actin mRNA. A slot-blot procedure, with the use of a digoxigenin-labeled cDNA probe complementary to sarcomeric actin mRNA, was used to measure the actin mRNA in 0.4 μg of total RNA. Details of the procedure have been described before (28). The specificity of the probe for actin mRNA was demonstrated by Northern blotting and by the absence of any signal with rRNA or yeast RNA. Relative intensities of the chemiluminescent hybridization signals were determined by densitometry of the X-ray film with a laser scanner (Molecular Dynamics).

Total myosin heavy-chain mRNA. A slot-blot procedure, with the use of a riboprobe that hybridizes with part of the coding region of the mRNAs encoding different isoforms of myosin heavy chain, was used to measure the total myosin heavy-chain mRNA in 0.4 μg of total RNA. Details of the procedure have been described before (28). The specificity of the probe for myosin heavy-chain mRNAs was demonstrated by Northern blotting and by the absence of any signal with rRNA, mouse liver RNA, or yeast RNA. Relative intensities of the hybridization signal were detected with a PhosphorImager as described in *Total mRNA*.

Isoform-specific myosin heavy-chain mRNA. Because of the limited amount of RNA available for analysis, we used PCR-based assays to determine whether exercise affected the relative concentrations of the mRNAs encoding different isoforms of myosin heavy chain. cDNA was synthesized by reverse transcription of 2 μg of total RNA from each sample, with oligo(dT) as the primer. The concentrations of myosin heavy-chain cDNAs were determined by competitive PCR assays. Primers (obtained from Integrated DNA Technologies, Coralville, IA) were chosen to amplify the 3′-untranslated regions of type 1, type 2a, and type 2x myosin heavy-chain cDNA sequences (Table 1). Automated sequencing by the University of Rochester Core Nucleic Acid Laboratory verified the identity of the PCR products. The internal standard DNAs were made by using the strategy described by Riedy et al. (21). This method produces DNA that is identical to the segment of sample cDNA being amplified, except that it has a short internal deletion (16–22 bases in this study) so that its PCR product can be differentiated from that of the sample cDNA. Twenty cycles of PCR were used to amplify the cDNAs and internal standards. The amount of standard DNA used for each assay was adjusted so that mean sample and standard product concentrations would be approximately the same (2 pg for type 1 cDNA assays, 1 pg for type 2a assays, 0.5 pg for type 2x assays). In all assays we used 1 μl of the reverse transcription solution (containing cDNA obtained from 33 ng

Table 1. *PCR primers*

Isoform	Forward Primer	Reverse Primer
Type 1	CTTTGCCACATCTTGATCTG AAGCCCCATGCTGGAGC*	TGCTTTATTCTGCTTCCTCC
Type 2a	ATGTCCTGATGCCATGG ATGTGACATCTTTGGTCATTTC*	CAAACTACCCTATGCTTTATTTCC
Type 2x	TTTATCTAACTGCTGAAAGGTGAC ATGTGAAAATCTTTGTC*	TCTCCAAAAGTCATAAGTACAAAATG

*These are the extensions added to the forward primers in order to produce standard DNA with 16–22 bases deleted from the full-length cDNA.

total RNA) as the source of myosin heavy-chain cDNA. PCR products were separated on a polyacrylamide gel, stained with SYBRgreen (Molecular Probes, Eugene, OR), and detected with a FluorImager (Molecular Dynamics). The ratio of sample to standard band intensity was calculated with ImageQuant software (Molecular Dynamics). Standard curves (see RESULTS) verified that sample-to-standard-product ratios were proportional to the initial concentration ratios of cDNA to standard DNA after 20 rounds of PCR amplification.

Data analysis. Values are presented as means ± 1 SE. Paired *t*-tests were used to determine the statistical significance of differences between mean values of the exercised and sedentary muscles. Two-tailed *P* levels are reported. Correlations between the stimulation of protein synthesis by exercise in each subject and the difference in RNA or mRNA levels between sedentary and exercised muscle are reported as the Pearson *R* coefficient.

RESULTS

The rate of myofibrillar synthesis was higher in the exercised muscle than in the sedentary muscle in all subjects. The stimulation of myofibrillar synthesis ranged from 8 to 58%, with a mean stimulation of 30 ± 6% ($P = 0.0016$).

The mean amounts of total RNA, mRNA (polyadenylated RNA), total myosin heavy-chain mRNA, and actin mRNA (Table 2) were not significantly different in sedentary and exercised muscles ($P = 0.10$ for total mRNA per tissue mass, $P > 0.40$ for all other variables). Figures 1–3 show the slot blots, which indicate some heterogeneity among the individual subjects in the difference in mRNA concentrations between sedentary and exercised muscles. However, differences in RNA and mRNA abundances between sedentary and exercised muscles in individual subjects did not correlate significantly with the stimulation of myofibrillar protein synthesis induced by exercise (Table 2).

Varying amounts of cDNAs corresponding to the 3′-untranslated regions of the myosin heavy-chain mRNAs were amplified by PCR in the presence of fixed amounts of internal standard DNAs. Standard curves indicated that the ratio of cDNA to standard after 20 cycles of PCR was reproducible and proportional to the initial ratio (Fig. 4). Table 3 shows relative concentrations of type 1, 2a, and 2x myosin heavy-chain cDNAs produced by reverse transcription of RNA extracted from sedentary and exercised muscles. Exercise did not affect the mean abundance of these mRNAs ($P > 0.25$), confirming the results of the slot-blot assay for total myosin heavy-chain mRNA. The mean myosin heavy-chain 2x mRNA abundance in the exercised muscle was about one-third less than in the control muscle, but the effect was variable among individuals and not statistically significant ($P = 0.26$).

The mean contribution of the various myosin heavy-chain mRNAs to the total myosin heavy-chain mRNA mass was estimated from the amount of internal stan-

Table 2. *Total RNA, total mRNA, myosin heavy-chain mRNA, and actin mRNA in sedentary and exercised muscles and correlation between individual %differences in RNA abundance and individual %differences in myofibrillar protein synthesis rates*

Variable	Sedentary Muscle	Exercised Muscle	Δ%RNA vs. Δ%Synthesis (*r*)
Total RNA, μg/mg tissue	0.54 ± 0.04	0.60 ± 0.06	+0.08
mRNA/tissue mass	100 ± 9	117 ± 6	+0.06
mRNA/total RNA	100 ± 9	110 ± 12	−0.01
Myosin heavy-chain mRNA			
Per tissue mass	100 ± 13	100 ± 5	−0.20
Per total RNA	100 ± 12	93 ± 8	−0.48
Actin mRNA			
Per tissue mass	100 ± 16	100 ± 9	−0.11
Per total RNA	100 ± 13	92 ± 5	−0.24

Values for sedentary and exercised muscles are means ± SE. All values except total RNA and *r* expressed as %mean value in sedentary muscle.

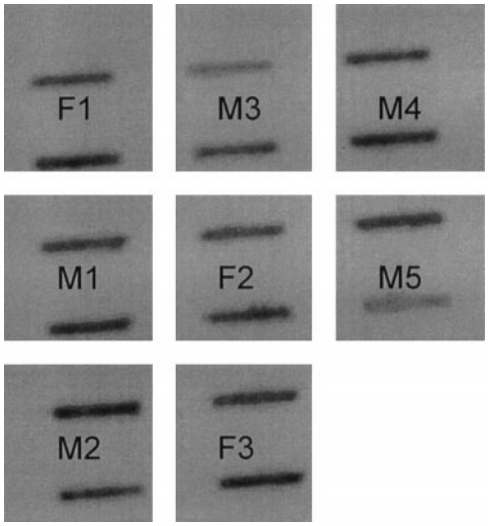

Fig. 1. Slot-blot determination of total mRNA. Each slot shows signal from ^{32}P-labeled $(dT)_{18}$ probe hybridized with 0.4 μg total RNA. Each panel shows results with use of RNA obtained from control (*top* band) and exercised muscle (*bottom* band) of a single subject (*subjects F1, F2, F3, M1, M2, M3, M4, M5*). F, female subject; M, male subject.

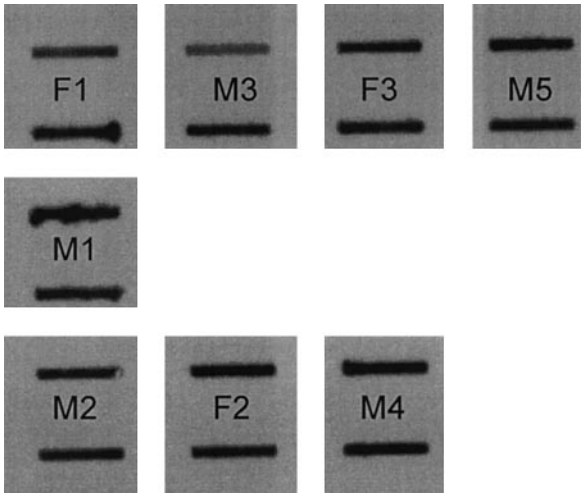

Fig. 2. Slot-blot determination of actin mRNA. Each slot shows signal from digoxigenin-labeled probe hybridized with 0.4 μg total RNA. Each panel shows results with use of RNA obtained from control (*top* band) and exercised muscle (*bottom* band) of a single subject.

dard DNA used in each assay (see METHODS) and the mean cDNA/standard DNA band intensity ratios. In sedentary muscles, type 1 myosin heavy-chain mRNA accounted for 58% of the total myosin heavy-chain mRNA, type 2a for 29%, and type 2x for 13%. In exercised muscles, the distribution was similar (61% type 1, 30% type 2a, 9% type 2x).

DISCUSSION

These results confirm the report of Chesley et al. (8) that, in human muscle, the stimulation of protein synthesis by resistance exercise does not require an increase in the concentration of total RNA. Total RNA consists primarily of rRNA. Hence it appears that the availability of ribosomes is not a rate-limiting factor in human muscle protein synthesis. This conclusion is consistent with the reports of Wernerman et al. (30, 31), which indicated that about one-half of the rRNA in human muscle is in the form of ribosomal subunits or monoribosomes. The increase in protein synthesis must result from recruitment of a greater proportion of the

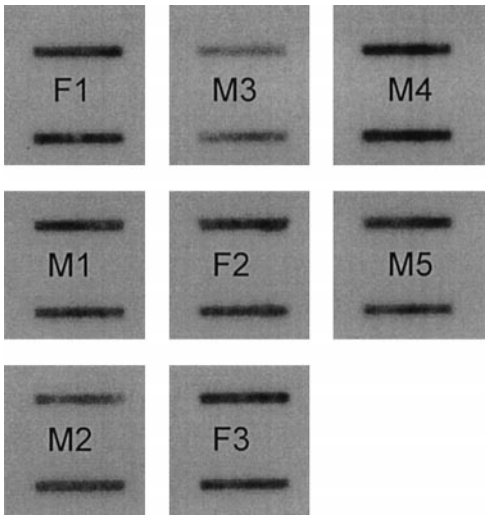

Fig. 3. Slot-blot determination of total myosin heavy-chain mRNA. Each slot shows signal from ^{32}P-labeled riboprobe hybridized with 0.4 μg total RNA. The probe is complementary to a segment of type 2a coding sequence that has high homology with other isoforms. Each panel shows results with use of RNA obtained from control (*top* band) and exercised muscle (*bottom* band) of a single subject.

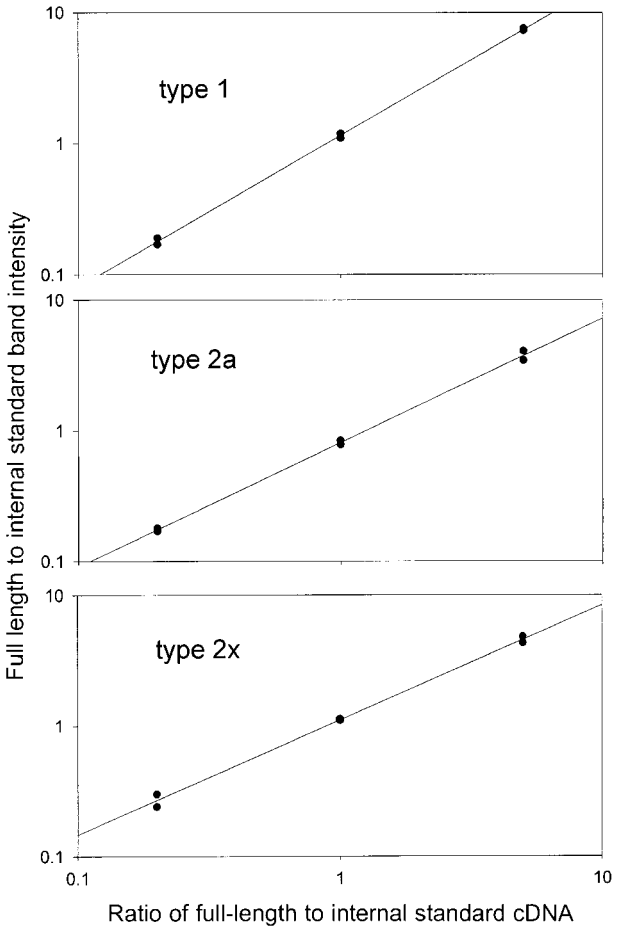

Fig. 4. Standard curves demonstrating that cDNA-to-internal standard product ratios are reproducible and proportional to initial ratios after 20 cycles of PCR. cDNA and internal standard DNA were produced by PCR and then quantified by ultraviolet absorbance. Various amounts of this cDNA were then amplified by 20 cycles of PCR in presence of a fixed amount of standard DNA. Duplicate analyses are shown at all points. Those that appear to be single points are overlapping duplicates.

Table 3. *Effect of exercise on concentrations of mRNAs encoding various isoforms of myosin heavy chain*

Myosin Heavy-chain Isoform	Sedentary Muscle	Exercised Muscle
Type 1		
Per tissue mass	100 ± 17	104 ± 13
Per total RNA	100 ± 12	100 ± 12
Type 2a		
Per tissue mass	100 ± 12	111 ± 14
Per total RNA	100 ± 10	100 ± 7
Type 2x		
Per tissue mass	100 ± 30	64 ± 16
Per total RNA	100 ± 25	75 ± 18

Values are means ± SE and are expressed as %mean value in sedentary muscle.

ribosomes for protein synthesis, from an enhanced rate of recycling of ribosomes after each round of peptide-chain elongation, from more rapid peptide-chain elongation by each ribosome, or from some combination of these possibilities.

An increased concentration of a particular mRNA species generally leads to an increase in the synthesis of the protein encoded by that mRNA. However, it is unclear whether or not the abundance of total mRNA is ever a determinant of changes in overall protein synthesis in muscle. In the present study, there was a consistent increase in myofibrillar protein synthesis in exercised muscles even though there was no significant change in the concentration of total mRNA or the mRNAs encoding the most abundant myofibrillar proteins (actin and myosin heavy chain). Thus the overall stimulation of protein synthesis appears to be mediated by posttranscriptional processes, although increased transcription of genes encoding translation factors cannot be excluded as a potential mechanism.

Mammalian muscle fibers have varying functional properties that depend on the relative expression of genes encoding metabolic enzymes and various isoforms of contractile proteins. Several methods of fiber classification have been described, but the most popular method relies on distinguishing fibers according to their myosin heavy-chain isoform expression. Fibers expressing mainly type 1 myosin heavy chain have the slowest contraction velocities. Type 2a, 2x, and 2b fibers have progressively faster contraction velocities. Human muscle fibers that have been classified as type 2b express type 2x myosin heavy chain rather than type 2b (9, 24). Properties other than maximal contraction velocity are related to fiber type, including rate of tension development, specific tension, phosphocreatine content, and ATP depletion during contractions (10, 22, 23). Thus changes in the pattern of myosin heavy-chain isoform expression are likely to have significant functional consequences. Previous research has demonstrated that resistance exercise can alter the pattern of myosin heavy-chain isoform expression (1, 12, 15–17, 20, 25–27) and that such effects can be induced at the mRNA level very rapidly (7, 13). However, changes in myosin heavy-chain isoform expression do not always occur during strength training (2). In the present study, we did not detect any consistent effect of three resistance exercise sessions on the concentrations of the mRNAs encoding the various myosin heavy-chain isoforms, although there was a trend toward reduced concentrations of type 2x mRNA.

A recent preliminary report indicated that resistance exercise of the quadriceps reduced the concentrations of type 1, 2a, and 2x myosin heavy-chain mRNAs (relative to glyceraldehyde-3-phosphate dehydrogenase mRNA) after only seven exercise sessions and reduced concentrations of type 1 and 2x mRNAs after a single exercise session (13). Type 2x mRNA declined more than the other types. Although our data are consistent with the possibility that type 2x mRNA is downregulated by resistance training, they do not confirm the finding that strength training rapidly reduces total myosin heavy-chain mRNA expression. Both membrane hybridization and PCR methods indicated that myosin heavy-chain mRNA concentrations did not decline in response to three resistance training sessions. The small decline in the average type 2x mRNA concentration had little effect on total myosin heavy-chain mRNA concentration, for this isoform is the least abundant.

Our method for determining myofibrillar synthesis does not distinguish the relative synthesis rates of the numerous myofibrillar proteins. In theory, it is possible that the increase in myofibrillar synthesis is related to enhanced production of proteins other than actin and myosin heavy chain and that mRNAs encoding other myofibrillar proteins were more abundant in exercised muscles. However, it is unlikely that there would be upregulation of the synthesis of other myofibrillar proteins without a similar effect on actin and myosin heavy chain, which comprise a large portion of the myofibrillar mass. A recent preliminary report indicated that both actin and myosin heavy-chain synthesis are increased within 2 wk of the initiation of a strength training program (11). Thus the present study provides strong evidence that the increase in myofibrillar protein synthesis induced by resistance training is mediated by more efficient mRNA translation.

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