Myosin heavy chain mRNA transform to faster isoforms in immobilized skeletal muscle: a quantitative PCR study

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Jänkälä, Heidi, Veli-Pekka Harjola, Niels Erik Petersen, and Matti Härkönen. Myosin heavy chain mRNA transform to faster isoforms in immobilized skeletal muscle: a quantitative PCR study. *J. Appl. Physiol.* 82(3): 977–982, 1997.—A quantitative polymerase chain reaction (PCR) method was used to measure the quantities of type I, IIa, IIx, and IIb myosin heavy chain (MHC) mRNA in total RNA preparations of the soleus, gastrocnemius, and plantaris muscles of normal and hindlimb-immobilized rats. Type IIx and even type IIb MHC mRNA were demonstrated at extremely low levels in normal soleus, $2.1 \pm 0.4 \times 10^5$ and $5.0 \pm 0.2 \times 10^5$ molecules of mRNA per microgram total RNA, respectively. Immobilization for 1 wk significantly altered the gene expression of MHC isoforms. In soleus, both type IIx and IIb MHC genes became significantly upregulated, 24-fold ($P < 0.005$) and 2.6-fold ($P < 0.05$), respectively. In gastrocnemius, the level of type IIa MHC mRNA decreased by 51% ($P < 0.01$) and the level of type IIx MHC mRNA increased by 140% ($P < 0.05$). In plantaris, the level of type IIa MHC mRNA decreased by 58% ($P < 0.005$). In conclusion, immobilization changed the MHC mRNA profile in three different types of skeletal muscle toward faster isoforms. The quantitative results permit reliable evaluation of changes in mRNA levels.

immobilization; number of myosin heavy chain mRNA molecules; quantitative reverse transcription-polymerase chain reaction

IMMOBILIZATION is widely employed in the treatment of musculoskeletal injuries. However, it rapidly leads to skeletal muscle atrophy and thereby delays recovery from trauma.

Skeletal muscle fibers have been divided into slow (type I) and fast (type II) fibers. In rat skeletal muscle, type II fibers can be further divided into IIa, IIx, and IIb fibers by histochemical myosin adenosinetriphosphatase (ATPase) staining or immunohistochemistry. Myosin heavy chain (MHC) isoforms are responsible for the differences in myosin ATPase activity and histochemical staining properties and influence the maximum velocity of contraction in distinct fiber types. One slow MHC isoform (type I MHC) and three fast MHC isoforms (types IIa, IIb, and IIx MHC) have been identified in rat skeletal muscle (4). Type I MHC dominates in muscle fibers that are primarily used for antigravity functions, i.e., maintaining posture. Type II MHC are expressed in muscle regions used during sustained locomotion (type IIa and IIx MHC) or high-power-output activity (type IIx and IIb MHC) (1).

All the MHC are encoded by distinct genes that are expressed in a tissue-specific and developmentally regulated manner (12, 23, 25). Changes in muscle activity alter MHC gene expression and phenotype and affect different types of muscles in characteristic ways (7, 16). Information concerning the early changes in disuse atrophy could be useful in the optimization of preventive and rehabilitative regimens with special attention to the most affected muscles.

Type I fibers are known to atrophy more than fast type II fibers during inactivity (16). Histochemical and immunohistochemical methods have shown that inactivity leads to a shift toward a faster muscle phenotype (17), but the information is primarily qualitative. The type IIx fibers are difficult to distinguish from type IIa and IIb fibers by the usual histochemical myosin ATPase reactions and thus were previously generally confused with the type IIb fibers. However, immunoblotting and electrophoretic analyses show that the type IIx fibers are numerous in most leg muscles (29).

Quantitative data concerning the changes in MHC mRNA levels might be used to predict changes in MHC composition and thus in muscle function. Therefore, the aim of this study was to analyze with the use of a quantitative polymerase chain reaction (PCR) technique the early effects of immobilization on the mRNA levels of all the major adult MHC in three functionally different hindlimb muscles: mixed fiber-type gastrocnemius; fast, phasic plantaris; and slow, postural soleus. The muscles were immobilized in the shortened position, because stretch is known to inhibit the changes resulting from inactivity.

MATERIALS AND METHODS

Experimental Animals

Eleven-week-old male Wistar rats were randomly assigned to control and experimental groups, with six rats in each group. Animals were housed in temperature-controlled rooms with 12:12-h light-dark periods and were provided with chow and tap water ad libitum. Immobilization for 1 wk was performed by bilateral hindlimb casting under ether anesthesia. The ankles were fixed in maximal plantar flexion, and the plaster cast was extended around the knee and hip joints, which were immobilized at approximated resting angles. Both hindlimb casts were connected by strips of plaster of paris dorsally, cranial to the tail, to prevent the legs from slipping out of the cast and to immobilize the hip joint. The animals were weighed before casting and after the casts had been removed under thiopental sodium anesthesia (50 mg/kg ip); they were killed thereafter by decapitation. Gastrocnemius, plantaris, and soleus muscles were rapidly excised and frozen in liquid nitrogen. All samples were stored at −75°C before analysis.

Analysis of MHC Isoform mRNA Levels

Muscle RNA isolation and cDNA synthesis. Total RNA was extracted from frozen gastrocnemius, plantaris, and soleus muscles by using a modification of the acid guanidium thiocyanate-phenol-chloroform extraction (RNAzol B, Tel-

Test, Friendswood, TX) (10). Frozen muscles were weighed and homogenized first manually in −20°C and then with Polytron (Kinematika, Littau, Switzerland), using 1 ml RNAzol B/25 mg muscle tissue. The total RNA concentration was assessed spectrophotometrically (Gene Quant, Pharmacia Biotech, Finland). First strand cDNA was generated by reverse transcription (RT; Superscript II, Life Technologies, Gaithersburg, MD) with both oligo-dT (Pharmacia Biotech, Finland) and random hexamer (Life Technologies) primers in a 40-μl reaction. The reaction contained 1 μg total cellular RNA and different known amounts of standard RNA.

PCR primers. Oligonucleotide primers complementary to selected regions of the rat genes encoding MHC I (see Fig. 4*A* in Ref. 24), MHC IIa (see Fig. 4*B* in Ref. 24), MHC IIb (see Fig. 4*C* in Ref. 24), and MHC IIx (see Ref. 12) were synthesized by the Institute of Biotechnology, University of Helsinki, Finland (Table 1). The length of the sequence they spanned was substantially greater than the size of a standard cDNA. Some of the 3′ primer of each primer pair was end-labeled with [^{32}P]ATP by using T4 polynucleotide kinase (Promega, Madison, WI).

Preparation and titration of internal standard RNA. To measure MHC gene mRNA levels, a known amount of an internal standard RNA was included in each RT reaction. Standard RNA was produced by in vitro transcription (Promega) by first constructing synthetic DNA templates that contained two primer pairs complementary to those used to amplify two cDNA of interest. DNA templates also contained sequences for the bacteriophage T7 promoter at the 5′ end for transcription into RNA and a polyA tail at the 3′ end to facilitate RT by oligo-dT (14). Because the concentrations of the standard DNA can adversely affect the amplification efficiency of the sample cDNA (Fig. 1), the optimal amount of standard RNA for each gene was defined by including varying amounts of standard RNA and total RNA in each RT-PCR. This was to ensure that the range of concentrations for both the sample cDNA and the standard DNA allows amplifications within the exponential range. The standard RNA preparation was divided into several tubes and stored at −75°C.

PCR conditions. The cDNA was amplified in a DNA thermal cycler (Perkin-Elmer) by using 4 μl of the RT products as template and the following reagents in a 100-μl reaction mixture containing 1× PCR buffer [10 mM tris(hydroxymethyl)aminomethane (Tris)·HCl, pH 8.8, at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100; Finnzymes, Finland], 200 μM deoxy nucleic acid triphosphate, 100 ng each 5′ and 3′ primers, and 2.5 U Dynazyme II DNA polymerase (Finnzymes). A trace amount of ^{32}P-labeled 3′ primer was added to provide ~1× 10^6 counts per min (cpm) per reaction. In this way, each synthesized DNA strand was radiolabeled. The first cycle started with a 4-min denaturation at 96°C. In the following cycles, the denaturation step lasted for 1 min, primer annealing lasted 1 min at 57–58°C, depending on the primer pair, and the synthesis step lasted 1 min at 72°C.

Table 1. *Oligonucleotide sequences of 5′ and 3′ primers for MHC isoforms*

mRNA	5′ Primer	3′ Primer	Product
MHC I	5′-gtc aag agc cgt gac att ggc-3′	5′-ctg cct gaa ggt gct gtt tca-3′	121 bp
MHC IIa	5′-taa aga gcc gcg agg ttc aca-3′	5′-gct tgc aag aac ttg ggc tct-3′	166 bp
MHC IIx	5′-tga tcg atc caa agc agg-3′	5′-ctc cca aag tcg taa gta-3′	88 bp
MHC IIb	5′-cac acc aaa gtc ata agc gaa-3′	5′-cct tga tat aca gga cag tga-3′	104 bp

MHC, myosin heavy chain; bp, base pairs.

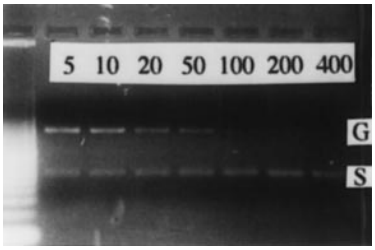

Fig. 1. Titration of amount of internal standard. Relative amount of internal standard affects amplification of sample cDNA. Nos. below each well indicate amount of standard included in each reverse transcription (RT) reaction. Values are expressed in picograms. Each reaction contains a 1-μg sample total RNA. *Left column*: a 10-base pair ladder is used as a weight marker. G, polymerase chain reaction (PCR) product of sample cDNA (gene); S, PCR product of internal standard cDNA (control).

Measurement of gene expression by quantitative PCR. To ensure that measurements were performed during the exponential phase of amplification, 10 μl of each PCR mixture was removed every fourth cycle during cycles 12–28. PCR products were electrophoresed on a gel containing 4.25% (wt/vol) NuSieveGTG (FMC Bioproducts, Rockland, ME) in Tris-acetate/EDTA buffer. Because the amplification products of the standard cDNA and the cDNA of interest were different in size, they could be separated electrophoretically. Products were visualized by ethidium bromide with indirect ultraviolet irradiation and excised from the gel. Radioactivity in each band was determined by Cerencov counting. The values from the cellular and standard bands were plotted on a logarithmic scale against the number of amplification cycles (Fig. 2). Parallel curves indicate that despite a difference in size, the

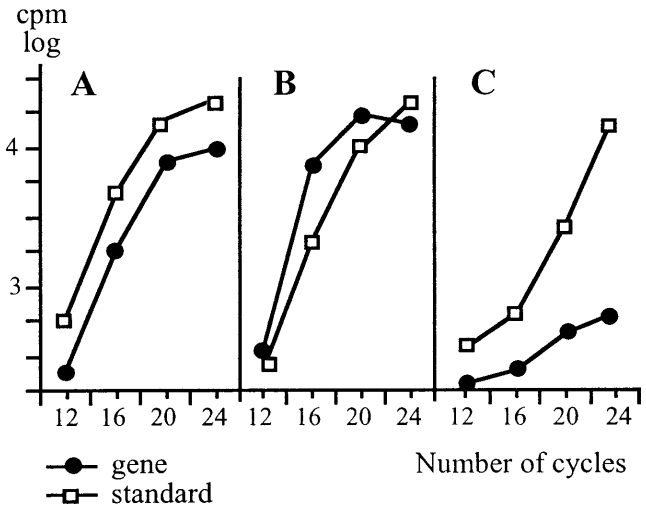

Fig. 2. Evaluation of amplification efficiencies in PCR. Amplification curves show no. of PCR cycles plotted against radioactivity [log counts per min (cpm)]. Every fourth cycle during *cycles 12–28*, 10 μl of each PCR reaction mixture were removed to ensure that measurements were performed during exponential and colinear phases of amplification. Aliquots were electrophoresed on a gel, and because of the difference in size, the 2 products could be separated. Products were then excised from the gel, and radioactivity was determined. *A*: amplification efficiencies of 2 DNAs are colinear, and no aliquots are needed. *B*: amplification slows down sooner for the gene than for the standard, and the reaction is not colinear, which is even more obvious in *C*, where the colinearity is lacking totally. The necessity for aliquots at appropriate intervals is therefore obvious.

Table 2. *Body and muscle weights*

Condition	Body, g	Soleus, mg	Gastrocnemius, mg	Plantaris, mg
Control	283 ± 9	103 ± 4	1,279 ± 54	242 ± 9
Immobilized	252 ± 9*	92 ± 3	1,083 ± 63	211 ± 12

Values are means ± SE. *$P < 0.05$ compared with control.

two templates were amplified with comparable amplification efficiencies for up to ~28 cycles. The mRNA levels of interest could then be calculated from the known amounts of internal standard by using values from the exponential cycles.

Quality control. Total RNA of the samples and internal standard cRNA were tested for DNA remnants by performing PCR and RT reactions without reverse transcriptase. As we expected, no amplification products were seen. To achieve this standard RNAzol B and in vitro transcription protocols had to be modified. PCR products and nonamplified samples were always handled in separate rooms. During measurements, PCR always included a negative control. When performing the RT and PCR and measuring the same sample (type IIb MHC mRNA from gastrocnemius at the level of 2.5×10^8 molecules of mRNA per μg total RNA) 10 times the coefficient of variation was 13.9%. The identity of each PCR product was confirmed by sequencing.

Statistical Analysis

All data are presented as means ± SE. The data were analyzed by nonparametric two-tailed Mann-Whitney *U*-test.

RESULTS

Body and Muscle Weights

After 1 wk of immobilization, the body weight decreased by 8.4%, whereas it increased by 9.5% in the control group ($P < 0.05$; Table 2). The weights of soleus, gastrocnemius, and plantaris muscles tended to be lower in the immobilization group (Table 2).

MHC mRNA Levels

The sum of type I, IIa, IIx, and IIb MHC mRNA (total MHC mRNA) in normal soleus was 6.34 and in immobilized soleus was 6.12×10^8 molecules of mRNA/μg total RNA. In plantaris, the sums were 9.92 and 9.75×10^8 molecules of mRNA/μg total RNA, in normal and immobilized rats, respectively. However, in gastrocnemius, total MHC mRNA increased by almost one-half after immobilization, from 4.64 to 6.83×10^8 molecules of mRNA/μg total RNA.

In slow soleus, type I MHC accounted for 97% and type IIa MHC accounted for only 3% of the total MHC mRNA present in normal rats (Table 3). Type IIx and IIb MHC accounted for <1 per mille of the mRNA (Table 3). However, the extremely low levels of type IIx and IIb MHC mRNA could be detected by quantitative PCR. In the immobilization group, the increase of 24-fold in type IIx MHC mRNA was most striking. Type IIb MHC mRNA increased by 2.6-fold (Fig. 3*B*). Despite these high relative increases in type IIx and IIb MHC mRNA, the total distribution of MHC mRNA in soleus was not affected (Table 3).

In mixed fiber-type gastrocnemius, type IIb and IIx MHC accounted for most of the total MHC mRNA in normal rats, 52 and 22%, respectively. Types I and IIa MHC each represented 13% of the mRNA (Table 3). Immobilization for 1 wk reduced the amount of type IIa MHC mRNA in gastrocnemius by one-half, and the type IIx MHC mRNA level rose 2.4-fold (Fig. 3*B*). After immobilization, type IIa MHC accounted for 5% and type IIx MHC accounted for 35% of the MHC mRNA, respectively, and the levels of type I and IIb MHC mRNA remained practically unchanged (Table 3).

In fast plantaris, type IIb MHC accounted for 69%, type IIx MHC was 13%, type IIa MHC was 12%, and type I MHC accounted for 6% of the total MHC mRNA in normal rats (Table 3). After immobilization, type I MHC mRNA decreased by one-third, and type IIx MHC mRNA tended to increase (Fig. 3*B*). The only statistically significant change in immobilized plantaris was the decrease of 58% in the level of type IIa MHC mRNA (Fig. 3*B*). This lead to a decrease in the relative proportion of type IIa MHC mRNA to 5% of the MHC mRNA and to an increase in the relative proportion of the fastest isoform, type IIb MHC mRNA, to 76% of the MHC mRNAs (Table 3). Thus immobilization caused a shift in the distribution of MHC mRNA toward faster isoforms.

DISCUSSION

Quantitative PCR Analysis of MHC Isoform mRNA

Here we describe a highly sensitive and specific method based on the approach proposed by Wang et al.

Table 3. *No. of molecules of specific MHC mRNA isoforms in muscles of control and immobilized rats*

Muscle/Condition	MHC I	MHC IIa	MHC IIx	MHC IIb
Soleus				
Control	$6.17 \pm 0.48 \times 10^8$	$1.65 \pm 0.57 \times 10^7$	$0.21 \pm 0.04 \times 10^6$	$0.50 \pm 0.02 \times 10^6$
Immobilized	$5.79 \pm 0.30 \times 10^8$	$2.66 \pm 0.55 \times 10^7$	$5.00 \pm 0.70 \times 10^6$ ‡	$1.30 \pm 0.10 \times 10^6$ *
Gastrocnemius				
Control	$6.15 \pm 0.54 \times 10^7$	$6.23 \pm 0.58 \times 10^7$	$1.00 \pm 0.15 \times 10^8$	$2.40 \pm 0.16 \times 10^8$
Immobilized	$5.47 \pm 0.31 \times 10^7$	$3.08 \pm 0.50 \times 10^7$ †	$2.42 \pm 0.60 \times 10^8$ *	$3.55 \pm 0.89 \times 10^8$
Plantaris				
Control	$6.04 \pm 0.98 \times 10^7$	$1.14 \pm 0.12 \times 10^8$	$1.30 \pm 0.20 \times 10^8$	$6.88 \pm 0.71 \times 10^8$
Immobilized	$3.91 \pm 0.86 \times 10^7$	$0.48 \pm 0.10 \times 10^8$ ‡	$1.44 \pm 0.19 \times 10^8$	$7.44 \pm 0.99 \times 10^8$

Values are expressed as no. of molecules of specific mRNA per μg total RNA (mean ± SE); $n = 6$ in control and immobilized groups. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.005$ compared with control.

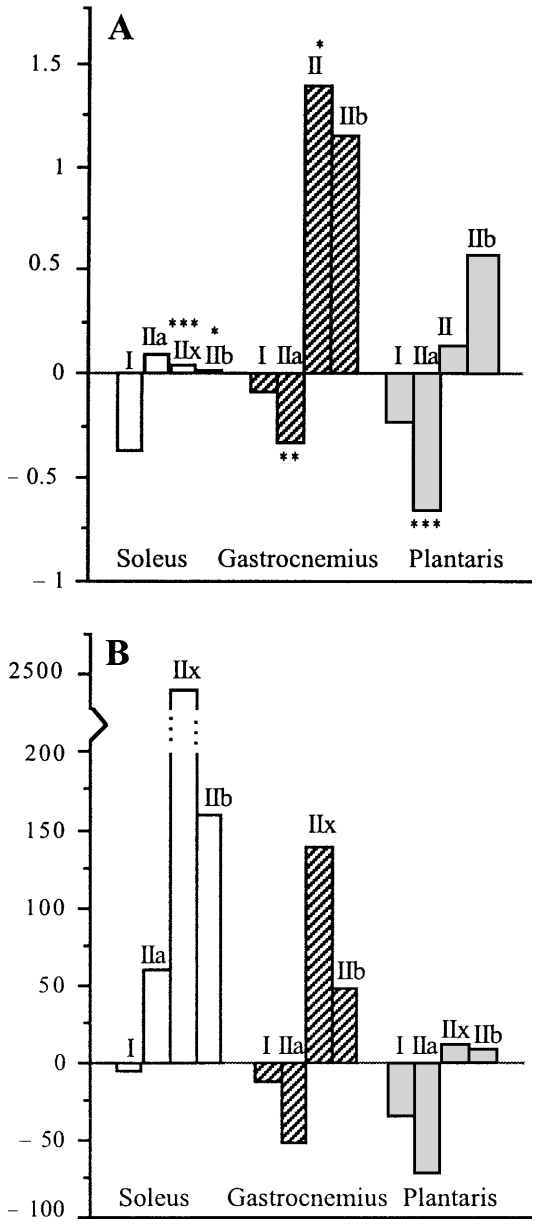

Fig. 3. Absolute and relative changes in type I, IIa, IIx, and IIb myosin heavy chain (MHC) mRNA expression after immobilization in soleus, gastrocnemius, and plantaris. *A*: values are expressed as no. of molecules of specific mRNA $\times 10^8$ per μg total RNA. *B*: values are expressed as %change relative to control level of MHC mRNA. Data were plotted from Table 3. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ compared with control.

(33) and modified by Feldman et al. (14) for the quantification of mRNA levels by using PCR and a synthetic RNA as an internal standard. The advantages of using the PCR-based methodology as opposed to conventional hybridization methods for quantification of mRNA levels (e.g., Northern blot, RNase protection, S1 nuclease mapping) are many: requirement of only a very small amount of sample RNA, easier comparison of expression levels, and higher sensitivity. Only 3–6 mg of tissue are needed (14), and the method enables direct mRNA quantification down to 10^4 molecules, being 1,000 times more sensitive than dot-blot assay (33). The high sensitivity is crucial for quantification of low mRNA levels and those present in small samples. The correlation is very good between mRNA levels determined by Northern blot analysis, RNase protection techniques, and quantitative PCR (8).

Contamination either with genomic DNA or previously amplified DNA can greatly affect the results because of the high degree of sensitivity (8). For these reasons, isolated sample RNA was tested for genomic DNA by performing the RT reaction in the absence of reverse transcriptase, after which PCR was performed and no amplification products were seen, as expected. The standard RNA isolation protocol had to be modified to remove all the genomic DNA. Because the internal standard cRNA was synthesized from DNA by in vitro transcription, the obtained cRNA was also tested for DNA remnants. To remove all the DNA remnants, >80 times the recommended amount of DNase enzyme was required in the in vitro transcription protocol.

The use of external or internal standards in mRNA quantification is controversial (28). We feel that the large interassay variations in the efficiency of the amplification process make it necessary to include an internal standard for quantification. With an internal standard in the same tube, variable effects due to differences in the conditions of RT or the PCR amplification will affect the yield of PCR product equally for the target mRNA and the standard cRNA. The purpose of aliquots is to detect the colinear amplification, as seen in Fig. 2. A limitation of this method is the need to estimate the concentration range of the cRNA of interest. Differences in tissue handling and RNA processing can potentially change the mRNA levels. The RT step must also be assumed to have occurred with equal efficiency.

Because it is customary to correlate the quantity of mRNA to the total RNA in quantitative PCR as well as, for instance, Northern blot analyses, the possible effects of changes in total RNA content have to be borne in mind. However, despite the decrease of 38% in the RNA content of immobilized soleus reported previously (3), the magnitude and varying directions of changes in mRNA levels reported in the present paper cannot be explained solely as a result of changes in the amount of total RNA.

MHC genes carry a high degree of sequence homology (24). Careful selection of primers for PCR provides the specificity required for quantifying similar mRNA, which is further confirmed by the sequencing of the products. We found quantitative PCR an excellent method for analyzing the different MHC isoforms.

MHC mRNA Levels in Normal and Immobilized Skeletal Muscle

To our knowledge, this is the first report concerning the quantities of type I, IIa, IIx, and IIb MHC mRNA in total RNA preparations of rat skeletal muscles. By using a quantitative PCR method, we observed type IIx and IIb MHC mRNA in the soleus of normal rat. These were not observed with an in situ hybridization method in a previous study (12). Although MHC IIx isoform has

not usually been observed in soleus, there are reports in which a small amount of type IIx MHC has been demonstrated by using monoclonal antibodies in young (21) and young adult (30) rats. By using myosin ATPase histochemistry in adult male rats, Delp and Duan (11) have classified 9% of the muscle fibers in soleus as type IIx, but they stress that the results may only be applicable for rats of the same strain, age, and gender.

Peuker and Pette (28) have measured type I, IIb, and IId (~IIx) MHC mRNA in various rabbit muscles by using a modification of the RT-PCR method. Our results were in approximately the same range as theirs for gastrocnemius and soleus. However, type IIb MHC mRNA was expressed in rat soleus at a 370-fold higher level and in gastrocnemius at a 13-fold higher level than in the corresponding muscles of rabbit in their study. These are most likely species-specific differences.

When comparing the levels of specific MHC mRNA among soleus, gastrocnemius, and plantaris, the amount of type IIa MHC was quite consistent. The other isoforms showed remarkable differences among specific muscle types. Type I MHC was expressed in soleus at a 10-fold higher level, whereas type IIx and type IIb MHC were expressed at >400-fold lower levels than in the other two muscles. This is in accordance with the characteristic fiber-type distribution and MHC isoform profile of each muscle (27). The distribution of each MHC mRNA has been shown to match that of the corresponding protein at the single fiber level (12, 32). However, the study of MHC mRNA levels might be more useful than the study of the corresponding proteins during rapid transitional changes (9, 20, 26). We measured the mRNA levels from homogenized whole muscle, which precludes the possibility of examining the changes in specified fiber types. However, it eliminates the possible error brought about by local variations in the distribution of mRNAs within one muscle (12) and even along a muscle fiber (27) in a study of, for example, cross-sectional samples.

The present study shows that immobilization in shortened position for 1 wk significantly alters the gene expression of MHC isoforms. Slow, tonic muscles like soleus are known to be the most sensitive to immobilization (5, 16). Even the number of sarcomeres in series within fibers decreases after immobilization in soleus but not in gastrocnemius (18). This seems to be due to the difference in muscle architecture and reflects the muscle-length adaptation to meet the change in muscle length induced by immobilization in shortened position (18). When 3- to 7-day-old mice and rats were immobilized for 2–3 wk with soleus in shortened position, the expression of β-myosin (type I) mRNA assessed by in situ hybridization was reduced (17). It has been suggested in a S1 nuclease-mapping study that, at least in slow muscles, the normal activity pattern actively inhibits expression of the MHC IIb gene, and the rapid expression of MHC IIb mRNA during inactivity is prevented by passive stretch (22).

In this study, there was no clear change in total MHC mRNA in soleus and plantaris after immobilization. In gastrocnemius, however, total MHC mRNA increased by almost one-half. The level of type I MHC mRNA in soleus was not altered significantly. Both type IIx and IIb MHC genes became significantly upregulated. Similar trends in MHC mRNA have been observed recently by using slot-blot analysis after 14- and 30-day hindlimb suspension (1, 13). However, in contrast with hindlimb immobilization, hindlimb suspension eliminates loadbearing or isometric contractions completely, and the qualitative changes occur with a faster time course during suspension (15, 16, 19, 34). Because 1 wk seems to be a relatively short period, considering the changes in mRNA level (31), the changes in gene expression apparently become more pronounced after prolonged immobilization (6).

Type IIx MHC mRNA has been readily expressed in soleus after 1-wk administration of thyroid hormone and long-term high-frequency electrical stimulation (12). It has been concluded from the observations of hybrid fibers that the MHC transitions follow an obligatory pathway of MHC gene expression in the order I ⇄ IIa ⇄ IIx ⇄ IIb (2). However, even fibers with coexpression of type I and IIx MHC have been demonstrated during slow-to-fast transformation in rat soleus 15 days after spinal cord transection (30). Our data at the mRNA level already after 1-wk immobilization support the finding that type IIx MHC is the fast MHC gene primarily upregulated during slow-to-fast transition in soleus (30). This emphasizes the limited value of most of the previous studies at the mRNA or protein level where only type IIa and IIb MHC have been studied. It seems that type IIx MHC gene is rapidly and effectively induced during the MHC isoform and fiber-type transformation caused by varying stimuli (9). One reason for this can be the intermediate nature of type IIx MHC as a fast MHC.

As shown here, the decrease in the level of type IIa MHC mRNA and the increase in the level of type IIx MHC mRNA in gastrocnemius altered the distribution of MHC mRNA toward faster isoforms. In a previous study in which pooled muscle samples were analyzed by using S1 nuclease-mapping analysis after 5-day immobilization, there was an apparent decrease in the level of type I MHC mRNA and an increase in the level of type IIb MHC mRNA in both gastrocnemius and plantaris, type IIx MHC mRNA not being determined (22). However, in the present study, the only significant change in fast plantaris was a decrease by one-half in the level of type IIa MHC mRNA that put further emphasis on type IIb MHC as the predominant mRNA. The changes in gastrocnemius can be interpreted as a combination of the changes in slow and fast muscles, which seems reasonable concerning the mixed fiber-type composition of the muscle.

In conclusion, this study shows that immobilization in the shortened position rapidly alters the MHC mRNA profile in skeletal muscle toward faster isoforms. Different muscles vary in their response to disuse. In fast plantaris, in which the level of type IIb MHC mRNA is initially high, the slower type IIa MHC gene becomes downregulated, whereas in slow soleus type IIx and IIb MHC genes become remarkably

upregulated. The RT-PCR method employed gives quantitative results that enable evaluation of changes in mRNA levels more reliably, and even the smallest amounts of mRNA can be demonstrated. The method is applicable in very small tissue samples and, therefore, could be employed for analyzing clinical muscle biopsy specimens when studying changes in muscle function.

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