Modulation of MHC isoforms in functionally overloaded and exercised rat plantaris fibers

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Modulation of MHC isoforms in functionally overloaded and exercised rat plantaris fibers. J. Appl. Physiol. 83(1): 280–290, 1997.—The effects of 1 and 10 wk of functional overload (FO) of the rat plantaris with (FOTr) and without daily endurance treadmill training on its myosin heavy chain (MHC) composition were studied. After 1 and 10 wk of FO, plantaris mass was 22 and 56% greater in FO and 37 and 94% greater, respectively, in FOTr rats compared with age-matched controls. At 1 wk, pure type I and pure type IIa MHC fibers were hypertrophied in FO (39 and 44%) and FOTr (70 and 87%) rats. By 10 wk all fiber types comprising >5% of the fibers sampled showed a hypertrophic response in both FO groups. One week of FO increased the percentage of hybrid (containing both type I and type IIa MHC) fibers and of fibers containing embryonic MHC. By 10 wk, the percentage of pure type I MHC fibers was ~40% in both FO groups compared with 15% in controls, and the percentage of fibers containing embryonic MHC was similar to that in controls. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses showed an increase in type I MHC and a decrease in type IIb MHC in both FO groups at 10 wk, whereas little change was observed at 1 wk. These data are consistent with hypertrophy and transformation from faster to slower MHC isoforms in chronically overloaded muscles. The additional overload imposed by daily endurance treadmill training employed in this study (1.6 km/day; 10% incline) results in a larger hypertrophic response but appears to have a minimal effect on the MHC adaptations.

immunohistochemistry; gel electrophoresis; hypertrophy; fiber type conversion; fiber size

FUNCTIONAL OVERLOAD (FO) of the rat plantaris by removal of its major synergists results in hypertrophy and a shift in the contractile, biochemical, and metabolic properties toward those observed in a “slower” muscle (26). The extent of these adaptations may be related, in part, to the activity level of the rats after surgery. For example, it appears that the amount of hypertrophy is greater in rats that are exercised on a treadmill than in rats not exercised after FO (13, 25), although this is somewhat controversial (3). Thus one of the mechanisms regulating muscle hypertrophy may be the amount and/or type of activity that the overloaded muscle experiences after ablation of its synergists. This contention is supported by the observation that in cats, a relatively sedentary animal, FO of the plantaris has little effect on the mass of the muscle unless the cat is exercised. In addition, the largest amount of hypertrophy was found when the cats were subjected to high-intensity exercise, i.e., sprinting and jumping (26, 29).

On the basis of gel electrophoresis analyses, FO of the rat plantaris has been shown to result in increases in the percent composition of type I, IIa, and IIx and a decrease in type IIb myosin heavy chains (MHCs) (8, 10, 19, 23, 37, 38). The effects of exercise on the degree of conversion from fast to slow fibers in FO muscles, however, are equivocal. Riedy et al. (25) reported a similar percentage of slow and fast fibers, on the basis of myofibrillar adenosinetriphosphatase staining, after 13 wk of FO with and without treadmill training. In contrast, the increase in the percent composition of slow native myosin in the plantaris of FO rats that were allowed spontaneous running exercise for 11 wk was equal to the sum of the increases observed with exercise or FO alone (14). Thus it is not presently known whether the shift toward slower biochemical properties in a muscle responding with elevated neuromuscular activity (12, 29) to one functional stressor can be enhanced by imposing a second functional stressor. In other words, can MHC expression reflecting a fast-to-slow fiber type transition in an FO muscle be further regulated by regular endurance exercise? In addition, the effects of FO with and without training on the expression of MHC isoforms in plantaris muscle fibers have not been determined.

The objectives of the present study, therefore, were threefold: 1) to define the complement of MHC isoforms in the FO plantaris by using immunohistochemical and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) procedures; 2) to compare the adaptations in the MHC isoforms and fiber size at an early (1-wk) and a later (10-wk) stage after FO; and 3) to determine any effects of an additional overload imposed on the FO muscles by treadmill training.

MATERIALS AND METHODS
Experimental design and surgical procedures. Adult female Sprague-Dawley rats (initial mean body weight of ~180 g) were used. After 3 days of acclimatization to laboratory conditions, all rats were run on a motor-driven rodent treadmill at various speeds and grades for a few minutes per day for 1 wk. Rats that would not run on the treadmill or had high or low body weights were eliminated from the study. The rats then were assigned randomly to one of eight groups: 1) 1-wk control (Con1wk; n = 7); 2) 1-wk sham control (ConSh1wk; n = 5); 3) 1-wk FO (FO1wk; n = 9); 4) 1-wk FO plus treadmill training (FOTr1wk; n = 9); 5) 10-wk control (Con10wk; n = 7); 6) 10-wk sham control (ConSh10wk; n = 5); 7) 10-wk FO (FO10wk; n = 9); or 8) 10-wk FO plus treadmill training (FOTr10wk; n = 9). One week was chosen because it represents the peak period of the inflammatory response to the FO surgery (2), and the 10-wk
period was chosen because it represents the period during which both the hypertrophic and myosin adaptations plateau (38). All rats were given water and food ad libitum. The rats were housed in groups of 3–4 in standard rodent cages.

The plantaris muscle in both legs of the rats in the overloaded groups was functionally overloaded by surgical removal of its major synergists, i.e., the soleus and both heads of the gastrocnemius muscles, under anesthesia [ketamine (75 mg/kg body wt), zylazine (10 mg/kg body wt)] and aseptic conditions as described previously (30). In the Con rats, the same skin incisions and connective tissue and muscle manipulations as in the FO rats were performed without removal of the plantaris synergists. Rats were allowed 4 days of recovery before treadmill training was initiated.

FOTr-1wk Rats ran at 0.6–0.7 miles/h (mph) at a 10% incline for ~1 h for seven consecutive days. The speed of the treadmill for FOTr-10wk rats was progressively increased such that the rats were running at ~1 mph at a 10% incline for ~1 h during the last 4 wk of the training period. FOTr-10wk rats were trained 5 days/wk. All rats were killed by an overdose of pentobarbital sodium (Eutha-6) ~48 h after the last exercise session. The plantaris muscle was excised, trimmed of excess fat and connective tissue, and wet weighed. The muscle was placed on cork, gently stretched to approximate its in vivo length, and quickly frozen in isopentane cooled with liquid nitrogen. Serial cross sections (10 µm thick) from the midbelly of the muscle were taken on a Reichert-Jung 2800 Frigocut E cryostat microtome and placed on chrome/alum-coated slides in preparation for immunohistochemical analyses. Thirty cross sections, 20 µm thick, were placed in precooled (~20°C) microcentrifuge tubes and stored at −70°C in preparation for myofibrillar protein isolation. Because of differences in the fiber type distribution along the rostral-caudal axis of the highly compartmentalized rat plantaris (Roy and Edgerton, unpublished observations) and the reported differences in the adaptations to FO along the proximal-distal axis of a muscle (11), fibers from a deep region (close to the bone) in the midportion of the muscle were consistently chosen for analysis and comparison.

SDS-PAGE. Isolated myofibrils were prepared from whole muscle cross sections stored in microcentrifuge tubes according to Thomason et al. (36), except that sample volumes were reduced to 0.25 ml to accommodate the small sample size. The protein concentration of the final myofibrillar suspension was determined according to Bradford (5). Myofibrillar protein was then boiled in sample buffer (20) for 2 min at a final concentration of 0.25 mg/ml. MHCs were separated by SDS-PAGE according to Talmadge and Roy (34). The SDS-PAGE gels were stained with Coomassie blue, photographed, and scanned with a digital imaging system densitometer (IS-1000, Alpha Innotech) for the quantification of MHC isoforms.

Immunohistochemistry. Immunohistochemical analysis of MHC content in individual fibers was performed on serial sections by using a series of monoclonal antibodies (primary antibody) specific to rat MHC isoforms (see Table 1 for monoclonal antibody specificities). The avidin-biotin immunohistochemical procedure was used for the localization of primary antibody binding according to the instructions for kits PK-6102 and AK-5010 (Vector Laboratories, Burlingame CA). Phosphate-buffered saline was used as a buffer for all immunoglobulin G-class primary antibodies, and tris(hydroxymethyl)aminomethane-buffered saline as the buffer for all immunoglobulin M-class primary antibodies. A sample of ~100 fibers that were free from artifact and showed good staining for all antibodies was selected for single-fiber MHC composition analysis from the deep region of each muscle.

<table>
<thead>
<tr>
<th>Table 1. Monoclonal antibody specificity</th>
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<tr>
<td><strong>Mab</strong></td>
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<td>---------</td>
</tr>
<tr>
<td>71</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>D9</td>
</tr>
<tr>
<td>F6</td>
</tr>
<tr>
<td>Fast</td>
</tr>
<tr>
<td>Slow</td>
</tr>
<tr>
<td>Dev</td>
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Each monoclonal antibody (Mab) is bound to a specific myosin heavy chain (MHC) isoform as determined by Schiaffino et al. (31) and the suppliers’ instructions. Neo, neonatal; Emb, embryonic; Dev, developmental; +, positive reaction between Mab and MHC isoform; −, no reaction between Mab and MHC isoform. All Mabs used were immunoglobulin G class, except for Mabs F3 and D9, which were immunoglobulin M class.

Stained cross sections were photographed on an Olympus BH-2 microscope with a Nikon camera attachment. A fiber showing a reaction to a specific antibody was considered to be expressing that specific MHC isoform.

Statistical procedures. With the use of Student’s t-test, it was determined that there were no significant differences for any parameter between the control and sham rats at either of the time points. Therefore, the data from these two groups were combined and used as the control group for each time period. A two-way analysis of variance (duration × group) was used to test for overall statistical effects. Tukey’s post hoc test was used to determine which groups were significantly different from each other. Statistical significance was set at P ≤ 0.05 for all analyses.

RESULTS

Body and muscle weights. The mean body weight of the Con1wk rats was 27.2% larger than that of the Con1wk rats (Table 2). The body weights at either time point, however, were not significantly different across experimental groups. Compared with their respective controls, the absolute plantaris wet weights in the FO were 22 and 56% larger and those in the FO trained rats were 37 and 94% larger after 1 and 10 wk, respectively. Relative weights showed a similar pattern.

Fiber type distributions. The pattern of immunohistochemical staining of a representative area of the deep portion of the plantaris is shown for a Con1wk rat (Fig. 1, FOTr-1wk (Fig. 2), and FOTr-10wk (Fig. 3) rat. In the control plantaris, ~50% of the fibers contained a single MHC isoform (Figs. 1 and 4). After 1 wk of FO with and without training, the percentage of fibers containing only one of the four primary MHC isoforms was ~40% (Figs. 2 and 4). In addition, ~35% of the fibers, including some of the pure fibers represented in Fig. 4, in both FO1wk groups contained the embryonic MHC isoform (Fig. 5). Typically, the fibers that contained the embryonic MHC isoform also contained MHC isoforms of type I, type IIa, or both. After 10 wk of FO and training, very few fibers contained either type IIb or embryonic MHC isoforms, and the proportion of fibers with type I was increased (Fig. 3).
Table 2. Body and muscle weights

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Plantaris Wt, mg</th>
<th>%Change From Con</th>
<th>Plantaris Wt, mg/kg body wt</th>
<th>%Change From Con</th>
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<tr>
<td>Con1wk</td>
<td>11</td>
<td>221 ± 3</td>
<td>249 ± 4</td>
<td></td>
<td>1.132 ± 0.019</td>
<td></td>
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<tr>
<td>FO1wk</td>
<td>9</td>
<td>213 ± 2</td>
<td>303 ± 6*</td>
<td>+22</td>
<td>1.429 ± 0.032*</td>
<td>+26</td>
</tr>
<tr>
<td>FOTr1wk</td>
<td>9</td>
<td>220 ± 4</td>
<td>342 ± 14*</td>
<td>+37</td>
<td>1.553 ± 0.051*</td>
<td>+37</td>
</tr>
<tr>
<td>Con10wk</td>
<td>9</td>
<td>281 ± 6†</td>
<td>343 ± 10*</td>
<td></td>
<td>1.218 ± 0.023</td>
<td></td>
</tr>
<tr>
<td>FO10wk</td>
<td>7</td>
<td>271 ± 9†</td>
<td>534 ± 29*†</td>
<td>+56</td>
<td>1.972 ± 0.039*†</td>
<td>+62</td>
</tr>
<tr>
<td>FOTr10wk</td>
<td>7</td>
<td>283 ± 15†</td>
<td>664 ± 52*††</td>
<td>+94</td>
<td>2.348 ± 0.057*††</td>
<td>+93</td>
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Values are means ± SE; n, no. of rats. Wt, weight; Con, control; Con1wk and Con10wk, 1- and 10-wk controls, respectively; FO1wk and FO10wk, functionally overloaded rats at 1 and 10 wk, respectively; FOTr1wk and FOTr10wk, functionally overloaded treadmill-trained rats at 1 and 10 wk, respectively. *Significantly different from appropriate control, P ≤ 0.05; †significantly different from same group at 1 wk, P ≤ 0.05; ‡significantly different from FO group at same time period, P ≤ 0.05.

Fig. 1. Serial cross sections from deep region of a control (Con) rat plantaris muscle stained with monoclonal antibodies (MAb) against specific myosin heavy chain (MHC) isoforms (see Table 1 for specificities). A: slow (type I). B: fast (type II). C: MAb 71 (type IIa). D: MAb 35 (all except type IIx). E: MAb D9 (type IIx and IIb). F: MAb F3 (type IIb). G: MAb G6 (embryonic). H: Dev (embryonic). I: B6 (neonatal). I, a, b, and x: type I, IIa, IIb, and IIx MHC isoforms, respectively. Scale bar in I, 50 μm.
There were only subtle changes in the fiber type distribution in the 1-wk overloaded groups, and the adaptations were similar in the trained and untrained rats (Fig. 4). There was a decrease in the percentage of fibers containing only type IIa MHC in both FO groups compared with that in Con1wk rats. It appears that these fibers also began to express type I MHC because the percentage of fibers containing both type I and IIa MHC increased from 1% in Con1wk rats to 8 and 6% in the FO1wk and FOTr-1wk groups, respectively.

Compared with the Con1wk rats, the Con10wk rats had a higher percentage of fibers containing only type IIx MHC and a lower percentage of fibers containing both type IIa and IIx MHCs (Fig. 4). The percentage of pure type I fibers was increased from 15% in Con10wk rats to 40 and 42% in the FO10wk and FOTr-10wk rats, respectively. In addition, the percentage of pure type IIa and pure type IIx fibers was decreased in both FO groups. There also was a tendency for the percentage of type IIa + IIx fibers to be increased in both FO groups (P > 0.05).

Both FO10wk groups had a higher percentage of pure type I fibers than both FO1wk groups (Fig. 4). There was a clear tendency for the FO10wk rats to have a smaller percentage of fibers that contained some type IIb MHC than did the FO1wk rats, i.e., 6 and 2% for the FO10wk and FOTr-10wk rats vs. 16 and 15% for the FO1wk and FOTr-1wk groups, respectively.

A small percentage of fibers (2% or less) in the control groups contained some embryonic MHC (Fig. 5).
percentage rose to 37 and 39% in the FO_{1wk} and FO_{Tr-1wk} groups but was only ~1% in both FO_{10wk} groups.

Fiber Cross-Sectional Areas (CSAs). One week of FO resulted in a 39 and 44% increase in the CSA of pure type I MHC and pure IIa MHC fibers, respectively, compared with Con_{1wk} rats (Fig. 6). In the FO_{Tr-1wk} rats, the CSAs of these fiber types were elevated by 70 and 87%. In addition, there was a 27% increase in the CSA of the type IIa+IIx MHC fibers in the trained compared with Con_{1wk} rats.

The mean CSAs of all fiber types comprising >5% of the fibers sampled in both FO_{10wk} groups were larger than in the Con_{10wk} rats. The percent increase in CSA was the highest in the pure type I MHC fibers, i.e., 159 and 220% increases in the FO_{10wk} and FO_{Tr-10wk} groups, respectively. The mean CSAs of type I, IIa, and IIa+IIx MHC fibers (comprising >70% of the total population of fibers in both groups) were larger in FO_{Tr-10wk} rats than in FO_{10wk} rats.

The CSA of the fibers containing embryonic MHC was similar across groups after 1 wk of FO (Fig. 7). At 10 wk, these fibers were significantly larger in the FO_{Tr} compared with the control or FO rats.

SDS-PAGE analyses. Figure 8 shows the migration patterns of the four adult rat MHCs in SDS-PAGE gels of plantaris muscle myofibrils isolated from six individual animals representing the six treatment groups. Note that in the two control animals (lanes 1 and 4), the type IIx and IIb MHC isoforms are the most prominent. After 1 wk of FO [with or without training (lanes 2 and 3)], there is a small increase in the proportion of type I

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Fig. 3. Serial cross sections from deep region of a plantaris muscle, stained with MAb against specific MHC isoforms, from a 10-wk FO and trained (FO_{Tr-10wk}) rat (see Table 1 for specificities). A: slow (I). B: fast (II). C: MAb 71 (IIa). D: MAb 35 (all except IIx). E: MAb D9 (IIx and IIb). F: MAb F3 (IIb). G: MAb G6 (embryonic). H: Dev (embryonic). I: B6 (neonatal). Fiber 1, type I; fiber 2, type IIa; fiber 3, type IIa and IIx; and fiber 4, type IIx. Note that no fibers in this region contain either type IIb or embryonic MHC isoforms. Also note large cross-sectional areas of fibers compared with those in Figs. 1 and 2. Scale bar in I, 50 µm.
MHC. After 10 wk of FO [with or without training (lanes 5 and 6)], there is a large increase in the proportion of type I MHC, and type IIb MHC is the least prominent MHC isoform.

The plantaris muscle of the Con1wk rats was composed of 5, 11, 46, and 38% type I, IIa, IIx, and IIb MHC, respectively (Fig. 9). The only significant difference from control in either the FO1wk or FOTr-1wk groups was a doubling of the percent composition of type I MHC in the FO1wk group. The Con10wk rats had a significantly lower percentage of type IIb MHC than the Con1wk rats. Both the FO10wk and FOTr-10wk groups had a higher percentage of type I and a lower percentage of type IIb MHCs than either the Con10wk rats or their comparable group at 1 wk after surgery. In addition, the FOTr-10wk group had a higher percentage of type IIx MHC than did the FO10wk group.

DISCUSSION

The increases in plantaris muscle weight and fiber size are consistent with previous reports (see Ref. 26 for a review). At 1 wk, the largest increases in fiber size...
were observed in the pure type I and pure type IIa fibers, with the changes being somewhat greater in the trained compared with the untrained rats. These data suggest that the lower-threshold slow and fast motor units (fibers) were preferentially recruited during this initial period of overload, consistent with the size principle of motor unit recruitment (15). In addition, the rats walked more plantigrade than normal during the first few days after FO [called “waddling” by Gardiner et al. (12); Roy and Edgerton, unpublished observations], resulting in a longer “yield” phase per step and presumably higher muscle forces than normal (see Ref. 29 for kinetic measurements in cats after FO of the plantaris). Activation patterns (on the basis of intramuscular electromyographic recordings) of the rat plantaris after FO also indicate higher but not maximal recruitment levels after compared with before FO (12). The greater effect on fiber CSA in the trained rats suggests that the daily training regime resulted in the recruitment of additional motor units (fibers) and had an additional effect on the overload imposed on the muscle fibers. These data also show that by 1 wk after FO the

Fig. 6. Mean (±SE) cross-sectional area of each fiber type in Con (A), FO (B), and FOTr (C) rats at 1 (left) and 10 (right) wk after FO surgery. Missing error bars are contained within bar itself. *Significantly different from appropriate Con, \( P < 0.05; \) §significantly different from same group at 1 wk, \( P < 0.05; \) †significantly different from FO group at same time period, \( P < 0.05.\)

Fig. 7. Mean (±SE) cross-sectional area of fibers containing embryonic MHC in Con, FO, and FOTr rats at 1 (left) and 10 (right) wk after FO surgery. *Significantly different from appropriate Con, \( P < 0.05; \) §significantly different from same group at 1 wk, \( P < 0.05; \) †significantly different from FO group at same time period, \( P < 0.05.\)
size of the fibers had increased, as shown previously (16), indicating that the hypertrophy process had begun and that the increase in muscle weight was not only a reflection of an early inflammatory response and edema associated with surgical trauma (2).

Ten weeks of overload resulted in a significant increase in the size of all fiber types comprising at least 5% of the total population of fibers compared with either the aged-matched control or the comparable 1-wk group. In general, the percent increase in fiber sizes was equal to or larger than the percent increase in muscle mass, suggesting that functional hypertrophy, i.e., an increase in contractile elements, had occurred. This contention is consistent with the increases in the maximum tetanic tension of the rat plantaris reported after 9–12 wk of FO (17, 30). However, the increase in force production is not proportional to the increase in physiological CSA, and thus the specific tension of the plantaris is decreased (~10–15%) after periods of overload ranging from 30 to 240 days (17, 29). This decrease in specific tension could be reflecting a disproportionate increase in noncontractile elements and/or a decrease in the tension-generating capability of the contractile elements. Evidence for the involvement of both of these factors has been reported. Kandarian and White (16) have reported that decreases in connective tissue protein concentration and increases in interstitial space can explain, in part, the decreased specific tension in the early, but not the late (17), stages of FO. A lower specific tension in predominantly slow (soleus; ~15 N/cm²) compared with predominantly fast (plantaris and medial gastrocnemius; ~26 and 21 N/cm², respectively) extensor muscles has been reported (27, 30). Because one of the primary adaptations in the long-term overloaded plantaris muscle is an increase in the percentage of slow fibers, this factor could explain, at least in part, the decrease in specific tension observed in the FO10wk rats. After 10 wk of overload, the muscles and fibers in trained rats were significantly larger than in the untrained overloaded rats, clearly demonstrating that exercise enhanced the hypertrophic response of the overloaded plantaris. These data are consistent with the 70 and 99% increase in plantaris weight after overload and overload plus treadmill exercise, respectively, reported by Riedy et al. (25).

The adaptations in the MHC isoforms were consistent with the plantaris becoming a slower muscle (see Ref. 26 for a review). The results are consistent with the reported increases in the percentages of 1) type I MHC...
fibers (4, 7); slow native isomyosins (14, 38); and 5) slow myosin light chains (24, 38), as well as a decrease in the maximum rate of shortening (18, 21, 30) after overload of the rat plantaris. Comparisons of the adaptations observed at the fiber level in the deep region of the muscle after 1 and 10 wk of overload demonstrate the progressive nature of these changes. For example, after 1 wk of overload the plantaris showed an increase in the percentage of hybrid fibers, i.e., fibers that contained both type I and some fast MHC isoform (usually type IIa) but showed no change in pure type I fibers. By 10 wk after overload, the percentage of pure type I fibers was almost threefold higher in both overloaded groups compared with control. Combined with the decrease in pure type IIa and IIx MHC fibers, these data strongly suggest that there is a shift in MHC isoforms from type IIx to IIa to I MHC in the deep region of the muscle after overload. Combined with the overall decrease in type IIb MHC as reflected by gel electrophoresis of the entire cross section of the muscle (Fig. 9), these data suggest a shift from type IIb to IIx to IIa to I MHC after 10 wk of FO. The progressive nature of these adaptations has also been observed by Cerrato et al. (7), who reported minimal changes in the percentage of slow fibers, slow myosin protein, and β-MHC mRNA after 7 days of FO of the rat plantaris, whereas these values were at least twofold higher than control after ~4 wk of FO. Although these data are suggestive of a progression from type IIb to IIx to IIa to I MHC after FO, the possibility still exists that some fibers did not express each of the intermediate MHC types during the adaptation process.

The similarity in the fiber type percentage between exercised and nonexercised overloaded groups at either of the two time points indicates that the endurance training program used had little effect on the phenotypic properties of the fibers in the deep region of the overloaded plantaris. Similarly, gel analyses of the entire muscle cross section showed minimal differences in the percent MHC composition between trained and untrained overloaded muscles at either time point (Fig. 9), further substantiating that the endurance training program had a minimal effect on MHC composition of overloaded muscles. These data are consistent with the observation of no difference in the percentage of slow fibers in the plantaris after 13 wk of FO or FO plus treadmill training (25). These data, however, are in contrast to the reported additive effect of FO and voluntary exercise on the shift of the native isomyosin profile from fast to slow in the rat plantaris after 11 wk of overload of the plantaris and soleus (14). In the study of Gregory et al. (14), the rats were housed in cages having voluntary wheels, and the rats ran a minimum of 8 km/day, 7 days/wk. Thus in one 7-day period, the rats in the present study ran ~11.2 km (1.6 km/day x 7 days), whereas the rats in the Gregory et al. (14) study ran a minimum of ~56 km (8 km/day x 7 days). The differences in the MHC responses in the two studies could, therefore, be related to the total volume of exercise experienced by the FO rats.

In general, the gel analyses of whole muscle cross sections reflected adaptations in a direction similar to, although to a lesser magnitude, those observed for the immunohistochemical analyses of a sample of fibers from the deep region of the muscle. These apparent discrepancies between the gel and immunohistochemical analyses could reflect several factors. For example, although a fiber may contain multiple MHC isoforms as demonstrated immunohistochemically, the amount of newly expressed MHCs in hybrid fibers may be very small. In addition, the superficial region of the plantaris, a large proportion of the total cross section of the muscle, is composed predominantly of fast fibers that may have adapted somewhat differently to the overload stimulus. In any case, the gel analyses suggest that a shift from faster to slower MHC isoforms occurred in the superficial as well as the deep region of the muscle. In addition, a conversion from fast to slow fibers has been observed previously in both the deep (from 16 to 48%) and the superficial (from 3 to 19%) regions of the plantaris after 9–12 wk of overload (4). The changes in the percentage of total MHC observed in the present study after 10 wk of FO are consistent with the results of Fauteck and Kandarian (10) after 5 wk of FO. They found the following shifts from control to FO: 11 to 16% for type I, 14 to 24% for type IIa, 36 to 42% for type IIx, and 39 to 18% for type IIb.

The appearance of the embryonic MHC isoform in a high proportion of plantaris fibers sampled in both overloaded groups after only 1 wk of overload (~40% compared with ~2% in either control group) could reflect the reexpression of this developmental isoform in existing mature fibers or the presence of “new” fibers. On the basis of the similarity in the sizes of the fibers containing embryonic MHC and those fibers of the same type but not having this developmental MHC, the data suggest that the former possibility is more likely. Furthermore, relatively few fibers (~1%) contained embryonic MHC after 10 wk of overload, suggesting that the expression of this MHC is transient and may be related to the initial muscle fiber damage (probably due to the high forces associated with a prolonged and increased yield phase during each step) and inflammation associated with the sudden increase in the loading properties of the muscle after surgery (2). A second and more likely interpretation for the time course of the adaptations in the embryonic MHC composition is that the compensatory hypertrophy after overload is associated with an increase in the number of myonuclei to maintain a relatively constant nuclear domain (cytoplasmic volume per myonucleus). This process would involve satellite cell activation (1), cells that initially express embryonic MHC after fusion with either existing fibers or other satellite cells (9). After 10 wk of overload when the hypertrophic response has plateaued, these cells would most likely have switched to adult MHC isoform expression. The low percentage of fibers containing embryonic MHC at 10 wk after FO is consistent with the small amounts of embryonic and neonatal MHC mRNAs observed after 4 or 11 wk of overload of the plantaris (24). The contention that there
were no new fibers in the FO muscles is consistent with the report of similar total fiber counts after FO with or without endurance treadmill training for 4–40 wk for FO and control plantaris muscles (13).

Perspective. FO is an excellent model for studying the effects of increased activation and loading on the plasticity of skeletal muscle (see Ref. 26 for a review). The adaptations in fiber size, MHC type, and metabolic properties all strongly indicate that a muscle becomes larger and slower after FO. These adaptations have been shown to occur in a variety of muscles (e.g., Ref. 27) and in laboratory animal models other than the rat, e.g., the cat (6, 29, 35) and mouse (28). Because of the rapid time course of these adaptations, FO appears to be an appropriate model for studying the interrelationships among fiber size, metabolic potential, and myonuclear number in hypertrophying and transforming skeletal muscle fibers. Initial results in the cat (1) and preliminary results in the rat (22) indicate that the number of myonuclei (presumably from satellite cell activation and incorporation) increases either in proportion to or at a faster rate than the increase in fiber volume after FO. Thus the increased genetic machinery provided by the additional myonuclei may be one mechanism for increasing muscle protein synthesis during hypertrophy and/or fiber transformation. That treadmill training in FO rats resulted in an enhancement of the muscle hypertrophy but no additional conversion toward slower MHCs suggests that there may be a limit to the modulation of specific protein systems in an overloaded muscle.

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