

Time course adaptations in rat skeletal muscle isomyosins during compensatory growth and regression

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TSIKA, R. W., R. E. HERRICK, AND K. M. BALDWIN. *Time course adaptations in rat skeletal muscle isomyosins during compensatory growth and regression*. *J. Appl. Physiol.* 63(5): 2111–2121, 1987.—The purpose of this study was to ascertain the time course of change during both compensatory growth (hypertrophy) and subsequent growth regression on myosin isoform expression in rodent fast-twitch plantaris muscle in response to functional overload (induced by removal of synergists). Peak hypertrophy of the plantaris muscle (92%) occurred after 9 wk of overload. After 7 wk of overload regression (induced by a model of hindlimb unweighting), muscle weight returned to within 30% of control values. Myofibril protein content (mg/g muscle) remained relatively constant throughout the overload period but became significantly depressed relative to control values after 7 wk of regression. However, when expressed on a per muscle basis (mg/muscle) no differences existed at this time point ($t = 7$ wk regression). The distribution of native myosin isoforms in the myofibril protein pool of the overloaded plantaris muscle reflected a progressive increase (23% at $t = 9$ wk; $P < 0.001$) in the relative proportion of slow myosin (Sm). This change was also accompanied by increases in intermediate myosin (Im) as well as the repression of the fast myosin one (Fm₁) isoform ($P < 0.001$). These shifts in Sm and Fm₁ isoform expression were gradually reversed during the regression period, whereas Im remained elevated relative to control values. These adaptive changes in myosin isoform expression during both hypertrophy and regression were further supported by concomitant shifts in both myosin adenosinetriphosphatase (ATPase) activity (decreased during overload) and slow myosin light chain (SLC) expression. However, during regression the changes in myosin isoform expression and myosin ATPase were not as synchronous as they were during overload. Estimation of the mixed myosin heavy chain (MHC) half-life ($t_{1/2}$), using a linear model that assumes zero-order synthesis and first-order degradation kinetics, revealed $t_{1/2}$ values of ~19 and 10 days for the overload and regression periods, respectively. Collectively these data suggest that 1) skeletal muscle myosin isoforms and corresponding ATPase activity are in a dynamic state of change, although not completely synchronous, in response to altered muscle stress, and 2) the kinetics of change in the mixed MHC protein pool are slower during compensatory growth compared with regression of growth.

myosin; isomyosin expression; myosin adenosinetriphosphatase; turnover; hypertrophy

fiber cross-sectional area (14, 34). Other findings suggest that distinct fibers and regions of the overloaded muscle undergo functional (34, 35) and biochemical (3, 16, 29, 37, 38) adaptations consistent with a conversion of fast- to slow-twitch properties. Interestingly, weight-bearing activity appears to be an essential component of this adaptive response, because in its absence significant increases in muscle enlargement and corresponding shifts in both myosin isoform expression and myofibril adenosinetriphosphatase (ATPase) activity indicative of slow-twitch properties are dramatically reduced (37).

A variety of approaches have clearly established that both isotonic shortening velocity and myosin ATPase activity in a given skeletal muscle are related to its relative content of fast and slow myosin isoforms (4, 33). One of the most notable changes that occurs in an overloaded fast-twitch skeletal muscle, such as the plantaris, is a shift in its myosin isoform pattern. This is reflected by a striking increase in the relative content of both intermediate and slow myosin at the expense of a decrease in the percent of fast myosin content as seen by the repression of fast myosin one isoform (Fm₁) (16, 37, 38). Therefore the present study was designed to examine the time course of change in myosin isoform expression in the context of changes in myosin ATPase activity in functionally overloaded muscle. Furthermore, since this adaptation in isoform expression appears to be dependent on weight-bearing activity (37), it was of further interest to examine whether the removal of weight-bearing activity influences myosin expression in muscles with overload-induced increases in slow myosin expression. This was accomplished by subjecting rats after 12 wk of functional overload to the subsequent intervention of hindlimb suspension, which chronically eliminates weight-bearing activity. Therefore, by coupling these two models in a sequential time course fashion, it was possible to estimate the kinetics of change in the myosin protein pool as well as the change in myosin isoform expression and myosin ATPase activity during both compensatory growth and its regression. Based on these interventions, our findings suggest that a relationship exists between myosin isoform expression and ATPase activity in overloaded skeletal muscle, if a significant increase in the expression of slow myosin isoform (Sm) occurs.

METHODS

Experimental design and surgical and suspension procedures. Female Sprague-Dawley rats, initially weighing

WHEN A SKELETAL MUSCLE is functionally overloaded by the surgical removal of its synergists it adapts by increasing its mass and total protein content (mg/muscle) (37, 38), thereby resulting in an increase in both muscle and

140 g, were used as subjects to study the time course of change in isomyosin expression and myosin ATPase specific activity during compensatory growth and regression of compensatory growth. These data were ascertained in two independent but related experiments.

In *experiment 1* (compensatory growth) rats were weight matched and assigned to one of two groups, designated as normal control (NC, $n = 36$) and overload (OV, $n = 30$). During *experiment 1*, subgroups ($n = 6$) of the OV group were killed after 1, 3, 6, 9, and 12 wk of the overload perturbation. At these same time points, and in addition at $t = 0$, subgroups ($n = 6$) of normal controls were killed and served as a reference point during subsequent weight and biochemical analysis.

In *experiment 2* (regression of compensatory growth), which was performed after *experiment 1*, rats were weight matched and assigned to one of two groups, designated NC ($n = 25$) and overload + suspension (OV + SUS, $n = 25$). During *experiment 2*, rats assigned to the OV + SUS group were first subjected to 12 wk of overload and thereafter to hindlimb unweighting; subgroups ($n = 5$) were killed at 1, 2, 4, and 7 wk postsuspension. For comparison, NC rats were killed at these same time points, and in addition at $t = 0$, and as in *experiment 1* they served as a reference point during subsequent weight and biochemical analysis.

All rats were provided with food and water ad libitum and were housed at room temperature (24°C) with a 12-h light-dark cycle in either standard cages or cages designed for head-down tilt suspension.

Those rats designated for overload were anesthetized with ketamine hydrochloride (10 mg/100 g im), and the plantaris muscle was then overloaded bilaterally by surgical removal of its synergist gastrocnemius and soleus muscles as described by Baldwin et al. (3). After the overload surgery the rats were allowed to recover for 3 days before being housed in groups of three or four in standard cages. The rats were studied at the time points designated after surgery (see above).

The rats designated for suspension were prepared by a noninvasive tail traction procedure which allows for approximately one-third of the tail to remain uncovered, thereby allowing normal thermoregulatory processes to occur. The portion of exposed tail maintained normal coloring, indicating that blood flow was not compromised. The rats were placed in a restraining cage and were not anesthetized, so that the tail was easily manipulated. The skin of the tail was thoroughly cleaned and dried, and a light coat of tincture of benzoin was applied. The benzoin application was dried until tacky with a hair dryer. Next, strips of Fas-Trac (Fas-Trac of California, Van Nuys, CA) the width of the tail were threaded through a swivel harness and applied to four sides of the tail. The tail was then wrapped with a layer of Elastoplast bandage (Biersdorf, Norwalk, CT) followed by a layer of Hexcelite (Hexcel Medical Products, Dublin, CA). After the materials were dried, the rat was suspended from a hook and the swivel system was placed above a case to allow the rat to move 360°. The height of the hook was adjusted so that only the front legs were able to come in contact with the cage floor. The size of the cage was such

that the rats could easily reach food and water without being able to touch the sides of the cage with the hindlimbs. These rats were able to move about freely and were capable of grooming without difficulty. This form of suspension provides traction along the tail and does not cause any obvious lesions on the tail.

Tissue removal. For a given time point (see above), rats were weighed and given an anesthetic overdose of pentobarbital sodium (Nembutal, 100 mg/kg ip). The plantaris muscles were removed, rinsed with cold saline, trimmed clean of visible fat and connective tissue, and weighed. Muscle samples were stored in 100% glycerol at -20°C until analyzed biochemically at a later date. Because of the large number of muscle samples ($n = 232$) involved in this study and the sequential ordering of experimental periods, the biochemical analysis was done in two phases. Muscles from the experimental and control groups comprising the overload period (*experiment 1*) were analyzed simultaneously in the first phase. This was allowed by the analysis of experimental and control muscles of the regression period (*experiment 2*).

Myofibril and myosin extraction. Skeletal muscle myofibrils were prepared by a modification of the method of Solaro et al. (36). All homogenates and suspensions were maintained on ice and all buffers were at 4°C. Approximately 140 mg of tissue representing the belly of the muscle were initially rinsed free of glycerol and homogenized in 10 vol of a solution containing (in mM) 250 sucrose, 100 KCl, 20 imidazole, and 5 ethylenediaminetetraacetic acid (EDTA) (pH 6.8). The homogenate was centrifuged at 1,000 g for 10 min at 4°C, and the supernatant was discarded. The resulting myofibril pellet was rehomogenized in 10 vol of 175 mM KCl containing 0.5% Triton X-100 (pH 6.8) and processed through two additional washings that were designed to remove membrane-bound ATPases. After these washes the purified myofibril pellet was subjected to two more washes in a solution of 150 mM KCl and 20 mM imidazole (pH 7.0). The resulting pellet was then suspended in the same buffer, and the protein concentration was adjusted to 6 mg/ml, using the biuret method (15). This procedure is quantitative for myosin recovery because there is little evidence of loss of myosin in the washing process, based on electrophoretic analysis of the supernatants (unpublished observations). Furthermore our myofibril yields are consistently between 11 and 12% of the total muscle weight, in agreement with the 12% value commonly accepted (41).

Myosin, essentially free of actin, was prepared by ammonium sulfate [(NH₄)₂SO₄] precipitation. Muscle samples, weighing ~300 mg, were homogenized in a solution (1 g/19 ml) containing (in mM) 300 KCl, 150 K₂HPO₄, 10 ATP, 2 EDTA, and 2 dithiothreitol (pH 6.8). The homogenate was gently stirred for 30 min at 4°C. Nonsoluble particulates were removed from the homogenates by centrifugation for 15 min at 48,000 g and further by centrifugation at 160,000 g for 2 h. The resulting supernatant was fractionated at 33% (NH₄)₂SO₄ saturation by addition of 0.5 vol of homogenization buffer 100% saturated with (NH₄)₂SO₄. This mixture was centrifuged at 12,000 g for 20 min. The

resulting supernatant was further fractionated by mixing the recovered volume with 0.34 vol of the saturated $(\text{NH}_4)_2\text{SO}_4$ buffer. After centrifugation at 12,000 g for 20 min the precipitate was resuspended in a buffer (10 ml/g initial wt) consisting of 600 mM KCl and 10 mM imidazole (pH 6.8). The myosin suspension was dialyzed overnight in 1,000 vol of 10 mM imidazole (pH 6.8). The precipitate was collected and dissolved in 150 mM KCl and 30 mM tris(hydroxymethyl)aminomethane (Tris, pH 7.0) to a concentration of 3 mg/ml by the biuret assay (15).

Ca^{2+} -activated ATPase activity of the myosin was determined at 30°C in a 2-ml reaction system containing 50 mM Tris·HCl, 25 mM KCl, 10 mM CaCl_2 (pH 7.4), and 0.25 mg/ml protein. The reaction was initiated by the addition of ATP to a final concentration of 5 mM and terminated after 2 min with 1 ml of 10% trichloroacetic acid (TCA). Aliquots of protein-free filtrate were analyzed for inorganic phosphate (P_i) using the procedure of Fiske and Subbarow (9). An aliquot of the myosin suspension (3 mg/ml) was prepared for storage (-20°C) pending electrophoretic analysis by mixing 1 vol of suspension with 2 vol of a buffer consisting of 75% glycerol, 25 mM $\text{Na}_4\text{P}_2\text{O}_7$, 1 mM ethyleneglycol-bis(β -aminoethylether)- N,N' -tetraacetic acid (EGTA), and 0.5 mM 2-mercaptoethanol (pH 8.8). The resulting myosin suspension was diluted to a final concentration of 1 mg/ml.

Electrophoretic analysis of native myosin. The myofibril samples, prepared as described above, were diluted to a final protein concentration of 1 mg/ml. The native myosins were separated by the method of Hoh et al. (17, 20). Briefly, native polyacrylamide gel electrophoresis (PAGE) was performed on 6-cm-long gels that were 4% in total acrylamide, 2.5% in bis-acrylamide (expressed as percent of total acrylamide), 10% glycerol, and 20 mM sodium pyrophosphate (pH 8.8) at 4°C. The gels were run in a Pharmacia GE-2/4 apparatus with recirculation of a running buffer consisting of 20 mM sodium pyrophosphate, 2 mM cysteine, and 10% glycerol (pH 8.8). The electrophoresis apparatus was cooled by refrigeration at 4°C and additionally by immersed cooling coils connected to an external recirculation refrigeration bath. The temperature of the recirculating buffer was maintained at -1°C throughout electrophoresis. Gels were prerun at 90 V (15 V/cm) maintained constant for 30 min before sample application. Approximately 5 μg of protein were electrophoresed for 20 h at 90 V (15 V/cm) maintained constant. Gels were stained at the end of electrophoresis with a solution that was 0.1% Coomassie blue R-250, 30% isopropyl alcohol, and 10% glacial acetic acid for 2 h. The gels were destained by diffusion in a solution that was 20% methanol and 10% glacial acetic acid.

Sodium dodecyl sulfate-PAGE of myofibrils and myosin. Myosin light chains were analyzed using electrophoretic techniques involving denaturing conditions as described by the method of Laemmli (23). Samples were prepared from the myofibril suspensions (1 mg/ml) by a twofold dilution with a buffer consisting of 100 mM Tris·HCl, 5% glycerol, 4% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, and 0.05% bromphenol blue (pH 6.8)

at room temperature. Purified myosin standards prepared from fast-twitch white gastrocnemius and slow-twitch soleus muscle were also suspended in the above buffer and were electrophoresed to provide markers for fast and slow myosin light chain protein. The sample mixture was warmed to 100°C for 2 min. Light chains of myosin were analyzed in gels that were 13% in total acrylamide, 2.5% in bis-acrylamide (expressed as percent of total acrylamide). The gels were run at room temperature in an apparatus with a central cooling reservoir through which tap water circulated. Electrophoresis was carried out at constant current (40 mA/slab gel) until the dye front reached the end of the gel (~3.5 h). Gels were stained for 2 h at the end of electrophoresis with a solution consisting of 0.1% Coomassie blue R-250, 30% isopropyl alcohol, and 10% glacial acetic acid. The gels were destained by diffusion in a solution that was 20% methanol and 10% glacial acetic acid.

Scanning procedures. The gels were subsequently scanned using a Quick Scan densitometer (Helena Laboratories, Beaumont, TX) connected to an Apple computer modified with Adalab hardware and software (Interactive Microwave, State College, PA) to determine peak area densities of the protein bands. For these scans, a 35-mm record of the backlit gel was made on Technical Pan 2415 (Kodak) exposed at ASA 100 through a 55-mm macro lens (Nikkon) set at $f/22$ and developed 15 min at room temperature in PODA developer (Kodak bulletin P-255). For scanning, a secondary film record was made from the 35-mm record enlarged to full frame on 4 × 5-in. Kodak Commercial 4127 film developed in Kodak HC110 developer (dilution B) for 4 min at room temperature. This film combination gives extremely high resolution and "hard toe" characteristics, yielding true reproduction of the gel image. For quantitating the relative proportions of the native myosins the peak densities of the individual bands representing the individual isozymes were summated to give a total value representing 100% of the total myosin. Then the relative proportion of a given isozyme was determined on the basis of its density relative to the total summated densities. Likewise the relative proportion of an individual myosin light chain was obtained by its density relative to the total summated densities of the individual light chains.

Identification of myosin isoforms. Purified myofibril protein was run on pyrophosphate gels under nondissociating conditions to separate myosin into its native isoforms. In this study, we found that the myofibril protein isolated from the normal control plantaris muscle contained five different myosin isoforms. These myosin isoforms were labeled in order of decreasing migration into pyrophosphate gels as follows: Fm_1 , Fm_2 , Fm_3 , Im , and Sm . This labeling scheme is the same as that used by Hoh et al. (19) and Fitzsimons and Hoh (10) and was adopted only after we were able to repeat the results of Hoh et al. (19) (Fig. 1). On occasion, when analyzing the native isoform profile of the overloaded plantaris muscle, we saw a thin band that migrated just ahead of the major Sm band. This minor component, which is visible in Fig. 1, is labeled Sm_1 , using the terminology of Marechal et al. (24). Because of the inconsistency in the appearance

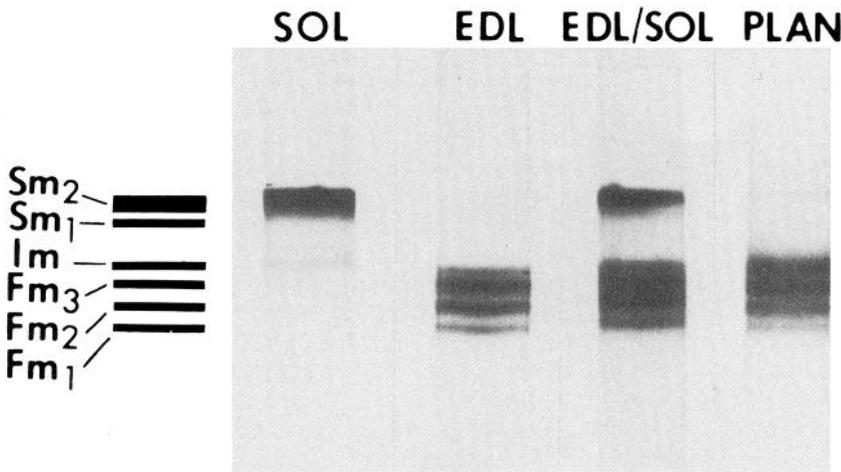


FIG. 1. Native isomyosin profile of typical slow- and fast-twitch skeletal muscle. Nomenclature is that used by Hoh et al. (19) and Marechal et al. (24). SOL, normal soleus; EDL, normal extensor digitorum longus; PLAN, normal plantaris. Sm₂ and Sm₁, slow myosin isoform in order of increasing mobility; Im, intermediate myosin isoform; Fm₃, Fm₂, and Fm₁, fast myosin isoforms in order of increasing mobility.

of this minor band we have elected to report here only one Sm isoform, which we have designated as Sm₂ (24). The light chain composition of the five native myosin isoforms, using the terminology of Hoh (18), are as follows: 1) slow myosin [Sm₂, consisting of slow light chains (SLC), SLC₁ and SLC₂], 2) fast myosin one [FM₁, consisting of fast light chains (FLC), FLC₂ and FLC₃], 3) fast myosin three (FM₃, consisting of FLC₂ and FLC₁), 4) fast myosin two (FM₂, existing as heterodimer of FLC₁, FLC₂, and FLC₃), and 5) intermediate myosin [Im, consisting of a mixture of fast and slow light chains (SLC₁, FLC₁, and FLC₂) (10, 16, 19, 39)].

Estimation of myosin content in skeletal muscle. Two different methods were developed to estimate the percent myosin content in the myofibril fraction [percent mixed myosin heavy chain (MHC)] quantitatively extracted from skeletal muscle (see *myosin and myofibril extraction*). Each was based on peak area densitometric analyses of protein bands derived from SDS-PAGE. This method of analysis is suitable for estimating myosin and myofibril protein content in gels, providing the following conditions are met: 1) myosin and myofibril protein are electrophoresed in the concentration range of 1–15 μ g (7, 8); 2) the staining dye, such as Coomassie blue, has a relatively uniform staining ratio for the major proteins comprising the myofibril fraction (28, 41); and 3) the sensitivity of the staining dye is sufficient to bind to proteins in the concentration range of 0.1 μ g, which is the estimated lower range of the minor protein components in the myofibril fraction. The staining dye used in this study was Coomassie blue R-250, which exhibits a sensitivity that will detect protein concentration as low as 10 ng (31), a sensitivity that is well within the lower limits of protein staining in the present study.

In the first method, SDS-PAGE was carried out in gels that were 10% in total acrylamide and 2.5% in bis-acrylamide (expressed as percent of total acrylamide), using known quantities of myofibril protein (2–4 μ g) run along with known quantities of purified myosin (0.25–1.0 μ g; see Fig. 2). Electrophoresis was carried out at a constant current of 40 mA for 2 h. After electrophoresis, a standard curve to be used for the estimation of myosin content in a given myofibril preparation was constructed

as follows: integral units of purified mixed MHC (y-axis) derived from scanning the lanes in which purified myosin (0.25–1.0 μ g) was electrophoresed were plotted against the quantity of purified myosin (μ g) loaded onto the gel (Fig. 2). In this manner, integral units obtained from scanning lanes in which a sample of myofibril was electrophoresed can be plotted against this standard curve and the unknown myosin content can be solved for. This method yielded an estimate of $48 \pm 4\%$ myosin in the myofibril protein of control plantaris muscle. This value is in general agreement with the results obtained by Yates and Greaser (41) in which MHC and actin were quantitated by amino acid analysis of the appropriate bands from SDS-PAGE.

A second approach was also used; it consisted of SDS-PAGE of 10–15 μ g of myofibril protein along with myosin standards on gels that were 14% in total acrylamide and 2.5% in bis-acrylamide (expressed as percent total acrylamide) to determine MHC content in the myofibril fraction. Electrophoresis was carried out at 40 mA (constant current) for ~3.5 h. The MHC component was expressed as a percent of the total myofibril fraction. This method yielded estimates of $39.5 \pm 0.5\%$ mixed MHC in myofibril protein ($n = 66$) of control plantaris muscles. These two approaches, as utilized herein, are in agreement with one another if one accounts for the observation that the myosin light chains account for ~8% of the myofibril protein (32).

Although the approach we have taken has its limitations in terms of the amount of protein that can be used for analysis, our findings on control muscles are in general agreement with other reports that have used a wide range of experimental approaches to quantify myosin in both whole muscle and the myofibril fraction. These include techniques of quantitative densitometric analysis of MHC in SDS-gel electrophoretograms (2, 6, 28), quantitation of Coomassie blue-stained proteins in polyacrylamide gels based on analysis of eluted dye (8), interference microscopy (21), nucleotide binding (25–27), and radioimmunoassay and isotopic dilution (7). Moreover, Yates and Greaser (41) found no evidence of substantial contaminants in the electrophoretically isolated MHC or actin bands from samples of whole muscle and myofibril

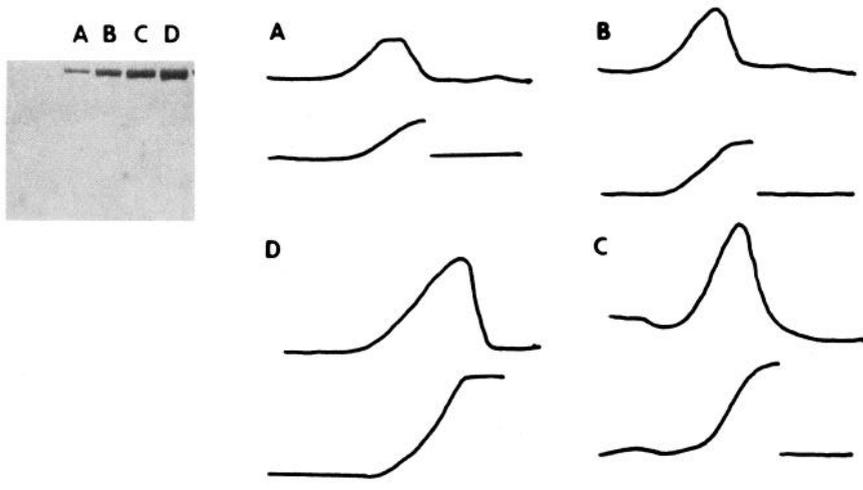
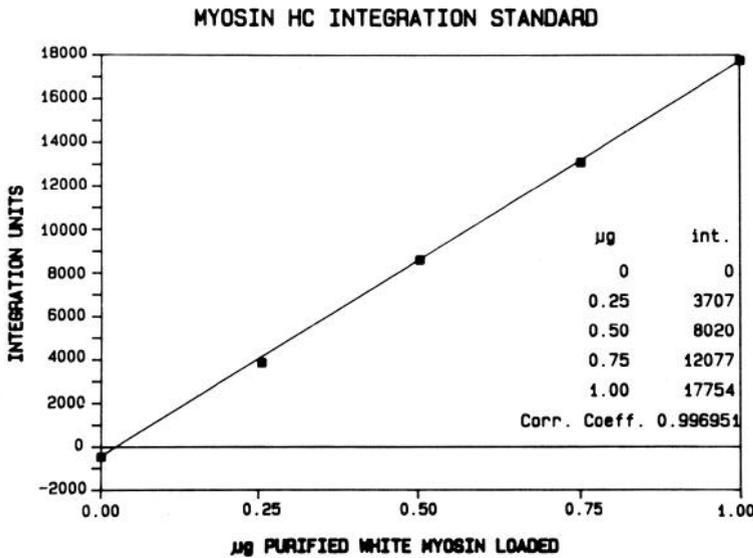


FIG. 2. Standard curve and densitometric scans of purified myosin heavy chains (HC). Standard curve was used for estimating myosin content in myofibril protein pool of plantaris muscle. *Top left*: HC standard curve representing from 0.25 μg of purified white myosin in well A to 1.0 μg in well D. *Top right*: densitometric scans representing density of protein in each band A-D with integrals located directly below each scan. *Bottom*: derived standard curve.



protein that would confound quantitative results. Furthermore, since there was no detectable alteration in the relative distribution of the protein bands comprising the myofibril pool among the various experimental groups examined using our technology, we feel justified in using the derived values reported in the present study on control and experimental muscles for estimating the myosin content per muscle to generate the kinetics of change in the myosin pool during the time course of skeletal muscle compensatory growth (hypertrophy) and its subsequent regression. This was obtained for each time point investigated by using the general equation

(mg myofibril protein/g muscle)

× percent mixed myosin heavy chain

× muscle weight (g) = mg mixed MHC/muscle.

Determination of myofibril and mixed MHC half-life.

To date at least three adult skeletal muscle sarcomeric MHC genes have been identified in rodents (22) and termed as slow/cardiac (β), fast type IIb, and fast type

IIa. Because our method of MHC separation on polyacrylamide gels was not designed to differentiate between these three MHC types we use the term mixed MHC when referring to half-life ($t_{1/2}$) values. To estimate $t_{1/2}$ of the mixed MHC and myofibril protein pools in response to the above perturbations, a linear model that assumes zero-order synthesis (k_s) and first-order degradation kinetics (k_d) was used (5). By applying regression analysis to a plot of $\ln(M_0 - M_{ss})$ vs. t , where M_0 is the mixed MHC or myofibril content at each time point and M_{ss} is the new steady-state mixed MHC or myofibril content of the experimental muscle, k_d was calculated ($k_d = -\text{slope}$). The k_d value was then inserted into the $t_{1/2}$ formula ($t_{1/2} = \ln 2/k_d$) and $t_{1/2}$ was calculated. To accurately assess changes during the compensatory growth and regression periods, NC values of mixed MHC or myofibril protein content were subtracted from those values obtained from the experimental muscles. This procedure was done at each time point studied ($t = 1, 3,$ and 6 wk etc.), thus resulting in a series of values that reflected only the effects of compensatory growth or

regression of compensatory growth. From this series of consecutive values, M_{ss} was ascertained.

Statistical analysis. Data were analyzed for homogeneity of variance using the Cochran *C* test, and all data were found to be homogeneous. A two-way analysis of variance was used to assess the between- and within-group differences over time. Student's *t* test for independent samples were used to assess differences between pairs of means at each time point. Data are reported as means \pm SE, and the lowest significance level was accepted at $P < 0.05$. Data significant to $P < 0.005$ and < 0.001 are reported as such.

RESULTS

Body and muscle mass. Body weight was not significantly different between experimental and control groups throughout the overload and regression periods with the exception of *wks* 6 and 4, respectively ($P < 0.005$ and $P < 0.05$; see Table 1). Normalized (muscle wt/body wt, mg/g) and absolute plantaris weight (mg) of the OV and OV + SUS groups were greater than control values throughout both the overload and regression periods ($P < 0.001$; Table 1). Peak plantaris muscle hypertrophy (mg/g) occurred after 9 wk of overload and amounted to 92%; whereas after 7 wk of regression of compensatory growth, plantaris weights returned to within 30% of the NC values. Throughout both the overload and regression periods, control values served as the reference for normalizing body weight, muscle weight, and myosin ATPase for each time point.

Protein content. The myofibril protein content (mg/g) of the OV and OV + SUS groups did not differ significantly from control values during both the overload and regression periods, with the exception that the OV + SUS group was reduced at *wk* 7 ($P < 0.005$; Table 2). However, when the myofibril protein content of the OV + SUS group was expressed on a per muscle basis [(mg myofibril protein/g) \times (mg muscle wt/1,000) = mg myofibril protein/muscle], no significant difference was found compared with control values (NC = 28.8 ± 1.19 mg/muscle vs. OV + SUS = 30.6 ± 3.82 mg/muscle). In fact, when myofibril protein content was expressed as milligram per muscle during the overload and regression periods the pattern was similar to that seen for mixed MHC expressed as milligram per muscle. (Data are not shown; however, estimates can be derived from Tables 1 and 2.) During overload the estimated mixed MHC content (mg/g) of the OV group, at *wk* 12, was greater compared with control values ($P < 0.005$), whereas that of the OV + SUS group was decreased at *wk* 7 of the regression period ($P < 0.005$; Table 2). Throughout overload the mixed MHC content per muscle (mg/muscle) of the OV group was greater than that of the controls ($P < 0.001$). However, by *wk* 4 of the regression period the mixed MHC content per muscle of the OV + SUS group was not significantly different from control values and, by *wk* 7, reached control values. Thus, with the exception of the last time point of the regression period, the mixed MHC content per muscle reflected the patterns of change in muscle mass (30% larger than NC at $t = 7$ wk) during both compensatory growth and regression of compensatory

TABLE 1. *Body and muscle weights*

	<i>n</i>	Body Wt, g	Plantaris Wt, mg	Normalized Wt, muscle wt/body wt
<i>Compensatory growth</i>				
<i>Wk</i> 0				
NC	6	140 \pm 1	140 \pm 4	1.05 \pm 0.07
OV	6	140 \pm 1	140 \pm 4	0.98 \pm 0.01
<i>Wk</i> 3				
NC	6	213 \pm 8	204 \pm 6	0.96 \pm 0.02
OV	6	208 \pm 3	315 \pm 5*	1.51 \pm 0.04*
<i>Wk</i> 6				
NC	6	250 \pm 7†	256 \pm 14	1.02 \pm 0.06
OV	6	218 \pm 5	366 \pm 15*	1.69 \pm 0.11*
<i>Wk</i> 9				
NC	6	270 \pm 6	249 \pm 6	0.92 \pm 0.03
OV	6	255 \pm 4	436 \pm 25*	1.77 \pm 0.10*
<i>Wk</i> 12				
NC	6	283 \pm 8	318 \pm 8	1.16 \pm 0.03
OV	6	283 \pm 8	434 \pm 27*	1.56 \pm 0.09*
<i>Regression of growth</i>				
<i>Wk</i> 0				
NC	5	262 \pm 9	320 \pm 8	1.23 \pm 0.02
OV + SUS	5	243 \pm 8	519 \pm 19*	2.13 \pm 0.09*
<i>Wk</i> 1				
NC	5	246 \pm 14	290 \pm 13	1.18 \pm 0.04
OV + SUS	5	231 \pm 12	450 \pm 16*	1.96 \pm 0.07*
<i>Wk</i> 2				
NC	5	249 \pm 14	281 \pm 14	1.12 \pm 0.04
OV + SUS	5	241 \pm 5	429 \pm 10	1.78 \pm 0.06*
<i>Wk</i> 4				
NC	5	290 \pm 9‡	327 \pm 10	1.13 \pm 0.03
OV + SUS	5	230 \pm 16	385 \pm 15†	1.69 \pm 0.06*
<i>Wk</i> 7				
NC	5	266 \pm 6	253 \pm 13	0.95 \pm 0.05
OV + SUS	5	273 \pm 8	330 \pm 20†	1.21 \pm 0.09‡

Values are means \pm SE; *n*, no. of rats. NC, normal control; OV, overload; SUS, suspension. Statistical significance for NC vs. experimental groups: * $P < 0.001$; † $P < 0.005$; ‡ $P < 0.05$.

tory growth (Table 2).

Myosin ATPase and myosin isoform expression. The size of the two experiments, i.e., compensatory growth (*experiment 1*) vs. regression of compensatory growth (*experiment 2*), necessitated the use of two different sets of animals. As previously discussed in METHODS, the biochemical analysis, i.e., ATPase analysis, for the two different experiments was done at different times. Therefore, differences seen in the ATPase values between the NC groups of the overload period at $t = 12$ wk and the NC group of the regression periods at $t = 0$ wk can be accounted for based on animal variation. However, there is consistency within the control groups of each experimental period. For the purpose of presenting a cohesive and complete time course analysis, these data are shown sequentially.

Compared with control values, myosin ATPase specific activity ($\mu\text{mol P}_i \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$) was depressed for the time points of *wk* 6–12 during the overload period ($P < 0.005$). However, during the regression period, myosin ATPase activity was significantly depressed, relative to control values, only through the *wk* 1 of suspension. By *wk* 2, myosin ATPase activity had returned to within 6% of control values.

The myosin isoform distribution of the OV group underwent striking changes (Table 3 and Fig. 3). By *wk*

TABLE 2. Protein content and myosin ATPase

	<i>n</i>	Myofibril Protein, mg/g	Mixed MHC, mg/g	Mixed MHC, mg/muscle	Myosin ATPase, $\mu\text{mol P}_i \cdot \text{mg myosin protein}^{-1} \cdot \text{min}^{-1}$
<i>Compensatory growth</i>					
<i>Wk 0</i>					
NC	6	94±5.6	37.9±2	5.2±2.1	0.866±0.060
OV					
<i>Wk 1</i>					
NC	6	107±6.7	40.3±2.3	5.4±2.3	0.834±0.037
OV	6	105±4	40.5±1.8	8.0±1.8*	0.795±0.060
<i>Wk 3</i>					
NC	6	116±2	43.7±1.0	8.8±1.0	0.883±0.035
OV	6	112±1.9	46.7±1.5	15.1±1.5*	0.818±0.065
<i>Wk 6</i>					
NC	6	110±3.7	42.5±1.9	10.8±1.9	0.810±0.023
OV	6	120±5.8	48.5±3.9	17.8±3.9*	0.692±0.024†
<i>Wk 9</i>					
NC	6	121±6.5	48.2±4.7	12.7±4.7	0.806±0.020
OV	6	116±5.8	49.2±3.2	22.9±3.2*	0.636±0.040‡
<i>Wk 12</i>					
NC	6	116±4	48.4±1.5	14.9±1.5	0.919±0.058
OV	6	121±7.5	55.7±1.6†	26.0±1.6*	0.667±0.041†
<i>Regression of growth</i>					
<i>Wk 0</i>					
NC	5	105±4.3	41.5±2.5	13.3±0.5‡	0.761±0.029
OV + SUS	5	98±12.0	40.6±5.5	20.5±2.3	0.584±0.018*
<i>Wk 1</i>					
NC	5	100±7.8	39.5±2.4	11.9±1.5‡	0.758±0.046
OV + SUS	5	98±5.9	39.3±3.9	17.5±2.3	0.645±0.020‡
<i>Wk 2</i>					
NC	5	111±7.4	41.8±2.3	11.7±0.9‡	0.729±0.024
OV + SUS	5	98±2.6	39.0±2.1	16.9±1.3	0.687±0.083
<i>Wk 4</i>					
NC	5	101±6.0	36.4±3.0	12.0±1.3	0.740±0.031
OV + SUS	5	91±3.0	35.6±0.3	13.3±0.6	0.718±0.049
<i>Wk 7</i>					
NC	5	115±5.0‡	47.8±2.5†	11.7±0.7	0.741±0.038
OV + SUS	5	92±6.0	36.6±2.6	11.8±1.7	0.726±0.039

Values are means ± SE; *n*, no. of rats. NC, normal control; OV, overload; SUS, suspension; MHC, myosin heavy chain; ATPase, adenosinetriphosphatase. Statistical significance for NC vs. experimental groups: * *P* < 0.001; † *P* < 0.005; ‡ *P* < 0.05.

1 Im approximately doubled in relative proportion, and it remained elevated throughout the remainder of the overload period. In addition, Sm progressively increased (23%) over control levels (*P* < 0.001). These increases in the relative content of Im and Sm occurred primarily at the expense of reductions in Fm₂ and Fm₁. In fact, the latter became undetectable 3 wk after overload (Table 3).

During the regression period, a partial reversal of the above isoform shifts occurred. Although there were gradual reductions in Sm expression and the reappearance of Fm₁ as the regression period progressed, the increase in Im, induced by the overload, was maintained. The changes in Sm expression were further supported qualitatively by myosin light chain analysis, suggesting increased and decreased staining density of the slow-type light chains during the overload and regression periods,

TABLE 3. Percent myosin isoform distribution in rat plantaris muscle

	<i>n</i>	Sm	Im	Fm ₃	Fm ₂	Fm ₁
<i>Compensatory growth</i>						
<i>Wk 0</i>						
NC	6	4.3±1.0	23.8±1.3	31.3±1.1	26±0.7	14.5±1.0
OV						
<i>Wk 1</i>						
NC	6	3.0±0.3	18.0±1.2	36.7±2.1	28.2±0.8	14.2±0.8
OV	6	2.5±0.3	42.0±0.5*	28.8±1.0†	18.2±1.4†	8.2±0.7†
<i>Wk 3</i>						
NC	6	2.7±0.6	19.3±1.0	34.5±1.6	28.7±1.3	14.8±0.9
OV	6	14.8±1.1*	42.7±0.7*	27.7±1.3‡	14.8±0.5*	0.0±0.0*
<i>Wk 6</i>						
NC	6	3.7±0.5	26.8±1.7	27.3±0.4	29.5±1.4	12.7±1.1
OV	6	18.3±0.8*	38.2±2.0*	29.3±0.8	14.2±1.3*	0.0±0.0*
<i>Wk 9</i>						
NC	6	2.5±0.3	21.0±0.8	31.8±1.3	31.5±2.6	13.8±0.5
OV	6	21.2±1.9*	36.0±2.4*	29.0±2.0	13.8±0.7*	0.0±0.0*
<i>Wk 12</i>						
NC	6	2.8±0.3	19.8±2.4	31.5±4.7	30.8±2.2	15.0±1.4
OV	6	22.8±1.5*	33.8±1.9*	26.3±1.2	17.0±1.7*	0.0±0.0*
<i>Regression of growth</i>						
<i>Wk 0</i>						
NC	5	3.4±0.4	23.0±2.2	31.2±1.4	27.8±2.0	14.6±1.9
OV + SUS	5	23.6±0.7*	32.6±0.4†	27.0±0.8‡	16.8±0.7*	0.0±0.0*
<i>Wk 1</i>						
NC	5	3.8±0.4	22.2±1.1	33.6±1.1	26.6±0.5	13.8±2.1
OV + SUS	5	18.6±0.9*	44.6±1.2*	25.6±0.7*	11.2±1.7*	0.0±0.0*
<i>Wk 2</i>						
NC	5	3.4±0.8	23.4±2.9	37.0±1.7	24.4±2.0	11.8±1.6
OV + SUS	5	17.2±1.5*	42.2±2.3*	25.4±1.1*	13.6±1.9†	1.4;0.9*
<i>Wk 4</i>						
NC	5	3.2±0.4	23.8±0.7	36.4±1.3	26.6±0.8	10.0±1.8
OV + SUS	5	15.6±1.0*	39.2±1.5*	28.2±0.2*	15.0±1.1*	2.0±1.2†
<i>Wk 7</i>						
NC	5	3.6±0.6‡	25.6±2.2	40.4±1.9	21.8±2.8	8.6±1.2
OV + SUS	5	8.2±1.4	39.0±2.7†	33.2±3.9	15.4±1.2	4.2±1.3‡

Values are means ± SE; *n*, no. of rats. Sm, slow myosin isoform; Im, intermediate myosin isoform; Fm₃, Fm₂, and Fm₁, fast myosin isoforms in order of increasing mobility. NC, normal control; OV, overload; SUS, suspension. Statistical significance for NC vs. experimental groups: * *P* < 0.001; † *P* < 0.005; ‡ *P* < 0.05.

respectively (Fig. 4). The failure to reverse the pattern of Im expression during regression of compensatory growth suggests an incomplete removal of stress on the OV + SUS muscle when the suspension intervention was subsequently imposed on the OV skeletal muscle (see DISCUSSION).

Mixed MHC and myofibril t_{1/2}. The *t_{1/2}* values for the mixed MHC and myofibril protein pools during the overload period were 18.8 and 27.7 days, respectively, whereas those for the regression period were 10.3 and 12.8 days, respectively. The generated *t_{1/2}* for both protein components reflected a slower turnover process during the overload compared with the regression period.

The limitations of this method are recognized by the authors; however, *t_{1/2}* values reported herein are used only as an estimate of the time frame in which either the myofibril or mixed MHC protein pools turnover due to the coordinate interaction of protein synthesis and degradation.

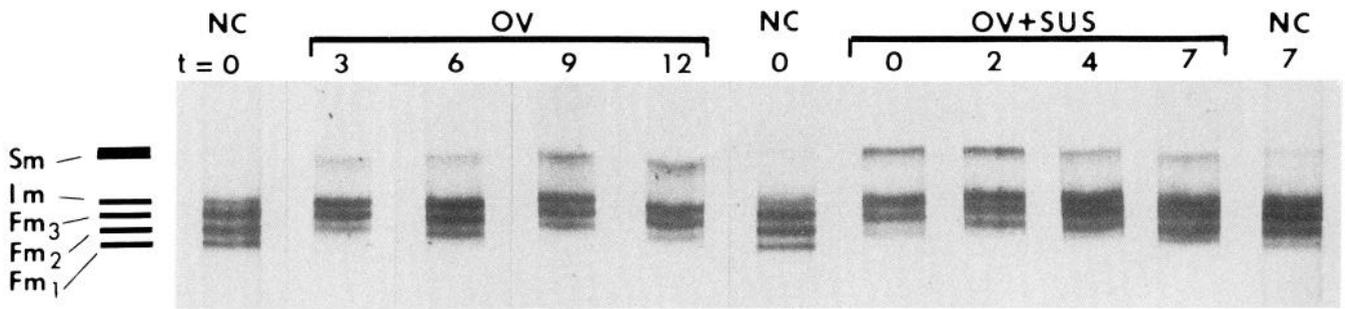


FIG. 3. Electrophoretic separation of native myosin isoforms. Representative gels of normal control (NC), overload (OV), and overloaded + suspended (OV + SUS) groups are shown from various time points ($t = 0, 3, 6$, etc.) of OV and regression periods. Note significant increase in Sm_2 and loss of Fm_1 during OV period, which is in contrast to gradual loss of Sm_2 and reexpression of Fm_1 during regression period. See Fig. 1 legend for definitions of abbreviations.

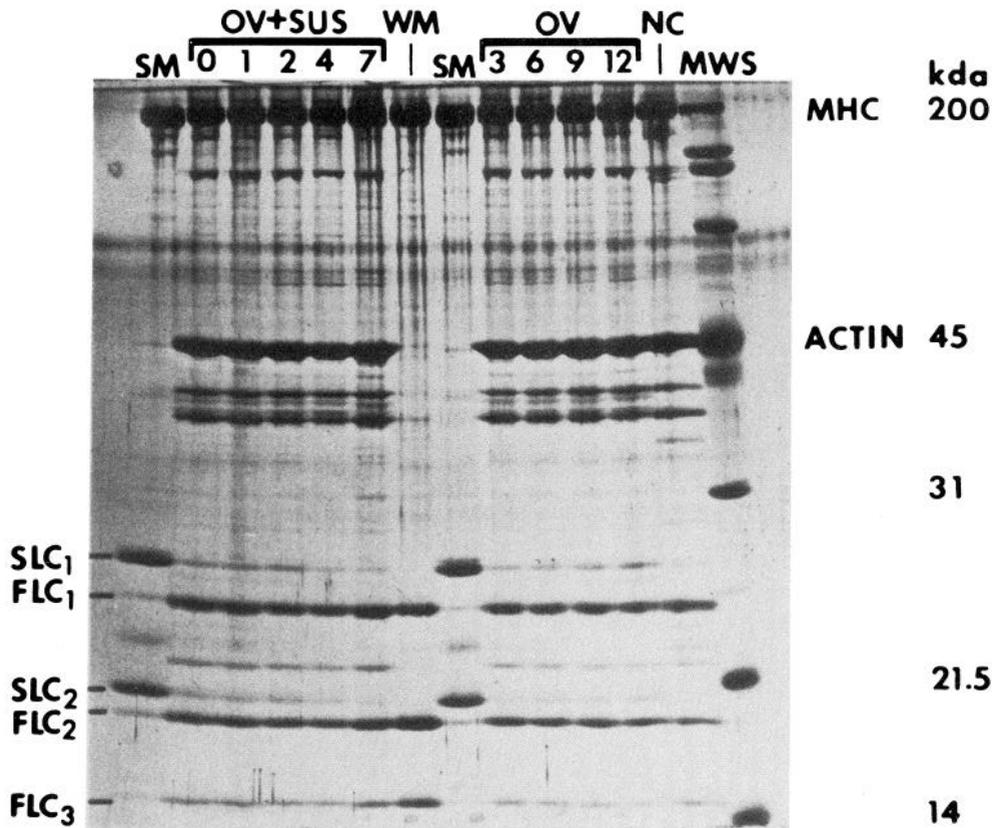


FIG. 4. Myosin light chain analysis was performed under denaturing conditions essentially as described by Laemmli (23). *Left-right*: representative samples of overload + suspension (OV + SUS) and OV myofibril protein from each time point of regression and OV periods. OV + SUS samples show qualitatively a gradual loss of slow-type light chains (SLC_1 , SLC_2), whereas OV samples reveal opposite trend, corroborating findings at native isoform level. FLC, fast light chain; SLC, slow light chain; WM and SM, white muscle and soleus muscle myosin standards, respectively; MWS, molecular weight standard; MHC, myosin heavy chain.

DISCUSSION

Little information exists concerning the plasticity of muscle enlargement processes and myosin ATPase and isoform expression in response to the sequential induction of functional overload and its removal. To gain insight into this process, we have combined sequentially two diverse models of altered skeletal muscle usage, which influence muscle mass in opposite fashions. These procedures were used in an attempt to manipulate mass and contractile protein expression without necessarily

changing the type of nerve innervating the muscle or by artificially stimulating the muscle.

The findings in the present study clearly show that it is possible to alter both mass and myosin isoform expression by altering the manner in which the animal performs weight-bearing activity. By artificially increasing the focus of weight-bearing stress on fast-twitch muscle it is possible to induce a transformation in contractile protein expression favoring myosin isoforms that appear to be more physiologically suitable for chronic weight-bearing

and locomotor activity (37, 38). Conversely, by imposing a model that is known to induce atrophy (loss of mass) of normal muscle it is possible to reverse this process to a significant extent, thereby further illustrating the importance of weight bearing as an important primary stimulus in the regulation of Sm expression.

The observed increase in both muscle mass and mixed MHC content (mg/muscle) of the OV group, throughout the overload period, probably represents an increased rate of protein synthesis in response to the overload. Although a direct measurement of the rates of total protein or myosin synthesis was not made in the present study, Noble et al. (30) have demonstrated increased rates of myosin synthesis in the overloaded rat plantaris muscle, whereas Goldspink et al. (11) have found increased rates of total protein synthesis in overloaded rat muscle. Furthermore, during the regression period the myofibrillar and mixed MHC content per muscle returned to control levels by *wk* 7, whereas muscle mass was still 30% greater than control values (see Tables 1 and 2). Most likely, this is the result of an increased rate of protein degradation in response to the decreased functional demand on the muscles. This is supported by the recent findings of Goldspink et al. (12) in which they reported increased rates of protein degradation in normal suspended and suspended + stretched hindlimb muscles of the rat. This response suggests that a preferential degradation of myofibril/myosin protein relative to total muscle mass occurred (see Tables 1 and 2).

Recent findings suggest a relationship between the maximal shortening velocity of a muscle fiber and its myosin isoform content (33). Our data on whole-muscle myosin ATPase specific activity and percent Sm isoform expression are generally consistent with these findings. By using regression analysis of normalized myosin ATPase specific activity vs. normalized percent Sm in whole muscle of the experimental animals, we observed a significant correlation between these two variables during both the overload and regression periods (Fig. 5). However, in establishing such a relationship at the whole-muscle level, it is apparent that to induce and maintain a significant depression in myosin ATPase specific activity, a significant increase in the expression of Sm must occur.

During the regression period the Sm content of the OV + SUS group gradually declined; but after 7 wk of regression of compensatory growth the level remained elevated above control values. Also the increase in Im induced by overload remained elevated throughout the regression period. These findings suggest that some form of stress is maintained on the overloaded muscle, even though the animals were unable to bear weight. We have found similar results in a previous study (37) in which animals were overloaded while simultaneously subjected to hindlimb suspension. Under these conditions there were small but significant increases in the relative expression of Sm, and this response was also accompanied by an even larger increase in Im. This response in myosin expression, when the models of overload and suspension are combined, is in contrast to conditions in which normal animals (skeletal muscles intact) are sub-

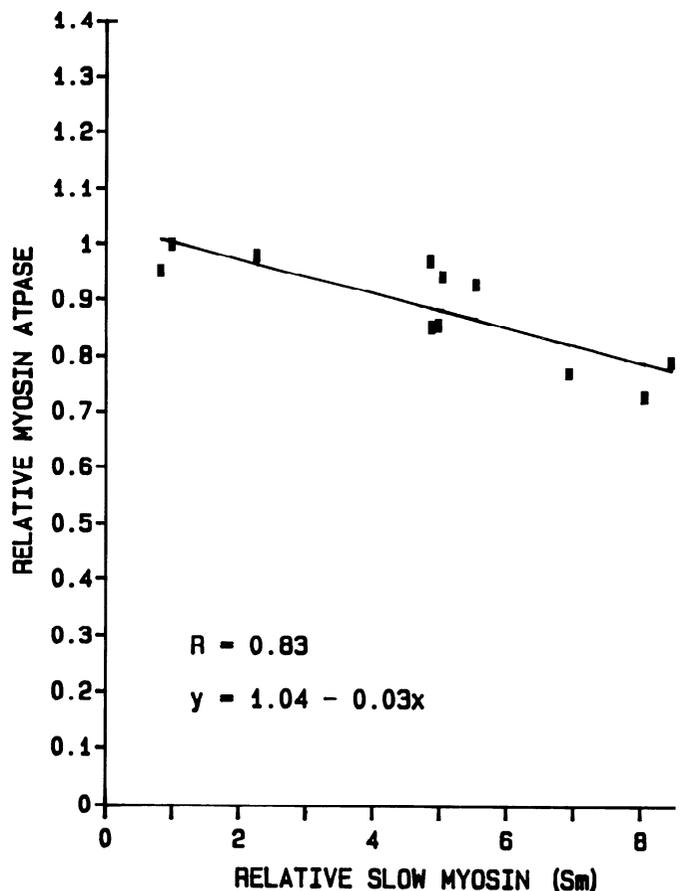


FIG. 5. Relationship between normalized myosin adenosinetriphosphatase (ATPase) and normalized percent slow myosin isoform (Sm), in whole rat plantaris muscle, representing overload and regression periods.

jected to hindlimb unweighting. Under the latter conditions, there is significant atrophy of the plantaris muscle and down regulation of Sm expression (37). Furthermore Im is not affected in the normal plantaris muscle when subjected to hindlimb unweighting. These data are consistent with the notion that some degree of stress, either active or passive, but not associated with weight bearing, is still manifest on the OV + SUS plantaris muscle.

A possible explanation for the above observation is that when a muscle such as the plantaris is overloaded by the procedure of synergist removal, the antagonistic muscles (e.g., tibialis anterior) exert a stretch on the overloaded muscle creating a chronic residual mechanical stress. Because of this low-level chronic stress (stretch) being placed on the muscle, it responds by increasing its relative expression of predominantly intermediate myosin (NC = 26% vs. OV + SUS = 39%) and slow myosin to a lesser degree (NC = 4% vs. OV + SUS = 8%) (see Table 3). If a chronic stretch is created by the antagonist (e.g., tibialis anterior), one might predict a loss in mass of the antagonist muscle because it would assume a shortened position. In support of this, previous studies have shown that when the tibialis anterior of the OV + SUS group was analyzed for wet weight, a 29% loss in mass was found (37, 38). Additionally, although these rats (OV + SUS) were not allowed to bear weight, they continue to use their legs in an active but unloaded fashion. This type of activity may contribute to this low-

level chronic mechanical stress, thereby increasing the relative expression of Im and Sm isoforms.

One interesting observation to evolve in the present study concerns the rapid time ($t = 1$ wk) in which Im is increased in relative content compared with appropriate control muscles during both the onset of compensatory growth and subsequent regression of compensatory muscle growth (see Table 3 and Fig. 3). Although the specific role of Im is not clear in terms of muscle function, it appears to be heavily expressed when the muscle is undergoing some transitional adaptation in fast-slow property. Moreover, we have observed that Im is normally heavily expressed in regions of skeletal muscle known to be involved in dynamic locomotor activity (39). Collectively these data suggests that Im may play an important functional role if it is indeed expressed in those motor units normally recruited with the major responsibility of performing repetitive limb movements while bearing a load. This functional role of Im would be in contrast to Sm, which appears to be most suitable for economizing isometric or more "static" contractile activity (13) and in contrast to the fast myosin isoforms, which are predominantly expressed in motor units recruited only for brief intense periods of contractile activity requiring high power output (40). Of further interest in the observation that during the overload period, Fm₁ expression became repressed during the time course of overload, whereas it soon reappeared (reexpressed) in the OV + SUS plantaris muscle by wk 2 of the regression period. These observations are in keeping with the aforementioned notion that during the overload period the functional responsibility of the majority of motor units of the plantaris muscle may be involved in greater static/dynamic usage. This chronic activity pattern could induce down regulation of Fm₁ while inducing up regulation of Im and Sm. In contrast, during the regression period in which the OV + SUS plantaris muscle is dramatically unloaded, the motor unit usage pattern would be expected to change in a fashion characteristic of reducing the static weight-bearing activity. In the context of these adaptations it is interesting to note that the electromyographic patterns of fast-twitch skeletal muscle reflect normal activity during hindlimb suspension (1). Thus during suspension the muscle remains involved in some form of dynamic activity but most likely with a reduced load.

In addition to the differential response of myosin isoform expression during the overload and regression periods, it is also of interest that the mixed MHC and myofibril protein $t_{1/2}$ values for these periods differed. The longer $t_{1/2}$ values observed during the overload period represent slower turnover kinetics for the myofibril and mixed MHC protein pools. In contrast, the regression period was found to have shorter $t_{1/2}$ values, reflecting more rapid kinetics of turnover.

In summary, by linking two diverse models of muscle usage in a sequential fashion, we have demonstrated striking changes in myosin isoform expressions of the rat plantaris muscle. The type and amount of isomyosin expressed, as well as the kinetics of turnover, appear to be determined by the degree of stress placed on the

plantaris, i.e., the functional role it assumes during both compensatory growth and its regression.

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REFERENCES

- ALFORD, E. K., R. R. ROY, P. C. CHIANG, AND V. R. EDGERTON. Hindlimb suspension effects on integrated electromyographic activity in selected rat hindlimb muscles (Abstract). *Physiologist* 28: 315, 1985.
- ARATA, R., AND Y. TONOMURA. Structure and function of the two heads of the myosin molecule. *J. Biochem. Tokyo* 80: 1353-1358, 1976.
- BALDWIN, K. M., V. VALDEZ, R. E. HERRICK, A. M. MACINTOSH, AND R. R. ROY. Biochemical properties of overloaded fast-twitch skeletal muscle. *J. Appl. Physiol.* 52: 467-472, 1982.
- BARANY, M. ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* 50: 197-218, 1967.
- BERLIN, C. M., AND R. T. SCHIMKE. Influence of turnover rates on the responses of enzymes to cortisone. *Mol. Pharmacol.* 1: 149-156, 1965.
- COOKE, R., AND K. FRANKS. All myosin heads form bonds with actin in rigor rabbit skeletal muscle. *Biochemistry* 19: 2265-2269, 1980.
- EVERETT, A. W., G. PRIOR, W. A. CLARK, AND R. ZAK. Quantitation of myosin in muscle. *Anal. Biochem.* 130: 102-107, 1983.
- FENNER, C., R. R. TRAUT, D. T. MASON, AND J. WIKMAN-COFELT. Quantification of Coomassie blue stained proteins in polyacrylamide gels based on analyses of eluted dye. *Anal. Biochem.* 63: 595-602, 1975.
- FISKE, C. H., AND Y. J. SUBBAROW. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375-400, 1925.
- FITZSIMONS, R. B., AND J. F. Y. HOH. Myosin isozymes in fast-twitch and slow-twitch muscles of normal and dystrophic mice. *J. Physiol. Lond.* 343: 539-550, 1983.
- GOLDSPINK, D. F., P. J. GARLICK, AND M. A. MCNURLAN. Protein turnover measured in vivo and in vitro in muscles undergoing compensatory growth and subsequent denervation atrophy. *Biochem. J.* 210: 89-98, 1983.
- GOLDSPINK, D. F., A. J. MORTON, P. LOUGHNA, AND G. GOLDSPINK. The effect of hypokinesia and hypodynamia on protein turnover and the growth of four skeletal muscles of the rat. *Pfluegers Arch.* 407: 333-340, 1986.
- GOLDSPINK, G., R. E. LARSON, AND R. E. DAVIES. The immediate energy supply and the cost of maintenance of isometric tension for different muscles in the hamster. *Z. Vergl. Physiol.* 66: 389-397, 1970.
- GOLLNICK, P. D., B. F. TIMSON, R. L. MOORE, AND M. REIDY. Muscular enlargement and number of fibers in skeletal muscles of rats. *J. Appl. Physiol.* 50: 936-943, 1981.
- GORNALL, A. G., C. J. BARDAWILL, AND M. M. DAVID. Determination of serum proteins by means of the biuret method. *J. Biol. Chem.* 177: 751-756, 1949.
- GREGORY, P., R. B. LOW, AND W. S. STIREWALT. Changes in skeletal-muscle myosin isozymes with hypertrophy and exercise. *Biochem. J.* 238: 55-63, 1986.
- HOH, J. F. Y. Light chain distribution of chicken skeletal muscle myosin isoenzymes. *FEBS Lett.* 90: 297-300, 1978.
- HOH, J. F. Y. Myosin isoenzymes in skeletal and cardiac muscle. In: *Cross-Bridge Mechanism in Muscle Contraction*, edited by H. Sugi and G. H. Pollack. Tokyo: Univ. of Tokyo Press, 1979, p. 489-498.
- HOH, J. F. Y., B. T. S. KWAN, C. DUNLOP, AND B. H. KIM. Effects of nerve cross-union and cordotomy on myosin isozymes in fast-twitch and slow-twitch muscles of the rat. In: *Plasticity of Muscle*, edited by D. Pette. New York: de Gruyter, 1980, p. 339-352.

20. HOH, J. F. Y., P. A. MCGRATH, AND R. I. WHITE. Electrophoretic analysis of multiple forms of myosin in fast-twitch and slow-twitch muscles of the chick. *Biochem. J.* 157: 87-95, 1976.
21. HUXLEY, H. E., AND J. HANSON. Quantitative studies on the structure of cross-striated myofibrils. I. Investigations by interference microscopy. *Biochim. Biophys. Acta* 23: 229-249, 1957.
22. IZUMO, S., B. NADAL-GINARD, AND V. MAHDAVI. All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science Wash. DC* 231: 597-600, 1986.
23. LAEMMLI, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature Lond.* 227: 680-685, 1970.
24. MARECHAL, G., K. SCHWARTZ, G. BECHERS-BLEUKX, AND E. GHINS. Isozymes of myosin in growing and regenerating rat muscles. *Eur. J. Biochem.* 138: 421-428, 1984.
25. MARSTON, S. The nucleotide complexes of myosin in glycerol-extracted muscle fibres. *Biochim. Biophys. Acta* 305: 397-412, 1973.
26. MARSTON, S. B., AND R. T. TREGGAR. Evidence for a complex between myosin and ADP in relaxed muscle fibres. *Nature New Biol.* 235: 23-24, 1972.
27. MARUYAMA, K., AND A. WEBER. Binding of adenosine triphosphate to myofibrils during contraction and relaxation. *Biochemistry* 11: 2990-2998, 1972.
28. MORIMOTO, K., AND W. F. HARRINGTON. Substructure of the thick filament of vertebrate striated muscle. *J. Mol. Biol.* 83: 83-97, 1974.
29. NOBLE, E. G., L. DABROWSKI, AND C. D. IANUZZO. Myosin transformation in hypertrophied rat muscle. *Pfluegers Arch.* 396: 260-262, 1983.
30. NOBLE, E. G., Q. TANG, AND P. B. TAYLOR. Protein synthesis in compensatory hypertrophy of rat plantaris. *Can. J. Physiol. Pharmacol.* 62: 1178-1182, 1984.
31. POEHLING, H.-M., AND V. NEUHOFF. Visualization of proteins with a silver "stain": a critical analysis. *Electrophoresis* 2: 141-147, 1981.
32. POTTER, J. D. The content of troponin, tropomyosin, actin and myosin in rabbit skeletal muscle myofibrils. *Arch. Biochem. Biophys.* 162: 436-441, 1974.
33. REISER, P. J., R. L. MOSS, G. G. GIULIAN, AND M. L. GREASER. Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. *J. Biol. Chem.* 260: 9077-9080, 1985.
34. ROY, R. R., K. M. BALDWIN, T. P. MARTIN, S. P. CHIMARUSTI, AND V. R. EDGERTON. Biochemical and physiological changes in overloaded rat fast- and slow-twitch ankle extensors. *J. Appl. Physiol.* 59: 639-646, 1985.
35. ROY, R. R., I. D. MEADOWS, K. M. BALDWIN, AND V. R. EDGERTON. Functional significance of compensatory overloaded rat fast muscle. *J. Appl. Physiol.* 52: 473-478, 1982.
36. SOLARO, R. J., D. C. PANG, AND F. N. BRIGGS. The purification of cardiac myofibrils with triton X-100. *Biochim. Biophys. Acta* 245: 259-262, 1971.
37. TSIKA, R. W., R. E. HERRICK, AND K. M. BALDWIN. Interaction of compensatory overload and hindlimb suspension on myosin isoform expression. *J. Appl. Physiol.* 62: 2180-2186, 1987.
38. TSIKA, R. W., R. E. HERRICK, AND K. M. BALDWIN. Effect of anabolic steroids on overloaded and overloaded suspended skeletal muscle. *J. Appl. Physiol.* 63: 2128-2133, 1987.
39. TSIKA, R. W., R. E. HERRICK, AND K. M. BALDWIN. Subunit composition of rodent isomyosins and their distribution in hindlimb skeletal muscles. *J. Appl. Physiol.* 63: 2101-2110, 1987.
40. WALMSLY, B., J. A. HODGSON, AND R. E. BURKE. Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J. Neurophysiol.* 41: 951-960, 1978.
41. YATES, L. D., AND M. L. GREASER. Quantitative determination of myosin and actin in rabbit skeletal muscle. *J. Mol. Biol.* 168: 123-141, 1983.