Force-velocity and power characteristics of rat soleus muscle fibers after hindlimb suspension

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McDonald, K. S., C. A. Blaser, and R. H. Fitts. Force-velocity and power characteristics of rat soleus muscle fibers after hindlimb suspension. *J. Appl. Physiol.* 77(4): 1609–1616, 1994.—The effects of 1, 2, and 3 wk of hindlimb suspension (HS) on force-velocity and power characteristics of single rat soleus fibers were determined. After 1, 2, or 3 wk of HS, small fiber bundles were isolated, placed in skinning solution, and stored at $-20°C$ until studied. Single fibers were isolated and placed between a motor arm and force transducer, functional properties were studied, and fiber protein content was subsequently analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Additional fibers were isolated from soleus of control and after 1 and 3 wk of HS, and fiber type distribution and myosin light chain stoichiometry were determined from SDS-PAGE analysis. After 1 wk of HS, percent type I fibers declined from 82 to 74%, whereas hybrid fibers increased from 10 to 18%. Percent fast type II fibers increased from 8% in control and 1 wk of HS to 26% by 3 wk of HS. Most fibers showed an increased unloaded maximal shortening velocity (V_o), but myosin heavy chain remained entirely slow type I. The mechanism for increased V_o is unknown. There was a progressive decrease in fiber diameter (14, 30, and 38%) and peak force (38, 56, and 63%) after 1, 2, and 3 wk of HS, respectively. One week of HS resulted in a shift of the force-velocity curve, and between 2 and 3 wk of HS the curve shifted further such that V_o was higher than control at all relative loads <45% peak isometric force. Peak absolute power output of soleus fibers progressively decreased through 2 wk of HS but showed no further change at 3 wk. The results suggest that between 2 and 3 wk the HS-induced alterations in the force-velocity relationship act to maintain the power output of single soleus fibers despite a continued reduction in fiber force.

contractile properties; slow-twitch fibers; myosin isozyme content

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MUSCLE ATROPHY and the loss of functional capacity are serious problems associated with spaceflight (27). During Skylab flights, astronauts experienced a 12% decrease in leg volume and a 20% decrease in muscle strength (3). The exact causes of the reduced muscle work capacity are unknown but could involve processes affecting the extent and frequency of motor unit activation, neuromuscular synaptic transmission, the coupling of muscle excitation and contraction, and the number and cycling rate of the force-generating cross bridges.

One animal model that has been developed to study the morphological and physiological changes in unloaded muscles is hindlimb suspension (HS). In the rat, HS has been found to elicit changes in muscle size, biochemistry, and function similar to those associated with zero gravity (0 G) (8–10, 12, 17, 23, 26). Both HS and 0 G induce the greatest changes in the slow antigravity muscles such as the soleus. For example, the relative atrophy observed with both HS and 0 G was soleus > gastrocnemius = plantaris > extensor digitorum longus (12, 23).

HS has also been found to alter the isometric and isotonic contractile properties of the intact soleus of the rat. Peak twitch and tetanic tensions, as well as twitch contraction and half-relaxation times, were reduced after HS (5, 9, 20). Furthermore, the maximal velocity of shortening, determined using both afterloaded (V_{max}) (5, 9) and unloaded (V_o) (5) contractions, was elevated after HS. These elevated speeds of shortening were associated with an increase in the relative amount of fast myosin isoforms (5, 9).

To assess the cellular basis of the functional changes induced by 0 G or HS, studies using single soleus fibers have been undertaken. HS caused a reduction in the size and peak tension-generating capacity of single fibers, which, in part, explained the drop in the peak tension of the intact soleus (9, 10, 24). Also, single-fiber V_o (10, 17) and adenosine triphosphatase (ATPase) activities (17) were elevated after HS. Although the percentage of soleus fast fibers increased from 4% in control to 29% after 3 wk of HS, a population of fibers showed an increased V_o and ATPase activity with no detectable fast myosin on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Additionally, no increase in the percentage of hybrid fibers was observed (17). These findings suggest that the altered functional capacity of the intact muscle after HS or 0 G is, at least in part, explained by cellular changes in the individual skeletal muscle fibers.

In vivo muscles shorten against a load, and thus the important functional property to move a load over time is power output, which comprises both force and velocity. The effect of HS on the time course of change in power output of single soleus fibers has not been characterized. Consequently, the purpose of this study was to examine the effect of 1, 2, and 3 wk of HS on the force-velocity relationship and power characteristics of individual slow-twitch fibers from the soleus. A second objective was to reevaluate the effect of HS on the soleus fiber type distribution, with particular emphasis on the relationship between fiber V_o and the myosin isozyme pattern.

MATERIALS AND METHODS

Animal care and suspension procedure. Male Sprague-Dawley rats (250–275 g) were obtained from Sasco (Madison, WI) and randomly divided into either the HS or cage control group. The HS rats were suspended for either 1, 2, or 3 wk by a tail harness attached to the proximal two-thirds of the tail as previously described in detail (9). The hindlimbs were elevated to prevent contact with any supportive surfaces. The forelimbs maintained contact with a grid floor, allowing the animal full range of motion. The rats were maintained on a diet of Purina rodent chow and water. The HS animals were fed ad libitum, whereas the control rats were pair fed to maintain body weights similar to those of the HS group. The two groups were main-

tained in separate rooms, both of which were maintained at 23°C with a 12:12-h light-dark cycle.

Muscle and single-fiber preparation. After 1, 2, or 3 wk of HS or cage control, the rats were weighed and anesthetized with pentobarbital sodium (50 mg/kg body wt ip). The soleus muscle was isolated, weighed, and divided into bundles of ~50–100 fibers. The bundles were tied to glass capillary tubes and stored at −20°C in a skinning solution containing (in mM) 125 K-propionate, 2 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 4 ATP, 1 MgCl, and 20 imidazole, as well as 50% (vol/vol) glycerol for up to 4 wk (10). On the day of an experiment, a bundle was transferred to a dissection chamber that contained relaxing solution composed of (in mM) 20 imidazole (pH 7.0), 7 EGTA, 5.4 $MgCl_2$, 10 caffeine, 4.7 ATP, 0.016 $CaCl_2$, and 14.5 creatine phosphate, as well as sufficient KCl to adjust ionic strength to 180 mM. A single fiber was carefully isolated from the bundle, and a segment (~2 mm) of the fiber was transferred to an experimental chamber containing relaxing solution. The fiber was mounted between a force transducer (model 400, Cambridge Technology, Cambridge, MA; sensitivity 2 mV/mg) and an isotonic DC torque motor (model 300H, Cambridge Technology), as described previously in detail (20). Fiber segments were exposed for 1 min to relaxing solution containing 0.5% Brij-58 (polyoxyethylene 20 cetyl ether; Sigma Chemical). This treatment disrupted sarcoplasmic reticulum membranes and improved the resolution of sarcomeres. The segment length was adjusted to a sarcomere spacing of 2.6 μm, and a Polaroid photograph was taken while the fiber was briefly suspended in air. From the photograph, the fiber diameter was measured at three equal-distant locations along the fiber and the average fiber diameter was determined. Fiber cross-sectional area was calculated by assuming a circular cross section.

Determination of peak force and tension. The outputs of the force and position transducers were amplified and sent to a Commodore 64 microcomputer via a universal input-output board (Microworld Computers, Lakewood, CO). Force in relaxing solution was monitored, and the fiber was activated by transfer into activating solution containing (in mM) 20 imidazole (pH 7.0), 7 EGTA, 5.4 $MgCl_2$, 10 caffeine, 4.7 ATP, 107.8 KCl, 14.5 creatine phosphate, and 7.0 $CaCl_2$. The pCa (−log [Ca^{2+}]) of the relaxing (pCa 9.0) and activating (pCa4.5) solution was determined as described by Metzger et al. (18). The sarcomere spacing during peak activation was 2.5 μm. Peak force (in N) was determined in each fiber by computer subtraction of the baseline force from peak active force. Peak tension (in kN/m^2) was calculated from the peak force and cross-sectional area.

Determination of V_o and force-velocity curve. V_o was obtained using the slack test method (7, 20) exactly as described previously (17). The fiber was maximally activated and then rapidly shortened to a predetermined length, such that tension fell to zero. The fiber shortened under zero load, taking up the slack, at which time tension began to redevelop. The time of unloaded shortening was determined by computer analysis. Several different length steps were used for each fiber, and the slack distance was plotted as a function of the duration of unloaded shortening. V_o [fiber length (fl)/s] was calculated by dividing the slope of the best-fit line by the segment length, and the data were normalized to a sarcomere length of 2.5 μm.

After the slack test, the force-velocity relationship was determined for each fiber by a procedure previously described by Julian and Moss (15). The fiber was fully activated in pCa 4.5 solution. After the development of peak force, the computer switched the motor control circuit from length to force control and the load on the fiber was rapidly stepped to a value less than peak force. Force was maintained constant at the load for 100 ms, during which time the length change was monitored.

Second and third steps to lower loads were applied and the length change monitored. Representative records are shown for a control and HS soleus fiber in Fig. 1. The computer calculated the velocity of shortening from the slope of the length change during the last 50 ms of each load step. The procedure was repeated four to six times such that 12–18 different loads were studied for each fiber. Loads were expressed as a percentage of peak force. V_{max} was calculated by the straight line form of the Hill equation $(P_o - P)/V = P/b + a/b$. Hyperbolic force-velocity curves were fit to the force-velocity data using the Hill equation $(P + a)(V + b) = (P_o + a)b$, where P is force during shortening at velocity V, P_o is the peak isometric force, and a and b are constants with dimensions of force and velocity, respectively (14).

Myosin analysis. After the functional measurements, the myosin heavy and light chain (MHC and MLC) compositions of each fiber were determined by SDS-PAGE (13). The fiber segments (~2 mm) were solubilized in 10 μl of 1% SDS-PAGE sample buffer composed of 6 mg/ml of EDTA, 0.06 M tris(hydroxymethyl)aminomethane, 1% SDS, 2 mg/ml of bromophenol blue, 15% glycerol, and 5% β-mercaptoethanol and stored at −80°C. To evaluate the MHC, 2.5 μl of sample buffer (~0.5 nl of fiber volume) were run on a minigel consisting of a 3.5% (wt/vol) acrylamide stacking gel and a 9.5% separating gel. The MLCs were analyzed by loading 7.5 μl of sample buffer (~1.5 nl fiber volume) to a minigel consisting of a 3.5% acrylamide stacking gel and a 12% separating gel. Gels were silver stained using the technique of Guilian et al. (13) and scanned on a scanning densitometer (CliniScan 2, Helena Laboratories, Beaumont, TX).

Fiber type distribution. An additional 200–300 fibers were isolated from the soleus skinned fiber bundles from control and after 1 and 3 wk of HS. In these experiments, the entire fiber (6–7 mm) was solubilized in 10 μl of 1% SDS sample buffer and stored at −80°C. The entire fiber (10 μl of sample buffer) was run on a Hoefer SE 600 gel system consisting of a 3.75% (wt/vol) acrylamide stacking gel and a 12% separating gel exactly as described by Guilian et al. (13). A representative subpopulation of fibers from control and after 1 and 3 wk of HS was scanned for the determination of MLC stoichiometry. The fibers were classified as fast, slow, or hybrid (fast and slow) on the basis of their MHC profile. In a subset of fibers isolated from control and after 2 wk of HS, fiber V_o and MHC and MLC profiles were determined on the same fiber. After the measurement of V_o, MHC was determined by loading one-half of the fiber on a SDS-PAGE gel using a 3.5% (wt/vol) acrylamide stacking gel with 5% separating gel at 4°C (16). The remaining portion of the fiber was used to assess MLC content by using a 12% SDS gel as described above.

Statistical analysis. A one-way analysis of variance was used to compare fiber diameter, peak force, peak tension, V_o, V_{max}, and peak power between the four groups. The force-velocity and power-load curves were analyzed with a two-way analysis of variance. A Student-Newman-Keuls test was used as a post hoc test to estimate the differences among means. $P < 0.05$ was chosen as significant.

RESULTS

HS induced significant atrophy of the soleus muscle. One, 2, and 3 wk of HS resulted in a 28, 44, and 56% decline, respectively, in the soleus muscle-to-body weight ratio. The time course of change in soleus fiber diameter and peak force and tension is presented in Table 1. Diameter and peak force of the slow type I fibers progressively declined with increasing duration of HS. Peak tension
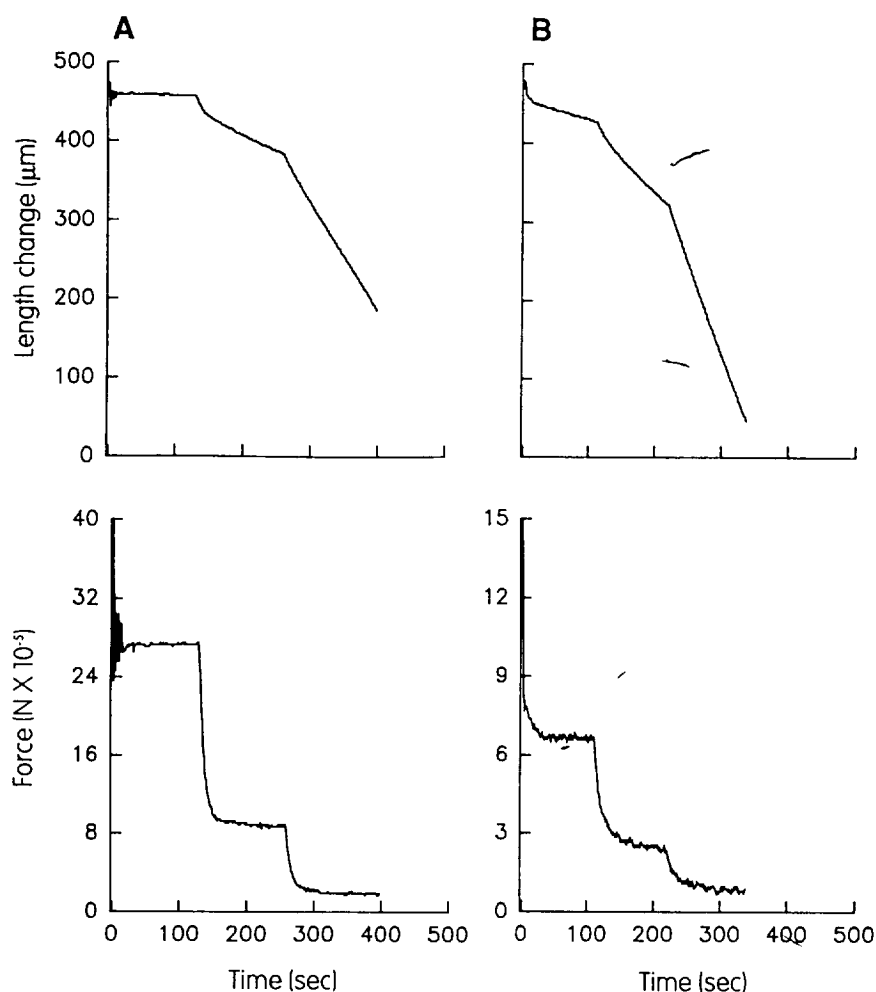
FIG. 1. Representative length and force records of soleus fibers from control and hindlimb suspension (HS) animals during force-velocity test. Shortening at each load was determined from slope of length change over last half of each load step. *A*: control. *B*: after 1 wk of HS.

TABLE 1. *Diameter, peak force, and peak tension of slow type I fibers from soleus of control and HS animals*

	n	Fiber Diameter, μm	Peak Force, $\times 10^{-5} N$	Peak Tension, kN/m^2
Control	20	66±2	36.8±1.6	107±4
1 wk of HS	19	57±1*	22.8±1.3*	89±3*
2 wk of HS	25	46±2*†	16.1±1.0*†	96±5
3 wk of HS	15	41±2*‡	13.5±1.1*†	102±6

Values are means ± SE; n, no. of fibers. HS, hindlimb suspension. * Significantly different from control, $P < 0.05$; † significantly different from 1 wk of HS, $P < 0.05$; ‡ significantly different from 2 wk of HS, $P < 0.05$.

TABLE 2. *Velocity and power characteristics of slow type I fibers from soleus of control and HS animals*

	n	V_o, fl/s	V_{max}, fl/s	Power, $\times 10^{-5} N \cdot fl \cdot s^{-1}$
Control	20	1.31±0.09	1.12±0.07	1.73±0.09
1 wk of HS	19	2.02±0.11*	1.72±0.15*	1.41±0.09*
2 wk of HS	25	1.59±0.07*†	1.47±0.08*	0.89±0.05*†
3 wk of HS	15	2.41±0.16*†‡	1.78±0.09*	0.96±0.08*†

Values are means ± SE; n, no. of fibers. V_o, maximum shortening velocity determined by slack test; V_{max}, maximum shortening velocity determined by straight line form of Hill equation for force-velocity relation; fl, fiber length. * Significantly different from control, $P < 0.05$; † significantly different from 1 wk of HS, $P < 0.05$; ‡ significantly different from 2 wk of HS, $P < 0.05$.

decreased significantly after 1 wk of HS but returned to the control level by 2 wk of HS.

The velocity and power of control and HS single soleus fibers are summarized in Table 2. The mean slow fiber V_o determined by the slack test was significantly elevated after 1 and 2 wk of HS and increased further at 3 wk of HS such that at this time point V_o was significantly higher than all other time points. The increased V_o occurred without the appearance of either the fast MHC or MLC. This can be clearly seen in Fig. 2 where the MHC (*A*) and MLC (*B*) profiles of soleus fibers from control (*lanes 4–12*) and after 2 wk of HS (*lanes 13–17*) are shown. For example, the fiber in *lane 14* contained only

slow myosin, yet its V_o was 2.14 fl/s. This velocity was higher than any of the control fibers shown, including the fast type IIa fiber in *lane 10* (1.97 fl/s) and the hybrid fiber in *lane 6* (1.54 fl/s). In this population of fibers, there was no correlation between the light chain (LC) $LC_{2s}/(LC_{1s} + LC_{2s})$ ratio and V_o. After 2 and 3 wk of HS, a few fast fibers were observed (10% at 2 wk and 25% at 3 wk). This population of fibers had an average diameter, peak force, tension, and V_o of 40 ± 3 μm, 9.4 ± 1.5 × 10⁻⁵ N, 73 ± 6 kN/m^2, and 4.27 ± 0.36 fl/s, respectively.

The force-velocity curves of the fibers from each group are shown in Fig. 3. After 1 and 2 wk of HS the curves were shifted significantly upward compared with the
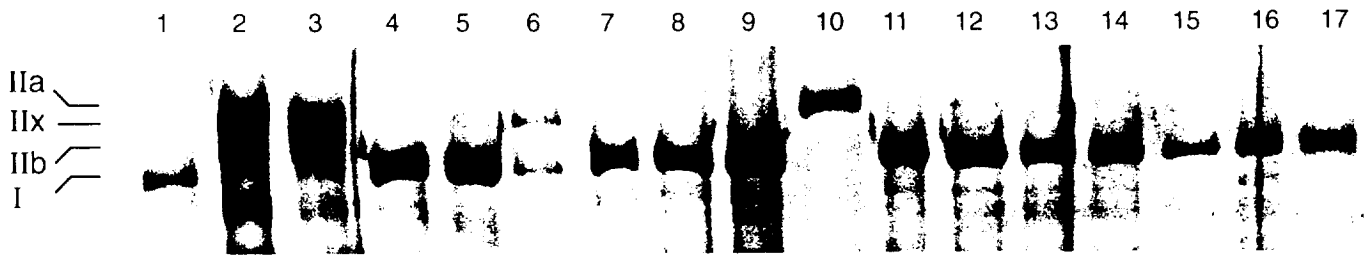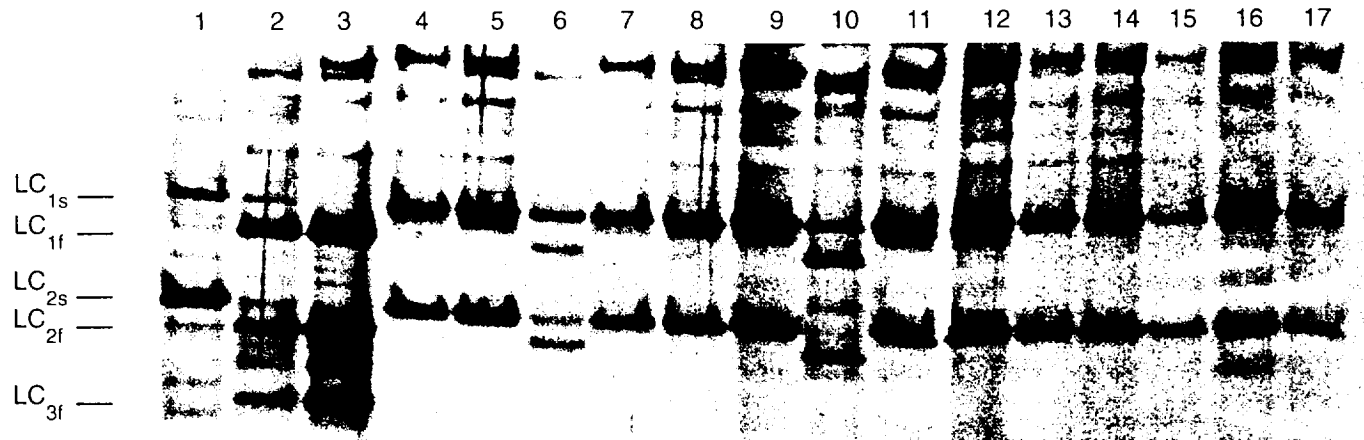
FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of single rat soleus fibers. *Lanes 1-3* are myosin standards prepared from soleus, red, and white regions of gastrocnemius, respectively. *Lanes 4-17* represent individual fibers, extracts of which were divided equally and assayed on 5% gel separating myosin heavy chain (MHC) isozymes (IIa, IIx, IIb, and I) (A) and 12% gel showing slow (LC_{1s} and LC_{2s}) and fast (LC_{1f}, LC_{2f}, LC_{3f}) myosin light chains (LC; B). *Lanes 4-12* and *13-17* are fibers from control and after 2 wk of HS, respectively. Fiber type and its maximal unloaded velocity of shortening [V_o; in fiber lengths (fl)/s] in each lane are as follows: *lane 4*, slow type I, 1.08; *lane 5*, slow type I, 1.27; *lane 6*, hybrid, 1.54; *lane 7*, slow type I, 1.01; *lane 8*, slow type I, 1.31; *lane 9*, slow type I, 1.74; *lane 10*, fast type IIa, 1.97; *lane 11*, slow type I, 1.45; *lane 12*, slow type I, 1.31; *lane 13*, slow type I, 1.49; *lane 14*, slow type I, 2.14; *lane 15*, slow type I, 1.84; *lane 16*, slow type I, 1.58; *lane 17*, slow type I, 1.83.

control values such that elevated velocities were observed at a given load. After 3 wk of HS, the force-velocity curve showed an additional upward shift toward the relationship observed for the fast type IIa fibers. The curve at 3 wk of HS was significantly different from those of the control and after 1 and 2 wk of HS.

Consistent with the reports of others (15, 21), V_{max} calculated using the straight line form of the Hill equation was less than V_o determined by the slack test (Table 2). V_{max} values at 1, 2, and 3 wk of HS were significantly higher than control values but not significantly different from each other.

The power curves of the slow fibers from each group are shown in Fig. 4. The absolute power output of a fiber was calculated by multiplying the fiber's absolute force times the velocity of shortening attained at that given force. The power output curve of a fiber was generated by making this calculation for each point of a fiber's force-velocity curve. The absolute power generated by control fibers was significantly greater than that generated by the HS fibers at all loads. The power output of the slow fibers at 1 wk of HS was significantly greater than that of the slow fibers at 2 and 3 wk of HS, but for loads >30% it was similar to the average values for the fast fibers at 2

and 3 wk of HS. The absolute power output was similar among the slow fibers at 2 and 3 wk of HS.

The mean peak fiber power outputs for the slow type I fibers are presented in Table 2. The peak fiber power output was significantly reduced after 1 wk of HS and continued to fall significantly through 2 wk of HS. However, by 3 wk of HS, peak power output had reached a plateau.

Representative SDS-PAGE gels of individual soleus fibers from control and after 1 and 3 wk of HS are shown in Fig. 5, and the fiber type distribution is summarized in Table 3. The number of slow fibers showed a progressive decrease with HS. In contrast, the percent fast fibers showed no change at 1 wk but was increased at 3 wk, and the hybrid population showed a transient increase at 1 wk of HS. The MLC stoichiometry was not significantly altered by HS. The $LC_{2s}/(LC_{1s} + LC_{2s})$ ratio was 0.512 ± 0.007 and 0.514 ± 0.014 in slow soleus fibers from control and after 3 wk of HS, respectively.

DISCUSSION

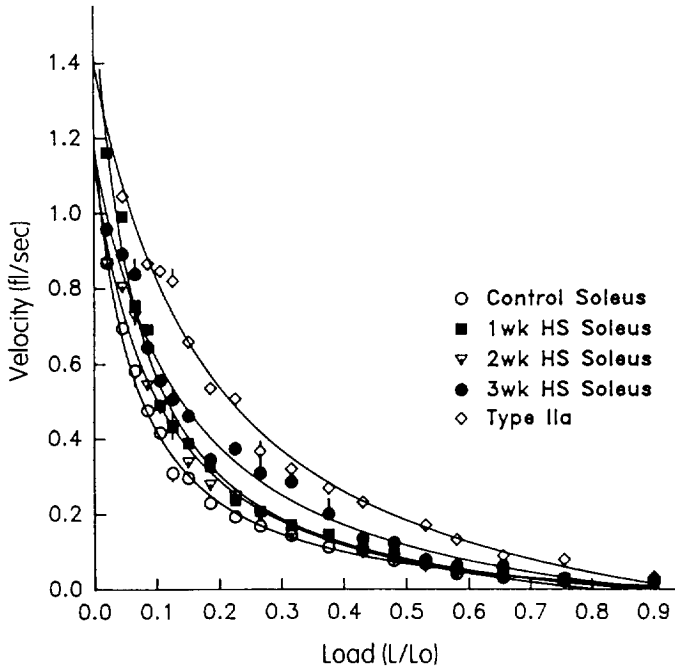Both space travel and land models of weightlessness result in changes that are muscle specific. There is prefer-

FIG. 3. Force-velocity relationship for single soleus fibers from control and after 1, 2, and 3 wk of HS. Each point represents mean ± SE. No. of fibers in each group is given in Table 2. Fast type IIa data represent means ± SE of 7 fibers (2 from 2 wk of HS and 5 from 3 wk of HS). Force or load (L) on x-axis is expressed as percent maximal load (L_o).

ential atrophy in muscles composed mainly of slow-twitch fibers relative to fast-twitch muscles (5, 9, 10, 12). Accordingly, functional changes induced by HS are most prevalent in slow-twitch muscles. For example, after HS, peak force-generating capacity was more dramatically reduced in the slow-twitch soleus muscle than in the fast-twitch muscles [extensor digitorum longus and superficial vastus lateralis (9) and plantaris (5)]. In addition, V_{max} of the soleus muscle was significantly increased after HS, whereas V_{max} of fast-twitch muscles was unaffected by HS (5, 9). Recently, single-fiber studies have been conducted in an attempt to understand the cellular processes responsible for the functional changes of the atrophied soleus. The force-generating capacity of single soleus fibers was reduced and V_o was elevated after HS (10, 17, 24). One objective of this study focused on the effects of varying duration of HS on the force-velocity relationship of single soleus muscle fibers and examined whether increased shortening speeds would offset decreases in fiber force to maintain fiber power output. The important findings were that 1) HS caused an alteration in the force-velocity relationship such that for a given relative fiber load the shortening velocity was increased; 2) the increase at 3 wk of HS was greater than at 1 and 2 wk of HS, which suggests that the HS-induced alteration in the force-velocity relationship had not reached a new steady state by 3 wk; 3) absolute power output of single soleus fibers decreased significantly after 1 wk of HS, continued to decline through 2 wk of HS, and reached an apparent steady state by 3 wk of HS; and 4) the percentage of fast fibers in the soleus increased to 26% by 3 wk of HS, yet the majority of the fibers showing an increased V_o contained only the slow type I myosin isozyme.

V_{max} values calculated from the force-velocity data are

in close agreement with others previously measured in rat skinned soleus fibers (1). These values were lower than V_o values determined by the slack test method. This is consistent with the previous reports of Julian and Moss (15) and Moss (20). They suggest that compression at the fiber ends may cause slight distortions and decreased shortening velocity under load and that force-velocity determination of V_{max} may miss the fast phase of shortening (15).

Locomotion involves dynamic contractions under varying degrees of load. The important functional property involved in the ability to move a load is power output. We constructed power curves of control and HS single soleus fibers from their respective force-velocity relationships. The fibers generated peak power at loads between 15 and 30% of P_o, which corresponds to ~15-35% of V_{max}. This is in agreement with previous animal and human studies of individual muscles where peak power was obtained at loads considerably below 50% of P_o and velocities ~25% of V_{max} (4, 25). HS did not affect the load and velocity at which the single fibers produce peak power. However, peak power and the power generated at all loads were dramatically reduced after 1 wk of HS and further declined by 2 wk of HS. By 3 wk of HS, soleus fibers exhibited power curves that were not different from fibers after 2 wk of HS. The power curves were similar even though absolute force continued to decline. The decreased force production of the fibers appeared to have been offset by an increased shortening velocity, such that power output between 2 and 3 wk of HS was maintained. These findings suggest that during muscle atrophy certain cellular changes occur that are functionally advantageous. In this case, the alteration of the force-velocity relationship resulted in the maintenance of power output
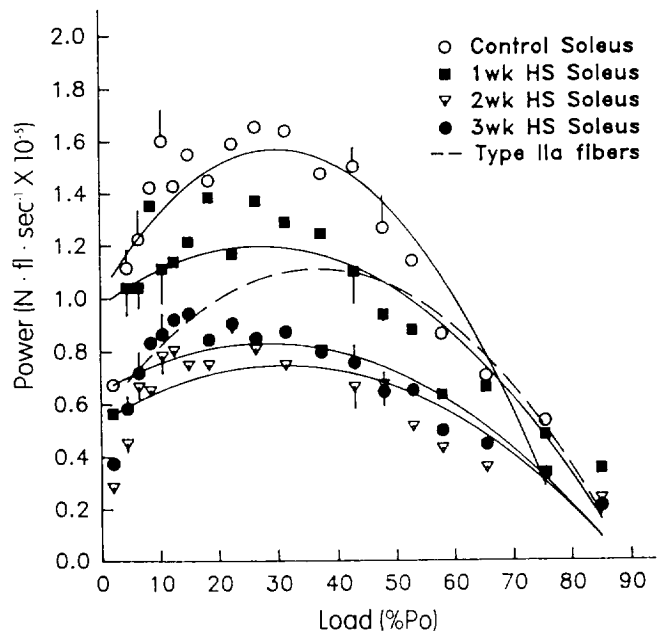

FIG. 4. Power curves for single soleus fibers from control and after 1, 2, and 3 wk of HS. Each point represents mean ± SE. No. of fibers in each group is given in Table 2. Dashed line, best fit for mean data for 7 fast type IIa fibers (2 from 2 wk of HS and 5 from 3 wk of HS); solid lines, best fits for slow fibers. For clarity, data points for type IIa fiber group were omitted. P_o, peak isometric force.
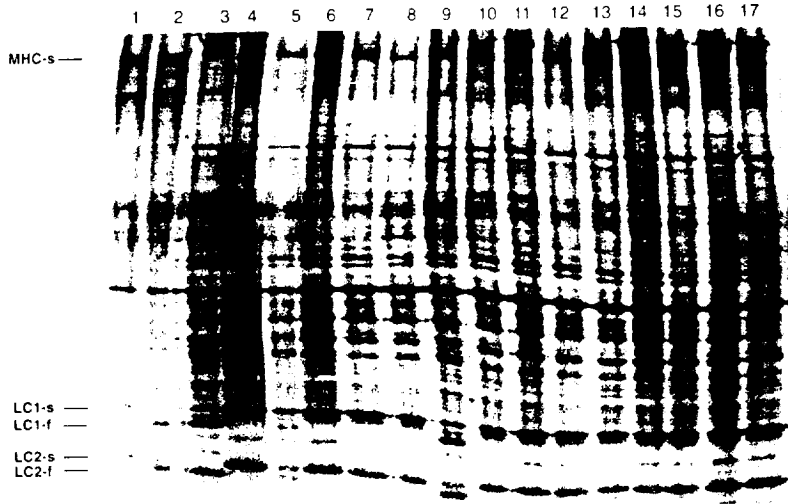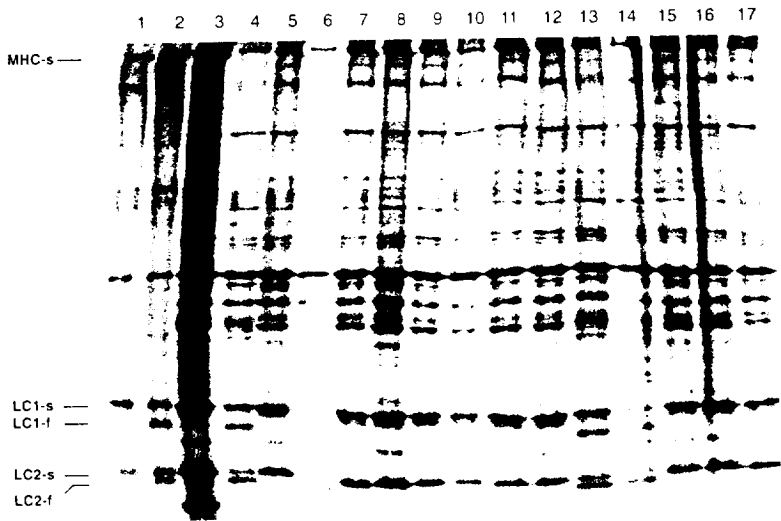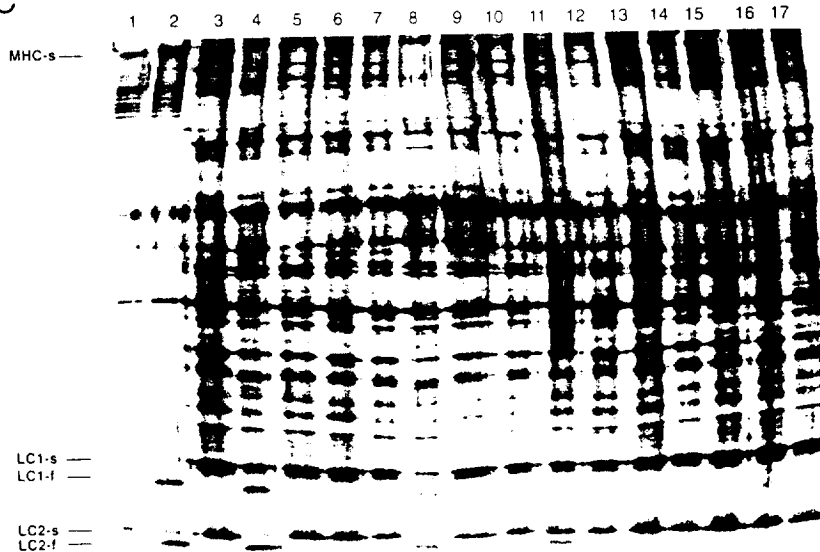
FIG. 5. Representative 12% SDS-PAGE gels of single soleus fibers from control (A) and after 1 wk of HS (B) and 3 wk of HS (C). Lanes 1 and 2, slow and fast myosin standards, respectively. MHC-s, slow MHC; LC1-s/LC2-s and LC1-f/LC2-f, slow and fast myosin LCs 1 and 2, respectively. All fibers are slow except 5A [lane 3 (fast), lanes 5 and 9 (hybrid)], 5B [lanes 6 and 14 (fast), lanes 4 and 13 (hybrid)], and 5C [lane 8 (fast), lanes 4 and 11 (hybrid)].

TABLE 3. *Fiber type distribution in soleus of control and after 1 and 3 wk of HS*

	n	%Slow	%Fast	%Hybrid
Control	195	82	8	10
1 wk of HS	271	74	8	18
3 wk of HS	246	69	26	5

n, No. of fibers assayed.

despite continued reductions in force. It is important to remember, however, that the power outputs of the fibers from animals after 2 and 3 wk of HS were markedly lower than control outputs.

The effect of 4 wk of HS on the absolute force-velocity relationship of the intact soleus was recently reported by Diffee et al. (5). Using their curve, we calculated peak soleus muscle power output to have declined ~50% after 4 wk of HS. The results from the present study indicate that single-fiber peak power output was reduced 49 and 45% after 2 and 3 wk of HS, respectively. Collectively, these findings suggest that the decrease in soleus muscle power output likely occurs within the first 2 wk of HS and that the decline can be primarily attributed to deficiencies at the single-fiber level.

A second objective of this study was to reevaluate the effect of HS on the soleus fiber type distribution and to assess the MLC stoichiometry. In a recent publication (17), we had observed HS to elicit a progressive increase in the number of fast fibers in the soleus from a control value of 4% to 7, 10, and 29% at 1, 2, and 3 wk of HS, respectively. As fibers transform from slow to fast, one would expect to observe at least a transient increase in the number of hybrid fibers (fibers containing both slow and fast myosin). However, <1% of the fibers showed a hybrid myosin profile (see Table 4 in Ref. 17). We hypothesized that hybrid fibers existed but went undetected on our gels due to low fast myosin content, which may have in part resulted from instability of the fast myosin with storage at $-80°C$. However, we recently observed that the fiber type composition of the soleus muscle from animals after 1 wk of HS was unaffected even by storage at $-20°C$ for 4 wk (unpublished observation). Thus, if hybrid fibers existed, they went undetected due to insufficient loading of the gel. In this study, we evaluated that possibility by conducting experiments in which the entire fiber (rather than a short 2-mm segment) was loaded on the SDS-PAGE gel. The result was a higher than expected hybrid content of 10% in control soleus that increased to 18% after 1 wk of HS. We conclude that the transient increase in hybrid fibers supports the hypothesis that the HS-induced increase in fast fibers results from a conversion of slow to fast fibers rather than a selective loss of slow fibers with synthesis of new fast fibers from satellite cells. The fast fibers likely consisted of fibers containing type IIa, IIa/IIx, and IIx MHC. This conclusion is based on the recent observations of Campione et al. (2) and Diffee et al. (6). Both groups found HS to increase fast myosin in the soleus, and the protein was identified as type IIa or IIx (or combination of IIa and IIx) but not type IIb MHC.

Although it is apparent that HS does induce a trans-

formation of fibers from slow to fast, a second subpopulation of slow fibers (~70% of the total number of slow fibers) showed an elevated V_o and fiber ATPase activity with no detectable fast myosin (17). Because the total number of fast fibers after 3 wk of HS was the same whether fiber segments (see Table 4 in Ref. 17) or the whole fiber was assayed (Table 3), the increased V_o is not likely to be due to the presence of undetectable amounts of fast myosin. Changes in the MLC stoichiometry might upregulate the myosin ATPase and thus increase V_o (22). However, after HS, we found no significant change in the $LC_{2s}/(LC_{1s} + LC_{2s})$ ratio and, furthermore, there was no correlation between the MLC ratio and the observed increase in fiber V_o. HS may induce the synthesis of a second, yet unidentified, slow MHC that comigrates with slow type I myosin on 12% SDS gels. Although we have observed two slow MHC bands on 5% SDS gels prepared from single fibers from control animals (unpublished data), such doublets have not been observed after HS (Fig. 2A). Another possibility is that HS might increase the spacing between the filaments of the myofibrillar lattice. Osmotic compression of the myofibrillar lattice in skinned fibers has been shown to reduce V_o, and thus it is possible that expansion of the filament spacing would increase V_o (19). Finally, alterations in the regulatory protein composition, particularly a shift in troponin T from slow to fast isoforms, could increase fiber V_o (11).

In conclusion, 1, 2, and 3 wk of HS caused a progressive decrease in rat soleus muscle fiber size and absolute force production. The power output of these fibers progressively declined through 2 wk of HS. However, this decline in fiber power output leveled off through 3 wk of HS despite a continued decline in absolute force-generating capacity. This result occurred due to the shift in the force-velocity relationship such that velocity was higher at the same relative workload. HS resulted in a transient increase in the number of hybrid fibers after 1 wk and an increase in fast fibers to 26% after 3 wk. A large percentage of slow fibers showed an increased V_o with no change in the MHC profile. The cause of the increased V_o in this population of fibers is currently unknown.

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