Effect of hindlimb unweighting on single soleus fiber maximal shortening velocity and ATPase activity

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McDONALD, K. S., AND R. H. FITTS. *Effect of hindlimb unweighting on single soleus fiber maximal shortening velocity and ATPase activity.* J. Appl. Physiol. 74(6): 2949–2957, 1993.—This study characterizes the time course of change in single soleus muscle fiber size and function elicited by hindlimb unweighting (HU) and analyzes the extent to which varying durations of HU altered maximal velocity of shortening (V_o), myofibrillar adenosinetriphosphatase (ATPase), and relative content of slow and fast myosin in individual soleus fibers. After 1, 2, or 3 wk of HU, soleus muscle bundles were prepared and stored in skinning solution at $-20°C$. Single fibers were isolated and mounted between a motor arm and a transducer, and fiber force, V_o, and ATPase activity were measured. Fiber myosin content was determined by one-dimensional sodium dodecyl sulfate- (SDS) polyacrylamide gel electrophoresis. After 1, 2, and 3 wk of HU, soleus fibers exhibited a progressive reduction in fiber diameter (16, 22, and 42%, respectively) and peak force (42, 48, and 72%, respectively). Peak specific tension was significantly reduced after 1 wk of HU (18%) and showed no further change in 2–3 wk of HU. During 1 and 3 wk of HU, fiber V_o and ATPase showed a significant increase. By 3 wk, V_o had increased from 1.32 ± 0.04 to 2.94 ± 0.17 fiber lengths/s and fiber ATPase from 291 ± 16 to $1,064 \pm 128$ $\mu M \cdot min^{-1} \cdot mm^{-3}$. The percent fibers expressing fast myosin heavy chain increased from 4% to 29% by 3 wk of HU, and V_o and ATPase activity within a fiber were highly correlated. However, a large population of fibers after 1, 2, and 3 wk of HU showed increases in V_o and ATPase but displayed the same myosin protein profile on SDS gels as control fibers. The mechanism eliciting increased fiber V_o and ATPase activity was not obvious but may have been due to increases in fast myosin that went undetected on SDS gels and/or other factors unrelated to the myosin filament.

muscle atrophy; contractile properties; myosin isozyme profile

HINDLIMB UNWEIGHTING (HU) has been demonstrated to elicit changes in skeletal muscle morphology, biochemistry, and physiology that closely mimic those that occur with spaceflight (5, 11, 12, 21, 27). HU, like spaceflight, has its most dramatic effect on antigravity postural muscles, such as the soleus. Documented HU-induced changes in the soleus include pronounced atrophy (11, 12, 28, 29), reduced work capacity (22), alterations in isometric and isotonic contractile properties (7, 11, 12), changes in myosin isozyme expression and distribution (7, 11, 25, 26, 29), and shifts in metabolic profile (10).

Published studies (7, 11) have reported HU to significantly alter both the isometric and isotonic contractile properties of the intact soleus. After 2 wk of HU, the soleus isometric contraction and half-relaxation times were decreased, whereas the maximal velocity of shortening (V_o), calculated from the force-velocity curve and the relative amount of fast myosin expression, increased (11). Because of the mixture of fiber types (85% slow-twitch type I and 15% fast-twitch type IIa) in the soleus (1), single-fiber studies have been undertaken to examine fiber specificity of these HU-induced changes. Gardetto et al. (12) found 2-wk of HU to cause soleus slow-twitch type I fibers to atrophy and produce less force (N and kN/m^2). Additionally, the slow-twitch soleus fibers exhibited a heterogeneous V_o, as determined by the slack test. One population had V_o values similar to control fibers, whereas another population showed a significant increase in V_o (28%). Reiser et al. (25) found that 4-wk of HU increased the average soleus fiber V_o (by 31%) and the relative amount of fast myosin heavy chain (MHC) present in small soleus fiber bundles. Because the V_o of a muscle is known to be correlated and likely rate limited by the myosin adenosinetriphosphatase (ATPase) activity (2, 3, 32), these studies collectively suggest that HU increases fiber ATPase and V_o by inducing the expression of fast myosin in a subpopulation of soleus type I fibers. The purpose of this work was to test this hypothesis by studying the time course of change in fiber V_o after 1, 2, and 3 wk of HU and to determine whether the elevated fiber V_o observed in individual fibers was correlated with an increased fiber ATPase and fast myosin isoform content. A second objective was to assess whether the extent of change in fiber V_o and ATPase was dependent on the duration of HU and to establish whether a new plateau in fiber function was reached by 3 wk of HU.

METHODS

Animal care and HU. Male Sprague-Dawley rats were obtained from Sasco (Madison, WI) and randomly divided into control and HU groups. Rats were maintained on a diet of Purina rodent chow and water. HU rats were fed ad libitum while the control rats were pair fed to maintain body weights comparable to the experimental group. The HU animals were housed in a room separated from the control rats to limit room noise, thereby keeping spontaneous muscle activity to a minimum. Both rooms were maintained at 22°C with a 12:12-h light-dark cycle. The HU animals were partially suspended for 1, 2,

or 3 wk with a tail harness, as previously described in detail (11). The hindlimbs were elevated so as not to make contact with any supportive surface. The forelimbs maintained contact with a grid floor, allowing the animal full range of motion.

Solutions. The composition of all the solutions used in the single-fiber experimental protocols was determined by using the computer program of Fabiato and Fabiato (9) and the apparent stability constants reported by Godt and Lindley (14) as described by Metzger et al. (23). The relaxing and activating solutions contained the following (in mM): 20 imidazole, 7 ethylene glycol-bis(β-amino-ethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 5.4 MgCl$_2$, 10 caffeine, and 4.7 ATP, as well as CaCl$_2$ to achieve pCa ($-$log [Ca^{2+}]) 9.0 (relaxing solution) or pCa 4.5 (activating solution). All solutions contained enough KCl to achieve an ionic strength of 180 mM and were adjusted to pH 7.0. The relaxing and activating solutions used to determine fiber ATPase activity contained 10 mM phospho*enol*pyruvate, 100 U/ml of pyruvate kinase, 100 U/ml of lactate dehydrogenase, 250 μM NADH, and 0.1 mM adenylate kinase inhibitor p^1,p^5-di(adenosine-5-)-pentaphosphate. NADH was prepared as 10 mM stock solution and standardized according to the method described by Lowry and Passoneau (20). The enzymes and substrates used in the ATPase assay were added on the day of the experiment.

Muscle preparation. After 1, 2, or 3 wk of HU, experimental and control rats were weighed and anesthetized with pentobarbital sodium (50 mg/kg body wt ip). The soleus and gastrocnemius muscles were isolated and weighed. The soleus was then placed in cold (4°C) relaxing solution, and 8–10 small bundles of ~1-2 mm in width (50–100 fibers) were prepared from regions throughout the muscle. Each bundle was stretched to in situ length, tied to a glass capillary tube with 4-0 surgical suture, and placed in skinning solution [containing (in mM) 125 K propionate, 2 EGTA, 4 ATP, 1 MgCl$_2$, and 20 imidazole, as well as 50% glycerol (vol/vol)] for storage at -20°C for $\leq$4 wk (24).

Single-fiber preparation. On the day of an experiment, a soleus muscle bundle was transferred to a dissecting chamber containing relaxing solution (4°C). A single fiber was isolated from the bundle and transferred to an experimental chamber containing relaxing solution. A fiber segment (~2 mm long) was mounted between a force transducer (model 400, Cambridge Technology, Cambridge, MA; sensitivity 2 mV/mg) and an isotonic direct current torque motor (model 300H, Cambridge Technology), as described previously in detail (24). Any fiber showing a high degree of striation nonuniformity or a damaged region was discarded.
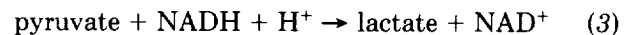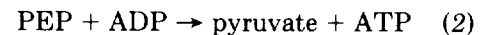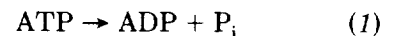
The fiber was observed and photographed through an Olympus microscope with an Olympus model PM-10AD photomicrographic system. The sarcomere length (SL) was initially adjusted to 2.6 μm in relaxing solution with a calibrated eyepiece micrometer. The fiber was then transferred to a chamber containing activating solution, and a Polaroid picture was taken to determine fiber SL during maximal activation. If necessary, the segment length was adjusted to obtain an SL of 2.5 μm. Another photograph was taken while the fiber was suspended

briefly in air to determine fiber diameter. The diameter was determined as the average of three measurements made along the length of the fiber. Segment length was determined by moving the microscope stage with a micrometer such that the fiber segment moved across the visual field of the eyepiece ($\times$400). The segment length was determined directly from the micrometer displacement. Fiber cross-sectional area was calculated assuming a circular cross section. All experiments were conducted at 15°C.

Determination of peak force and specific tension (P_o). The outputs of the force and position transducers were amplified and sent to a Commodore 64 microcomputer via a universal input-output board consisting of an eight-bit 10-KHz analog-to-digital converter (Microworld Computers, Lakewood, CO). Force in relaxing solution was monitored, and the fiber was then activated by transfer into pCa 4.5 solution. Peak force (N) was determined in each fiber by computer subtraction of the baseline force from peak active force. P_o (kN/m^2) was calculated from the peak force and fiber cross-sectional area.

Determination of V_o. V_o was obtained by using the slack test method (8, 12). The slack test involves measuring the amount of time required for an activated fiber to take up slack introduced at the motor end. The fiber is maximally activated and then shortened a predetermined length such that force falls to zero. The fiber then shortens under zero load until the slack is taken up, at which time force begins to redevelop. Several length steps were used for each fiber, and the slack distance was plotted vs. the duration of unloaded shortening (i.e., the time between the onset of slack and the redevelopment of force) (Fig. 1). V_o [fiber lengths (fl)/s] is calculated by dividing the slope of the best fit line by the segment length, and the data were normalized to an SL of 2.50 μm.

Simultaneous measurement of force and ATPase activity. The myofibrillar ATPase activity of single skinned muscle fibers was determined fluorometrically by enzymatically coupling ADP production to the oxidation of NADH by a modification of the technique described by Griffiths et al. (16). The following cascade of reactions was used

$$ATP \rightarrow ADP + P_i \qquad (1)$$

$$PEP + ADP \rightarrow pyruvate + ATP \qquad (2)$$

$$pyruvate + NADH + H^+ \rightarrow lactate + NAD^+ \qquad (3)$$

where PEP is phospho*enol*pyruvate. *Reactions 2 and 3* are catalyzed by pyruvate kinase and lactate dehydrogenase, respectively. As the reaction scheme shows, the production of 1 mol ADP results in the oxidation of 1 mol NADH (which fluoresces) to NAD (which does not fluoresce). NADH was excited by passing light from a halogen lamp (21 V, 150 W) through a 340-nm filter. The emitted light from the fiber was filtered at 470 nm and detected by a photomultiplier tube (EMI, Gencom, Plainview, NY). The signal was sent to a current-to-voltage amplifier and subsequently measured and recorded by a microcomputer (Commodore 64). The change in NADH fluorescence with concentration was linear over the range employed in this study.

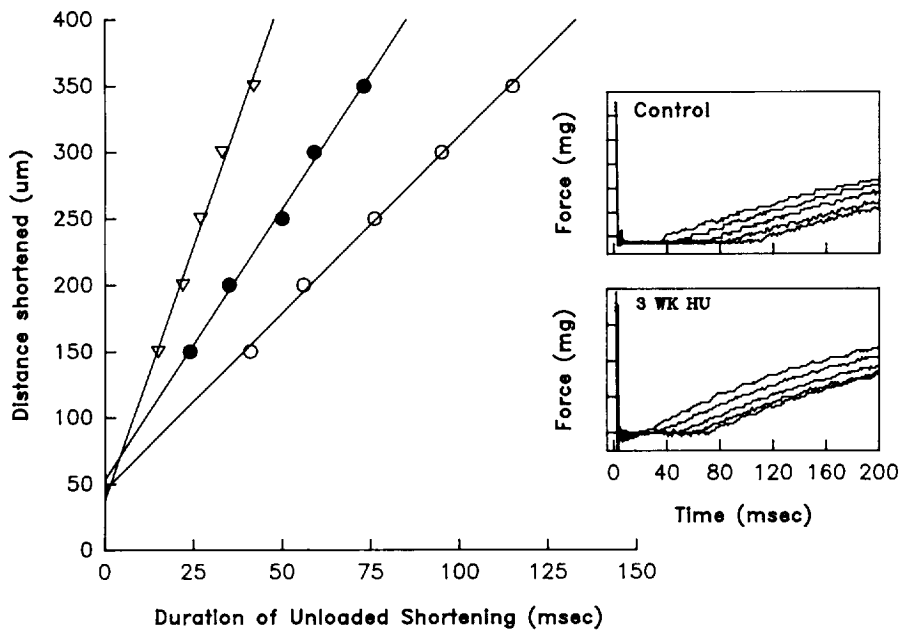After the measurement of V_o (slack test), the fiber was

FIG. 1. Slack test determination of maximal shortening velocity (V_0). Duration of unloaded shortening after imposed slack is plotted vs. length change for control soleus fiber (O), 3 wk of hindlimb unweighting (HU) soleus fiber (●), and representive fast type IIa fiber isolated from red gastronemius (∇). V_0 was determined by slope of best-fit line and normalized for fiber length. *Inset:* 5 superimposed force records for control soleus fibers and soleus fibers from animals after 3 wk of HU immediately after fiber was slacked.

incubated in relaxing solution (pCa 9.0) containing the necessary enzymes and substrates for 30 min. After incubation, NADH fluorescence was monitored for 5 min and the resting fiber ATPase calculated from the decrease in fluorescence. On activation in high Ca^{2+} (pCa 4.5), force and NADH fluorescence were again monitored for an additional 5 min. The ATPase activity was calculated by subtracting the resting rate from the activated rate and was expressed as micromolar per minute per millimeter cubed. The units were calculated assuming a circular fiber cross section. The calculated fiber ATPase represents primarily myofibrillar ATPase, as sarcoplasmic reticulum and mitochondrial ATPases make an undetectable contribution to the total activity under these experimental conditions (19).

Myosin analysis. After the physiological measurements, the MHC and myosin light chain (MLC) compositions of each fiber were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (13). The fibers were solubilized in 10 μl of 1% SDS sample buffer and stored at $-80°C$. For some fibers, the MHCs and MLCs were analyzed separately with a Mini-Protean II dual-slab cell (Bio-Rad Laboratories, Richmond, CA; 70 × 80 × 1 mm). To evaluate the MHC, ~0.5 nl (≈0.5 μg) of fiber volume was run on a minigel consisting of a 3.5% (wt/vol) acrylamide stacking gel and a 9.5% separating gel (200 V for 90 min). The MLC was analyzed by loading ~1.5 nl of fiber volume onto a minigel consisting of a 3.5% acrylamide stacking gel and a 12% separating gel (200 V for 50 min). For other fibers, both the MHC and MLC were analyzed on the same gel with a Hoefer model SE600 gel cell (Hoefer Scientific Instruments, San Fransisco, CA; 160 × 180 × 0.75 mm). Approximately 1 nl of fiber volume was loaded to a large gel consisting of a 3.5% acrylamide stacking gel and a 12% acrylamide separating gel (38 mA for 5 h). All gels were silver stained with the technique of Giulian et al. (13) and scanned on a scanning densitometer (CliniScan 2, Helena Laboratories, Beaumont, TX).

Statistical analysis. A one-way analysis of variance was

used to compare rat body weight, soleus weight, soleus-to-body weight ratio, soleus fiber diameter, force, P_0, V_0, and ATPase activity across the four different groups. A Tukey's test was used as a post hoc test to estimate differences among means. $P < 0.05$ was chosen as significant. Regression analysis was used to determine the correlation between fiber V_0 and ATPase.

RESULTS

Time course of changes in soleus muscle size, fiber diameter, and force-generating capacity. The soleus-to-body weight ratio and fiber diameter both progressively declined during 1, 2, and 3 wk of HU (Tables 1 and 2). These two parameters showed more dramatic changes between 0 and 1 wk of HU and 2 and 3 wk of HU than between 1 and 2 wk of HU. One, 2, and 3 wk of HU resulted in the soleus-to-body weight ratio decreasing 29, 39, and 55%, respectively, from control values, whereas fiber diameter fell 16, 22, and 42%, respectively.

The time courses of change of peak absolute force and P_0 in single skinned soleus fibers are shown in Table 2. The absolute force generated by single soleus fibers fell 42, 48, and 72% after 1, 2, and 3 wk of HU, respectively. The continuous decline in force through 3 wk of HU did not persist when expressed as per fiber cross-sectional

TABLE 1. *Animal characterization*

	n	Body Wt, g	Soleus Wt, mg	Soleus/Body Wt, mg/g
Control	9	353±5	172±5	0.49±0.02
HU				
1 wk	8	303±10*	105±4*	0.35±0.01*
2 wk	6	326±14	96±7*	0.30±0.03*
3 wk	4	398±11*†‡	89±6*	0.22±0.01*†

Values are means ± SE; n, no. of animals. HU, hindlimb unweighted. * Significantly different from control $(P < 0.05)$; † significantly different from 1 wk of HU $(P < 0.05)$; ‡ significantly different from 2 wk of HU $(P < 0.05)$.

TABLE 2. *Diameter, peak force, and P_o of soleus fibers from control and HU animals*

	n	Fiber Diameter, μm	Force, $\times 10^{-5}$ N	P_o, kN/m²
Control	104	69±1	39.6±0.8	106±2
HU				
1 wk	53	58±1*	23.0±0.8*	87±2*
2 wk	69	54±2*	20.5±1.2*	88±2*
3 wk	45	40±1*†‡	10.9±0.7*†‡	85±4*

Values are means ± SE; n, no. of fibers. P_o, peak tetanic tension. * Significantly different from control ($P < 0.05$); † significantly different from 1 wk of HU ($P < 0.05$); ‡ significantly different from 2 wk of HU ($P < 0.05$).

area. P_o dropped 18% after 1 wk of HU but did not significantly change further after 2 and 3 wk of HU (Table 2).

Effect of HU on V_o, ATPase activity, and myosin isozyme expression. V_o and ATPase activities were measured on the same fiber with the slack test method and fluorometrically, as described in METHODS. The time courses of changes in mean V_o and ATPase values are shown in Table 3. One week of HU resulted in a 46% increase in V_o and a 55% increase in ATPase activity (Table 3). V_o and ATPase showed only a small additional increase between 1 and 2 wk of HU; however, after 3 wk of HU both V_o and ATPase showed a significant increase from 2 wk of HU (Table 3). The values of V_o and ATPase after 3 wk of HU were 2.2- and 3.8-fold greater, respectively, than control values. The fiber ATPase activity was highly correlated with fiber V_o. Figure 2 shows the relationship between V_o and ATPase of fibers isolated from control animals and from animals after 1 and 2 wk of HU. The relationship is linear with a correlation coefficient of 0.89. When fiber V_o and ATPase values from animals after 3 wk of HU were included, the correlation coefficient was 0.81. These values are shown in Fig. 2 (*inset*).

The distributions of fiber V_o values obtained from single fibers from control animals and animals after 1, 2, and 3 wk of HU are shown in Fig. 3. The control soleus fibers exhibited a normal distribution with a mode of 1.1 fl/s. HU shifted the distribution to more elevated speeds. The fiber populations from animals after 1 and 2 wk of HU had similar distributions; however, at 2 wk of HU, there was a greater percentage of fibers with V_o values between 2.0 and 4.0 fl/s. Three week of HU resulted in a

TABLE 3. *V_o and ATPase activities of fibers from control and HU animals*

	n	V_o, fl/s	n	ATPase Activity, μM·min⁻¹·mm⁻³
Control	102	1.33±0.05	48	302±17
HU				
1 wk	51	1.94±0.09*	24	468±25*
2 wk	64	2.01±0.10*	28	493±29*
3 wk	43	2.96±0.18*†‡	17	1,144±125*†‡

Values are means ± SE; n, no. of fibers. V_o, maximal shortening velocity; fl, fiber length; ATPase, adenosinetriphosphatase. * Significantly different from control ($P < 0.05$); † significantly different from 1 wk of HU ($P < 0.05$); ‡ significantly different from 2 wk of HU ($P < 0.05$).
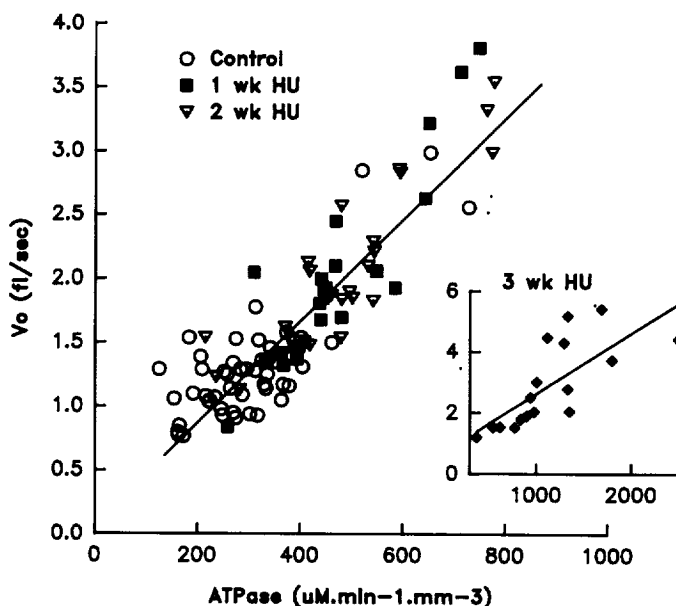

FIG. 2. Relationship between fiber V_o and ATPase activity shown as results of individual experiments. Correlation coefficient for all data points = 0.89. *Inset*: fiber V_o vs. ATPase activity after 3 wk of HU; when 3-wk data are included, correlation coefficient = 0.81. fl, fiber length.

more even distribution of fibers over a V_o range of 1.2 to 5.2 fl/s. At all three durations of HU, fibers with V_o values similar to control values were present.

The vast majority of fibers (>99%) expressed an SDS-PAGE myosin profile of either all slow MHC and MLC (type I fibers) or only fast MHC and hybrid MLC (LC_{1s}, LC_{1f}, LC_{2f}, and on occasion LC_{2s}) (type IIa fibers) (Table 4, Figs. 4 and 5). The remaining fibers expressed various combinations of slow and fast MHC and MLC (e.g., slow MHC, slow or hybrid MLC, and LC_3 or both slow and fast MHC and either slow or hybrid MLC; data not shown). Interestingly, <1% of the fibers displayed hybrid or transitional MHC patterns (containing both types I and II MHC). The percentage of type IIa fibers, as determined by MHC, increased from 4% of control values to 7, 10, and 29% at 1, 2, and 3 wk of HU, respectively (Table 4). The type IIa fibers had V_o values ranging from 1.90 to 5.74 fl/s. At 1, 2, and 3 wk of HU there was a large number of fibers with V_o values between 1.4 and 3.5 that expressed the same myosin pattern as control fibers (Fig. 5). On the basis of densitometric scanning, the myosin pattern of these fibers consisted of 100% type I MHC and an MLC_{1s}-to-MLC_{2s} ratio of 1.60 ± 0.06 and 1.55 ± 0.08 for fibers from control and HU animals, respectively.

DISCUSSION

The purpose of this investigation was to examine the time course of HU-induced changes in soleus muscle fiber size and function and to quantitatively analyze the extent to which varying durations of HU alter V_o, myofibrillar ATPase, and the relative content of slow and fast myosin in individual soleus fibers. The results demonstrate a progressive decrease in soleus-to-body weight ratio, fiber diameter, and absolute force and a progressive increase in V_o and ATPase activity. For each of these parameters the changes from 0 to 1 wk of HU and from 2
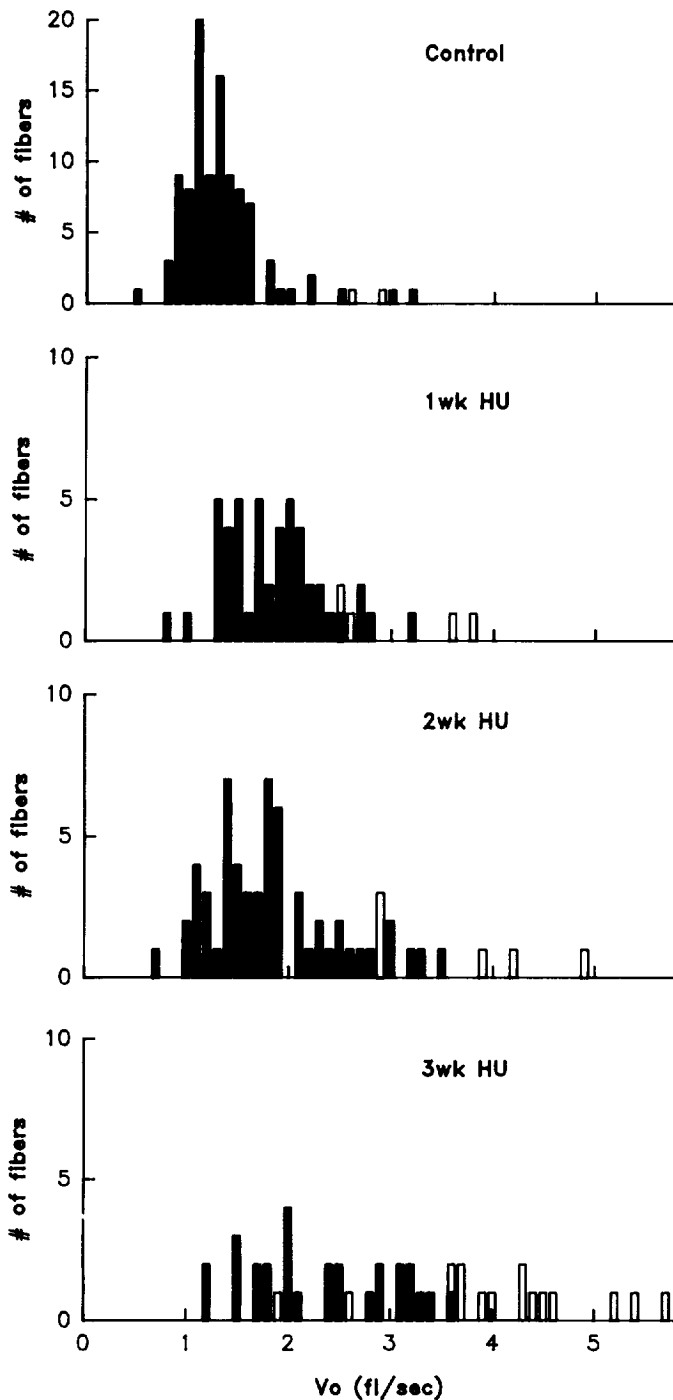
FIG. 3. Distribution of V_o for individual soleus fibers from control animals and animals after 1, 2, and 3 wk of HU. Ordinate, no. of fibers obtained for given V_o from control and HU groups. Open bars, type IIa (determined by SDS-PAGE) fibers.

to 3 wk of HU were more dramatic than between 1 and 2 wk of HU. The percentage of fibers expressing only fast MHC progressively increased with longer periods of HU, and V_o and ATPase activity within a fiber were highly correlated. However, a large population of fibers analyzed after 1, 2, and 3 wk of HU exhibited elevated V_o and ATPase activity relative to control fibers but contained only slow myosin based on SDS gel analysis.

Increasing durations of HU have been shown to cause

significant and progressive decreases in the peak force and P_o in the whole soleus muscle (7, 11). In agreement, single-fiber studies, including this study, found both peak force and P_o to be reduced after HU (12, 25). Gardetto et al. (12) observed a 28% drop in P_o of slow-twitch soleus fibers after 2 wk of HU, and Reiser et al. (25) found that 4 wk of HU induced a 12% decline in fiber P_o. This difference (2 vs. 4 wk) could be attributed to the variability in the extent of HU-induced muscle atrophy that has been documented to occur between laboratories and even within the same laboratory (11), or it could reflect an actual recovery of P_o with prolonged periods of HU. We found that P_o significantly decreased after 1 wk of HU and showed no further change through 2-3 wk of HU. This plateauing of P_o suggests that after 1 wk of HU fiber cross-sectional area and force-generating cross bridges decline proportionately. Alternatively, the plateau of P_o with increasing duration of HU could result from more force per cross bridge in the presence of fewer cross bridges per fiber cross section. This latter possibility is currently being tested by determining the effect of HU on the relationship between force and stiffness.

V_o of limb skeletal muscle is hypothesized to be dependent on the rate of ATP hydrolysis by the actomyosin ATPase (2). This is supported by findings of a high correlation between V_o and myofibrillar ATPase in limb skeletal muscles (2, 3, 32). In agreement, our results indicate that in all groups studied fiber V_o is highly correlated with fiber ATPase activity. The fiber ATPase activities reported here are very similar to those previously reported on whole soleus muscle. If we assume 1 µg protein/mm fiber, our value for control soleus fibers was 0.92 $\mu mol \cdot s^{-1} \cdot g$ protein^{-1}. The Ca^{2+}-activated myosin ATPase activity of Bárány and Close (3), Unsworth et al. (32), and Diffee et al. (7), when corrected for temperature ($Q_{10} = 2$) and expressed as micromoles P_i per second per gram (assuming one-half of the fiber's protein by weight is myosin), were 1.75, 1.68, and 1.50, respectively. The

TABLE 4. *MHCs and MLCs in single fibers determined by SDS-PAGE*

		MHC		
	n	I	I/IIa	IIa
Control	85	94	2	4
HU				
1 wk	41	93	0	7
2 wk	48	90	0	10
3 wk	31	68	3	29

		MLC		
		I	I w/LC$_{3f}$	IIa
Control	83	93	2	5
HU				
1 wk	39	92	0	8
2 wk	43	93	0	7
3 wk	42	60	7	33

Values are expressed as percent fiber type; n, no. of fibers. MHC, myosin heavy chain; MLC, myosin light chain. Type I fibers contained slow MHC and slow MLCs (LC$_{1s}$ and LC$_{2s}$). Type IIa fibers contained fast MHC and hybrid MLCs (LC$_{1s}$, LC$_{1f}$, LC$_{2s}$, and LC$_{2f}$). Type I fibers with LC$_{3f}$ (I w/LC$_{3f}$) contained LC$_{1s}$, LC$_{2s}$, and LC$_{3f}$.

MHC-f
MHC-s

ACTIN —

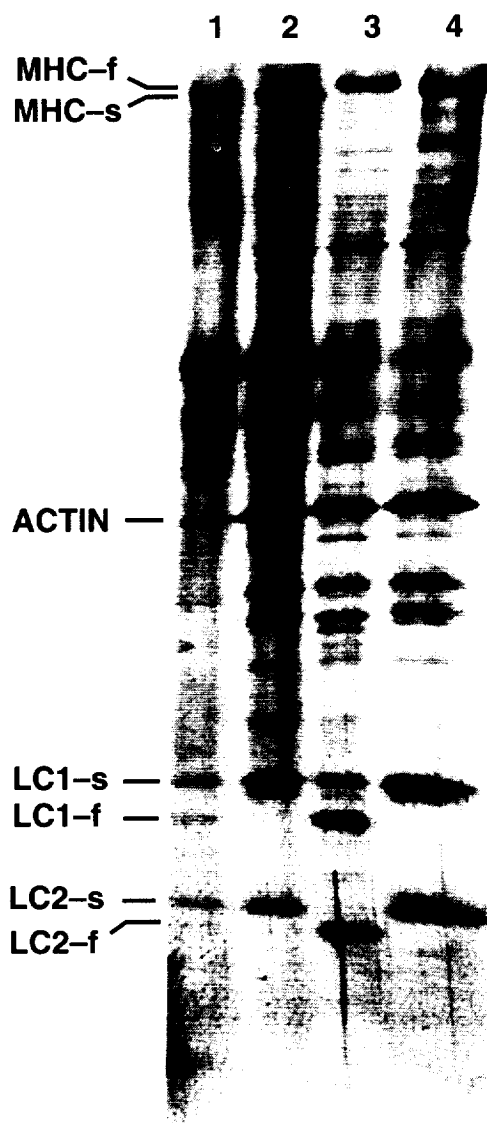LC1-s —
LC1-f —

LC2-s —
LC2-f

FIG. 4. Twelve percent SDS-PAGE showing typical myosin heavy chain (MHC) and myosin light chain (LC) patterns observed in single fibers throughout study. Lanes 2 and 4, samples from control soleus and soleus after 2 wk of HU, respectively. Control fiber, V_o = 1.40 fl/s; fiber after 2 wk of HU, V_o = 1.00 fl/s. Both fibers express myosin characteristic of type I fibers (slow MHC and slow MLC). Lane 3, sample of soleus fiber after 2 wk of HU with V_o = 4.9 fl/s, showing myosin pattern of type IIa fiber (fast MHC and hybrid MLC). Lane 1, both fast (f) and slow (s) myosin standards isolated from red gastrocnemius and soleus, respectively.

slightly higher values in the whole muscle studies may be because of the contribution of the 15% type IIa fibers in the soleus (1).

To compensate for the loss of cross bridges due to fiber atrophy, we expressed the ATPase data per fiber volume ($\mu M \cdot min^{-1} \cdot mm^{-3}$). When expressed per fiber volume, fiber V_o were closely matched with fiber ATPase activity (Fig. 2). The disproportionate (3.6- vs. 2.2-fold) increase in ATPase activity compared with V_o after 3 wk of HU may indicate a less-efficient myofibrillar ATPase or the calculated fiber volume may underestimate the actual volume at small fiber diameters.

V_o and ATPase values of fast-twitch fibers from the

red and white gastrocnemius are reported to be ~3.5- and 6-fold higher than those of the slow-twitch soleus fibers (12; unpublished observations). Increasing durations of HU caused a progressive shift in fiber V_o and ATPase values intermediate between slow-twitch type I and fast-twitch type IIa fibers. However, even after 3 wk of HU, soleus fibers were present with V_o and ATPase values similar to control values. The cellular basis for these functionally unresponsive cells is unknown. Because fibers developed from primary myotubes have been shown to be unresponsive to denervation, whereas secondary myotubule-derived fibers are responsive (18), it is possible that the population of fibers with unaltered V_o developed from slow primary myotubes. Following this argument, the HU-induced changes in activity and work load may cause secondary myotubule-derived fibers to adapt toward their initial, or default, fast phenotype (18). Another possibility is that the nonresponsive fibers are innervated by the smallest most tonically active α-motoneurons, the firing pattern of which may remain virtually unaltered with HU.

All the soleus fibers in this study expressed almost exclusively one of two SDS-PAGE myosin protein patterns. One contained only slow MHC and slow MLCs (LC_{1s} and LC_{2s}); the other contained fast MHC and hybrid light chains (LC_{1s}, LC_{1f}, LC_{2f}, and on occasion LC_{2s}). These correspond to the MHC and MLC patterns of the Sm_2 and Im native isomyosins described by Tsika et al. (31) and correspond to the slow type I and fast type IIa fiber types, respectively. The percentage of fibers that expresses Im patterns (or type IIa) progressively increased through 1, 2, and 3 wk of HU. In agreement, histochemical staining patterns show a greater type IIa-to-type I fiber ratio after muscle atrophy induced by either HU (6, 28) or spaceflight (21). A part of the increased type IIa percentage after HU in this study can be attributed to the low control type IIa percent. In the control muscles, the fast type IIa fibers are considerably smaller and thus less likely to be isolated compared with the slow type I fibers. After HU, all fibers are small and equally difficult to isolate. Nevertheless, the control muscles would be expected to contain $\leq 15\%$ type IIa fibers, and we observed, based on MHC analysis, 29% after 3 wk of HU (Table 4). This increase in type IIa fibers may result from the selective loss of slow fibers, the selective loss of slow fibers with the synthesis of new fast fibers from satellite cells, and/or the conversion of slow fibers to fast fibers. Because HU does not change the actual number of fibers and apparently does not trigger either satellite cell proliferation or myotube formation, the first two possibilities seem unlikely (6, 28; D. A. Riley, personal communication).

An interesting group of fibers in this study was the large population (~70% of type I fibers from HU animals) at 1, 2, and 3 wk of HU that had elevated V_o (≥ 1.4 fl/s) and ATPase values but expressed the same myosin protein profile on SDS gels as the control fibers. The ranges of V_o and ATPase activity for all (control and experimental) type I fibers were 0.50–3.60 fl/s and 125–1,010 $\mu M \cdot min^{-1} \cdot mm^{-3}$, respectively. Reiser et al. (26) have shown a strong quantitative correlation between V_o and MHC composition in single rabbit soleus fibers, and
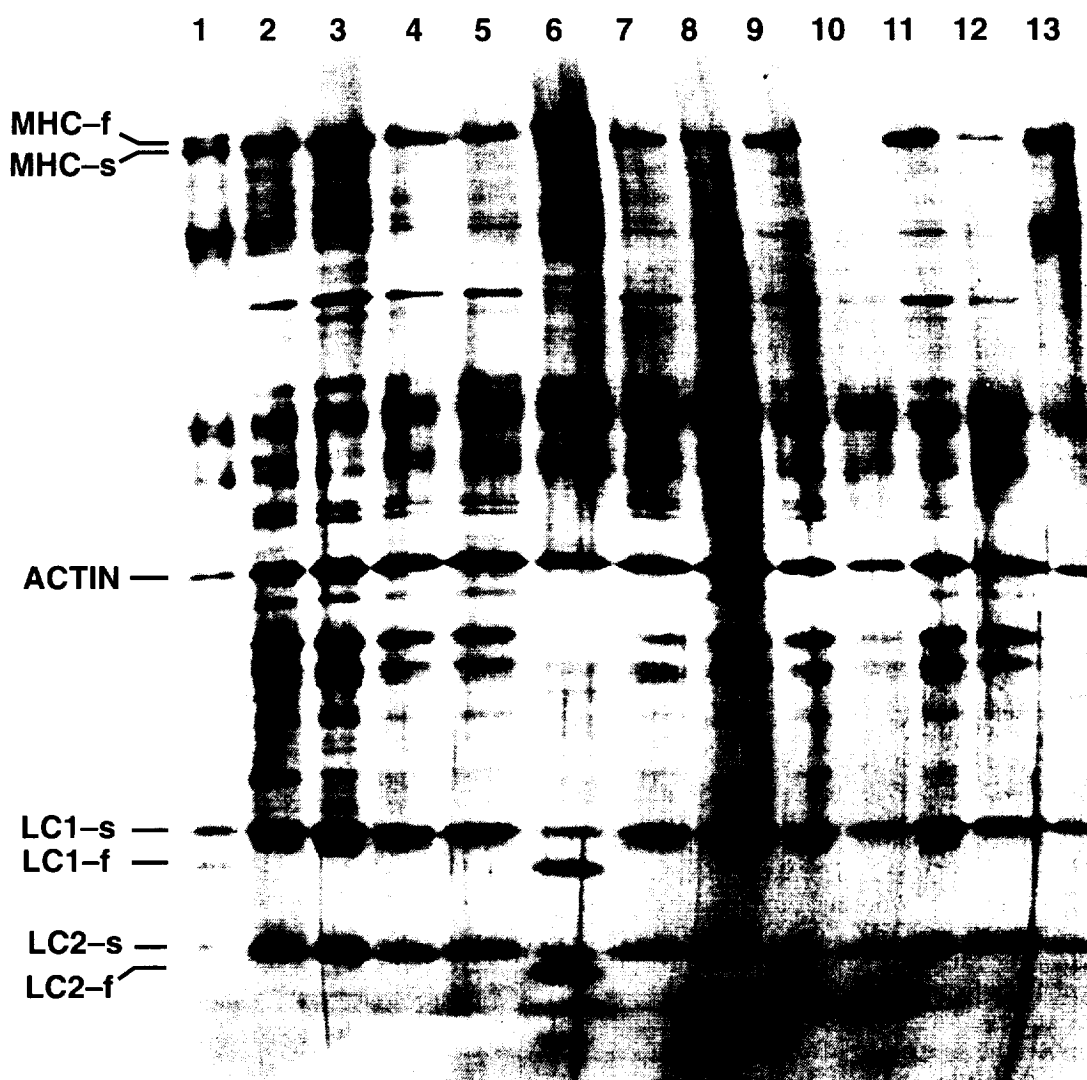
FIG. 5. Twelve percent SDS-PAGE of fibers isolated from control animals and animals after 1, 2, and 3 wk of HU. *Lanes 1, 6,* and *13,* both fast and slow myosin standards. Type of soleus fiber and its V_0 in each lane are as follows: 1 wk of HU, 3.2 (*lane 2*); control, 1.2 (*lane 3*); 1 wk of HU, 1.98 (*lane 4*); 1 wk of HU, 2.02 (*lane 5*); 2 wk of HU, 1.87 (*lane 7*); control, 1.1 (*lane 8*); 2 wk of HU, 1.91 (*lane 9*); 3 wk of HU, 1.96 (*lane 10*); control, 2.01 (*lane 11*); 3 wk of HU, 2.91 (*lane 12*).

Thomason et al. (29) demonstrated a similar relationship between MHC and myofibrillar ATPase activation in a wide spectrum of muscles. Additionally, Reiser et al. (25) reported that the HU-induced increase in single soleus fiber V_0 was correlated with an increase in the relative amount of fast myosin. We hypothesized that an increase in both fiber V_0 and ATPase with HU would result from an incorporation of fast myosin in a normally slow fiber. However, no evidence of fast myosin on one-dimensional SDS-PAGE gels was found in a large population of fibers from animals after 1, 2, and 3 wk of HU that exhibited elevated V_0 and ATPase activity. If fibers transform from slow to fast, it seems reasonable, given the relatively long half-life of myosin, to expect a gradual transition of the myosin isoform. Thus with SDS gel analysis, one should observe hybrid fibers containing both slow and fast MHC. This speculation is supported by the greater percentage of soleus fibers with histochemical staining patterns characteristic of the IIc fibers (such fibers presumably contain both slow and fast MHC) after

HU (6) and spaceflight (21) and by immunohistochemical data demonstrating hybrid fibers that cross-react to both type I and II myosin antibodies after zero gravity (Riley, personal communication).

Our laboratory (11), Reiser et al. (25), and Diffee et al. (7) have demonstrated that HU increases the amount of fast myosin in the soleus or bundles of soleus fibers. Additionally, in a previous single-fiber analysis we observed hybrid fibers, but it was not established that HU statistically increased their appearance relative to the control group (12). In this work, <1% of the fibers showed a hybrid myosin profile, and the single-fiber analysis of Reiser et al. showed slow and fast fibers but no hybrids after HU (Fig. 5). Consequently, although it seems logical to expect HU to increase the appearance of fibers containing both slow and fast myosin (hybrid fibers), this expectation has not been convincingly established. The possibility exists that fast myosin may be present but in amounts too low for our gel system to detect. A dilution analysis has indicated that (assuming 1 μg protein/mm

fiber and one-half of fiber's protein by weight is myosin) our gel system is sensitive to ~25 ng fast mysoin. For a 2-mm fiber segment, the total myosin by weight is ~1 μg. Therefore, the functionally adapted fibers displaying only slow myosin could not have contained more than 2.5% fast myosin. It seems unlikely that such a small increase in fast myosin could in some cases more than doubled fiber V_o and ATPase activity. A second possibility is that fast myosin may be less stable than slow myosin and degrade with storage even at $-80°C$. If this is true, a hybrid fiber with an intermediate V_o may appear after some period of storage as a homogeneous slow type I fiber when analyzed on an SDS gel. Preliminary data utilizing immunostaining of single fibers demonstrated fiber V_o and ATPase increased before the appearance of fast myosin (30). This result suggests that either factor besides the MHC modulates V_o and the fiber ATPase activity (15, 17) or that HU induces the synthesis of a second, yet unidentified, slow MHC that comigrates with slow type I myosin on SDS gels but contains higher ATPase activity. The altered V_o may involve alterations in thick filament proteins other than myosin (such as C- or X-protein). C- or X-protein is an integral component of the thick filament and has been considered an organizer of thick-filament structure. However, recent evidence suggests that C-protein also modulates contractile function. The extraction of C-protein significantly increased V_o in the low-velocity phase of shortening during submaximal activation of fast-twitch fibers (17). It is not known whether changes in C- or X-protein content affect the V_o of slow type I fibers or whether the concentration of these proteins change with HU. The relative importance of nonmyosin factors vs. increases in the fast or a second slow myosin isoform in producing the increased V_o and whether significant numbers of hybrid fibers exist after HU are important questions currently under investigation in our laboratory.

In conclusion, the time course of 3 wk of HU on the size, peak force, P_o, V_o, and ATPase activity of single soleus fibers has been characterized. HU induced a progressive increase in fiber V_o that was likely caused, at least in part, by an increase in the fiber's myofibrillar ATPase activity. The HU-induced increases in V_o and ATPase were associated with the presence of a greater percentage of fast type IIa fibers. However, these functional changes also occurred in fibers containing the same myosin isozyme (or one that comigrates with it on the SDS gel) that is expressed in control soleus fibers.

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