Fatigability and blood flow in the rat gastrocnemius-plantaris-soleus after hindlimb suspension

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McDONALD, K. S., M. D. DELP, AND R. H. FITTS. *Fatigability and blood flow in the rat gastrocnemius-plantaris-soleus after hindlimb suspension.* J. Appl. Physiol. 73(3): 1135–1140, 1992. —The purpose of this study was to test the hypothesis that hindlimb suspension increases the fatigability of the soleus during intense contractile activity and determine whether the increased fatigue is associated with a reduced muscle blood flow. Cage-control (C) and 15-day hindlimb-suspended (HS) rats were anesthetized, and either the gastrocnemius-plantaris-soleus (G-P-S) muscle group or the soleus was stimulated (100 Hz, 100-ms trains at 120/min) for 10 min in situ. In the G-P-S preparation, blood flow was measured with radiolabeled microspheres before and at 2 and 10 min of contractile activity. The G-P-S fatigued markedly at this stimulation frequency, and the differences between C and HS animals were not significant until the 9th min of contractile activity. In contrast, the stimulation resulted in faster rates and significantly larger amounts of fatigue in the soleus from HS than from C animals. The atrophied soleus showed significant differences by 1 min of stimulation (C = 70 ± 1% vs. HS = 57 ± 2% of peak train force) and remained different at 10 min (C = 64 ± 4% vs. HS = 45 ± 2% peak train force). Relative blood flow to the soleus was similar between groups before and during contractile activity (rest: C = 20 ± 3 vs. HS = 12 ± 3; 2 min: C = 128 ± 6 vs. HS = 118 ± 4; 10 min: C = 123 ± 11 vs. HS = 105 ± 11 ml · min^{-1} · 100 g^{-1}). In conclusion, these results established that 15 days of HS increased the fatigability of the soleus, but the effect was not caused by a reduced muscle blood flow.

muscle fatigue; muscle atrophy

THE BIOCHEMICAL AND PHYSIOLOGICAL properties of limb skeletal muscle have been shown to adapt to a variety of experimental conditions (9, 13, 18). Among these is the microgravity encountered with spaceflight (18, 27). Studies on skeletal muscles from rats orbited in COSMOS biosatellites and Spacelab 3 reveal multifaceted deterioration, involving muscle fiber atrophy, degeneration of motor innervation, muscle fiber segmental necrosis and central-core lesions, and disruption of the microvasculature (18, 27). These changes suggest a spaceflight-induced reduction in muscle work capacity. To study the cellular and molecular mechanisms responsible for these changes, numerous models have been used to mimic the hypokinesia (reduced number of contractions) and hypodynamia (reduced force of contractions) associated with weightlessness (23). One frequently studied model is hindlimb suspension (HS), which has been observed to produce atrophy, central corelike lesions (in 30% of soleus fibers), alterations in the enzymatic and contractile properties, and a reduced peak power in individual rat soleus muscle fibers (6, 7, 9, 10, 20, 28, 29).

Fell et al. (6) and Winiarski et al. (29) reported 1 and 4 wk of HS to have no effect on the fatigability of the soleus muscle. However, the train stimulation frequency used by Fell et al. (45/min) and Winiarski et al. (60/min) elicited only minimal fatigue and, thus, may not have been intense enough to uncover differences in the fatigue patterns of control and experimental groups. Witzmann et al. (30) employed a higher stimulation frequency (110 trains/min) and observed greater fatigability of the rat soleus after 42 days of hindlimb immobilization (HI) compared with the control. The effect of high-frequency stimulation on the fatigability of the soleus after HS has not been characterized.

HI has been shown to increase the extent of glycogen and ATP decline and yield a higher lactate content in the soleus during contractile activity (30). Additionally, the glucose uptake capacity and muscle fiber glycogen content were increased in the soleus after HS (11, 12), whereas the specific activity of phosphofructokinase and lactate dehydrogenase of single soleus fibers was elevated after 4 wk of HI and HS (7). These findings suggest that both models induce a shift in the atrophied soleus toward an increased reliance on glycogen metabolism. This shift occurs despite an increase in the oxidative enzyme capacity of single fibers isolated from the soleus after both HI and HS (7). An increased fatigability and dependence on glycogen metabolism in the atrophied soleus despite higher concentrations of aerobic enzymes could be explained by a reduced tissue blood flow. Consequently, the purpose of this study was to test the hypothesis that HS increases the fatigability of the soleus during intense contractile activity and that the increased fatigue is associated with a reduced muscle blood flow.

MATERIALS AND METHODS

Animal care and suspension procedure. Male Sprague-Dawley rats (250–275 g) were obtained from Sasco (Madison, WI) and randomly assigned to either the HS or cage-control group. The hindlimbs of the HS animals were elevated for 15 days with use of a harness attached to the proximal two-thirds of the tail, as previously described in detail (9). The height of suspension was adjusted to prevent the hindlimbs from contacting supportive surfaces. The forelimbs maintained contact with a

grid floor, which allowed the animals to move about to obtain food and water. The HS animals were fed Purina rat chow and water ad libitum, whereas the control rats were pair fed to maintain weights similar to those of the HS group. Both groups were housed at 23°C with a 12:12-h light-dark cycle.

Surgical procedures. After 15 days, the HS and control rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). A Silastic catheter (0.6 mm ID, 1.0 mm OD) was surgically implanted in the ascending aorta via the right carotid artery, as previously described (16). This catheter was subsequently used for recording arterial pressure and the infusion of radiolabeled microspheres for blood flow measurements. A second polyurethane catheter (0.36 mm ID, 0.84 mm OD) was inserted ~4 cm into the tail caudal artery, as described by Chiueh and Kopin (3) with several modifications (4), to withdraw blood for the blood flow measurements.

Muscle preparation and stimulation. After catheter implantation the left gastrocnemius-plantaris-soleus (G-P-S) muscle group was prepared for in situ stimulation. The muscle group was exposed and dissected free of surrounding tissue, with its blood and nerve supply left intact. Silk thread (4-0) was secured to the distal end of the Achilles tendon, and a small loop was tied for subsequent attachment to the force transducer. The rat was placed on a fixed platform within a 50 × 50 × 90-cm Plexiglas chamber maintained at 35°C, and the hindlimb was secured as previously described (30). The leg was immobilized at the ankle by a U-bolt fastened to the base of the platform. The skin that had been dissected free from the leg was pulled over a flange on each side of the platform and secured by stainless steel wound clips. The muscle and nerve preparation was kept moist with a gauze pad saturated with rat Ringer solution (in mM: 137 NaCl, 4 KCl, 1 $MgCl_2$, 1 KH_2PO_4, 12 $NaHCO_3$, and 2 $CaCl_2$). In a second group of HS and control animals the soleus, composed primarily of slow-twitch type I fibers (2), was freed of surrounding tissues, with the blood and nerve supply of the G-P-S muscle group remaining intact. The isolation of the soleus involved dividing the gastrocnemius into its medial and lateral heads and carefully pulling back the two heads of the gastrocnemius and the plantaris such that their contribution to soleus force production was minimal. A silk thread loop (4-0) was tied to the distal tendon, the rat was transferred to the Plexiglas chamber, and the leg was fixed to the support, as described above.

Before the contractile properties were measured, the preparation was allowed to thermoequilibrate for 20 min. Muscle temperature for the G-P-S and soleus was estimated by inserting a needle thermistor into the biceps femoris and lateral head of the gastrocnemius, respectively. Core temperature was monitored via a rectal probe. A multichannel telethermometer (Yellow Springs Instruments) displayed the inputs of both probes. The muscle and body temperatures averaged 35.2 ± 0.2 and 37.5 ± 0.1°C, respectively.

The muscles were stimulated indirectly through the distal portion of the cut sciatic nerve, which was isolated in the gluteal region and drawn into a suction-stimulating electrode. Each muscle preparation was adjusted to its optimal length at which maximal twitch and tetanic force (newtons) was achieved. Twitch contractions were elicited by supramaximal (1.5-V) square-wave pulses of 0.1-ms duration produced by a Grass S48 stimulator. Tetanic contractions were generated by supramaximal stimulation (2-s duration) at 100 Hz. During the isometric contractions, the force transducer (model FT10C, Grass Instruments) output was amplified and displayed on a pin recorder (model 735-1320, Narco Physiograph).

Fatigue studies. Isometric train force (P_{tr}) of the G-P-S or soleus was recorded at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min of contractile activity. The muscles were stimulated with 100-ms trains of 100 Hz at a train rate of 120/min. The train duration was selected to obtain high but not peak tetanic force, because prolonged tetanic contraction might impede blood flow and prevent detection of flow differences between control and HS animals. This stimulation protocol has previously been shown to elicit fatigue and high blood flow in the G-P-S muscle group (17). Muscle blood flow was measured before contractile activity (resting flow) and during the 2nd and 10th min of contraction. Blood flow measurements were made only in the G-P-S preparation.

Blood flow measurements. Radiolabeled (^{85}Sr, ^{113}Sn, and ^{153}Gd) microspheres (New England Nuclear) with a 15-μm diameter were used for blood flow measurements, as previously described (16). The microspheres were suspended in 0.9% physiological saline containing <0.01% Tween 80, mixed for 10 min in a Mettler Electronics ultrasonicator, and vortexed for 1 min before infusion. A 0.2-ml aliquot containing 500,000 spheres was infused into the aorta and the catheter flushed with warm saline (37°C). The infusion process required ~30 s and thus was begun 15 s before each measurement period. Radioactivity of the samples was measured with a gamma counter (Packard Auto Gamma 5780), and flows were computed (IBM-PC computer) from counts per minute and tissue wet weights. To ensure that microsphere-blood mixing was adequate, flows to right and left kidneys were compared for each animal. Mixing was considered sufficient if kidney flows were within 15% of each other.

For the blood flow measurements, the entire soleus and plantaris muscles were assayed, whereas the gastrocnemius was divided into white, red, and mixed portions. The white and red portions were obtained by pooling samples obtained from the superficial and deep regions of both heads of the gastrocnemius, respectively. The mixed portion consisted of the remaining gastrocnemius tissue.

Central hemodynamic measurements. Mean arterial pressure and heart rate were recorded from the carotid catheter just before each microsphere infusion with use of a pressure transducer (model RP-1500, Narco Bio-Systems) and recorder (model 735-1320, Narco physiograph). Only mean arterial pressure was reported because of the damping effect of the catheters. Heart rates were determined from the pulsatile pressure recordings.

Data analysis. Exponential curves of the form of $y_t = Ae^{-t} + C$, where y_t is developed force at *time t*, A is a constant of the slope function, and C is the asymptote of developed force, were fit to the time patterns of fatigue,

TABLE 1. *Tissue weights*

Tissue	Tissue Wt, mg		Tissue Wt/Body Wt, mg/g	
	C	HS	C	HS
Soleus	185±5	106±6*	0.52±0.01	0.32±0.02*
Plantaris	445±14	355±15*	1.25±0.03	1.07±0.03*
Gastrocnemius	2,147±55	1,661±68*	6.05±0.19	5.03±0.12*
Left kidney	1,166±45	1,270±40	3.29±0.17	3.85±0.07*
Right kidney	1,168±44	1,303±46	3.31±0.15	4.00±0.10*

Values are means ± SE of 8 observations. C, control group; HS, 15-day hindlimb-suspended group. * HS significantly different from C, $P < 0.05$.

and the curves were analyzed by t test statistics. A two-way analysis of variance was used to compare heart rates, mean arterial pressures, and blood flows within tissues between control and HS groups and across rest and 2 and 10 min of contractile activity. When significant differences occurred, a Student's unpaired two-tailed t test was used as the post hoc test to determine statistical significance between groups. The 0.05 level of probability was set for statistical significance. Reported values represent means ± SE.

RESULTS

Tissue weights. Significant reductions in the soleus, plantaris, and gastrocnemius muscle wet weights occurred as a result of 15 days of HS (Table 1). Consistent with observations by others (6, 9, 10), the soleus muscle demonstrated the greatest atrophy after HS. The absolute soleus muscle weight was 43% lower than control after HS (185 ± 5 vs. 106 ± 6), whereas the soleus muscle-to-body weight ratio was 38% lower than controls (0.52 ± 0.02 vs. 0.32 ± 0.02). The muscle weight-to-body weight ratios of the plantaris and gastrocnemius after HS were 14 and 17% lower than control values, respectively. The elevated kidney-to-body weight ratio of HS animals can likely be attributed to the selective loss of muscle mass.

Fatigue studies. The fatigue patterns of both the soleus and G-P-S are shown in Fig. 1. The initial P_{tr} of the G-P-S muscle group was significantly lower after 15 days of HS (C = 18 ± 1 vs. HS = 15 ± 1 N). Similarly, the initial soleus P_{tr} for control (2.02 ± 0.09 N) and HS (1.10 ± 0.07 N) animals was significantly different. However, when expressed per gram tissue, the soleus P_{tr} was not significantly different between groups (control = 11.13 ± 0.34 vs. HS = 11.29 ± 0.74 mN/mg tissue). The initial P_{tr} values averaged 71 ± 1 and 73 ± 2% of peak tetanic tension produced by the soleus from control and 15-day HS animals, respectively. All the contractile activity curves are characterized by a biphasic response, with an initial (1–2 min) rapid fall in force followed by a steady-state phase in which force is maintained or falls slightly. During the 10-min train stimulation period the soleus of the control and HS rats fatigued an average of 34 and 52% from their initial train force, respectively. Except for the first 30 s, the percent decline in tension was significantly greater in the HS group throughout the stimulation protocol. Exponential curves of the form $y_t = Ae^{-t} + C$ were fit to the individual fatigue patterns. The equation describing the soleus fatigue pattern in the control animals was $y_t = 34e^{-t} + 66$ and in the HS rats was $y_t = 52e^{-t} + 48$.

The equations describing the G-P-S muscle group fatigue patterns were $y_t = 69e^{-t} + 31$ for controls and $y_t = 73e^{-t} + 27$ for HS animals. The curves were significantly different between groups; however, the percent decline in force was not significantly greater in the HS group until 9 min of activation.

Blood flow measurements. The absolute blood flows (ml/min) and blood flows expressed per 100 g tissue weight ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) during rest and isometric contractile activity are presented in Table 2. The absolute blood flow to the soleus was significantly lower in the HS than in the control animals at all three time points. However, when normalized to muscle weight, soleus blood flow was not different between groups at rest or during contractile activity (Table 2).

During contractile activity both the absolute and normalized blood flow to the plantaris and white gastrocnemius were significantly lower in the HS animals (Table 2). The absolute flows of the red gastrocnemius (2 min) and mixed gastrocnemius (2 and 10 min) were significantly lower in the HS than in the control animals (Table 2). However, when expressed per tissue weight, the blood flow to these tissues was not different between groups.

Heart rate and blood pressure. Mean heart rate and arterial blood pressure values for control and HS animals during rest and contractile activity are presented in Table 3. Both heart rate and mean arterial pressure remained stable during contractile activity and were not different between groups.

DISCUSSION

The purpose of this study was to test the hypothesis that 15 days of HS reduces the blood flow and increases the fatigability of the slow-twitch soleus muscle during intense contractile activity. The results indicate that after 15 days of HS the soleus had a reduced resistance to fatigue; however, soleus blood flow per 100 g muscle was

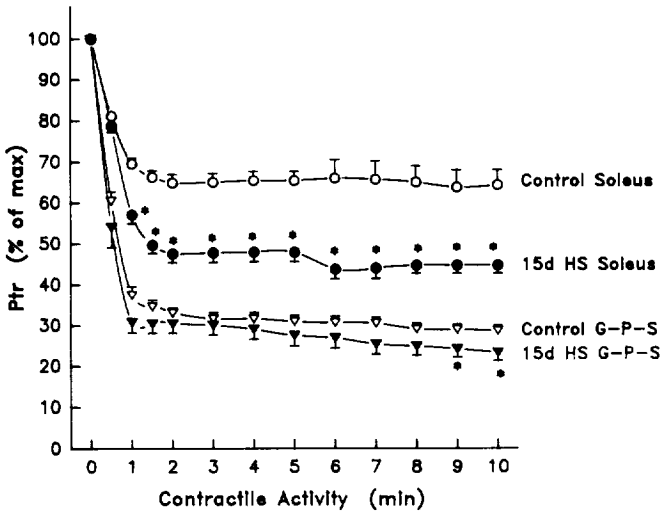

FIG. 1. Effect of 15 days of hindlimb suspension (HS) on peak train force (P_{tr}) relative to initial values during contractile activity in soleus and gastrocnemius-plantaris-soleus (G-P-S) muscle group. Each point is mean ± SE for 8 observations. *Control vs. 15-day HS, $P < 0.05$.

TABLE 2. *Blood flows in tissues and organs during rest and contractile activity*

Tissue	Blood Flow			
	ml · min^{-1} · 10^{-2}		ml · min^{-1} · 100 g^{-1}	
	C	HS	C	HS
Soleus				
Rest	3.7±0.5	1.3±0.3*	20±3	12±3
2 min	23.6±1.2	12.5±0.8*	128±6	118±4
10 min	22.6±1.9	10.8±0.9*	123±11	105±11
Plantaris				
Rest	2.9±1.0	2.2±0.6	6±2	7±2
2 min	66.0±10.0	33.0±3.8*	146±18	94±11*
10 min	74.3±12.0	33.8±5.5*	165±22	97±17*
Red gastrocnemius				
Rest	2.2±0.8	1.5±0.4	23±9	18±6
2 min	26.6±2.5	19.5±1.7*	253±26	232±16
10 min	27.0±3.7	20.0±2.6	273±41	236±24
White gastrocnemius				
Rest	1.7±0.3	1.5±0.4	11±2	12±4
2 min	10.3±2.0	4.1±0.8*	65±12	32±6*
10 min	9.8±1.7	4.4±0.9*	64±11	33±7*
Mixed gastrocnemius				
Rest	22.2±5.0	20.6±6.8	12±3	15±5
2 min	271.0±33.0	161.0±20.0*	144±17	113±15
10 min	312.0±32.0	168.0±27.0*	168±18	120±20
Left kidney				
Rest	618.0±56.0	714.0±96.0	526±34	559±70
2 min	768.0±126.0	816.0±100.0	645±86	638±67
10 min	686.0±95.0	806.0±83.0	611±88	635±61
Right kidney				
Rest	655.0±53.0	731.0±98.0	554±30	555±65
2 min	809.0±135.0	836.0±11.0	673±91	634±65
10 min	714.0±108.0	868.0±81.0	629±96	663±50

Values are means ± SE of 8 observations. * HS significantly different from C, $P < 0.05$.

not altered. These findings suggest that the increased fatigability of the atrophied soleus cannot be accounted for by a reduced blood flow to soleus muscle cells.

The stimulation protocol selected was known to elicit high muscle blood flow and significant fatigue in G-P-S muscles of control animals. Fitts and Holloszy (8) and Witzmann et al. (30) demonstrated that a train stimulation frequency of 110/min elicited a significant decline (~30%) in the P_{tr} of the soleus after 30 min of contractile activity. Mackie and Terjung (17) showed that a train stimulation frequency of 120/min caused both significant fatigue of the G-P-S muscle group and high blood flow to these muscles. The muscle blood flow at rest and during contractile activity reported in this study for the soleus and red and white gastrocnemius (Table 2) was essentially identical to that observed by Mackie and Terjung in the anesthetized rat hindlimb (see Ref. 17, Fig. 5).

The resting soleus blood flow in the control group was lower than that in awake standing rats (20 ± 3 vs. 36 ± 5 ml · min^{-1} · 100 g^{-1}) but higher than that measured during acute (5 min) HS in conscious animals (20 ± 3 vs. 8 ± 1 ml · min^{-1} · 100 g^{-1}) (19). The somewhat lower muscle blood flow in the anesthetized rat than in the awake standing rat was not surprising in that the hindlimbs of the anesthetized rat were unloaded. After HS, the resting soleus blood flow in the anesthetized rat was reduced by 40% compared with the control group. Although not statistically significant, this change was consistent with the observation that HS significantly reduced the soleus blood flow in the awake standing rat (19). The hindlimb muscle blood flows elicited with electrical stimulation in this study and that of Mackie and Terjung (17) were higher than those observed during treadmill walking at 15 m/min (19) but lower than those obtained during heavy treadmill exercise (1, 15). Mackie and Terjung found hindlimb muscle blood flow to decrease with stimulation frequencies <120/min. Consequently, it seems reasonable to suggest that the hindlimb blood flows elicited by contractile activity in this study were the peak flows obtainable during in situ electrical stimulation. Although 15 days of HS did not affect soleus blood flow, the flow to the white gastrocnemius was significantly depressed during contractile activity. This finding differs from the in vivo conditions in which flow to the white gastrocnemius (and other fast-twitch glycolytic muscle) was increased after HS both at rest and during treadmill walking (19). We hypothesized that this increased flow was mediated by a downregulation in sympathetic-mediated vasoconstriction (19). The reduced flow in the white gastrocnemius during intense in situ contractile activity suggests greater vasoconstriction in this muscle after HS. The possibility exists that metabolic factors (such as an increased H^+) triggered a chemoreflex, causing vasoconstriction (22). Thus this work and our previous study (19) suggest that HS modifies the sympathetic-mediated vascular tone in both active and inactive white fast-twitch muscle.

After 15 days of HS the soleus muscle was significantly less resistant to fatigue during 10 min of electrical stimulation. In contrast, Fell et al. (6) found 1 wk of whole body suspension to yield no differences in fatigue between atrophied and control soleus. Additionally, Winiarski et al. (29) found 4 wk of HS not to affect the fatigue index (ratio of force developed after 2 min of stimulation to maximum force) in the soleus. These differences are likely attributed to the variations in stimulation protocols. The higher train stimulation frequency used in the present study (120/min) revealed fatigue differences between control and atrophied soleus not observed with the lower frequencies of 45 and 60 trains/min employed by Fell et al. and Winiarski et al., respectively. Other possibilities for the discrepancy in soleus fatigability could include *1*) duration of HS, *2*) age and sex of the rats, and *3*) different experimental techniques.

Several possibilities could explain the increased fatigability of the soleus after HS despite no difference in blood flow. Kandarian et al. (14) recently observed a twofold increase in the relative interstitial volume of the soleus after 4 wk of HS, a change that could increase the diffu-

TABLE 3. *Heart rate and blood pressure*

Condition	Heart Rate, beats/min		Mean Arterial Blood Pressure, mmHg	
	C	HS	C	HS
Rest	379±14 (8)	398±14 (8)	125±6 (8)	121±5 (8)
2 min	380±15 (8)	399±9 (8)	124±9 (8)	114±5 (8)
10 min	389±13 (7)	411±11 (8)	126±5 (7)	117±7 (8)

Values are means ± SE for no. of observations in parentheses.

sion distance for oxygen exchange between the capillary and muscle cell. However, extracellular volume was unaltered immediately after 2 wk of HS, and interstitial edema did not develop until >12 h of hindlimb reloading (D. A. Riley, personal communication). Furthermore, Desplanches et al. (5) observed that capillary density was increased after HS, and this plus the smaller fiber diameter associated with HS should increase the surface-to-volume ratio and thus reduce the diffusion distances. A second possibility is an HS-induced decrease in responsiveness to sympathetic activation. HS may cause a chronic stimulation of sympathetic drive, yielding a downregulation of adrenergic receptors. In fact, Overton and Tipton (25) found 9 days of HS to blunt the mesenteric artery pressor response to exogenous sympathomimetic agents. Additionally, we recently found 15 days of HS to attenuate the redistribution of visceral blood flow normally observed during exercise (19). These findings could be caused by a reduced number and/or sensitivity of α-receptors after HS. The consequence of this may be a decreased cellular response to sympathetic drive, which could inhibit the mobilization of free fatty acids (FFA), thus reducing their delivery, uptake, and oxidation by skeletal muscle. The working muscle would then be more dependent on the oxidation of carbohydrates for energy production.

A reduced liberation of FFA and their subsequent delivery and oxidation by the working muscle may have contributed to an increased fatigue and glycogen usage during the second steady-state portion of the stimulation period. However, the initial rapid phase of fatigue most likely occurred before significant amounts of FFA could be mobilized from adipose cells, delivered, and oxidized in working muscle. Thus, additional factors must be responsible for the augmented early phase of fatigue in the atrophied soleus.

The more rapid fall in peak train force in the atrophied soleus may involve neural and/or excitation-contraction coupling processes. Fitts and Holloszy (8) observed a similar contractile response of the soleus to both direct and indirect stimulation after fatigue. This suggests that the fatigue induced in the control soleus was not due to alterations in neuromuscular transmission. However, the effect of HS on neuromuscular transmission is unknown. Additionally, the atrophied muscle may be less effective at coupling excitation and contraction. The effects of unweighting on the excitation-contraction coupling process (e.g., T-tubular charge movement and sarcoplasmic reticulum Ca^{2+} release) have not been investigated.

Another possibility for the increase in hindlimb muscle fatigability despite no difference in blood flow may be a lower blood and/or muscle oxygen-carrying capacity after HS. A reduction in erythrocyte concentration has been reported after HS (26). To our knowledge, there are no studies that have measured the effect of weightlessness on myoglobin concentration.

Finally, the greater fatigability of the soleus after HS could be in part mediated by an increased type IIa-to-type I fiber ratio (5). During contractile activity, predominantly fast-twitch limb muscles are known to fatigue more rapidly and generate higher lactates, H^+, and inorganic phosphate (30). Both of the latter two ions have been shown to reduce peak force (21, 24).

Even though the isolated soleus exhibited greater fatigue after HS, the fatigue pattern of the G-P-S muscle group was not different between groups until the 9th min of activation. The fiber mass of the G-P-S contains ~65% fast-twitch glycolytic fibers (2). Therefore the G-P-S fatigue pattern likely reflects the response of this fiber type, a fiber known to depend primarily on anaerobic metabolism and thus unlikely to be affected by changes in substrate or oxygen delivery. Why the fatigue pattern of the G-P-S differed between groups late in the bout of activation is unknown. One possibility is that by the 9th min of activation the force output of the gastrocnemius and plantaris may have sufficiently declined such that the difference in soleus force becomes significant.

The observation that the fatigue pattern of the G-P-S was not different between groups (except in the final minute of activation) despite significantly lower blood flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) to the plantaris and white gastrocnemius suggests that the maintenance of force output was not limited by blood flow to the G-P-S in either group.

In conclusion, after 15 days of HS, the soleus muscle exhibited marked atrophy and fatigued to a greater extent than control soleus. However, despite the increased fatigability of the atrophied soleus, the blood flow per gram muscle was similar to control values. This result suggests that the greater fatigability of the atrophied soleus was not caused by a reduced blood flow.

The authors are grateful to Barbara DeNoyer for help in the preparation of the manuscript, Dr. Robert B. Armstrong for advice on the design of the blood flow experiments, and Dr. Naveen Bansel for statistical analysis consultation.

This study was supported by National Aeronautics and Space Administration Grant NAG 2-212.

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Received 19 September 1991; accepted in final form 14 April 1992.

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