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

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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.

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-inducible 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
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 arte-
rial pressure and the infusion of radiolabeled micro-
spheres for blood flow measurements. A second polyure-
thane catheter (0.36 mm ID, 0.84 mm OD) was inserted
4 cm into the tail caudal artery, as described by Chieueh
and Kopin (3) with several modifications (4), to with-
draw blood for the blood flow measurements.

Muscle preparation and stimulation. After catheter im-
plantation 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 subse-
quent 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 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 MgCl2, 1 KH2PO4, 12 NaHCO3, and 2
CaCl2). 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 in-
 tact. The isolation of the soleus involved dividing the
gastrocnemius into its medial and lateral heads and care-
fully 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 Plex-
iglas 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 esti-
 mated by inserting a needle thermistor into the biceps
femoris and lateral head of the gastrocnemius, respec-
tively. Core temperature was monitored via a rectal
probe. A multichannel telemetherometer (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-stimulat-
ing 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. Te-
tanic contractions were generated by supramaximal stim-
ulation (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 (Ptr) 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 stim-
ulated 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 con-
traction might impede blood flow and prevent deter-
mination 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 (58Sr, 113mSn,
and 153Gd) microspheres (New England Nuclear) with a
15-μm diameter were used for blood flow measurements,
as previously described (16). The microspheres were sus-
pended in 0.9% physiological saline containing <0.01%
Tween 80, mixed for 10 min in a Mettler Electronics ul-
trasonicator, 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. Radioac-
tivity 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 kid-
neys were compared for each animal. Mixing was consid-
ered 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 gas-
 trocnemius was divided into white, red, and mixed por-
tions. The white and red portions were obtained by pool-
ing samples obtained from the superficial and deep re-
 gions of both heads of the gastrocnemius, respectively.
The mixed portion consisted of the remaining gastrocne-
mus 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 physio-
graph). Only mean arterial pressure was reported be-
cause of the damping effect of the catheters. Heart rates
were determined from the pulsatile pressure recordings.

Data analysis. Exponential curves of the form of
where yt 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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue Wt, mg</th>
<th>Tissue Wt/Body Wt, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>HS</td>
</tr>
<tr>
<td>Soleus</td>
<td>185±5</td>
<td>106±6*</td>
</tr>
<tr>
<td>Plantaris</td>
<td>445±14</td>
<td>355±15*</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>2,147±55</td>
<td>1,661±68*</td>
</tr>
<tr>
<td>Left kidney</td>
<td>1,186±45</td>
<td>1,270±40</td>
</tr>
<tr>
<td>Right kidney</td>
<td>1,168±44</td>
<td>1,303±46</td>
</tr>
</tbody>
</table>

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

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 P0 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 P0 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 P0 was not significantly different between groups (control = 11.13 ± 0.34 vs. HS = 11.29 ± 0.74 mN/mg tissue). The initial P0 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·min⁻¹·100 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...
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 120/min caused both significant fatigue for the G-P-S muscle group and high blood flow and significant fatigue in this study and that of Mackie and Terjung (17) were higher than those observed during treadmill exercise (1, 15). Mackie and Terjung found hindlimb muscle blood flow to increase 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. 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 diffusion.

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Blood Flow</th>
<th>Characteristic</th>
<th>Control (C)</th>
<th>HS (C)</th>
<th>Hypoxia (HS)</th>
<th>Control (C)</th>
<th>HS (HS)</th>
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<tr>
<td></td>
<td>ml·min⁻¹·10⁻²</td>
<td>ml·min⁻¹·100 g⁻¹</td>
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<tr>
<td>Rest</td>
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<tr>
<td>2 min</td>
<td>23.6±1.2</td>
<td>22.6±1.9</td>
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<tr>
<td>10 min</td>
<td>22.6±1.9</td>
<td>22.6±1.9</td>
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<tr>
<td>Plantaris</td>
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<td>Rest</td>
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<td>4.0±1.0</td>
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<tr>
<td>10 min</td>
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<tr>
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<tr>
<td>Rest</td>
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<td>Mixed gastrocnemius</td>
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<td>Left kidney</td>
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<tr>
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<td>10 min</td>
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<tr>
<td>Right kidney</td>
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<tr>
<td>Rest</td>
<td>655.0±53.0</td>
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<td>2 min</td>
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<tr>
<td>10 min</td>
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</table>

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

### TABLE 3. Heart rate and blood pressure

<table>
<thead>
<tr>
<th>Condition</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Blood Pressure, mmHg</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>HS</td>
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<tr>
<td>Rest</td>
<td>379±14 (8)</td>
<td>398±14 (8)</td>
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<td>2 min</td>
<td>380±15 (8)</td>
<td>399±9 (8)</td>
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<tr>
<td>10 min</td>
<td>389±13 (7)</td>
<td>411±11 (8)</td>
</tr>
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</table>

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 Ca²⁺ 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·min⁻¹·100 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.

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REFERENCES


