Human soleus single muscle fiber function with exercise or nutrition countermeasures during 60 days of bed rest

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Trappe S, Creer A, Minchev K, Slivka D, Louis E, Luden N, Trappe T. Human soleus single muscle fiber function with exercise or nutrition countermeasures during 60 days of bed rest. Am J Physiol Regul Integr Comp Physiol 294: R939–R947, 2008. First published December 19, 2007; doi:10.1152/ajpregu.00761.2007.—The soleus muscle has been consistently shown to atrophy more than other leg muscles during unloading and is difficult to protect using various exercise countermeasure paradigms. However, the efficacy of aerobic exercise, a known stimulus for oxidative adaptations, has not been tested in combination with resistance exercise (RE), a known hypertrophic stimulus. We hypothesized that a concurrent exercise program (AE + RE) would preserve soleus fiber myosin heavy chain (MHC) I size and function during 60 days of bed rest. A secondary objective was to test the hypothesis that a leucine-enriched high protein diet would partially protect soleus single fiber characteristics. Soleus muscle biopsies were obtained before and after bed rest from a control (BR; n = 7), nutrition (BRN; n = 8), and exercise (BRE; n = 6) group. Single muscle fiber diameter (Dia), peak force (P0), contractile velocity, and power were studied. BR decreased (P < 0.05) MHC I Dia (−14%), P0 (−38%), and power (−39%) with no change in contractile velocity. Changes in MHC I size (−13%) and contractile function (−30%) from BRN were similar to BR. BRE decreased (P < 0.05) MHC I Dia (−13%) and P0 (−23%), while contractile velocity increased (P < 0.05) 26% and maintained power. These soleus muscle data show 1) the AE + RE exercise program maintained MHC I power but not size and strength, and 2) the nutrition countermeasure did not benefit single fiber size and contractile function. The divergent response in size and functional MHC I soleus properties with the concurrent exercise program was a unique finding further highlighting the challenges of protecting the unloaded soleus.

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tory subjects (18, 27). The nutrition countermeasure, however, did not benefit single muscle fiber size or contractile function and actually promoted additional muscle loss of the thigh compared with the control group (54, 55).

This study presents the soleus single muscle fiber contractile properties from the WISE 60 days of bed rest study. In contrast to the vastus lateralis findings (54), the slow-twitch muscle fibers from the soleus were not completely protected by the exercise program. This novel finding of different responses to exercise at the cellular level between an upper and lower leg muscle is discussed along with implications for future prescription models to protect muscles from different regions in the human body.

METHODS

Subjects

Twenty-four healthy women underwent 60 days of 6° head-down tilt bed rest. Of the 24 women involved in the study, 21 completed the pre- and post-muscle biopsy subjects. Subjects were divided into bed rest only (BR; n = 7), bed rest + nutritional countermeasure (BRN; n = 8), or bed rest + exercise countermeasure (BRE; n = 6) groups. Subjects in the BR group were 34 ± 1 y, 163 ± 2 cm, and 57 ± 1 kg, with a body mass index (BMI) of 21 ± 1 kg/m². Subjects in the BRN group were 29 ± 1 y, 170 ± 2 cm, and 62 ± 2 kg, with a BMI of 21 ± 1 kg/m². Subjects in the BRE group were 33 ± 1 y, 164 ± 3 cm, and 58 ± 3 kg, with a BMI of 22 ± 1 kg/m².

Screening for potential volunteers took place at the Institut de Médecine et de Physiologie Spatiale (MEDES) clinic and involved an interview, general medical examination, and psychological evaluation. Subjects were selected into each group by the MEDES staff based on their medical and psychological profile. Before the screening, each potential volunteer was informed of all procedures and potential risks associated with the experimental testing. Informed consent was then obtained from each volunteer. The study was approved by Human Use Committees in France and the United States (Johnson Space Center and Ball State University).

Countermeasures to Bed Rest

A summary of the concurrent resistance and aerobic exercise program and time commitment of the regimen is shown in Table 1. Subject setup and configuration of the devices was not included in the time estimates as this varies greatly from bed rest to spaceflight.

Resistance training protocol. The BRE group trained the calf muscles using calf press (CP) exercises on an inertial ergometer (1–3, 55). The inertial ergometer is a gravity-independent resistance exercise device incorporating a flywheel mechanism that allows for concentric and eccentric force development over a full range of motion for a given muscle group. For the current study, the flywheel device was positioned so that all thigh and calf exercises were completed with the subject in the 6° head-down tilt position as shown and described previously (1, 2). Resistance exercise was scheduled for each subject approximately every third day (2–3 days/wk) beginning on day 2 of bed rest for a total of 19 sessions. Ten minutes of light supine cycling and submaximal CP repetitions were completed as warm-up. The CP exercise consisted of 4 sets of 14 maximal concentric and eccentric repetitions. There were 2 min of rest between sets. Force and flywheel rotational velocity were measured, and work and power were calculated throughout each repetition (1–3). This exercise protocol was similar to a previous 90-day study conducted in males (2, 52). Data compiled at the end of both bed rest campaigns provided a profile of the exercise sessions. For the CP sessions, 73% were conducted as planned, 22% were reduced effort, and 5% were missed.

Aerobic training protocol. The lower body negative pressure (LBNP) treadmill device used for this study was similar to that described previously (9, 33, 57). The treadmill was positioned vertically so that all treadmill exercise activity was performed with the subject in a horizontal (zero degree) position. Two to four days per week, exercise subjects performed 40 min of exercise ranging from 40 to 80% of prebed rest VO₂peak, followed by 10 min of resting LBNP (33, 57). During the course of the 60-day bed rest, 29 exercise sessions were prescribed for each BRE subject. A few exercise sessions were missed due to illness, joint pain, and soreness from prior exercise. Of the exercise sessions performed, the mean exercise time was 50 ± 2 min. Across all exercise sessions completed, the average LBNP was 52 ± 3 mmHg, which corresponded to a mean loading of 1.0 ± 0.1 body wt.

Nutritional countermeasure. All meals were prepared for all three groups by the MEDES dietary staff, with controlled amounts of total energy and macronutrients (carbohydrate, fat, and protein) as well as sodium, potassium, calcium, and fluid intake. All three groups received similar diets during the bed rest period, with the protein composition maintained at ~1.0 g·kg body wt⁻¹·day⁻¹. The BR and BRE groups continued to receive this amount of protein during the bed rest period, while the BRN group received ~1.45 g·kg body wt⁻¹·day⁻¹. In addition, the BRN group received 3.6 g/day of free leucine, 1.8 g/day of free valine, and 1.8 g/day of free isoleucine equally divided over the three meals of the day. Thus, the total protein intake for the BRN group was ~1.6 g·kg body wt⁻¹·day⁻¹. To compensate for the additional increase in energy intake from protein in the BRN group, carbohydrate content was reduced during the bed rest period. Energy intake for all three groups was adjusted downward during bed rest due to the reduced energy expenditure compared with the prebed rest period. Also during bed rest, the BRE group received an additional amount of energy intake equal to the energy expended during the exercise training sessions.

Muscle Biopsy

A muscle biopsy (4) was obtained from the soleus of each subject before bed rest and on day 59 of bed rest. The muscle biopsy was performed on day 59 to avoid interfering with other testing procedures being performed on the final day of bed rest before subject reembulation.

A portion of the sample was sectioned into several longitudinal pieces, placed in cold skinning solution (see below), and stored at −20°C for later analysis of single muscle fiber physiology. After a single muscle fiber experiment, each single fiber was analyzed for MHC and myosin light chain (MLC) composition as described below. All single muscle fiber physiology experiments were completed within a 4-wk period of the muscle biopsy.

Table 1. Summary of muscle contractions performed and time commitment for the concurrent exercise-training regimen

<table>
<thead>
<tr>
<th>Muscle contraction</th>
<th>Time commitment</th>
</tr>
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<tbody>
<tr>
<td>RE (thigh) = 4 × 7 × 19 sessions = 532 contractions</td>
<td>Exercise performed on 48 out of 60 days (~80%)</td>
</tr>
<tr>
<td>RE (calf) = 4 × 14 × 19 sessions = 1,064 contractions</td>
<td>RE = 56 min total contraction time (thigh = 23 min; calf = 33 min)</td>
</tr>
<tr>
<td>AE = 100 steps/min (estimate) × 40 min × 29 sessions = 208,800 (104,400 per leg)</td>
<td>AE = −24.2 h of activity (range = 40–80% VO₂peak)</td>
</tr>
<tr>
<td>−106,000 dynamic contractions performed per muscle</td>
<td>Exercise constituted −1.75% of total bed rest time</td>
</tr>
</tbody>
</table>

RE, resistance exercise; AE, aerobic exercise.
Skinning, Relaxing, and Activating Solutions

The skinning solution contained (mM): 125 K propionate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl₂, 20.0 imidazole (pH 7.0), and 50% (vol/vol) glycerol. The compositions of the relaxing and activating solutions were calculated using an interactive computer program described by Fabiato and Fabiato (20). These solutions were adjusted for temperature, pH, and ionic strength using stability constants in the calculations (23). Each solution contained the following (mM): 7.0 EGTA, 20.0 imidazole, 14.5 creatine phosphate, 1.0 free Mg²⁺, and 4.0 free MgATP, KCl, and KOH to produce an ionic strength of 180 mM and a pH of 7.0. The relaxing and activating solutions had a free Ca²⁺ concentration of pCa 9.0 and pCa 4.5, respectively (where pCa = −log Ca²⁺ concentration).

Single Muscle Fiber Physiology Experiments

For each experiment, a 2- to 3-mm muscle fiber segment was isolated from a muscle bundle and transferred to an experimental chamber filled with pCa 9.0 solution. Each end of the fiber was then securely fastened between a force transducer (model 400A, Cambridge Technology) and a direct current torque motor (model 308B, Cambridge Technology) as described by Moss (35). The apparatus was mounted on a microscope (Olympus BH-2) so that the fiber could be viewed (∼800×) during an experiment. With the use of an eyepiece micrometer, sarcomeres along the isolated muscle segment length were adjusted to 2.5 μm and the fiber length was determined. All single muscle fiber experiments were performed at 15°C.

Unamplified force and length signals were sent to a digital oscilloscope (Nicolet 310, Madison, WI) enabling muscle fiber performance to be monitored throughout data collection. Analog force and position signals were amplified (Positron Development, dual differential amplifier, 300-DIF2, Ingelwood, CA), converted to digital signals (National Instruments) and transferred to a computer (Gateway, Irvine, CA) for analysis using customized software. Servo-motor arm and isotonic force clamps were controlled using a computer interfaced force-position controller (Positron Development, force controller, 300-FC1).

Single Muscle Fiber Analysis

Individual muscle fibers were analyzed for diameter, peak force (P₀), maximal unloaded shortening velocity and force-power characteristics. Detailed descriptions and illustrations of these procedures have been previously published by our laboratory (50, 53).

Single fiber diameter. A video camera (Sony CCD-IRIS, DXC-107A) connected to the microscope and interfaced to a computer allowed viewing on a computer monitor and storage of the digitized images of the single muscle fibers. After the fiber was mounted between the force transducer and lever arm system with sarcomeres spaced to 2.5 μm, fiber diameter was determined from a captured computer image taken with the fiber briefly suspended in air (<5 s).

Fiber width (diameter) was determined at three points along the segment length of the captured image using National Institutes of Health public domain software (Scion Image, release Beta 4.0.2, for Windows).

Single fiber P₀. The outputs of the force and position transducers were amplified and sent to a microcomputer via a Lab-PC+ 12 bit data acquisition board (National Instruments). Resting force was monitored, and then the fiber was maximally activated in pCa 4.5 solution. P₀ was determined in each fiber by computer subtraction of the baseline force from the P₀ in the pCa 4.5 solution.

Single fiber shortening velocity. Fiber shortening velocity (V₀) was measured by the slack test technique as described by Edman (19). Four different activation and length steps [150, 200, 250, and 300 μm; each ≤15% of fiber length (FL)] were used for each fiber, with the slack distance plotted as a function of the duration of unloaded shortening. Fiber V₀ (FL/s) was calculated by dividing the slope of the fitted line by the fiber segment length and the data were normalized to a sarcomere length of 2.5 μm.

Single fiber power. Submaximal isotonic load clamps were performed on each fiber for determination of force-velocity parameters and power. Each fiber segment was fully activated in a pCa 4.5 solution and then subjected to a series of three isotonic load steps. This procedure was performed at various loads so that each fiber was subjected to a total of 15–18 isotonic contractions. Force and shortening velocity data points derived from the isotonic contractions were fit using the hyperbolic Hill equation (28). Fiber peak power was calculated from the fitted force-velocity parameters (P₀, Vmax, and a/P₀, where a is a force constant and Vmax is the y-intercept). Absolute power (μN·FL−1·s⁻¹) was defined as the product of force (μN) and shortening velocity (FL/s). Normalized power (W/l) was defined as the product of normalized force and shortening velocity.

Composite peak power. Composite peak power, estimated from all fiber types studied, was calculated as has been previously described in detail (52). Briefly, we implemented a weighted single fiber value system based on the MHC composition of the fibers studied from each volunteer. The resulting composite peak power value theoretically reflects the average for the entire muscle (in this case the soleus muscle). The composite estimates allowed us to include the hybrid fibers in the analysis in an attempt to make a link between the myocellular functional results and the whole muscle results in this investigation. This procedure was conducted for peak power for each individual and then averaged to represent the BR, BRN, and BRE groups.

MHC and MLC Determination

After the single muscle fiber physiology experiments, each fiber was solubilized in 80 μl of 1% SDS sample buffer and stored at −20°C until assayed (62). Briefly, samples were run overnight at 4°C on a Hoefer SE 600 gel electrophoresis unit (San Francisco, CA) utilizing a 3.5% (wt/vol) acrylamide stacking gel with a 5% (MHC) or 12% (MLC) separating gel. After electrophoresis, the gels were silver stained as described by Giulian et al. (22). A computer-based image system (Alpha Innotech, ChemImager 4000, San Leandro, CA) was used to quantify the relative density of each MLC band.

Whole Muscle Strength and Size

To document muscle strength and size changes, isometric and dynamic muscle tests were performed before and after bed rest in all three groups. MRI was conducted to determine size of the calf muscles before and after bed rest in all three groups. A more complete report on methodology, muscle size, and performance from the same subjects of the current investigation is presented elsewhere (55).

Statistical Analysis and Calculations

Changes in muscle strength and size, as well as MHC I single fiber variables [diameter, P₀, P₀/cross-sectional area (CSA), V₀, Vmax, peak power, and normalized peak power] from pre- to postbed rest in BR, BRN, and BRE were determined using a two-way ANOVA with repeated measures. Only MHC I fibers were analyzed statistically due to the low number of other fiber types studied in the soleus muscle. However, data from the fiber types with relatively low yield are presented. Significance was set at P < 0.05, and a Tukey’s post hoc test was used when significance was noted. All data are means ± SE.

RESULTS

Contractile function and subsequent MHC/MLC analysis were completed on 533 soleus muscle fibers (Table 2). A small number of MHC I/IIa/IIx (n = 3) and no MHC IIx fibers were examined in the pre- and postbed rest phase of the study.
respectively. When corrected for cell size (Po/CSA), MHC I (P shown in Table 4. There were no differences in MHC I Vo with Single Muscle Fiber Po and Po/CSA/H11021 13% BRE).

BRE, bed rest

P increased (S H11021 39% in BR (P = 0.05) by 46% in BR and 39% in BRN following bed rest. Calf muscle power was reduced (P < 0.05) by 29% in BR and 28% in BRN following bed rest. Bed rest resulted in a decrease (P < 0.05) in MHC I Po decreased 39% in BR (P = 0.05) by 46% in BR and 39% in BRN following bed rest. BRE had a decrease (P < 0.05) in calf muscle size (−8%) but maintained whole muscle power. Complete whole muscle results are presented elsewhere (55).

DISCUSSION

This phase of the WISE 60-day bed rest study reports the myocellular profile (size, contractile function, and MHC/MLC isoforms) of the soleus. The main finding was that the concurrent exercise prescription was not completely effective for MHC I soleus fibers (Fig. 2). This is in contrast to the vastus lateralis muscle (54) showing preservation (size and function) of MHC I and Ila fibers with this exercise regimen. Secondly,
the nutritional intervention did not provide any benefit for MHC I soleus fiber size and contractile function.

A similar degree of atrophy (~14%) was observed in the soleus MHC I and the MHC I vastus lateralis fibers (54) fibers from the control (BR) subjects. These are the first human data to show that the MHC I fibers atrophy to a similar extent with long-term unloading in these two leg muscles. In contrast, the MHC I fibers from the soleus and vastus lateralis muscles responded differently to the cumulative effects of 48 (RE = 19 and AE = 29) training sessions (Table 1) during the 60-day bed rest period. The concurrent exercise program did not protect MHC I soleus fiber diameter as shown by the similarities to the control group, while vastus lateralis MHC I fiber diameter was protected by the exercise program (54). These results show that the loading and subsequent adaptation from the exercise sessions differ between upper and lower leg muscles.

The basis for the differential adaptations that occurred in the MHC I fibers from the vastus lateralis and soleus is currently unknown. One possibility may be related to the protein synthesis response after exercise, which is greater in the vastus lateralis compared with the soleus (56). This finding suggests that the vastus lateralis has greater anabolic potential in response to resistance exercise compared with the soleus. Fuel stores could also be a contributing factor. Several studies (6, 44) have shown a greater reliance on glucose for energy in unloaded muscle. Further, soleus muscle glycogen use is greater during running compared with the vastus lateralis (15). As the bed rest period progressed, it is possible that the increased glycolytic flux and reliance on glycogen during treadmill exercise may have resulted in chronically low soleus muscle glycogen levels. Low muscle glycogen levels have been shown to downregulate the typical exercise response of key signaling intermediates (Akt/mTOR; Ref. 16) and the basal expression of genes (10) that are involved with muscle growth and remodeling. While low muscle glycogen levels may attenuate growth, it is also associated with the upregulation of genes responsible for endurance training adaptations (38), which may have been beneficial for the treadmill exercise. It is also possible the soleus muscle began the study in a more aerobically conditioned state compared with the vastus lateralis due to daily activities (postural support and walking) that heavily rely on the soleus muscle. A better conditioned muscle has an attenuated response in protein synthesis (29, 37), signaling (13), and gene induction (12) events involved with the regulation of muscle growth following exercise. Collectively, the current and previous studies highlight the unique nature of the exercise response between muscle groups (i.e., soleus and vastus lateralis) and warrant further investigation.

While the static (size and strength) properties of the MHC I soleus fibers were not maintained in the exercise group, the parameter of power was preserved with exercise. A disconnect between cell size and power has also been observed after run training with a decrease in cell size (~21%) and increased power output (+56%) of the MHC I gastrocnemius muscle fibers (51). In the current investigation, the preservation of MHC I soleus power can most likely be attributed to the 26% increase in $V_{\text{max}}$. This was an interesting observation given that cell size was reduced and unloaded shortening velocity, another index of shortening speed, was unchanged. The $V_{\text{max}}$ data are obtained while the fiber is under tension while $V_{o}$ is measured when the fiber is completely unloaded.

### Table 5. Single muscle fiber peak power and peak power normalized to cell size types from BR, BRN, and BRE before and after 60 days of bed rest

<table>
<thead>
<tr>
<th></th>
<th>MHC I</th>
<th>MHC I/IIa</th>
<th>MHC IIa</th>
<th>MHC IIa/IIx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Peak power, $\mu$N·FL$^{-1}·s^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>6.4 ± 0.8 †</td>
<td>3.9 ± 0.4 †</td>
<td>13.1 ± 3.1</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>BRN</td>
<td>9.4 ± 1.0</td>
<td>6.6 ± 0.4 †</td>
<td>NA</td>
<td>10.6 ± 1.3</td>
</tr>
<tr>
<td>BRE</td>
<td>11.2 ± 1.4</td>
<td>10.3 ± 1.3</td>
<td>13.6 ± 2.5</td>
<td>12.9 ± 1.5</td>
</tr>
<tr>
<td>Norm power, W/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>1.00 ± 0.12</td>
<td>0.84 ± 0.08</td>
<td>2.94 ± 0.71</td>
<td>1.09 ± 0.05</td>
</tr>
<tr>
<td>BRN</td>
<td>1.39 ± 0.10</td>
<td>1.34 ± 0.05</td>
<td>NA</td>
<td>2.36 ± 0.34</td>
</tr>
<tr>
<td>BRE</td>
<td>1.62 ± 0.25</td>
<td>1.80 ± 0.11</td>
<td>2.06 ± 0.35</td>
<td>2.01 ± 0.18</td>
</tr>
</tbody>
</table>

Norm. power, peak power normalized to cell size. *P < 0.05 from pre; †P = 0.09 from pre; ‡P < 0.05 from BRE.
these two measures parallel each other in human studies (7, 52, 59). It should be considered, however, that the V\text{max} data more closely relates to how the fiber behaves in vivo (i.e., shortening while under tension). In the current study, all resistance and running exercise training involved dynamic muscle actions. Approximately 106,000 dynamic muscle contractions were performed during the 60-day exercise-training program when running and resistance exercise are combined (see Table 1). Thus, it is possible that the isotonic nature of the contractions performed for the exercise program used in this study specifically targeted the isotonic properties of the MHC I muscle fibers during bed rest.

To further examine a potential mechanism for the increase in single muscle fiber shortening velocity of the exercise group, we analyzed the MLC composition of the MHC I fibers before and after bed rest. Changes in the expression of these proteins have been noted with years of distance running in humans (61) and endurance training in rodents (42) and are thought to play a role in fine-tuning or modulating contraction speed of the fiber (45, 46). The idea was that the dynamic contractions employed in the countermeasure program during bed rest, and in particular the running portion of the training program, may have altered the MLC profile. In the current study, we did not observe any alterations in MLC chain composition in any of the groups after bed rest. Perhaps more exercise volume is necessary to remodel the MLC profile given the amount of endurance exercise previously noted (42, 61) when changes were evident. In support of this, a high intensity, low volume resistance exercise program does not alter MLC composition (53), lending support to the concept that MLC alterations may require a greater exercise volume. No change in MLC composition indicates that other aspects of muscle contraction mechanics (i.e., Ca\textsuperscript{2+} kinetics and ATPase activity) or architecture may be modulating the increased V\text{max} observed with bed rest in the exercise group.

Although soleus MHC I fiber size and function were not completely maintained in the exercise group during bed rest, calf muscle power was maintained (55). Maintaining calf muscle performance despite of a loss in muscle mass has been noted previously (2, 43). The disconnect between muscle size and power is most likely related to the plasticity of both the nervous system and intrinsic properties of the muscle, which have been shown to be dynamically altered with unloading (11). The plasticity of the nervous system would also lend itself to improved technique on the exercise device and improved neural control (i.e., recruitment and synchronization of motor

### Table 6. Single fiber myosin light chain composition of soleus MHC I fibers from BR, BRN, and BRE before and after 60 days of bed rest

<table>
<thead>
<tr>
<th>MLC Isomorph</th>
<th>Pre</th>
<th>Post</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR MLC\textsubscript{1s}</td>
<td>0.52±0.01</td>
<td>0.50±0.03</td>
<td>-4%</td>
</tr>
<tr>
<td>BR MLC\textsubscript{2s}</td>
<td>0.40±0.01</td>
<td>0.41±0.03</td>
<td>+2%</td>
</tr>
<tr>
<td>BR MLC\textsubscript{3s}</td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
<td>0%</td>
</tr>
<tr>
<td>BRN MLC\textsubscript{1s}</td>
<td>0.54±0.01</td>
<td>0.51±0.01</td>
<td>-5%</td>
</tr>
<tr>
<td>BRN MLC\textsubscript{2s}</td>
<td>0.38±0.01</td>
<td>0.42±0.01</td>
<td>+10%</td>
</tr>
<tr>
<td>BRN MLC\textsubscript{3s}</td>
<td>0.09±0.00</td>
<td>0.07±0.01</td>
<td>-13%</td>
</tr>
<tr>
<td>BRE MLC\textsubscript{1s}</td>
<td>0.51±0.01</td>
<td>0.50±0.01</td>
<td>-2%</td>
</tr>
<tr>
<td>BRE MLC\textsubscript{2s}</td>
<td>0.41±0.01</td>
<td>0.42±0.01</td>
<td>+2%</td>
</tr>
<tr>
<td>BRE MLC\textsubscript{3s}</td>
<td>0.08±0.01</td>
<td>0.09±0.01</td>
<td>+12%</td>
</tr>
</tbody>
</table>

The relative contribution of each myosin light chain (MLC) band is represented as a fraction of the entire MLC band region.
units) that would benefit whole muscle performance. We did not isolate the cellular and whole muscle contributions of the gastrocnemius, which would have also played a role in overall calf muscle performance. This is emphasized from short-term (17 day) spaceflight showing the contractile properties of the gastrocnemius (60) are less affected than the soleus muscle (59). Changes in the cellular profile of the muscle were also modified and most likely contributed to whole muscle performance. There was a modest shift in soleus MHC isoforms from slow to fast (Table 2), with a 10% increase in hybrid muscle fibers that were of the MHC IIa/IIX phenotype. This would result in a faster and more powerful muscle when all fiber types are taken into consideration as supported by the composite power calculations (Fig. 1). This would aid crewmembers when performing brief high power output oriented tasks in space, but not advantageous for long duration tasks such as exploration of a planet surface that may require several hours of walking. The shift in soleus cellular structure and function observed in 60 days may be exacerbated when extended for space travel to other planets (i.e., Moon and Mars) that could result in risk to the well-being of crewmembers.

Since the combined resistance and aerobic exercise program did not preserve MHC I cell size and the soleus contains a large proportion of these oxidative fibers, other modes of exercise should be established. Resistance exercise during unloading favors fast-twitch fiber adaptation (52), while a higher volume of running may be counteractive for preserving MHC I fiber size (26, 51). Thus, an increase in resistance exercise or running may not be ideal candidates for the soleus muscle. While isometric contractions have not proven completely effective for preserving muscle size and function (24), it is worth considering that static type muscle contractions may be necessary, in combination with dynamic contractions, for a complete preservation of the soleus. This is supported by data showing that wearing an anti-G penguin suit with elastic load elements that provide a load equivalent to ~50–70% of body wt protects the size and force properties of the soleus MHC I fibers with long duration bed rest (64). Another consideration is that load activities with a low-to-moderate oxidative requirement, such as walking, performed at greater frequencies would strike a balance between load and duration for the soleus MHC I fibers. Exploration of the planet surface (Moon or Mars) may provide enough stimulus to maintain the size, contractile function, and oxidative profile of soleus MHC I fibers, leaving only resistance exercise to protect the fast-twitch muscle fibers, which takes very little time (2, 52).

We observed a similar degree of muscle atrophy in the soleus muscle between the control (−29%) and nutrition groups (−28%). The whole muscle findings are supported by the single fiber data showing nearly identical atrophy of the MHC I fibers (−14%) between the nutrition and control groups. These data are supported by recent studies of moderate duration bed rest (28 days) showing no protection of calf muscle mass (36) and soleus MHC I fiber size (21) when supplemented with an essential amino acid and carbohydrate countermeasure. Animal studies (47) also corroborate this finding, showing that a high protein diet did not sustain protein synthesis nor prevent a reduction in muscle growth in rats that were hindlimb suspended for 21 days. Taken together, these data do not support the use of a nutrition program alone to protect the soleus muscle/triceps surae during moderate and long duration bed rest.

Perspectives and Significance

This study highlights myocellular adaptations of the soleus to a combined resistance and aerobic training program while in bed for 60 days and adds insight for future exercise prescription models for space. While the addition of aerobic exercise to resistance exercise proved complimentary and completely protective for the vastus lateralis (54), single muscle fiber properties of the soleus, in particular the MHC I fibers, did not achieve the same level of protection. The fact that MHC I fibers from the vastus lateralis and soleus muscle atrophy to a similar extent with long duration unloading but respond differently to the same exercise loading program was an interesting and novel finding. These data suggest that unique and specific exercise regimens for the upper (vastus lateralis) and lower leg (soleus) may be necessary to protect human skeletal muscle during extended unloading periods. Our impression is that the difference in the vastus lateralis and soleus muscle response to the concurrent exercise program is not a volume of exercise issue per se, but more related to the type of muscle contractions performed. All of the muscle contractions performed as part of this investigation (>100,000 per muscle group over 60 days) were dynamic in nature. However, given that the soleus is suited for postural support, static type contractions of longer duration to more closely mimic the typical load on Earth in a 1-g environment may be required. We do not recommend that static load contractions be performed in lieu of dynamic contractions. Rather, it is likely that a proper combination of static and dynamic contractions will be necessary to protect the quantitative (size) and qualitative (intrinsic speed and power) properties of the soleus muscle fibers. The last decade of exercise countermeasure research has significantly reduced the time necessary to protect most muscle groups (<2% total time in the unloaded state) for crewmembers. While static contractions of longer duration may be necessary for the soleus muscle, these could easily be implemented with a device that would not interfere with crewmembers during their typical working days in space.

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REFERENCES


54. Trappe SW, Creer A, Slivka D, Minchev K, Trappe TA. Single muscle fiber function with concurrent exercise or nutrition countermeasures during 60 days of bed rest in women. *J Appl Physiol*, In press.


