Human vastus lateralis and soleus muscles display divergent cellular contractile properties

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Luden N, Minchev K, Hayes E, Louis E, Trappe T, Trappe S. Human vastus lateralis and soleus muscles display divergent cellular contractile properties. Am J Physiol Regul Integr Comp Physiol 295: R1593–R1598, 2008. First published September 24, 2008; doi:10.1152/ajpregu.90564.2008.—The purpose of this study was to investigate potential differences in single-fiber contractile physiology of fibers with the same myosin heavy chain isoform (MHC I and MHC IIA) originating from different muscles. Vastus lateralis (VL) and soleus biopsies were obtained from 27 recreationally active females (31 ± 1 yr, 59 ± 1 kg). A total of 943 single fibers (MHC I = 562; MHC IIA = 381) were isolated and examined for diameter, peak tension (Po), shortening velocity (Vo), and power. The soleus had larger (P < 0.05) fibers (MHC I +18%; MHC IIA +19%), higher MHC I Vo (+13%), and higher MHC I Po (+18%) compared with fibers from the VL. In contrast, fibers from the VL had higher (P < 0.05) specific tension (MHC I +18%; MHC IIA +20%), and MHC I normalized power (+25%) compared with the soleus. There was a trend for MHC IIA soleus fibers to have higher Vo [MHC IIA +13% (P = 0.058)], whereas VL MHC IIA fibers showed a trend for higher normalized power compared with soleus fibers [MHC IIA +33% (P = 0.079)]. No differences in absolute power were detected between muscles. These data highlight muscle-specific differences in single-fiber contractile function that should serve as a scientific basis for consideration when extending observations of skeletal muscle tissue from one muscle of interest to other muscles of origin. This is important when examining skeletal muscle adaptation to physical states such as aging, unloading, and training.

skeletal muscle; muscle plasticity

APPLICATION OF THE NEEDLE biopsy technique has allowed scientists to better characterize human skeletal muscle over the course of the last four decades (2, 3). Some of the more commonly investigated muscles include the gastrocnemius, deltoid, vastus lateralis, and soleus. From this research the functional diversity of skeletal muscle has been well documented. For instance, the three primary myosin heavy chain (MHC) isoforms identified in human skeletal muscle (30) (i.e., MHC I, MHC IIA, and MHC IIX) display contrasting metabolic (27a) and contractile properties (5). Consequently, whole muscle, which is composed of multiple MHC isoforms, has the ability to perform within a functional continuum influenced by relative fiber-type distribution. Although fiber-type distribution can substantially vary between muscles from an individual (19) (e.g., soleus and vastus lateralis), little is known about the functional differences that may exist within a fiber-type population from different muscles of origin (4).

Physical activity has repeatedly been shown to induce alterations in single muscle fiber contractile physiology (11, 15, 20, 31, 34, 36, 40). Therefore, two leg muscles with distinctly different daily loading demands (9), much like the vastus lateralis and soleus, could have disparate contractile profiles. To our knowledge, only two human single-fiber studies have made a direct functional comparison between fibers containing the same MHC isoform obtained from different muscles (17, 41). While the original inquiry did not detect differences between vastus lateralis and soleus MHC I muscle fibers from a relatively small fiber population (17), subtle differences have been identified between MHC I fibers of the gastrocnemius and soleus (41). Thus, whether functional differences exist among muscle fibers with the same MHC isoform from two leg muscles with different loading regimes remains unanswered.

Our laboratory has studied the effects of multiple interventions on soleus and vastus lateralis single muscle fiber function. These studies have provided us with the unique opportunity to combine data from a large number of women (n = 27; >900 individual human single fibers) in an effort to make a direct comprehensive comparison of the single muscle fiber function between soleus and vastus lateralis muscles. Because of dissimilar daily activity patterns between the soleus and vastus lateralis muscles and their differential responses to unloading (32, 37, 38) and exercise (39), we tested the hypothesis that both size and contractile function differ between vastus lateralis and soleus single fibers, within MHC I and IIA fiber populations.

METHODS

Experimental Design and Subject Profile

This investigation compared single-fiber contractile properties between vastus lateralis and soleus muscles. The design included women who were part of previous research conducted in our laboratory, where both the soleus and vastus lateralis skeletal muscles were concurrently investigated. One subgroup was previously examined for their whole muscle and single-fiber functional response to 60 days of bed rest (32, 37, 38). The single-muscle fiber data included in this investigation were derived from the pre bed rest time point and were generated as part of a much larger study conducted at the Institute for Space Physiology and Medicine in Toulouse, France. The second subgroup included females recruited from Ball State University and the local area.

A total of 27 females underwent the muscle biopsy procedure and were included in the analysis. The subjects were 31 ± 1 yr, 165 ± 1 cm, and 59 ± 1 kg, and they were recreationally active (VO2max ~40 ml·kg−1·min−1). Before testing, the bed rest subgroup completed a consent form adhering to the guidelines of the Human Use Committees of the participating institutions in France and the United States (Johnson Space Center and Ball State University), and the other subgroup completed an informed consent approved by the Ball State University Institutional Review Board.

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Muscle Biopsy

Muscle biopsies were obtained from the vastus lateralis and soleus muscles (2). A portion of each muscle sample was sectioned longitudinally into several pieces and placed in cold skinning solution (see below) and stored at −20°C for later analysis of single muscle fiber physiology. Following a single muscle fiber experiment, each single fiber was analyzed for MHC composition, as described in Single Muscle Fiber MHC Isoform Analysis.

Skinning, Relaxing, and Activating Solutions

The skinning solution contained (in mM): 125 potassium propionate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl₂, and 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 (10). These solutions were adjusted for temperature, pH, and ionic strength using stability constants in the calculations (13). Each solution contained (in mM): 7.0 EGTA, 20.0 imidazole, 14.5 creatine phosphate, 1.0 free Mg²⁺, 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⁺] of pCa 9.0 and pCa 4.5, respectively (where pCa = −log [Ca²⁺]).

Single Muscle Fiber Experimental Setup

On the day of an experiment, a 2- to 3-mm muscle fiber segment was isolated from a muscle bundle and transferred to an experimental chamber filled with cold relaxing solution. Each end of a fiber was securely fastened between a force transducer (model 400A; Cambridge Technology, Watertown, MA) and a DC torque motor (model 308B; Cambridge Technology), as described by Moss (25). The apparatus was mounted on a microscope (Olympus BH-2; Tokyo, Japan), so that the fiber could be viewed (∗800) during an experiment. All single-fiber experiments were performed at 15°C.

For each single muscle fiber experiment, a fiber with a compliance [calculated as fiber length (FL) divided by y-intercept] >10% and/or a decrease in peak force (Po) of >10% was discarded and not used for analysis. The within-fiber test/retest of a single muscle fiber in our laboratory for the measurements of diameter, Po, contractile velocity [maximal unloaded shortening velocity (Vo)], and power were <1%. The coefficient of variation for the force transducer and servomechanical lever mechanism during the 3-yr period in which we examined single muscle fiber function was <1%.

Single Muscle Fiber Analysis

Single-fiber size. Size refers to single-fiber diameter and any subsequent calculations to estimate cross-sectional area or fiber volume. The sarcomere spacing for each muscle fiber was adjusted to 2.5 μm with an eyepiece micrometer. A video camera (CCD-IRIS, DXC-107A; Sony) connected to the microscope and interfaced to a computer allowed viewing on a computer monitor and storage of the digitized images of the muscle fibers during the experiment. Fiber diameter was determined from a captured computer image taken with the fiber briefly suspended in air (<3 s) (37, 38). Fiber width (diameter) was determined at 3 points along the length of the captured computer image using National Institutes of Health public domain software (Scion Image, release Beta 4.0.2, for Windows). Fiber cross-sectional area (CSA) was calculated from the mean width with the assumption that the fiber forms a cylindrical cross section when suspended in air (23).

Force determination (Po and Po/CSA). The output of the force and position transducers were amplified and sent to a microcomputer via a lab-PC + 12-bit data acquisition board (National Instruments, Austin, TX). Resting force was monitored, and then the fiber was maximally activated in pCa 4.5 solution. Po was determined for each fiber by computer subtraction of the force baseline from the peak in the pCa 4.5 solution (43). Po is also expressed relative to fiber size (Po/CSA).

Unloaded Vo. Fiber Vo was measured by the slack test as described by Edman (7). Four different activation and length steps (150, 200, 250, and 300 μm; each ≤15% of fiber length) were used for each fiber, with the slack distance plotted as a function of the duration of unloaded shortening. Fiber Vo (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 2.50 μm.

Single-fiber power (peak power, normalized power, and Vmax). Submaximal isotonic load clamps were performed on each fiber for determination of force-power parameters. Each fiber segment was fully activated and then subjected to a series of isotonic load steps. This procedure was performed at various loads with a total of 15–18 isotonic contractions. Force and shortening velocity data points derived from the isotonic contractions were fit by the hyperbolic Hill equation (18). Only individual experiments in which r² was ≥0.98 were accepted. Fiber peak power was calculated from the fitted force-velocity parameters Po, Vmax, and aPo, where Po is a force constant and Vmax is the y-intercept. Absolute power (μN·FL/s) 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.

Single Muscle Fiber MHC Isoform Analysis

After single muscle fiber physiology experiments were completed, each fiber was solubilized in 80 μl of 1% SDS sample buffer and stored at −20°C until assayed. Briefly, samples were run overnight at 4°C on a Hoefer SE 600 gel electrophoresis unit (San Francisco, CA) using a 3.5% (wt/vol) acrylamide stacking gel with a 5% separating gel. After electrophoresis, the gels were silver stained as described by Giulian et al. (12). MHC isoforms were identified according to migration rate.

Statistical Analysis

Single muscle fiber physiological variables (diameter, force, normalized force, unloaded shortening velocity, Vmax, absolute power, and normalized power) and MHC distribution were analyzed with a paired t-test. For the single-fiber physiology parameters, all of the fibers studied within each individual were aggregated to represent a mean value for both MHC I and MHC IIa fibers. Because of the minimal number of hybrid and pure MHC IIx studied, analyses were restricted to MHC I and IIa fibers. Significance was set at P < 0.05. All data are presented as means ± SE.

RESULTS

Single Muscle Fiber Number and MHC Composition

A total of 943 single fibers from the soleus (n = 392) and vastus lateralis (n = 551) were observed (Table 1). The percentage of MHC I fibers studied in the soleus (74%) was greater (P < 0.05) than in the vastus lateralis (50%). The percentage of MHC IIa fibers studied also differed (P < 0.05) between the soleus (20%) and vastus lateralis (40%). Similarly, the soleus contained a lower percentage of total hybrids (5%) compared with the vastus lateralis (10%) (P < 0.05).

Approximately 20 single fibers/subject were successfully examined from the vastus lateralis, whereas ~15 single fibers/subject were studied from the soleus. While 27 total subjects were biopsied, no MHC I fibers were studied from 1 subject’s vastus lateralis (MHC I VL vs. Sol comparison; n = 26 subjects), and there were no MHC IIa fibers examined from the soleus of 7 individuals (MHC IIa VL vs. Sol comparison; n = 20 subjects).
Table 2. Single muscle fiber size and contractile summary for MHC I and MHC IIA fibers of the vastus lateralis and soleus

<table>
<thead>
<tr>
<th>MHC/Muscle</th>
<th>Diameter, μm</th>
<th>Po, mN</th>
<th>Po/CSA, kN/m²</th>
<th>Vo, FL/s</th>
<th>Vmax, FL/s</th>
<th>Power, μN·FL/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL</td>
<td>80.1±2.1</td>
<td>0.55±0.03</td>
<td>109.0±4.2*</td>
<td>0.99±0.04</td>
<td>0.73±0.03</td>
<td>8.6±0.7</td>
</tr>
<tr>
<td>Soleus</td>
<td>93.1±1.9*</td>
<td>0.62±0.02*</td>
<td>92.6±4.4</td>
<td>1.09±0.04*</td>
<td>0.77±0.03</td>
<td>9.6±0.7</td>
</tr>
<tr>
<td>MHC IIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL</td>
<td>77.6±3.0</td>
<td>0.70±0.05</td>
<td>149.6±6.7*</td>
<td>3.16±0.11</td>
<td>2.73±0.12</td>
<td>46.2±4.1</td>
</tr>
<tr>
<td>Soleus</td>
<td>88.6±2.8*</td>
<td>0.80±0.06</td>
<td>130.4±5.6</td>
<td>3.51±0.20*</td>
<td>2.91±0.21</td>
<td>53.6±6.1</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. *P < 0.05 and †P = 0.058 between the vastus lateralis (VL) and soleus. MHC, myosin heavy chain.
along with potentially undetectable or unidentified MHC isoforms (24). The existence of factors other than MHC ATPase activity contributing to single-fiber contractile function is apparent, and on the basis of the current findings, should be investigated between muscles of origin.

Normalized power is particularly representative of single muscle fiber function because it takes into account contraction speed, force production, and cell volume. One intriguing finding was that the MHC I vastus lateralis fibers produced more normalized power than fibers from the soleus, with a statistical trend ($P = 0.079$), supporting the same theme in MHC IIa fibers. Both vastus lateralis and soleus MHC I and IIa normalized power observed in the current study fell within the range of values previously reported by our laboratory (MHC I range $1.00–1.91$ W/l, MHC IIa range $4.45–12.49$ W/l), with the vastus lateralis single-fiber normalized power fitting in the upper end of the range. In the current study, the elevated normalized power of the vastus lateralis fibers appears to be the result of higher force generation per unit size compared with the soleus fibers. Although these findings are in contrast to the well-characterized relationship between size and strength (11, 22, 31, 33, 42), modifications to this relationship have been demonstrated. For instance, altered specific tension at the cellular level has been documented following acute interventions such as unloading (20, 32, 35, 37) and run training (15, 34).

The most intuitive explanation for differences between soleus and vastus lateralis single-fiber physiology is that the soleus is “better conditioned” than the vastus lateralis as a result of chronic loading (9). In support of this concept, data suggest that 1) exercise training skeletal muscle attenuates the increase in protein synthesis following an acute bout of exercise (26) and 2) the soleus does not increase protein synthesis to the magnitude of the vastus lateralis following exercise (39). Taken together, the soleus could be considered more “trained” than the vastus lateralis. The relatively large soleus fibers ($\sim 19\%$) in the current study, consistent with earlier reports (1, 8, 41), are another indication that soleus fibers have adapted to chronic loading. The significantly higher unloaded shortening velocity of MHC I fibers ($\sim 13\%$) along with the trend ($P < 0.058$) for higher MHC IIa ($\sim 13\%$) unloaded shortening velocity from the soleus also fits the theory of a more conditioned muscle, since shortening velocity has been shown to be elevated in response to aerobic training (29, 34, 36).
43). Further corroboration is provided by a recent cross-sectional investigation demonstrating that gastrocnemius muscle fibers from collegiate runners are faster (shortening velocity) and larger in diameter than gastrocnemius fibers from recreationally active individuals (16).

The hypothesis that the soleus is simply more trained than the vastus lateralis is attractive, but the specific tension data lend subtle support to the presence of intrinsic differences between muscles. Specifically, increased physical activity has been shown to increase specific tension (15, 34), whereas unloading has induced the opposite (20, 32, 35, 37). In the current study, the specific tension values observed in the fibers from both muscles are within the range previously reported by our laboratory (MHC I range = 72–126 kN/m², MHC IIa range = 90–154 kN/m²). However, the “more conditioned” soleus fibers produced less specific tension than the vastus lateralis fibers, which is in direct contrast to the physical activity-specific tension relationship described above. It is, therefore, impossible to completely discount potential factors independent of activity level that may contribute to muscle-specific differences in single-fiber physiology.

The current findings contribute to the concept of human skeletal muscle heterogeneity and are supported by earlier data showing that MHC I soleus fibers display different contractile physiology compared with gastrocnemius fibers (41). However, these results are in disagreement with a report that is most comparable to the current study (17). Specifically, Harridge et al. (17) demonstrated that single-fiber contractile function, within the same MHC isoform, is similar between triceps brachii, soleus, and vastus lateralis muscles. The divergent findings between the current study and Harridge et al. may be attributed to obvious disparities such as gender, training status, and total number of observed single fibers. Of these factors, the most likely explanation for the lack of agreement is the relatively small number of fibers investigated by Harridge et al. (sum of soleus and VL, MHC I, and MHC IIa = 52 fibers). The small number of observed fibers is in contrast to the exceptionally large number of fibers investigated in the current study (each of the 943 fibers is represented in Fig. 3).

Perspectives and Significance

This investigation highlights differences in size and contractile function among single muscle fibers containing the same MHC isoform obtained from the vastus lateralis and soleus. When fiber-type distribution is taken together with single-fiber size and function, distinctly different profiles are evident that likely impact skeletal muscle performance and locomotion. It is unknown whether our findings are the result of intrinsic differences between muscles or that the divergent profiles reflect muscle-specific loading patterns. Though the mechanisms underlying these differences remain unknown, thin to thick filament ratios have been shown to influence shortening velocity (27), while myosin concentration has been hypothesized to impact specific tension (4). Although both represent viable targets for examination, myosin concentration warrants particular consideration, given that mixed muscle data from a subset of the current subjects show that the vastus lateralis contains a higher concentration of myofibrillar protein than the soleus (21). To date, skeletal muscle heterogeneity has been recognized within the context of activity-induced plasticity, as well as inherent differences in muscle fiber type and variability in force-velocity characteristics among a given MHC isoform (4). The current investigation extends the idea of skeletal muscle heterogeneity to include differences in myofilament contractile function between human leg muscles identified in this study (vastus lateralis and soleus). These data further our understanding and appreciation for skeletal muscle diversity and serve as a scientific basis for consideration when characterizing and making generalizations about skeletal muscle tissue in humans.

GRANTS

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