Neuromuscular responses of the superficial quadriceps femoris muscles: muscle specific fatigue and inter-individual variability during severe intensity treadmill running

Haley C. Bergstrom1, Terry J. Housh2, Taylor K. Dinyer1, M. Travis Byrd1, Nathaniel D.M. Jenkins3, Kristen C. Cochrane-Snyman4, Pasquale J. Succi1, Richard J. Schmidt2, Glen O. Johnson2, Jorge M. Zuniga5,6

1Department of Kinesiology and Health Promotion, University of Kentucky, Lexington, USA; 2Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, USA; 3Department of Health and Human Performance, Oklahoma State University, Stillwater, USA; 4Department of Kinesiology, California State University, Fresno, USA; 5Department of Biomechanics, University of Nebraska-Omaha, Omaha, USA; 6Facultad de Ciencias de la Salud, Universidad Autonoma de Chile, Chile

Introduction

Neuromuscular responses including muscle activation and action potential conduction velocity are reflected in the time and frequency domains of the electromyographic (EMG) signal and have also been used to characterize fatigue during dynamic exercises, including cycle ergometry and treadmill running1-3. De Luca1 described changes in the EMG signal as myoelectric manifestations of fatigue that result in a ‘slowing’ of the surface EMG signal, causing an increase in the time, and a decrease in the frequency domains. During fatiguing cycle ergometry exercise, an increase in EMG amplitude (AMP) reflects the fatigue-induced recruitment of additional motor units, increases in firing rate, and/or synchronization4, while a decrease in EMG mean power frequency (MPF) reflects a...
reduction in the muscle fiber action potential conduction velocity and changes in the shape of the waveform\(^6\). It has been suggested that the fatigue induced changes in EMG AMP and EMG MPF are the result of the accumulation of metabolic byproducts (i.e., inorganic phosphate, hydrogen, ammonium, and potassium ions) that cause decreases in membrane excitability\(^6\), excitation-contraction coupling involving \(\mathrm{Ca}^{2+}\) release and re-uptake from the sarcoplasmic reticulum, myofibrillar \(\mathrm{Ca}^{2+}\) sensitivity for binding with troponin, actin-myosin binding, and ATP production and breakdown\(^7\)-\(^10\). These specific fatigue-induced responses have been shown to be intensity\(^2\)-\(^6\), muscle\(^14\)-\(^16\), and mode\(^12\)-\(^14\) specific.

Intensity specific responses have previously been reported that include greater positive slope coefficients for EMG AMP and greater negative slope coefficients for EMG MPF over time for exercise intensities in the severe domain compared with the heavy domain\(^2\)-\(^11\). Thus, it has been suggested that the severe intensity domain is characterized by a greater increase in motor unit activation, as well as greater decreases in action potential conduction velocity than the heavy domain\(^2\)-\(^11\). In addition, dissociation in neuromuscular responses for the superficial quadriceps muscles have been reported for constant power output\(^5\) and incremental cycle ergometry\(^16\) as well as incremental treadmill running\(^4\), however, linear increases in EMG AMP for all three superficial quadriceps muscles have also been reported for incremental treadmill running\(^7\). Typically, the rectus femoris (RF) has been shown to demonstrate an earlier onset of fatigue and lower neuromuscular fatigue threshold, compared with the vastus lateralis (VL) and vastus medialis (VM)\(^14\)-\(^16\). It has been suggested these intensity, muscle, and mode specific responses are related to differences in biomechanical properties, muscle architecture (biarticular versus monarticular), and muscle fiber type composition\(^13\)-\(^19\).

Recently, two distinct zones have been identified within the severe exercise intensity domain based on different metabolic responses at exhaustion\(^20\)-\(^24\). The severe intensity zone 1 (SIZ\(_1\)) is defined as intensities between critical velocity (CV) and 50%\(\Delta\) (\(\Delta =\) difference between CV and \(\dot{\mathrm{V}}\mathrm{O}_2\text{peak}\)) where exhaustion may occur at \(\dot{\mathrm{V}}\mathrm{O}_2\) values less than \(\dot{\mathrm{V}}\mathrm{O}_2\text{peak}\). The severe intensity zone 2 (SIZ\(_2\)) is defined as intensities >50%\(\Delta\) but less than 175% CV where \(\dot{\mathrm{V}}\mathrm{O}_2\) values reach \(\dot{\mathrm{V}}\mathrm{O}_2\text{peak}\) at exhaustion\(^20\)-\(^21\). There is evidence\(^7\)-\(^22\) that neuromuscular responses for the VL, including EMG AMP and EMG MPF, differ between SIZ\(_1\) and SIZ\(_2\) during cycle ergometry. Specifically, an earlier onset of changes in the EMG signal was reported for SIZ\(_2\) versus SIZ\(_1\), during rides to exhaustion\(^22\). Currently, it is unknown if there are distinct neuromuscular responses (EMG AMP and EMG MPF) during treadmill running to exhaustion in SIZ\(_1\) and SIZ\(_2\), that are dependent on the muscle examined. In addition, although composite (i.e., mean or average) responses are typically used to draw conclusions about motor unit activation strategies and neuromuscular fatigue, there are many factors that affect the EMG signal that may result in great variability in individual responses\(^23\). Inter-individual variability in patterns of responses has been previously reported for cycle ergometry\(^12\) and treadmill running\(^24\)-\(^25\), and thus, it has been suggested EMG signals should be examined on a subject-by-subject basis. No previous studies, however, have examined the individual variability in EMG responses across the superficial quadriceps muscles during exhaustive treadmill running in the SIZ\(_1\) and SIZ\(_2\). Therefore, the purposes of this study were to: 1) examine the time course of changes and patterns of responses in EMG AMP and EMG MPF responses for the superficial quadriceps muscles (VL, RF, and VM) during treadmill runs to exhaustion within the lower (SIZ\(_1\)) and upper (SIZ\(_2\)) intensity zones of the severe domain; and 2) determine if the individual subject patterns of responses were consistent with the composite responses. We hypothesized that: 1) the EMG AMP and EMG MPF responses for the superficial quadriceps muscles would be consistent with fatigue in both zones, but the time course of changes analyses would indicate an earlier onset of neuromuscular fatigue in SIZ\(_2\), than SIZ\(_1\); 2) the RF, responsible for thigh flexion and leg extension, would demonstrate greater fatigue than the VL and VM; and 3) there would be variability in the individual patterns of responses (linear, quadratic, and cubic) that may not reflect the composite model, but most subjects would demonstrate characteristics of neuromuscular fatigue during exhaustive exercise in the SIZ\(_1\) and SIZ\(_2\).

**Methods**

**Experimental procedures**

The subjects visited the laboratory on 5 separate occasions, with a minimum of 24-48 hours between each visit. During the first visit, an incremental treadmill test was performed to determine the \(\dot{\mathrm{V}}\mathrm{O}_2\text{peak}\) and velocity at \(\dot{\mathrm{V}}\mathrm{O}_2\text{peak}\) (\(\dot{\mathrm{V}}\mathrm{O}_2\text{peak}\)). The CV was determined from slope of the total distance versus time to exhaustion \((\mathrm{T}_{\text{lim}})\) relationship for 4 constant velocity runs performed during visits 2 – 5. The constant velocity runs performed at the lowest and the highest velocities for the determination of the CV were within the SIZ\(_1\) and SIZ\(_2\), respectively. The EMG AMP and EMG MPF (from the VL, RF, and VM) and \(\mathrm{T}_{\text{lim}}\) were examined during SIZ\(_1\) and SIZ\(_2\) runs.

**Subjects**

Ten moderately trained, recreational runners (4 men and 6 women; mean±SD age= 23.2±3.3 years, height= 174 ± 8 cm, weight= 69±13 kg) completed this study. Moderately trained was defined as running 16 to 48 km·wk\(^{-1}\) most weeks during the previous 6-months. The subjects took part in one, or a combination, of the following physical activities; running (n=11), cycling (n=7), weight lifting (n=7), and recreational sports (e.g., volleyball, badminton, Jiu Jitsu) (n=3). All subjects were instructed to abstain from caffeine consumption for 4 h prior to each session and from exercise the day of testing. These subjects were from a large data set that included multiple independent and dependent variables. There was no overlap, however, for the neuromuscular variables in this study and previously published papers\(^20\)-\(^26\)-\(^27\). The subjects had no known cardiovascular, pulmonary,
metabolic, muscular and/or coronary heart disease. This study was approved by the University Institutional Review Board for Human Subjects and all subjects completed a health history questionnaire and signed a written informed consent document before testing.

**Determination of the peak values**

Each subject performed a graded exercise treadmill test (GXT) to exhaustion (Precor Inc., Bothell, WA USA) to determine the V\textsubscript{O\textsubscript{2peak}} and vV\textsubscript{O\textsubscript{2peak}}. Prior to the test, each subject completed a 3 min warm-up on the treadmill at a velocity of 4.8 km·h\textsuperscript{-1} and 0% grade, followed by a 3 min passive recovery. Following the warm-up, each subject was fitted with a nose clip and breathed through a 2-way valve (Hans Rudolph 2700 breathing valve, Kansas City, MO, USA). Expired gas samples were collected and analyzed using a calibrated TrueMax 2400 metabolic cart (Parvo Medics, Sandy, UT, USA). The gas analyzers were calibrated with room air and gases of known concentration prior to all testing sessions. The O\textsubscript{2}, CO\textsubscript{2}, and ventilatory parameters were recorded breath-by-breath and expressed as 20 s averages\textsuperscript{28}. The GXT began at a treadmill velocity of 6.4 km·h\textsuperscript{-1} and 0% grade. Thereafter, the velocity was increased by 1.6 km·h\textsuperscript{-1} every 2 min to 14.4 km·h\textsuperscript{-1} and 0% grade. Following the 14.4 km·h\textsuperscript{-1} stage, the velocity was no longer increased, however, the treadmill grade was increased by 2% every 2 min until the subject could no longer maintain the running velocity and grasped the handrails to signal exhaustion. The V\textsubscript{O\textsubscript{2peak}} was defined as the highest 20 s average V\textsubscript{O\textsubscript{2}} value recorded during the test. The velocities performed at 0% grade (6.4 to 14.4 km·h\textsuperscript{-1}) were plotted against V\textsubscript{O\textsubscript{2peak}} and the regression equation derived was used to determine the vV\textsubscript{O\textsubscript{2peak}}.

**Determination of critical velocity**

Four, constant velocity, randomly ordered treadmill runs at velocities ranging from 79–103% of the vV\textsubscript{O\textsubscript{2peak}} were performed on separate days. This range of velocities was selected so that each subject could complete 3 to 20 min of exercise before exhaustion\textsuperscript{29}. Prior to the start of the run, each subject practiced getting on and off the treadmill at the velocity associated with that run to become familiarized with that velocity. In addition, each subject completed a self-paced walking or jogging 5 min warm-up, followed by 3 min of passive rest. The treadmill was then set to the selected velocity at 0% grade. Timing for each treadmill run began when the subject released the handrails (usually 2–3 s after getting on the treadmill) and was terminated when the subject grasped the handrails to signal exhaustion. Critical velocity was determined from the 2-parameter, linear total distance (TD) versus T\textsubscript{lim} model\textsuperscript{30}. Critical velocity was defined as the slope of the linear relationship between the TD and T\textsubscript{lim} from the four constant velocity runs. The V\textsubscript{O\textsubscript{2}}, EMG AMP, and EMG MPF responses were recorded during each of the constant velocity runs.

**Determination of severe exercise intensity zones**

Two distinct intensity zones have been previously identified within the severe domain\textsuperscript{20}. The severe intensity domain zone 1 (SIZ\textsubscript{1}) includes intensities between critical velocity (CV) and 50%∆ (Δ = difference between CV and V\textsubscript{O\textsubscript{2peak}}), where exhaustion may occur below V\textsubscript{O\textsubscript{2peak}}. The severe intensity domain zone 2 (SIZ\textsubscript{2}) includes intensities > 50%∆ but < 175% CV, where V\textsubscript{O\textsubscript{2peak}} is reached at exhaustion. For this study, the lowest and the highest velocities used to derive the CV were intensities within the SIZ\textsubscript{1} and SIZ\textsubscript{2} for each subject. Specifically, the lowest running velocity for each subject was performed between CV and approximately 50%∆ and was defined as the SIZ\textsubscript{1} run. The highest running velocity for each subject was performed between approximately 50%∆ and 175% of CV and was defined as the SIZ\textsubscript{2} run.

**Electromyographic measurements**

The EMG signals were measured from the VL, RF, and VM on the dominant leg during the SIZ\textsubscript{1} and SIZ\textsubscript{2} runs. Prior to electrode placement, the skin at each site was shaved, carefully abraded, and cleaned with alcohol. A bipolar surface electrode (circular 24 mm, Kendall disposable EMG electrodes, Covidien LTD; Gosport Hampshire, UK) arrangement (2.0 cm center-to-center) was placed based on the recommendations from the SENIAM Project for EMG electrodes placement\textsuperscript{31}. Specifically, a reference line was drawn over the VL, 66% of the distance between the anterior superior iliac spine (ASIS) and the lateral superior border of the patella. In addition, the electrode-placement site was located 5 cm lateral to the reference line so that the electrodes were over the VL muscle\textsuperscript{32}. A goniometer (Smith & Nephew Rolyan, Inc., Menomonee Falls, WI) was used to orient the EMG electrodes at a 20° angle to the reference line to approximate the pennation angle of the muscle fibers for the VL\textsuperscript{33}. The electrodes the RF were placed on the midline of the segment at 50% of the distance between the ASIS of the pelvis and the superior part of the patella\textsuperscript{31}. For the VM, the electrodes were placed 80% of the distance between the ASIS and the joint space in front of the anterior border of the medial collateral ligament and oriented at a 53° angle to approximate the pennation angle of the muscle fibers\textsuperscript{31}. The reference electrode was placed over the iliac crest. The EMG signal was amplified (gain: ×1,000) using differential amplifiers (Free EMG 300, BTS, Milan, Italy, bandwidth = 10-500 Hz).

**Signal processing**

The raw EMG signals were digitized at 1,000 Hz, stored in a personal computer (MacBook Pro OS X, version 10.6.8, Apple Inc., Cuperino, CA) for subsequent analysis and processed with custom program, written with LabVIEW programming software (version 7.1, National Instruments, Austin, TX). The EMG signals were bandpass-filtered (zero phase shift, fourth-order Butterworth) at 10–500 Hz. Continuous 10 s epochs for the EMG AMP (microvolts root mean square, μVrms) and EMG MPF (MPF in Hz) were calculated. For the MPF

http://www.ismni.org
analyses, each data segment was processed with a Hamming window and a discrete Fourier transform (DFT) algorithm in accordance with the recommendations of Hermens et al.\textsuperscript{31}. The MPF was selected to represent the power spectrum on the basis of the recommendations of Hermens et al.\textsuperscript{31} and was calculated as described by Kwatny et al.\textsuperscript{34}.

**Statistical analyses**

Mean differences among $\dot{V}O_2^{\text{peak}}$ and $\dot{V}O_2$ at exhaustion for the 4 constant velocity runs were examined using a one-way repeated measures ANOVA and Bonferroni corrected pairwise comparisons. Analyses were performed for the composite (defined as the mean of all of the subjects) and individual neuromuscular responses during the SIZ\textsubscript{1} and SIZ\textsubscript{2} runs. The neuromuscular responses were normalized as a % change from the initial 10% values to observe the pattern of responses over time. The first 10% of the EMG signals was omitted from analyses to account for changes in neuromuscular activity as the subject adjusted to treadmill velocity. Time was normalized as a percentage of the Tlim to account for the differences in times to exhaustion among the participants\textsuperscript{2} and 10 data points were used in the analyses (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% of Tlim). Separate polynomial regression analyses (linear, quadratic, or cubic) were used for the composite and individual data to determine the normalized (% change from the initial 10% values) EMG AMP and EMG MPF responses versus %Tlim (10-100%) for the VL, RF, and VM during constant velocity runs in SIZ\textsubscript{1} and SIZ\textsubscript{2}. For the individual and composite, normalized polynomial regression analyses, a positive relationship was defined by a 100% Tlim value above baseline and a negative relationship was defined by a 100% Tlim value below baseline. Twelve separate one-way repeated measures (RM) ANOVAs 1 (neuromuscular measurement (EMG AMP and MPF for the VL, RF, and VM) x 10 (%Tlim for SIZ\textsubscript{1} and SIZ\textsubscript{2} runs (10, 20, 30, 40, 50, 60, 70, 80, 90, 100%)) were used to examine changes in the neuromuscular parameters at every 10% of Tlim. Post-hoc Student Newman-Keuls (SNK) tests were then used to determine the time course of changes among the variables. The SNK test was chosen because it is designed to analyze the time course of changes in repeated measure variables\textsuperscript{35-38}. All statistical analyses were performed with Statistical Package for the Social Sciences software (v.19.0, IBM SPSS Inc., Chicago, Illinois, USA).

**Results**

**Characteristics of SIZ\textsubscript{1} and SIZ\textsubscript{2}**

Table 1 includes the mean (± SD) and range of values for the subject demographics as well as the $\dot{V}O_2$ and velocity variables from the GXT, CV test, SIZ\textsubscript{1} and SIZ\textsubscript{2} runs. The mean (± SD) $\dot{V}O_2$ values at exhaustion for the 4 runs used to determine the CV were, 2.97±0.90 L·min\textsuperscript{-1}, 3.05±0.94 L·min\textsuperscript{-1}, 3.11±0.98 L·min\textsuperscript{-1}, and 3.19±1.02 L·min\textsuperscript{-1}, respectively, from the lowest to highest velocity. The one-way RM ANOVA indicated there were significant differences (F=12.406, p<0.001, $\eta^2_p=0.580$) among the $\dot{V}O_2^{\text{peak}}$ and $\dot{V}O_2$ values at exhaustion for the 4 constant velocity runs. The follow-up pairwise comparisons indicated the $\dot{V}O_2$ values at exhaustion for the lowest two velocities were significantly less (p=0.019 and p=0.018) than $\dot{V}O_2^{\text{peak}}$ (3.28±1.02 L·min\textsuperscript{-1}), but the $\dot{V}O_2$ values at exhaustion for the higher two velocities were not different (p=0.371 and p=0.103) from $\dot{V}O_2^{\text{peak}}$.

**Composite Responses in SIZ\textsubscript{1}**

The SIZ\textsubscript{1} (86±5% $\dot{V}O_2^{\text{peak}}$) run occurred at 34±8 %Δ (109±3 %CV) and the Tlim was 17.73±2.63 min. The one-way RM ANOVAs indicated there were significant differences among time points for the VL EMG AMP (F=3.920, $\eta^2_p=0.303$, p=0.028) and RF EMG MPF (F=2.469, $\eta^2_p=0.215$, p=0.015), but no significant differences among time points for the VL EMG MPF (F=1.767, $\eta^2_p=0.164$, p=0.163), RF EMG MPF (F=1.431, $\eta^2_p=0.137$, p=0.266), VM EMG AMP (F=0.880, $\eta^2_p=0.089$, p=0.530), and VM EMG MPF (F=0.703, $\eta^2_p=0.072$, p=0.514). The composite polynomial regression analyses for the VL indicated there was a positive, linear relationship for EMG AMP ($r^2=0.846$, p<0.001), that significantly increased from the 10% time point at 100% of Tlim, and a negative, linear relationship for EMG MPF ($r^2=0.656$, p=0.004), but no differences between any of the time points and the initial 10% time point (Figure 1). For the RF, there was a positive, linear relationship for EMG AMP ($r^2=0.847$, p<0.001), but no

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>23.2 ± 3.3</td>
<td>19 – 28</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>69.1 ± 13.2</td>
<td>54.9 – 92.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.1 ± 8.0</td>
<td>162.6 – 190.5</td>
</tr>
<tr>
<td>$\dot{V}O_2^{\text{peak}}$ (L·min\textsuperscript{-1})</td>
<td>3.28 ± 1.02</td>
<td>2.14 – 5.38</td>
</tr>
<tr>
<td>$\dot{W}O_2^{\text{peak}}$ (km·hr\textsuperscript{-1})</td>
<td>15.2 ± 1.4</td>
<td>13.5 – 18.0</td>
</tr>
<tr>
<td>CV (km·hr\textsuperscript{-1})</td>
<td>12.1 ± 1.5</td>
<td>9.8 – 14.3</td>
</tr>
<tr>
<td>50%Δ (km·hr\textsuperscript{-1})</td>
<td>13.7 ± 1.4</td>
<td>11.6 – 15.7</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, $\dot{V}O_2$ (km·hr\textsuperscript{-1})</td>
<td>13.2 ± 1.5</td>
<td>10.6 – 15.1</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, $\dot{V}O_2$ end (L·min\textsuperscript{-1})</td>
<td>2.97 ± 0.90*</td>
<td>1.84 – 5.00</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, (%Δ)</td>
<td>34 ± 8</td>
<td>21 – 43</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, (%CV)</td>
<td>109 ± 3</td>
<td>105 - 113</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, Tlim (min)</td>
<td>17.73 ± 2.63</td>
<td>14.06 – 22.23</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, (%CV)</td>
<td>124 ± 5</td>
<td>119 - 132</td>
</tr>
<tr>
<td>SIZ\textsubscript{1}, Tlim (min)</td>
<td>6.57 ± 0.78</td>
<td>5.98 – 7.92</td>
</tr>
</tbody>
</table>

*Significantly lower than the $\dot{V}O_2^{\text{peak}}$ from the GXT (p=0.019), Δ= the difference between the CV and velocity at $\dot{V}O_2^{\text{peak}}$.

http://www.ismni.org

H.C. Bergstrom et al.: Neuromuscular responses in the severe intensity domain
differences between any of the time points and the initial 10% time point, and a negative, linear relationship for EMG MPF ($r^2=0.852$, $p<0.001$), which significantly decreased from the 10% time point at 80% and continued to 100% of $T_{\text{lim}}$ (Figure 1). For the VM, there was a negative, cubic relationship for the EMG AMP ($R^2=0.848$, $p=0.013$) and a negative, quadratic relationship for the EMG MPF ($R^2=0.648$, $p=0.024$), but no differences between any of the time points and the initial 10% time point for either the AMP or MPF (Figure 1).

**Composite responses in SIZ2**

The SIZ2 ($98\pm 4\%\ V\dot{\text{VO}}_2\text{peak}$) occurred at $96\pm 17\%\Delta$ ($124\pm 5\%\text{CV}$) and the $T_{\text{lim}}$ was $6.57\pm 0.78$ min. The one-way RM ANOVAs indicated there were significant differences among time points for the VL EMG AMP (F=2.306, $\eta^2_p=0.204$, $p=0.024$) and VL EMG MPF (F=2.958, $\eta^2_p=0.247$, $p=0.006$), but no significant differences among time points for the RF EMG AMP (F=3.001, $\eta^2_p=0.250$, $p=0.094$), RF EMG MPF (F=1.954, $\eta^2_p=0.178$, $p=0.180$), VM EMG AMP (F=1.935, $\eta^2_p=0.177$, $p=0.077$), and VM EMG MPF (F=0.844, $\eta^2_p=0.086$, $p=0.541$). The polynomial regression analyses indicated there was a positive, quadratic relationship for the VL EMG AMP ($R^2=0.684$, $p=0.019$), but no differences between any of the time points and the initial 10% time point, and a negative, cubic relationship for VL EMG MPF ($R^2=0.844$, $p=0.01$), significant at 100% of $T_{\text{lim}}$ (Figure 1). For the RF, there was a positive, cubic relationship for the EMG AMP ($R^2=0.953$, $p=0.021$) and negative, linear relationship for the EMG MPF ($r^2=0.805$, $p<0.001$), but no differences between any of the time points and the initial 10% time point for either the AMP or MPF. For the VM, there was a negative, quadratic relationship for the EMG AMP ($R^2=0.645$, $p=0.017$), but no differences between any of the time points and the initial 10% time point, and no significant relationship for EMG MPF ($p=0.604$) (Figure 1).

**Individual responses in SIZ1 and SIZ2**

The order of the model fit (1st= linear, 2nd= quadratic, 3rd= cubic) as well as the $r^2$ or $R^2$ values for the VL, RF, and VM neuromuscular responses for each subject during the SIZ1 and SIZ2 runs are presented in Tables 2 and 3. The EMG AMP and EMG MPF responses versus $T_{\text{lim}}$ relationships were plotted for each subject for the SIZ1 and SIZ2 runs and are presented in Figures 2 and 3.
This study examined the neuromuscular responses for the superficial quadriceps muscles during exhaustive treadmill running in two distinct zones within the severe intensity domain, SIZ1 and SIZ2. These zones are defined by specific metabolic responses, where \( VO_2 \) may (SIZ1) or may not (SIZ2) reach \( VO_2 \)peak at exhaustion. In this study, the \( VO_2 \) at exhaustion for the SIZ1 run was less than \( VO_2 \)peak and was performed at 34±8% \( \Delta \), while the \( VO_2 \) at exhaustion for the SIZ2 run, was not different from \( VO_2 \)peak and was performed at 124±5% CV. These findings were consistent with the \( VO_2 \) responses and relative intensities (%\( \Delta \) and CV) previously used to define the SIZ1 and SIZ2 and indicated the neuromuscular parameters examined in this study reflected responses from two distinct zones (SIZ1 and SIZ2) within the severe intensity domain.
Composite responses in SIZ$_1$ and SIZ$_2$

We hypothesized the EMG AMP and EMG MPF responses for each muscle would be consistent with neuromuscular fatigue in both zones, but the time course of changes analyses would indicate an earlier onset of neuromuscular fatigue in the SIZ$_2$ than SIZ$_1$, and the RF, a bi-articular muscle, would demonstrate greater fatigue than the VL and VM. The current findings, however, did not fully support this hypothesis. The patterns of responses in EMG AMP and EMG MPF for the VL and RF were consistent with neuromuscular fatigue in the SIZ$_1$ and SIZ$_2$, but those for the VM were not (Figure 1). In addition, although the RF tended to demonstrate greater fatigue, the time course of changes analyses identified a limited number of significant differences among time points, likely as a result of large inter-individual variability in response to fatiguing treadmill running (Tables 2 and 3; Figures 2 and 3).

The dissociation in neuromuscular responses for the superficial quadriceps muscles is consistent with constant...
power output cycle ergometry\textsuperscript{16} and treadmill running\textsuperscript{24} as well as incremental treadmill running\textsuperscript{14}, however, linear increases in EMG AMP for all three superficial quadriceps muscles have also been reported for incremental treadmill running\textsuperscript{17}. In the present study, the VL and RF demonstrated similar motor unit activation strategies (positive, linear relationships for EMG AMP in SIZ\textsubscript{1} and positive, cubic and quadric relationships in SIZ\textsubscript{2}). The RF, however, demonstrated greater fatigue (significant decreases in EMG MPF from 80–100% $T_{\text{lim}}$) in SIZ\textsubscript{1} compared to the VL or VM, while the RF and VL demonstrated a similar level of fatigue in the SIZ\textsubscript{2} (Figure 1). The patterns of responses for the VL and RF in the SIZ\textsubscript{2} and SIZ\textsubscript{1} likely indicated fatigue induced increases in motor unit activation (recruitment and/or firing rate; EMG AMP) and a slowing of the motor unit action potential conduction velocity (EMG MPG)\textsuperscript{6,16}. For the RF in particular, the negative responses in EMG MPF (motor unit action potential) corresponded to the positive responses in EMG AMP (motor

---

**Figure 3.** Polynomial regression analyses for the individual electromyographic amplitude (EMG AMP) and mean power frequency (MPF) responses of the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) versus time to exhaustion ($T_{\text{lim}}$) for the severe intensity zone 2 (SIZ\textsubscript{2}). The EMG signals were normalized as a percent change from the 10% time point and time was normalized as a percent of the $T_{\text{lim}}$. See Table 3 for a description of the order (linear, quadratic, cubic) of the relationship. A solid line indicates a significant relationship and a dotted line indicates no significant relationship.
unit activation) at 60% of Tlim in the SIZ2. The VM, however, did not demonstrate the same fatigue characteristics as the VL and RF, as evidenced by the cubic relationship for EMG AMP and quadratic relationship for EMG MPF that remained near baseline during the exhaustive run in SIZ1 and the non-significant relationship for EMG MPF in SIZ2. These findings suggested treadmill running at a 0% grade may be more dependent on the VL and RF for force production than the VM. Thus, the current findings indicated there were muscle specific responses to exhaustive treadmill running. The differences in fatigability among muscles may be related to the muscle architecture and fiber type characteristics of the VL, RF, and VM.

The biarticular nature of the RF and its role in both thigh flexion and leg extension during treadmill running as well as its fiber type characteristics likely explain its greater fatigability in SIZ2. In fact, there is evidence from magnetic resonance imaging that ~74% of the total muscle volume of the RF was activated during horizontal treadmill running, while only 53% of the monoarticular vastus muscle group (VL, VM and vastus intermedius) contributed to force production. Furthermore, the RF is characterized by a higher percentage, relative to the vastus muscle group, of fast-twitch glycolytic fibers, which rely heavily on anaerobic energy production and would contribute to greater metabolic byproduct accumulation. Thus, the muscle specific responses and greater fatigability of the RF to exhaustive treadmill running in the SIZ2, are likely explained by differences in muscle architecture (biarticular versus monoarticular) and fiber type characteristics between the RF and vastus muscle group.

Previous studies have demonstrated intensity specific neuromuscular responses to dynamic, constant power output exercise in the severe domain. In the present study, there were differences in the patterns of responses between exhaustive runs performed in the SIZ1 and SIZ2. Specifically, changes in the nature of the relationships for EMG AMP and EMG MPF occurred at ~60% Tlim for RF and VL during the SIZ2 run (Figure 1), versus a positive, linear relationship from the start of the SIZ2 run (Figure 1). The divergent patterns of responses between the intensity zones may be a reflection of the competing influences of metabolic byproducts and decreases in the global motor unit firing rate as a result of difference in total time accumulated at each epoch and the relative intensity at the start of the run. For the SIZ2 run, 60% of Tlim reflected ~4 minutes of exercise, compared with ~10.5 minutes in SIZ1. During the initial 60% of Tlim (~4 min) in the SIZ2, it is possible the decrease in the EMG AMP reflected a decrease in the global motor unit firing rate from the recruitment of higher threshold motor units with lower firing rates. In addition, the shorter duration of exercise in SIZ2 likely allowed for less time for byproduct accumulation to alter motor unit activation or the action potential conduction velocity. These findings indicated intensity specific neuromuscular patterns of responses within and between muscles of the superficial quadriceps. Therefore, the current findings indicated neuromuscular fatigue should be examined and characterized on a muscle and intensity specific basis within the severe intensity domain.

Inter-individual variability

We hypothesized there would be variability in the individual patterns of responses (linear, quadratic, and cubic) that may not reflect the composite model, but most subjects would demonstrate characteristics of neuromuscular fatigue during exhaustive exercise in the SIZ1 and SIZ2. The presence of inter-individual variability in neuromuscular responses has previously been reported for variable power output cycle ergometry, incremental treadmill running, and constant velocity running. For example, during variable power output cycle ergometry, only 19 to 56% and 25 to 31% of the subjects demonstrated the same patterns of responses as the composite for EMG AMP and EMG MPF, respectively. In addition, Zuniga and Malek reported that 78% of the individual responses reflected the composite responses for EMG AMP versus running velocity during incremental exercise, but all of the subjects demonstrated a positive relationship. In addition, it was reported that the individual patterns of responses were consistent across muscles. The current findings, however, demonstrated greater inter-individual variability in response for EMG AMP and EMG MPF than previously reported and individuals did not demonstrate consistent patterns across muscles (Tables 2 and 3; Figures 2 and 3). In this study, 10 to 20% of the subjects for the VL and RF, and none of the subjects for the VM, demonstrated the same patterns of responses as the composite for EMG AMP in the SIZ1 and SIZ2. There was, however, an overall increase in EMG AMP for 20 to 60% of the subjects for the VL, RF, and VM in both zones. For the EMG MPF, 10 to 20% of the subjects for the VL, RF, VM in the SIZ1 and 40 to 60% of the subjects for the RF and VM, but none of the subjects for the VL, in the SIZ2 demonstrated the same patterns of responses as the composite. There was an overall negative relationship for 20 to 60% of the subjects for the VL, RF, and VM in the SIZ2. These findings indicated there were dissociations between the composite and individual responses and support the recommendation of others that in conjunction with composite results, EMG signals should be examined on a subject-by-subject basis. The greater inter-individual variability in this study compared to cycle ergometry and incremental treadmill running may be due to a number of factors including changes in stride length/frequency, muscle activation patterns that differ between individuals as well as the influence of training status of the subject on muscle activation. The large inter-individual variability reported in this study indicated that individual responses should be reported and considered in conjunction with composite findings to examine neuromuscular fatigue and make inferences about motor control strategies.
Summary

In the current study, the composite patterns of responses in EMG AMP and EMG MPF for the VL and RF were consistent with neuromuscular fatigue in the SIZ1 and SIZ2, but those for the VM were not. Thus, treadmill running may be more reliant on the VL and RF than the VM during fatiguing runs at 0% grade. In addition, the composite as well as the individual responses for the RF indicated greater neuromuscular fatigue than the VL or VM. The biarticular nature and fiber type characteristics of the RF may explain the earlier onset of neuromuscular fatigue compared with the VL and VM, especially at the lower severe intensity (SIZ1). In addition, the composite patterns of responses for each muscle differed between the SIZ1 and SIZ2, but the time course of changes analyses identified a limited number of significant differences among time points, likely as a result of large inter-individual variability observed in this study.

There are several limitations in the current study regarding the ability to address the specific nature of the inter-individual variability in neuromuscular fatigue. This study examined a combined sample of men and women and did not include direct comparisons between sexes. In addition, the subjects in this study were all of a similar training status and there were no assessments of biomechanical differences in running technique or changes across fatigue levels. Furthermore, this study did not include any direct assessments of muscle architecture or size. Therefore, future studies should compare the neuromuscular fatigue responses between men and women to determine if there are sex differences in the onset of fatigue during severe intensity runs. In addition, these responses should be examined across a range of fitness levels and include biomechanical (e.g., stride length and frequency) and muscle architecture assessments to determine the effects of these factors on the rate of neuromuscular fatigue and their contribution to the inter-individual variability observed in this study.

Acknowledgements

HCB was a substantial contributor to data acquisition, analysis, and interpretation, and drafting the manuscript. TKD, MTB, and HCB contributed to revising the work and approved the final submission of this manuscript.

References

18. Edgerton VR, Smith JL, Simpson DR. Muscle fiber
type populations of human leg muscles. Histochem J 1975;7:259-266.