A comparison of exercise type and intensity on the noninvasive assessment of skeletal muscle mitochondrial function using near-infrared spectroscopy

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Ryan TE, Brizendine JT, McCully KK. A comparison of exercise type and intensity on the noninvasive assessment of skeletal muscle mitochondrial function using near-infrared spectroscopy. J Appl Physiol 114: 230–237, 2013. First published November 15, 2012; doi:10.1152/japplphysiol.01043.2012.—Near-infrared spectroscopy (NIRS) can be used to measure muscle oxygen consumption (mVO2) using arterial occlusions. The recovery rate of mVO2 after exercise can provide an index of skeletal muscle mitochondrial function. The purpose of this study was to test the influence of exercise modality and intensity on NIRS measurements of mitochondrial function. Three experiments were performed. Thirty subjects (age: 18–27 yr) were tested. NIRS signals were corrected for blood volume changes. The recovery of mVO2 after exercise was fit to a monoeponential curve, and a rate constant was calculated (directly related to mitochondrial function). No differences were found in NIRS rate constants for VOL and ES exercises (2.04 ± 0.57 vs. 2.01 ± 0.59 min⁻¹ for VOL and ES, respectively; P = 0.317). NIRS rate constants were independent of the contraction frequency for both VOL and ES (VOL: P = 0.166 and ES: P = 0.780). ES current intensity resulted in significant changes to the normalized time-tension integral (54 ± 11, 82 ± 7, and 100 ± 0% for low, medium, and high currents, respectively; P < 0.001) but did not influence NIRS rate constants (2.02 ± 0.54, 1.95 ± 0.44, 2.02 ± 0.46 min⁻¹ for low, medium, and high currents, respectively; P = 0.771). In summary, NIRS measurements of skeletal muscle mitochondrial function can be compared between VOL and ES exercises and were independent of the intensity of exercise. NIRS represents an important new technique that is practical for testing in research and clinical settings.

NIRS; mitochondrial capacity; oxidative metabolism

NONINVASIVE TECHNIQUES to assess skeletal muscle function are important for the study of neuromuscular-related pathologies as well as sports performance. In vivo assessments of skeletal muscle oxidative metabolism are commonly made using phosphorus magnetic resonance spectroscopy (3, 21). Near-infrared spectroscopy (NIRS) devices can provide information about the changes in tissue oxygenation based on the oxygen-dependent absorption characteristics of infrared light in the 600–900 nm range. The main absorbing compounds in regard to skeletal muscle physiology are hemoglobin and myoglobin. NIRS has been used to measure skeletal muscle blood flow (4, 35), muscle oxygenation (34, 38), and muscle oxygen consumption (16, 26, 40, 42). Repeated arterial occlusions have been used after exercise to assess the recovery of muscle oxygen consumption (mVO2) as an index of mitochondrial function (33). This approach has been modified using blood volume-corrected algorithms to remove potential artifacts due to small shifts in blood volume during repeated arterial occlusions (39). Postexercise recovery kinetics of phosphocreatine (PCr) (22, 27, 37) and mVO2 (33, 39) have been measured using 31P-MRS and NIRS, respectively. For each of these techniques, short durations of exercise are used to either deplete PCr or increase mVO2, and the subsequent recovery of PCr/mVO2 is dependent on the availability of oxygen (17) and the mitochondrial capacity (37). 31P-MRS measurements have been validated against mitochondrial enzyme activities (25, 27) and State 3 respiration from isolated mitochondria (24). These cross-validations remain to be established with NIRS. Postexercise recovery of PCr follows a monoexponential time course after submaximal exercises and has been found to be independent of the work intensity (31). Similarly, the fundamental recovery kinetics of pulmonary VO2 also appears to be independent of the work intensity (36). However, the effect of work intensity on the recovery kinetics of mVO2 has not been investigated.

Two approaches have been used to exercise muscles for the measurement of mitochondrial function: voluntary (VOL) and electrically stimulated (ES) contractions. VOL contractions result in an ordered muscle fiber recruitment from smaller fibers to larger fibers, known as the Henneman size principle (18). Unlike voluntary muscle contractions, ES muscle contractions result in a nonselective, synchronous recruitment of muscle fibers (14). The recruitment of muscle fibers using transcutaneous ES is also spatially dependent upon the placement of electrodes. Transcutaneous ES can also result in incomplete muscle activation due to limitations in pain thresholds for individuals with intact sensory nervous systems, as the electrical current will also cause action potentials in sensory neurons. Changes in ES current amplitude may result in differing pools of motor units activated, with higher currents recruiting deeper motor units. Therefore, submaximal ES currents could influence NIRS measurements of mVO2. Heterogeneities in fiber compositions within a muscle have been found in both rodent and human muscles, which due to differences in oxidative capacity of slow and fast fibers may also influence metabolic measurements (5, 19). These heterogeneities are less pronounced in human muscles and therefore are less likely to affect metabolic measurements in the average human. Despite these potential differences, ES is often employed in experimental situations in an attempt to produce comparable amounts of muscle activation between groups or between tests.

The purpose of this study was to determine the influence of exercise modality (VOL vs. ES) and exercise intensity on NIRS measurements of skeletal muscle oxygen consumption (mVO2) and skeletal muscle mitochondrial function (recovery rate of mVO2 after exercise). Three experiments were performed in this study. The first experiment compared voluntary exercise and electrical stimulation exercise modalities for
NIRS measurements of mitochondrial function. The second experiment examined the influence of exercise intensity (i.e., frequency of contraction) on the recovery kinetics of muscle oxygen consumption for both VOL and ES exercises. The third experiment examined the influence of changes in electrical stimulation current amplitude on NIRS mitochondrial measurements. We hypothesized that VOL and ES exercise would result in similar NIRS time constants for mitochondrial function. Furthermore, we also hypothesized that the rate of recovery of muscle oxygen consumption would be independent of workload (i.e., contraction frequency and electrical current) performed for both VOL and ES exercises.

MATERIALS AND METHODS

Subjects

A total of 30 subjects (13 male, 17 female) were tested in this study. Healthy college-aged subjects (age: 18–27 yr) were recruited from the local university and community. The study was conducted with the approval of the Institutional Review Board at the University of Georgia (Athens, GA), and all subjects gave written, informed consent before testing.

Experimental Procedures

Each subject was placed on a padded table, supine, with both legs extended (0° of flexion). The right foot was placed into a home-built pneumatic exercise device. To limit motion artifact in the NIRS signal, the foot was strapped firmly to the exercise device using nonelastic Velcro straps proximal to the base of the fifth digit, with the knee supported. The NIRS optode was placed at the level of the largest circumference of the medial gastrocnemius and secured with Velcro straps and biadhesive tape. Two aluminum foil electrodes (2 in. × 4 in.) attached to a Theratouch 4.7 stimulator (Rich-mar, Inola, OK) were placed on the skin, one proximal and one distal to the NIRS optode. A rapid-inflating blood pressure cuff (Hokanson E20 cuff inflator; Bellevue, WA) was placed proximal to the NIRS optode, above the knee joint.

For each experiment, testing occurred on one visit to the laboratory. Adipose tissue thickness (ATT) was measured at the site of the NIRS optode using B-mode ultrasound (LOGIQ e; GE HealthCare, USA). ATT has influences on NIRS signals that can affect measurements of mVO2 (23, 39, 43). ATT was used to determine the interoptode spacing for each individual. The shallow interoptode distance was set approximately twice the ATT. The deeper interoptode distance was always 1 cm greater than the shallow interoptode distance. Subjects were instructed not to consume caffeine or tobacco on the day of the test or to consume alcohol or perform moderate or heavy physical activity for at least 24 h.

NIRS Device

NIRS signals were obtained using a continuous-wave NIRS device (Oxymon MK III, Artinis Medical Systems, Netherlands), which consists of two channels (two equivalent pulsed light sources, two avalanche photodiode detectors, shielding from ambient light), uses intensity-modulated light at a frequency of 1 MHz and laser diodes at three wavelengths (905, 850, and 770 nm) corresponding to the absorption wavelengths of oxyhemoglobin (O2Hb) and deoxyhemoglobin (HHb), with an autosensing power supply. NIRS data were collected at 10 Hz.

Study 1: VOL vs. ES Exercise Modalities

The purpose of study 1 was to examine the influence of ES and VOL exercises on measurements of mVO2 and the recovery rate of mVO2. All testing was performed in a single visit to the laboratory lasting approximately 1 h. ES and VOL exercise was performed at various contraction frequencies. The contraction frequencies for VOL and ES exercises were randomized within the visit.

Baseline measurements. Resting muscle oxygen consumption was assessed from the decrease in muscle oxygenation, which accompanies an arterial occlusion by way of inflating a blood pressure cuff to 250–300 mmHg. Four resting measurements were made using 10 s of arterial occlusion. The resting oxygen consumption was calculated using simple linear regression with the first 8 s of each occlusion (80 data points).

Voluntary exercise protocol. Subjects performed voluntary plantarflexion exercise (VOL) against a pneumatic exercise ergometer for 15 s at 0.33, 0.5, 1.0, and 2.0 Hz in random order. Immediately after completion of each of the exercise protocols, a blood pressure cuff was inflated to ∼250–300 mmHg for 5 s to measure oxygen consumption. mVO2 was calculated using simple linear regression with the first 3 s of each occlusion (30 data points). To measure the recovery of oxygen consumption after voluntary exercise, all subjects had a series of 10–18 brief (5–10 s) arterial occlusions after the contraction frequency of 1.0 Hz. The repeated arterial occlusion protocol was designed to optimize the time resolution (given the compressor capacity) while minimizing any discomfort to the participants. The repeated occlusions were performed as follows: cuffs 1–5 (5 s on/5 s off), cuffs 6–10 (5 s on/10 s off), and cuffs 11–15 (10 s on/20 s off). Care was taken to maintain tissue oxygen saturation above 30% so that low oxygen tensions, which may influence NIRS measurements of mVO2, were avoided. Pilot studies performed found that the duration/interval of the cuff occlusions did not influence the recovery rate (unpublished data).

Electrical stimulation protocol. Twitch electrical stimulation was performed using 15 s of continuous electrical stimulation (pulse duration/interval = 200/50 μs) and was administered at the following frequencies in random order: 2.0, 3.0, 4.0, 5.0, and 6.0 Hz. The current intensity was adjusted for each individual to produce twitch contractions at the maximal tolerable level. Immediately following each bout ES, a blood pressure cuff was inflated for 5 s to measure the rate of oxygen consumption using simple linear regression with the first 3 s of each occlusion (30 data points). A series of 10–18 brief (5–10 s) arterial occlusions were applied to measure the rate of recovery of mVO2 back to resting levels after 15 s of electrical stimulation at 4.0 Hz. The repeated arterial occlusion protocol is described above (see Voluntary Exercise Protocol).

Ischemia/hyperemia calibration. An ischemia/hyperemia calibration was used to normalize the NIRS signals. After 5 s of ES exercise at 4 Hz, a blood pressure cuff was inflated to 250–300 mmHg for 3–6 min (or until the NIRS signals plateaued). This value represents zero oxygenation in the tissue under the NIRS probe. Upon release of the blood pressure cuff, the hyperemic response causes an overshoot in the NIRS signal, with the maximum representing 100% oxygenation. The short-duration ES exercise was used to increase mVO2 (without creating an oxygen debt) to minimize the duration of the ischemic cuff and any discomfort to the participants. A calibration was performed for all tests, both VOL and ES. This calibration was used to scale NIRS signals to this maximal “physiological” range, thus allowing comparisons between individuals with differing ATT and/or heme concentrations.

Study 2: Influence of Contraction Frequency

Six healthy subjects (three male, three female; age = 23.2 ± 2.2 years) were used to examine the effects of the amount of contractile activity on the recovery of mVO2. This group performed both VOL and ES exercise. VOL exercise consisted of plantarflexion exercise against a pneumatic exercise ergometer for 15 s at 0.33, 0.5, 1.0, and 2.0 Hz. ES exercise consisted of 15 s of continuous electrical stimulation (pulse duration/interval = 200/50 μs) at the following frequencies: 2.0, 3.0, 4.0, 5.0, and 6.0 Hz. Each exercise was coupled with a
series of 10–18 brief (5–10 s) arterial occlusions to measure the recovery of mVO\(_2\) after exercise. The repeated arterial occlusion protocol is described above (see Voluntary Exercise Protocol). VOL and ES exercises were performed in a random order and 5 min rest was given between exercises. Pneumatic resistance (for VOL exercise) and current intensity (for ES exercise) remained constant. Exercise intensity was increased using the frequency of contractions or twitches.

**Study 3: Influence of ES Current Amplitude**

Ten healthy subjects (three male, seven female; age = 21.2 ± 1.4 years) were recruited to examine the influence of changes in ES current amplitude on the recovery kinetics of mVO\(_2\). This group of individuals performed an ES experiment that consisted of three mVO\(_2\) recovery measurements. Resting muscle oxygen consumption measurements were made as described above. Following the resting measurements, participants underwent a stimulation current check, which involved a gradual ramped increase in current amplitude until each subject signaled a maximal tolerable level. Three recovery tests were performed, in a random order, and the current was adjusted to provide changes in the time tension integral (isometric equivalent of work) of ~75% and ~50% of the maximal tolerable current. A force transducer (Rice Lake Weighing Systems, Wisconsin, Model: RL20001-T6–250) connected to a Biopac MP100A-CE (Biopac Systems, Santa Barbara, CA) was used to measure the time-tension integral (TTI) of ES exercises. The force transducer was calibrated using a series of weights with known masses and demonstrated a linear output. Force data were sampled at 200 Hz, exported, and analyzed using custom-written routines in Matlab v. 7.13.0.564 (The Mathworks, Natick, MA).

**Calculation of Muscle Oxygen Consumption**

The determination of mVO\(_2\) and the rate constant for mVO\(_2\) has been reported previously (39). mVO\(_2\) was calculated as the slope of change in O\(_2\)Hb and HHb during the first 3 s (30 data points) of the arterial occlusion using simple linear regression. The postexercise repeated measurements of mVO\(_2\) were fit to a monoeponential curve according to the formula below:

\[
y(t) = \text{End} - \Delta \times e^{-k \cdot t}
\]

For this equation, \(y\) represents relative mVO\(_2\) during the arterial occlusion, End is the mVO\(_2\) immediately after the cessation of exercise, Delta is the change in mVO\(_2\) from rest to end exercise, \(t\) is time, and \(k\) is the fitting rate constant. After muscle activation, the recovery of mVO\(_2\) to resting levels occurs exponentially with a rate constant \((k)\) that represents the muscle maximal oxidative capacity, as previously demonstrated using PCR recovery kinetics (25, 27).

**Correction for Blood Volume**

NIRS data were analyzed using custom-written routines for Matlab v. 7.13.0.564 (The Mathworks, Natick, MA). NIRS data were corrected for blood volume changes as previously described (39). A blood volume correction factor \((\beta)\) was calculated for each data point during an arterial occlusion using the equation below:

\[
\beta(t) = \frac{[O_2\text{Hb}(t)]}{([O_2\text{Hb}(t)] + [\text{HHb}(t)])}
\]

where \(\beta\) is the blood volume correction factor, \(t\) represents time, O\(_2\)Hb is the oxygenated hemoglobin/myoglobin signal, and HHb is the deoxygenated hemoglobin/myoglobin signal. Each data point was corrected using its corresponding \(\beta\) according to Eqs. 3 and 4 below.

Once \(\beta\) was defined, its application to the raw NIRS data is shown below:

\[
O_2\text{Hb}_c(t) = O_2\text{Hb}(t) - [\text{HHb}(t) \times (1 - \beta)]
\]

where O\(_2\)Hb and HHb are the corrected oxygenated and deoxygenated hemoglobin/myoglobin signals, respectively; \(\beta\) is the blood volume signal from the NIRS device; and \(t\) is the blood volume correction factor; and \(t\) is time. In Eq. 3, the raw O\(_2\)Hb signal is corrected by subtracting the proportion of the blood volume \([\text{HHb} \times (1 - \beta)]\) change attributed to O\(_2\)Hb. Similarly, in Eq. 4, the raw HHb signal is corrected by subtracting the proportion of blood volume \([\text{HHb} \times \beta]\) change attributed to HHb.

**Statistical Analysis**

Data are presented as means ± SD. Statistical analyses were performed using SPSS 19.0 (IBM, Armonk, NY). Statistical analysis of VOL vs. ES exercise, contraction frequencies for both ES and VOL, and changes in ES current were performed using repeated-measures ANOVA. When a significant difference was detected, a subsequent comparison of means was performed using the Bonferroni test. The relationship between two variables was analyzed by least squares regression analysis. Significance was accepted when \(P < 0.05\).

**RESULTS**

All subjects completed testing with no adverse events. The physical characteristics of all participants are shown in Table 1. Resting mVO\(_2\) for all participants was 0.33 ± 0.18% s\(^{-1}\). The NIRS mVO\(_2\) kinetic parameters from all three studies are shown in Table 2.

**Voluntary vs. Electrical Stimulation Exercise**

A representative NIRS test for the recovery of mVO\(_2\) following VOL exercise is shown in Fig. 1A, with the monoeponential curve fit shown in Fig. 1B. Twenty subjects (10 male, 10 female) performed both VOL and ES exercise to measure the recovery of mVO\(_2\). NIRS rate constants for the recovery of mVO\(_2\) for VOL contractions (frequency = 1.0 Hz) and ES contractions (frequency = 4.0 Hz) were not statistically different [2.04 ± 0.57 vs. 2.01 ± 0.59 min\(^{-1}\)] for VOL and ES, respectively; \(F(1,19) = 1.058, P = 0.317, \eta^2 = 0.053\]. There was also no difference in the recovery of mVO\(_2\) for the wider NIRS interoptode distance [2.03 ± 0.56 vs. 2.11 ± 0.52 min\(^{-1}\)] for VOL and ES, respectively; \(F(1,19) = 1.170, P = 0.293, \eta^2 = 0.058\]. We also found good agreement between channels (interoptode spacing) for the rate constants of VOL \(F(1,19) = 103.955, P < 0.0001, r = 0.92\] and ES \([F(1,19) = 99.063, P < 0.0001, r = 0.92\] exercises. Furthermore, linear regression analyses show good agreements between VOL and ES exercise for both channel 1 \([F(1,19) = 29.762, P < 0.0001, r = 0.78\] and channel 2 \([F(1,19) = 15.684, P = 0.001, r = 0.682\]. Figure 2 shows the relationship between NIRS rate constants for VOL and ES from channel 1.

**Mean rates of mVO\(_2\)** for the different contraction frequencies of both VOL and ES exercises are shown in Fig. 3. mVO\(_2\) increased linearly in proportion to the contraction frequency.
for both VOL and ES exercise. Peak mVO₂ was significantly greater with VOL contractions [7.53 ± 2.23 vs. 4.33 ± 1.57% s⁻¹] for VOL and ES exercise, respectively; F(1,19) = 36.782, P < 0.0001, η² = 0.671].

**Table 2. mVO₂ kinetic parameter estimates for all tests**

<table>
<thead>
<tr>
<th></th>
<th>Baseline, %/s</th>
<th>Amplitude, %/s</th>
<th>Tc, s</th>
<th>Tc95CI, s</th>
<th>k, min⁻¹</th>
<th>k95CI, min⁻¹</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>VOL</td>
<td>0.37 (0.17)</td>
<td>4.47 (2.26)</td>
<td>30.8 (7.1)</td>
<td>3.1</td>
<td>2.05 (0.55)</td>
<td>0.24</td>
<td>0.97 (0.02)</td>
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<tr>
<td>ES</td>
<td>0.38 (0.16)</td>
<td>3.16 (1.23)</td>
<td>32.5 (9.1)</td>
<td>3.9</td>
<td>1.98 (0.53)</td>
<td>0.23</td>
<td>0.96 (0.04)</td>
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<tr>
<td><strong>Study 2</strong></td>
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<td>VOL</td>
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<tr>
<td>0.33 Hz</td>
<td>0.35 (0.26)</td>
<td>2.42 (1.14)</td>
<td>30.8 (10.2)</td>
<td>8.1</td>
<td>2.07 (0.46)</td>
<td>0.37</td>
<td>0.94 (0.06)</td>
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<tr>
<td>0.50 Hz</td>
<td>0.40 (0.30)</td>
<td>3.58 (1.20)</td>
<td>29.8 (6.7)</td>
<td>5.3</td>
<td>2.08 (0.38)</td>
<td>0.30</td>
<td>0.96 (0.01)</td>
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<tr>
<td>1.00 Hz</td>
<td>0.35 (0.25)</td>
<td>6.00 (1.36)</td>
<td>31.1 (6.5)</td>
<td>5.2</td>
<td>1.99 (0.34)</td>
<td>0.27</td>
<td>0.98 (0.01)</td>
</tr>
<tr>
<td>2.00 Hz</td>
<td>0.34 (0.18)</td>
<td>7.36 (1.08)</td>
<td>35.5 (7.2)</td>
<td>5.7</td>
<td>1.79 (0.30)</td>
<td>0.24</td>
<td>0.96 (0.04)</td>
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<tr>
<td>ES</td>
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<tr>
<td>2.0 Hz</td>
<td>0.22 (0.15)</td>
<td>1.81 (0.22)</td>
<td>33.7 (9.4)</td>
<td>7.5</td>
<td>1.88 (0.46)</td>
<td>0.37</td>
<td>0.95 (0.03)</td>
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<td>3.0 Hz</td>
<td>0.24 (0.13)</td>
<td>3.09 (0.60)</td>
<td>35.1 (12.8)</td>
<td>10.2</td>
<td>1.87 (0.56)</td>
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<td>4.0 Hz</td>
<td>0.34 (0.23)</td>
<td>3.81 (0.56)</td>
<td>33.5 (12.3)</td>
<td>9.8</td>
<td>1.98 (0.66)</td>
<td>0.53</td>
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<td>5.0 Hz</td>
<td>0.31 (0.16)</td>
<td>4.52 (0.30)</td>
<td>32.3 (11.3)</td>
<td>9.0</td>
<td>2.02 (0.57)</td>
<td>0.46</td>
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<tr>
<td>6.0 Hz</td>
<td>0.33 (0.19)</td>
<td>5.03 (0.31)</td>
<td>34.4 (12.8)</td>
<td>10.2</td>
<td>1.92 (0.58)</td>
<td>0.46</td>
<td>0.98 (0.02)</td>
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<tr>
<td><strong>Study 3</strong></td>
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<tr>
<td>Lowest current</td>
<td>0.23 (0.07)</td>
<td>3.22 (1.00)</td>
<td>32.3 (7.7)</td>
<td>4.7</td>
<td>1.95 (0.44)</td>
<td>0.27</td>
<td>0.98 (0.01)</td>
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<td>Medium current</td>
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<td>30.9 (7.1)</td>
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<td>2.04 (0.51)</td>
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<td>0.98 (0.01)</td>
</tr>
<tr>
<td>Highest current</td>
<td>0.18 (0.10)</td>
<td>3.35 (1.15)</td>
<td>32.5 (9.3)</td>
<td>5.7</td>
<td>1.97 (0.51)</td>
<td>0.32</td>
<td>0.98 (0.01)</td>
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</table>

Values are presented as means (SD). Abbreviations: VOL, voluntary; ES, electrical stimulation; Baseline, baseline (resting) mVO₂; Amplitude, delta mVO₂ from resting to end-exercise; Tc, fitting time constant; k, fitting rate constant; 95CI, 95% confidence interval; R², goodness of fit.

**Contraction Frequency**

Representative exponential fits (data points excluded for visual purposes) for various contraction frequencies of both VOL and ES exercises from a single participant are shown in Fig. 4, A and B. NIRS rate constants for the recovery of mVO₂ were independent of the contraction frequency for both VOL [F(1,5) = 2.621, P = 0.166, η² = 0.344] and ES exercise [F(1,5) = 1.148, P = 0.780, η² = 0.017].

**Electrical Stimulation Current Amplitude**

Time-tension integrals (isometric equivalent of work performed) were measured at three different intensities of ES. TTI were normalized to the maximal tolerable current and expressed as a percentage. Average currents used in this study were 45 ± 11, 61 ± 12, and 82 ± 14 mA for the low, medium, and high current intensities. Average TTI for each intensity were 5.7 ± 2.0, 6.4 ± 2.1, and 7.1 ± 2.2 s, respectively.

Values are presented as means (SD). Abbreviations: VOL, voluntary; ES, electrical stimulation; Baseline, baseline (resting) mVO₂; Amplitude, delta mVO₂ from resting to end-exercise; Tc, fitting time constant; k, fitting rate constant; 95CI, 95% confidence interval; R², goodness of fit.
using various intensities of both VOL and ES exercises. We found no difference in NIRS recovery rates of mVO₂ between VOL and ES exercises. In contrast, a recent study from our lab (29) compared skeletal muscle oxidative metabolism, using phosphorus magnetic resonance spectroscopy (31P-MRS), between VOL and ES protocols in able-bodied controls. Both exercise modalities resulted in similar end-exercise Pi/PCr values and intracellular pH, but the time constant for phosphocreatine (PCr) resynthesis was faster with VOL exercise. Similarly, Vanderthommen et al. (44) also reported slower PCr time constants (~40% slower) after ES compared with VOL. These studies suggest that mitochondrial capacity was lower when measured with ES compared with VOL exercise. In contrast, we found good agreement between NIRS measured recovery rates of mVO₂, suggesting that NIRS measurements of mitochondrial function can be made with both ES and VOL exercises, although ideally comparisons between groups should be made with only one type of exercise.

The discrepancies between the findings of the present study and previous studies (29, 45) are most likely attributable to methodological differences in the exercise modalities. ES exercise results in a synchronous recruitment of all fiber types within a given region in proximity to the electrode placement. Subjects in the present study performed rapid dynamic contractions of plantar flexor muscles for VOL exercise. This type of rapid exercise should result in greater activation of fast-glycolytic motor units (6, 13, 15), which more closely resembles motor unit recruit of ES. In contrast, VOL exercises like those performed in the study by Vandethommen et al. (45) consisted of long-duration (~5 min) bouts of quadriceps exercise at forces less than 60% maximum isometric voluntary contraction. This type of VOL exercise likely recruited a

DISCUSSION

The primary finding of these studies was that NIRS measurements of mitochondrial function were comparable for voluntary and electrical stimulation exercises and that the recovery of mVO₂ was independent of the exercise intensity. Using blood-volume corrected NIRS signals as previously described (39), skeletal muscle mitochondrial function was assessed

![Diagram](http://example.com/diagram.png)

**Fig. 3.** Rate of mVO₂ at varying frequencies of VOL (A) and ES (B) contractions. VOL exercise resulted in significantly greater peak mVO₂ compared with ES (see RESULTS). Data presented at mean ± SD (error bars). Closed circles represent resting mVO₂. Relationships were calculated using simple linear regression. (n = 20).

and high currents, respectively. Normalized TTI for lowest current, medium current, and maximal tolerable current trials were 54 ± 11, 82 ± 7, and 100 ± 0%, respectively (Fig. 5A). Normalized TTI were significantly different for each trial \( F(2,9) = 124.775, P < 0.001, \eta^2 = 0.933 \). The initial (end-exercise) mVO₂ were \( 2.56 \pm 1.44, 3.64 \pm 0.85, \) and \( 3.79 \pm 0.60\% \ s^{-1} \) for lowest current, medium current, and maximal tolerable current trials (Fig. 5B). Initial mVO₂ from the lowest current trial was statistically different than the medium current \( F(1,9) = 12.061, P = 0.007, \eta^2 = 0.573 \) and from the maximal tolerable current \( F(1,9) = 9.53, P = 0.013, \eta^2 = 0.514 \). There was no difference in the initial mVO₂ between the medium and maximal tolerable current trials \( F(1,9) = 0.59, P = 0.463, \eta^2 = 0.061 \). Despite changes in the TTI and initial mVO₂, NIRS rate constants were not different between trials \( 2.02 \pm 0.54, 1.95 \pm 0.44, 2.02 \pm 46 \text{ min}^{-1} \) for lowest current, medium current, and maximal tolerable current trials, respectively; \( F(2,9) = 0.264, P = 0.771, \eta^2 = 0.028 \) (Fig. 5C).

**Fig. 4.** Representative recovery curves for mVO₂ at varying contraction frequencies for both VOL (A) and ES (B) exercises from a single individual. Notice the initial rate mVO₂ increases in proportion to contraction frequency, but all fits appear monoexponential. Data points were omitted for visual purposes (curve fitting was \( R^2 > 0.96 \) for all tests).
smaller percentage of fast-glycolytic fibers, which have lower oxidative capacities, compared with the ES exercises. The potential differences in motor unit recruitment between the VOL and ES exercises in the study may be reflective of the differences in PCr recovery rates. In the study by McCully et al. (29), the motor unit recruitment patterns for maximal isometric voluntary contractions of the quadriceps muscles are unclear. Although previous studies suggest orderly recruitment of motor units during isometric contractions (32), fast-glycolytic fibers would not be recruited until higher force thresholds. Because force and EMG measurements were not made in the study by McCully et al., the extent of fast-glycolytic fibers activated remains unclear, which makes comparisons between VOL and ES exercises difficult.

The VOL exercise in this study resulted in greater mVO2 rates (peak mVO2) compared with twitch ES exercise. It is difficult to equate the energy cost of VOL and ES contraction as the VOL contractions likely produced higher levels of force and greater depolarization frequencies during VOL exercise (>15 Hz) compared with the twitch ES (2–6 Hz). The greater peak mVO2 with VOL exercise is consistent with high ATP cost, and thus oxygen cost, of tetanic contractions compared with twitch contractions (11, 41). The mVO2 increased linearly in proportion to the contraction intensity for VOL and ES. This is consistent with early studies by Wallace Fenn demonstrating linear relationships between the work performed and heat liberated by muscle contractions (7, 8). Similarly, Hood and colleagues (20) found a linear relationship between muscle VO2 and twitch contraction frequency (up to 60 Hz) and tetanic contraction frequencies (up to 90 tetanic contractions per minute). The authors of this study suggested that the slope of the relationship between mVO2 and twitch/tetanus frequency represents the oxygen cost per twitch/tetanus. The difference in the estimated oxygen cost for VOL was ~4.4-fold greater compared with twitch ES exercises in this study. This finding is in line with the sevenfold difference in oxygen cost reported by Hood et al., given the greater heterogeneity of rodent skeletal muscle fiber compositions (i.e., greater percentage of fast-glycolytic muscle fibers). These findings support the validity of NIRS measurements of mVO2 using arterial occlusions.

The recovery of mVO2 following low- to moderate-intensity exercises appears exponential in nature, which is consistent with a first-order metabolic system, where the recovery of mVO2 is entirely dependent on ATP production by oxidative phosphorylation (17, 30). We found that the NIRS rate constants for the recovery of mVO2 were independent of the contraction frequency for both VOL and ES. These results are consistent with previous studies. Ozyener et al. (36) found similar recovery time constants for pulmonary VO2 following moderate- and high-intensity cycling exercise. Meyer showed that the recovery rate of PCr was also independent of the exercise intensity (30). Higher-intensity exercises have been reported to produce PCr recovery kinetics of a higher order (12). Even in these conditions, the contributions of glycolysis to PCr resynthesis were small (~7%). Higher-order recovery kinetics and the pH sensitivity have not been tested using NIRS. Previous studies have used NIRS to measure the recovery of muscle oxygenation (1, 2, 9, 10, 28). In these studies, the recovery of NIRS signals (Hb, O2Hb, Hb difference, etc.) represents the balance between oxygen delivery and oxygen consumption by the mitochondria. In this study, the application of short arterial occlusions allows for isolated measurements of mVO2. Careful consideration of these methodological differences should be taken when making direct comparisons between the mVO2 kinetics measured in the current study and studies that measure the offset kinetics of oxygenation.

We found no difference in the NIRS rate constants, despite ~20% and ~45% decreases in the TTI with lowering ES intensities. The initial mVO2 was influenced by changes in ES current. The nonlinearity of the relationship between mVO2 and TTI is a result of the relatively shallow sampling depth of the NIRS device. Both medium and high current amplitudes used in study 3 most likely resulted in complete activation of the NIRS sampled tissue, whereas the lowest current only results in partial activation of this muscle tissue. Thus the increase in TTI from the medium to high current trials is a result of activation of muscle deeper than the NIRS device is able to measure, not an increase in the force production from the NIRS sampled tissue. The recovery kinetics of mVO2 after exercise were independent of amount of activation and represent only the recovery of activated muscle. Because higher-intensity ES may cause discomfort in some individuals, supramaximal ES intensities may not be feasible, especially in frail or diseased populations. These results suggest that submaximal ES intensities tolerated in subjects with normal sensory feeling are capable of providing adequate increases in mVO2 to measure the recovery kinetics and that small changes in the ES intensity do not influence the recovery of mVO2.
Clinical Significance

NIRS devices can accurately and reliably measure mitochondrial function after voluntary exercise or electrical stimulation using repeated arterial occlusions. NIRS devices are cost-effective and expose participants to little, if any, danger during testing. These measurements can be made with different intensities of both electrical stimulation and voluntary exercise. Recovery measurements of mVO₂ can be made as long as there is a modest increase in metabolic rate (8–10 fold above resting). We have found that 10–20 s of low- to moderate-intensity exercise provides a reasonable increase in mVO₂, without causing discomfort to participants. Furthermore, this study suggests that voluntary contractions produce a larger increase in mVO₂ compared with twitch electrical stimulation. Using a rapid-inflating blood pressure cuff inflator is vital to the success of this technique. Repeated arterial occlusions require a cuff inflator with a large enough reservoir to ensure adequate pressures are reached quickly. The duration and interval of arterial occlusions can be adjusted to optimize the measurement while minimizing any discomfort for the participants. Higher frequencies of cuffing may be necessary for more fit individuals with faster recovery of mVO₂. In contrast, individuals with reduced mitochondrial function will have a slower recovery of mVO₂, thus reducing the need for higher-frequency occlusions. Motion artifact can be detected with NIRS. Participants should be placed into exercise ergometers or devices to limit motion artifact, to ensure accurate NIRS measurements.

Limitations and Assumptions

NIRS devices provide information about relative changes in the tissue oxygenation. The pathlength of NIR light is unknown in this study, as well as most studies using NIRS. Time-resolved and frequency-domain (phase modulation) NIRS devices provide estimates of the pathlength of NIR light, as well as the scattering and absorption characteristics. Despite this additional information, these devices are still influenced by adipose tissue thickness (23). These limitations have made the quantification of NIRS signals difficult. In this study, we normalized NIRS signals to a “maximal physiological” range by use of an ischemia/hyperemia calibration. This calibration method is based on assumptions that the ischemia results in complete deoxygenation (dissappearance of O₂Hb) and that the hyperemia results in complete reoxygenation (disappearance of HHb). Absolute values (milliMolar oxygen concentration) cannot be obtained directly using this method. Continuous-wave NIRS devices, like the one used in this study, only measure changes in light attenuation, and it is assumed that these changes reflect changes in the hemoglobin/myoglobin concentration. Structural of geometric changes in the shape of the muscle could result in misinterpretations of changes in muscle oxygenation. We placed participants in ergometers to minimize motion artifacts that can result in changes in the path of NIR light. After correcting for changes in blood volume, the change in oxygenation during arterial occlusions represents only muscle VO₂ (or mitochondrial respiration).

Conclusions

In summary, NIRS measurements of mitochondrial function can be made with both VOL and ES exercises. The intensity of both ES and VOL exercises does not influence the measurement of the recovery of mVO₂. The recovery of mVO₂ after low- to moderate-intensity exercise appears to follow a mono-exponential function, and measurements can be made with reasonably small increases in mVO₂. NIRS represents an important new methodology for assessing muscle energetics, and future studies should aim to apply NIRS measurements to various populations of interest.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


