Skeletal Muscle Metabolism in Endurance Athletes with Near-Infrared Spectroscopy

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ABSTRACT

BRIZENDINE, J. T., T. E. RYAN, R. D. LARSON, and K. K. MCCULLY. Skeletal Muscle Metabolism in Endurance Athletes with Near-Infrared Spectroscopy. Med. Sci. Sports Exerc., Vol. 45, No. 5, pp. 869–875, 2013. Purpose: To determine whether near-infrared spectroscopy (NIRS) measurements of muscle mitochondrial function could detect the expected differences between endurance-trained athletes \((n = 8)\) and inactive subjects \((n = 8)\). Methods: Muscle oxygen consumption \((\text{mV} \text{O}_2)\) of the vastus lateralis was measured with continuous-wave NIRS using transient arterial occlusions. The recovery rate of \(\text{mV} \text{O}_2\) after electrical stimulation was fit to an exponential curve, with the time constant \((T_j)\) used as an index of mitochondrial capacity. Whole-body peak oxygen uptake was determined by indirect calorimetry during a continuous ramp protocol on a cycle ergometer. Results: Whole-body peak oxygen uptake values for endurance-trained and inactive controls were 73.5 ± 9.1 and 33.7 ± 5.9 mL kg\(^{-1}\) min\(^{-1}\), respectively \((P < 0.001)\). The recovery rates of \(\text{mV} \text{O}_2\) after exercise for endurance training were 18.4 ± 3.2 and 18.8 ± 2.5 s, whereas those for inactive controls were 32.4 ± 5.2 and 34.9 ± 5.9 s for the shallow and deep channels, respectively \((P < 0.001\) for comparison between groups). Resting \(\text{mV} \text{O}_2\) was 0.52%/s\(^{-1}\) ± 0.22%/s\(^{-1}\) for endurance athletes and 0.77%/s\(^{-1}\) ± 0.82%/s\(^{-1}\) for inactive controls \((P = 0.42)\). Conclusions: The recovery rates of \(\text{mV} \text{O}_2\) after exercise in endurance athletes were almost twofold faster than inactive subjects measured with NIRS, consistent with previous studies using muscle biopsies and magnetic resonance spectroscopy. Our results support the use of NIRS measurements of the recovery of oxygen consumption to assess muscle oxidative capacity. Key Words: NIRS, MITOCHONDRIAL CAPACITY, ELECTRICAL STIMULATION, OXIDATIVE METABOLISM, ENDURANCE TRAINING, MAXIMAL OXYGEN UPTAKE, COMPETITIVE CYCLISTS

Oxidative metabolism is the primary method of energy production in skeletal muscle, and changes in oxidative muscle metabolism have been shown to be useful in understanding muscle function in both healthy and diseased conditions \((15,19,27)\). In the past two decades, noninvasive methods have been developed to evaluate skeletal muscle oxidative metabolism \((27)\). Magnetic resonance spectroscopy \((\text{MRS})\) has the ability to measure active forms of high-energy phosphorus metabolites and intramuscular pH \(\text{in vivo} (6)\). With MRS, mitochondrial capacity can be assessed by the kinetics of phosphocreatine \((\text{PCr})\) resynthesis after exercise \((23,25)\). However, MRS is costly and has limited availability. Near-infrared spectroscopy \((\text{NIRS})\) is an optical method that uses wavelengths in the range of 600–900 nm for the measurement of skeletal muscle tissue oxygenation and hemodynamics \((8,9,16)\). Muscle oxygen consumption \((\text{mV} \text{O}_2)\) can be measured with NIRS with the application of venous and arterial occlusions \((1,14,26,31)\).

Motobe et al. \((26)\) originally described a transient arterial occlusion technique with NIRS in the forearm to assess mitochondrial capacity. Ryan et al. \((28)\) developed a modified approach to the repeated transient arterial occlusion method that corrects for small changes in apparent blood volume during the ischemic periods. The advantage of the NIRS approach to measuring muscle mitochondrial function is the lower cost and wider spread availability.

The purpose of this study was to evaluate the ability of NIRS measurements to detect differences in mitochondrial function. This was performed by testing two groups of participants: one group with high muscle oxidative capacity based on self-reported history of strenuous endurance training and another group with lower muscle oxidative capacity based on a lack of strenuous endurance training \((17,18,29)\). We hypothesized that NIRS measured recovery of muscle oxygen consumption after exercise would be faster in endurance-trained cyclists \((i.e., \text{lower time constant for } \text{mV} \text{O}_2 \text{ recovery from exercise})\) compared with untrained individuals and that the differences between the two groups would be consistent with previously published results using other methods of assessing muscle mitochondrial function.

MATERIALS AND METHODS

Subjects. Sixteen subjects \((12 \text{ men and } 4 \text{ women})\) were tested in this study. Subjects were selected to have either a...
high (endurance trained) or a low (inactive) muscle oxidative capacity. The endurance-trained group consisted of college-aged cyclists, all of whom were in the middle of their competitive season. Inactive individuals did not perform regular exercise more than once per week. Participant classification was confirmed based on VO\textsubscript{2peak} measurement. Endurance-trained athletes were individuals classified with VO\textsubscript{2peak} measurement higher than 60 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} for males and 55 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} for females. Inactive subjects were classified as those less than 45 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} for males and 40 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} for females. 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.** Testing occurred on one visit to the laboratory. Subjects were instructed not to consume caffeine or tobacco on the day of the test or to use alcohol or perform moderate or heavy physical activity for at least 24 h before testing. NIRS testing was performed before measuring whole-body oxygen uptake (VO\textsubscript{2peak}).

**Whole-body peak oxygen uptake.** Whole-body peak oxygen uptake (VO\textsubscript{2peak}) was used to characterize the metabolic differences between the two groups. VO\textsubscript{2peak} was determined by indirect calorimetry during a continuous ramp protocol to exhaustion. Ramp protocols have been shown to produce similar results compared with traditional incremental protocols (34). Pedal rate was “self-selected” in this study; however, an inability to pedal higher than 50 RPM for any duration exceeding 30 s was used as a sign of exhaustion only. Participants pedaled on an electrically braked cycle ergometer (Lode BE, Groningen, Netherlands) beginning at a low workload (50–200 W, depending on fitness level) for 3 min. After the first 3 min, workload was increased by 1 W every 2 s until exhaustion. Oxygen consumption and carbon dioxide production were measured continuously by open-circuit spirometry and analyzed using a ParvoMedics metabolic measurement system (model TrueMax 2400; ParvoMedics, Sandy, UT) that was calibrated before each experimental run. HR was monitored by a Polar HR monitor (Polar Beat, Port Washington, NY). RPE were assessed during the last 10 s of every minute during the ramp protocol using Gunnar Borg’s 6–20 RPE scale (5). For the test to be considered an acceptable measurement of physiological VO\textsubscript{2peak}, two of the following criteria had to be met: 1) RER >1.1, 2) maximal HR within 10 beats of age-predicted maximum, and (3) RPE ≥18.

**NIRS testing.** NIRS testing was performed as previously described (28). Each subject was placed on a padded table with both legs extended (0° of flexion). The right or left foot was placed into a home-built device to limit motion artifact during data collection (Fig. 1A). The foot was then strapped firmly to the device using nonelastic Velcro straps proximal to the base of the fifth digit, with the knee supported. The NIRS optode was placed on the vastus lateralis, approximately two-thirds of the way down from its origin (greater trochanter) to its insertion (patella), and secured with Velcro straps and biadhesive tape. NIRS signals were obtained using a continuous-wave NIRS device (Oxymon MK III; Artinis Medical Systems, Zetten, The Netherlands), which consists of two channels (two equivalent pulsed light sources and 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 (O\textsubscript{2}H\textsubscript{b}) and deoxyhemoglobin, with an autosensing power supply (approximately 40 W at 110–240 V). The probe was set for two source-detector separation distances after the measurement of adipose tissue thickness (ATT). ATT was measured at the site of the NIRS optode using B-mode ultrasound (LOGIQe; GE Healthcare, Waukesha, WI). The deep source-detector pair separation distance was always 1 cm greater than the shallow. NIRS data were collected at 10 Hz.

Four aluminum foil electrodes (2 × 2 in.) attached to a Theratouch 4.7 stimulator (Rich-mar, Inola, OK) were placed on the skin, two proximal and two distal to the NIRS optode. A straight segmental blood pressure cuff (SC12D

![Figure 1](http://www.acsm-msse.org)
Cuff; Hokanson, Bellevue, WA) was placed proximal to the NIRS optode with enough separation to prevent mechanical influence from inflation.

Resting measurements of muscle oxygen consumption was assessed by inflation of the blood pressure cuff (250–300 mm Hg) for 30 s once, followed by three short 10-s cuff inflations. Twitch electrical stimulation was performed using 15 s of continuous electrical stimulation (biphasic pulse, duration/interval = 200/50 μs) and was administered at 4.0 Hz. The current intensity was adjusted for each individual to produce twitch contractions at the maximal tolerable level. Force was not recorded in this study during stimulation. Immediately after each bout of electrical stimulation, a series of 10–18 brief (3–10 s) arterial occlusions using a blood pressure cuff were applied to measure the rate of recovery of mVO₂ back to resting levels. The repeated arterial 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). Finally, to normalize the NIRS signal, a 3- to 5-min arterial occlusion was applied to completely deoxygenate the tissue under the optode (i.e., 0% oxygenation), and the peak hyperemic response upon release of the cuff was used to indicate 100% oxygenation (Fig. 1B).

Calculation of muscle oxygen consumption. mVO₂ was calculated as the slope of change in O₂Hb and deoxyhemoglobin during the arterial occlusion using simple linear regression. This measurement was made at rest and repeated several times after exercise. The postexercise repeated measurements of mVO₂ were fit to a monoexponential curve according to the following formula:

\[ y = \text{End} - \Delta \text{Delta} \times e^{-t/T_e} \]  

For this equation, \( y \) represents relative mVO₂ during the arterial occlusion, \( \text{End} \) is the mVO₂ immediately after the cessation of exercise, Delta is the change in mVO₂ from rest to end exercise, and \( T_e \) is the fitting time constant.

Statistical analysis. Data are presented as means ± SD. Statistical analyses were performed using the Statistical Package for the Social Sciences (version 19.0; IBM®, Armonk, NY). Comparisons between endurance-trained and inactive subjects were made using the Student unpaired t-test for measurements of oxidative recovery and VO₂peak. The relationship between two variables was analyzed using least squares regression analysis. Significance was accepted when \( P < 0.05 \). An a priori power calculation was performed using G*Power 3 (Heinrich Heine, Düsseldorf, Germany) and yielded a total sample size of 6, a group sample size of 3, based on a t-test with a 50% difference in mitochondrial capacity between two independent groups, \( \alpha = 0.05 \), and power \( (1 - \beta) = 0.8 \).

RESULTS

All subjects were able to complete testing with no adverse events. The physical characteristics of participants are shown in Table 1. Table 2 contains variables related to whole-body oxygen uptake, such as VO₂peak expressed as the traditional VO₂peak:body mass ratio (mL·kg⁻¹·min⁻¹), in absolute terms (L·min⁻¹), RER, RPE, HR, and average end work level for both endurance-trained athletes and inactive controls.

NIRS measurements. Resting oxygen consumption of the vastus lateralis was measured by NIRS during arterial occlusion (~270 mm Hg). Resting mVO₂ was 0.52%·s⁻¹ ± 0.22%·s⁻¹ for endurance-trained athletes and 0.77%·s⁻¹ ± 0.82%·s⁻¹ for inactive participants. The difference in resting oxygen consumption between endurance-trained and inactive participants was not statistically significant (\( P = 0.42 \)). We also compared resting mVO₂ from the long resting arterial occlusion (~30 s) with the average of three resting mVO₂ values from 10-s arterial occlusions. There were no differences in either group (\( P = 0.56 \) and 0.53, for endurance trained and inactive, respectively).

End-exercise recovery of mVO₂. Representative post-exercise mVO₂ recovery curves from a single subject in each group are shown in Figure 2A. The initial end-exercise mVO₂ (% calibration) values for each group were recorded and were statistically different between groups (ET mVO₂ = 5.15, IC mVO₂ = 3.74, \( P = 0.03 \)). Time constants (\( T_e \)) representing the recovery of mVO₂ in endurance-trained athletes after electrical stimulation were 18.4 ± 3.2 and 18.8 ± 2.5 s for shallow and deep channels, respectively. In the inactive group, the recovery time constants were 32.4 ± 5.2 and 34.9 ± 5.9 s for shallow and deep channels. The differences between the endurance-trained group and the inactive group were statistically significant for both channels (\( P < 0.001 \)). For endurance-trained athletes, the average interoptode distances for the shallow and deep channels were 3 and 4 cm, respectively (range, 3–4 cm), whereas the average interoptode distances for the inactive group were 3.25 ± 0.27 and 4.25 ± 0.27 (range, 3–4.5 cm). There was no difference in the time constant for the recovery of mVO₂ between shallow and deep channels for both endurance-trained and inactive groups (\( P = 0.79 \) and 0.38, respectively). Figure 2B illustrates the average measurements of mitochondrial capacity with NIRS.
DISCUSSION

The principal finding of this study was that endurance-trained athletes had approximately twice the mitochondrial capacity of inactive controls as measured with NIRS. Mitochondrial capacity was measured as the recovery rate of mVO$_2$ and assumed to be inversely related to the time constant, analogous to the recovery of PCr using MRS (11,21,23). Assessments of oxidative capacity using the rate of PCr recovery measured with $^{31}$P-MRS has also produced similar results. Both track athletes and competitive rowers have been observed to have PCr recovery rates approximately twice as fast as untrained controls in their respective active muscle tissue (22,24). McCully et al. (24) measured the kinetics of PCr resynthesis after submaximal exercise in distance runners and untrained controls. The time constant ($T_c$) values of PCr recovery after submaximal exercise in distance runners and controls were 17.5 ± 2.7 and 31.4 ± 6.8 s, respectively. In the current study, the NIRS $T_c$ values in endurance-trained cyclists and inactive controls were 18.8 ± 2.5 and 34.9 ± 5.9 s, respectively. This suggests a good agreement between NIRS $T_c$ and PCr $T_c$ results for trained and inactive subjects.

Relative differences between endurance-trained and inactive subjects in this study were also similar to differences reported using biochemical assays of muscle biopsy tissue. For example, citrate synthase activity between well-trained runners and untrained controls was compared by Holloszy and Coyle (18) in 1984 and was twofold higher in well-trained runners. Gollnick et al. (12) found twofold greater succinate dehydrogenase activity between several different-trained groups, including cyclists, compared with untrained groups. Similarly, Blomstrand et al. (2) reported a 90% higher oxoglutarate dehydrogenase activity in well-trained runners compared with untrained controls. When muscle mitochondrial capacity is normalized between trained and inactive participants, the present study is in good agreement with the relative differences across the different methods (Fig. 3).

McCully et al. in 1994 demonstrated a close relationship between the kinetics of PCr recovery and O$_2$Hb; however, there are marked methodological differences between that article and the current article. The aforementioned article by McCully et al. evaluated the recovery of oxygen saturation after exercise using NIRS. Under these conditions, the rate of recovery reflects both the delivery of oxygen (reactive and exercise hyperemia) as well as the oxygen debt for both endurance-trained and inactive humans. Figure 2C shows the corresponding VO$_{2\text{peak}}$ and NIRS time constant values for all participants.

TABLE 2. Peak oxygen uptake variables.

<table>
<thead>
<tr>
<th>Group</th>
<th>VO$_{2\text{peak}}$ (mL·kg$^{-1}$·min$^{-1}$)</th>
<th>VO$_{2\text{peak}}$ (L·min$^{-1}$)</th>
<th>RER</th>
<th>RPE</th>
<th>HR (bpm)</th>
<th>End Work Level (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endurance (n = 8)</td>
<td>73.5 ± 9.1*</td>
<td>5.2 ± 0.9*</td>
<td>1.1 ± 0.1</td>
<td>19 ± 0.8</td>
<td>189.6 ± 6.8</td>
<td>452.5 ± 70.9*</td>
</tr>
<tr>
<td>Inactive (n = 8)</td>
<td>33.7 ± 5.9</td>
<td>2.4 ± 0.8</td>
<td>1.3 ± 0.1</td>
<td>18.6 ± 0.9</td>
<td>193.9 ± 11.3</td>
<td>212.3 ± 56.2</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.

*P < 0.05.
The recovery rate measurements of mitochondrial capacity have been made after either voluntary exercise or electrical stimulation, and both approaches have their advantages. This study used electrical stimulation because it provided a consistent stimulus between different groups of participants. During voluntary exercise, differences in strength as well as activation of synergistic or antagonistic muscles may influence the metabolism of the muscle of interest. Electrical stimulation provides uniform (nonselective) activation of all muscle fibers in the proximity of the electrode. Short duration exercise used in this study is similar to that used in previous studies to evaluate mitochondrial function (32,33), which illustrates measurements of mitochondrial function do not require steady-level exercise and do not show a “warm-up” effect. This short duration of exercise is used to produce a stimulus for mitochondrial respiration (presumably through an increase in ADP). If the offset kinetics are exponential, as the recovery of mV O₂ from exercise in our articles appear to be, the differences in metabolic rate at the start of the recovery measurements do not influence the recovery rate (25). The initial end-exercise mV O₂ was greater in the endurance-trained group. This difference could be due to two factors: greater mitochondrial density of the NIRS sampled tissue or the lower currents in the inactive controls (ET = 96 ± 17 and IC = 65 ± 8) may have resulted in incomplete activation of NIRS sampled tissue. We feel the differences are more likely due to the differences in mitochondrial density, as the NIRS sampling area is relatively shallow and moderate currents should be adequate for full activation of the sampling area. Nonetheless, the recovery kinetics only represents activated tissue and thus does not require complete activation.

One of the key aspects to this study was the two-step inclusion criteria of participants into the two groups. Individuals were recruited based on self-reported training activity of either endurance training as a competitive race cyclist or training at most a few days of the week. Participants were then selected for the two groups based on the VO₂peak test results. Target VO₂peak values for the endurance-trained group were higher than 60 mL·kg⁻¹·min⁻¹ for males and 55 mL·kg⁻¹·min⁻¹ for females. The target values for the inactive subjects were less than 45 mL·kg⁻¹·min⁻¹ for males and less than 40 mL·kg⁻¹·min⁻¹ for females. Although the reason for the differences between groups could be both genetic and environmental, the two-step selection process did result in clear differences in VO₂peak and time constant between groups.

The limitations to continuous-wave NIRS includes a handful of potentially confounding factors that include unknown optical path length, absorption, and scattering coefficients as well as the interaction of adipose tissue with NIR light (15,27,28). The measurement of path length, absorption, and scattering coefficients has been facilitated through the development of frequency and time domain NIRS devices (3,8,9). Despite new developments in NIRS devices, ATT remains a consideration when monitoring muscle oxygenation. The estimated depth of penetration with continuous-wave NIRS is one-half the interoptode distance. To account for this, we measured ATT at the site of measurement before commencement and then set the interoptode distance on our device accordingly. With the large range of ATT in our participants (3.2–9.2 mm), we used a physiological ischemic calibration for the calculation of muscle oxygen saturation and consumption in each subject. This allowed us to report our measurements of mV O₂ as a percentage change per unit time as well as compare individuals by reducing the influence of adipose tissue and skin overlying individual muscle. Absolute changes were not reported in this study as previous studies have clearly illustrated these measurements to be influenced by subcutaneous fat thickness, independent of any NIRS signal coming from the skeletal muscle. This is true for both continuous-wave NIRS devices (30) and time-resolved NIRS (20). In previous studies by Van Beekveld et al. (30) and Ryan et al. (28), the quantification of mV O₂ using the differential path length factor was influenced by ATT, and with the application of an ischemic calibration, this influence was abolished. In addition, this study used two interoptode separation distances, which enabled us to compare measurements of muscle oxygen consumption at a shallow and deep penetration depth. As in our previous study (28), we did not find a
significant influence of optode distance (sample depth) on muscle metabolic measurements.

In summary, the NIRS time constant for the recovery of mVO2 in endurance-trained athletes was approximately twofold higher than in inactive subjects. These results are consistent with the expected differences in muscle oxidative capacity between these groups based on previous studies with other methods and support the use of NIRS and repeated arterial occlusion to measure mitochondrial function.

The ease of use of this method provides the potential for it to be used in studies of different human subjects.

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The authors report no conflict of interest.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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