A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy

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Ryan TE, Southern WM, Reynolds MA, McCully KK. A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. J Appl Physiol 115: 1757–1766, 2013. First published October 17, 2013; doi:10.1152/japplphysiol.00835.2013.—The purpose of this study was to cross-validate measurements of skeletal muscle oxidative capacity made with near-infrared spectroscopy (NIRS) measurements to those made with phosphorus magnetic resonance spectroscopy (31P-MRS). Sixteen young (age = 22.5 ± 3.0 yr), healthy individuals were tested with both 31P-MRS and NIRS during a single testing session. The recovery rate of phosphocreatine was measured inside the bore of a 3-Tesla MRI scanner, after short-duration (~10 s) plantar flexion exercise as an index of skeletal muscle oxidative capacity. Using NIRS, the recovery rate of muscle oxygen consumption was also measured using repeated, transient arterial occlusions outside the MRI scanner, after short-duration (~10 s) plantar flexion exercise as another index of skeletal muscle oxidative capacity. The average recovery time constant was 31.5 ± 8.5 s for phosphocreatine and 31.5 ± 8.9 s for muscle oxygen consumption for all participants (P = 0.709). 31P-MRS time constants correlated well with NIRS time constants for both channel 1 (Pearson’s r = 0.88, P < 0.0001) and channel 2 (Pearson’s r = 0.95, P < 0.0001). Furthermore, both 31P-MRS and NIRS exhibit good repeatability between trials (coefficient of variation = 8.1, 6.9, and 7.9% for NIRS channel 1, NIRS channel 2, and 31P-MRS, respectively). The good agreement between NIRS and 31P-MRS indexes of skeletal muscle oxidative capacity suggest that NIRS is a valid method for assessing mitochondrial function, and that direct comparisons between NIRS and 31P-MRS measurements may be possible.

mitochondrial capacity; 31P-MRS; oxidative metabolism; mitochondrial function

Traditionally, mitochondrial function has been studied using methods that can be classified into two categories: invasive (ex vivo) or noninvasive (in vivo) approaches. Ex vivo approaches involve a small biopsy of muscle tissue to measure enzyme concentrations or activity levels (11, 14), isolated mitochondrial preparations (5), or permeabilized muscle fiber preparations (10). Until recently, in vivo approaches to studying mitochondrial function have been limited to the use of magnetic resonance spectroscopy (MRS) to study changes in phosphorus (31P) metabolites during exercise and the recovery postexercise (4). The most widely used 31P-MRS assessment for mitochondrial function is the recovery rate of phosphocreatine (PCr) following exercise. Using the assumption of equilibrium for the creatine kinase reaction, the recovery rate of PCr after exercise is a function of mitochondrial ATP production, which has been validated against in vitro measurements of enzyme activity (19, 21, 29) and high-resolution respirometry (18).

Alternatively, near-infrared spectroscopy (NIRS) has been used to investigate muscle blood flow and oxidative metabolism both at rest and during different types of exercises. It has been shown by our laboratory and others that oxidative metabolism, measured by NIRS, increases linearly with exercise intensity (32, 36). Previous studies have compared the recovery of O2Hb (oxygenated hemoglobin/myoglobin) and/or HHbolHHb, where HHb represents deoxygenated hemoglobin/myoglobin after exercise with the recovery kinetics of PCr (13, 23). Interestingly, the findings of these studies are contradictory. McCully et al. (23) reported “good agreement” between the time constants for the recovery of PCr and O2Hb after submaximal exercise intensities, but not for the recovery after maximal exercise intensities. Although there was a strong correlation in the study by McCully et al., they did report that the time constants for the recovery of O2Hb were faster than PCr in all participants. Surprisingly, Hanada et al. (13) did not find a correlation between the time constants of PCr and O2Hb recovery in either healthy controls or patients with heart failure. These somewhat conflicting results can be explained by the fact that the recovery of O2Hb is not actually a measurement of oxidative metabolism, but rather a combination of oxygen delivery and oxygen consumption. For these reasons, our laboratory and others have recently utilized a novel, in vivo approach to measuring skeletal muscle mitochondrial function using NIRS (26, 27, 33). This approach uses NIRS in combination with a rapid cuff inflation system (used to block oxygen delivery and venous return) to measure kinetic changes in skeletal muscle oxygen consumption (mVO2) after submaximal exercise. This approach is different from that used by McCully et al. (23) and Hanada et al. (13) because the arterial occlusions isolate oxygen consumption from oxygen delivery, whereas the recovery of NIRS signals without arterial occlusions represents...
the recovery of an oxygen debt and is influenced by both the rate of oxygen consumption and the rate of oxygen delivery. Similar to PCr recovery, the recovery of mVO$_2$ (using arterial occlusions) after exercise should be a function of mitochondrial ATP production and, therefore, has the potential to be used as a measure of skeletal muscle oxidative capacity (26). This NIRS approach has been shown to be reproducible (33) and independent of exercise intensity (32), detects the expected differences between untrained and trained individuals (3), as well as paralyzed and nonparalyzed individuals (8), and can track changes in mitochondrial function with exercise training and detraining (34). In the present study, we cross-validated NIRS measurements of skeletal muscle oxidative capacity with $^{31}$P-MRS measurements of skeletal muscle oxidative capacity in a population of young healthy adults. Because the repeatability of measurements can affect the level of agreement, we also assessed the repeatability of both NIRS and $^{31}$P-MRS measurements.

MATERIALS AND METHODS

Participants

Sixteen healthy participants (10 men, 6 women), ages 19–30 yr, were tested in this study. 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.

Study Design

NIRS and $^{31}$P-MRS testing was performed on all participants (in random order) in a single visit to the University of Georgia BioImaging Research Center (Athens, GA). The NIRS and $^{31}$P-MRS testing protocols took ~30 min each.

NIRS Experimental Protocol

NIRS testing performed in this study is similar to that describe in previous studies (32, 33). Each subject was placed on a padded table with both legs extended (0° of knee flexion). The participant’s dominant foot was placed into a home-built nonmagnetic pneumatic exercise device, similar to that used in previous studies (23, 39). The foot was strapped firmly to the exercise device using nonelastic Velcro straps and adhesive tape. A blood pressure cuff (Hokanson SC12D, Bellevue, WA) was placed proximal to the NIRS optode above the knee joint. The blood pressure cuff was connected to a rapid-inflation system (Hokanson E20, Bellevue, WA). Adipose tissue thickness (ATT) was measured at the site of the NIRS optode using B-mode ultrasound (LOGIQ e; GE HealthCare). Participants were asked 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 the test.

The test protocol consisted of two measurements of resting mVO$_2$ by way of inflation of a blood pressure cuff (250–300 mmHg) for 30 s. For the recovery measurements, 10 s of plantar flexion exercise were performed at a given resistance [pneumatic resistance in the form of pounds per square inch (psi)] to increase mVO$_2$. The resistance was set during a familiarization session before testing to a level that would produce the optimal balance between force and speed of contractions. For example, the participants were expected to perform plantar flexions at the highest psi (air resistance) that would allow for a minimum of 2 contractions/s. The average resistance was 19 ± 4 psi (range = 12–25 psi). Previous studies have shown short-duration exercise produces similar rates of PCr resynthesis (39). Following the plantar flexion exercise, a series of 10–18 brief (5–10 s) arterial occlusions were applied to measure the rate of recovery of mVO$_2$ back to resting levels. There was a small time delay between the end-exercise and initial arterial occlusion (~2 s) due to movement of the exercise ergometer as the pressure dissipated from the air cylinder. To maximize our ability to measure the recovery of mVO$_2$ while minimizing the discomfort to participants, the duration between arterial occlusions began at 5 s and extended to 20 s by the end of the repeated occlusions (i.e., 5 s on/5 s off for cuffs 1–6, 7 s on/7 s off for cuffs 7–10, 10 s on/15 s off for cuffs 11–14, and 10 s on/20 s off for recovery cuffs 15–18). The exercise and repeated cuffing procedure was repeated a second time, with ~5–7 min in between the first and second exercise/recovery bouts.

An ischemic calibration procedure was performed before the recovery measurements and used to scale the NIRS $\text{O}_2$Hb, HHb, and Hbdiff signals to the maximal physiological range, as previously described (32).

NIRS device. NIRS signals were obtained using a continuous-wave NIRS device (Oxymon MKIII, Artinis Medical Systems). The probe was set to have two source-detector separation distances (between 30

<table>
<thead>
<tr>
<th>Physical characteristics of the participants</th>
<th>Optode Distance, mm</th>
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<tbody>
<tr>
<td></td>
<td>Channel 1</td>
</tr>
<tr>
<td>Men 10</td>
<td>179.1 ± 6.4</td>
</tr>
<tr>
<td>Women 6</td>
<td>168.1 ± 9.6</td>
</tr>
<tr>
<td>Total 16</td>
<td>174.9 ± 9.2</td>
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</tbody>
</table>

Values are means ± SD; n, no. of subjects. ATT, adipose tissue thickness. Near-infrared spectroscopy (NIRS) optode distances used and their corresponding frequency of use are shown for informational reasons. Statistical comparisons between sexes were not performed.

Fig. 1. Recovery of phosphocreatine (PCr) and muscle oxygen consumption (mVO$_2$) after short-duration plantar flexion exercise. Values are mean of all participants (n = 16) and all trials ± SD.
Fig. 2. Repeatability and reproducibility of phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) and near-infrared spectroscopy (NIRS) data. Correlations between trials are shown for $^{31}$P-MRS (A), NIRS channel 1 (C), and NIRS channel 2 (E). For A, C, and E, the dashed lines represent the 95% confidence intervals for the regression equation. Corresponding Bland-Altman plots are shown for $^{31}$P-MRS (B), NIRS channel 1 (D), and NIRS channel 2 (F). For Bland-Altman plots, dotted lines represent the 95% limits of agreement.
and 45 mm), with the smallest source-detector distance set to approximately twice the ATT. The second source-detector distance was always 1 cm greater than the first. NIRS data were collected at 10 Hz.

**Calculation of mVO₂**

mVO₂ was calculated as the slope of change in the HbO₂ signal (HbO₂ = O₂Hb - HHb) during the arterial occlusion using simple linear regression. mVO₂ was expressed as a percentage of the ischemic calibration per unit time. This measurement was made at rest and repeated a number of times after exercise. The postexercise repeated measurements of mVO₂ were fit to a monoexponential curve according to the formula below:

\[ y = \text{End} - \Delta \times e^{-kt} \]  

For this equation, \( y \) is 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 \( k \) is the fitting rate constant, \( t \) is the time.

**Correction for blood volume.** NIRS data were analyzed using custom-written routines for Matlab version 7.13.0.564 (The Mathworks, Natick, MA). NIRS signals were corrected for changes in blood volume using the following method, as previously described (33).

**Calculation of signal-to-noise ratio.** The quality of NIRS data was determined by calculating a signal-to-noise ratio (SNR). The SNR was calculated for each arterial occlusion on all data. The signal was calculated as the change in the NIRS signal during the chosen measurement period of an arterial occlusion and is, therefore, a function of the duration of the occlusion and the rate of oxygen consumption. The noise was calculated as the standard deviation of 600 data points (60 s).

**31P-MRS Experimental Protocol**

Subjects were tested in a 3-Tesla whole-body magnet (GE Healthcare, Waukesha, WI). 1H and 31P radio-frequency dual-surface coil (Clinical MR Solutions, Brookfield, WI) was placed over the medial gastrocnemius muscle of the subject’s dominant leg. The size of the 31P coil was 13 cm \( \times \) 13 cm, placed orthogonal to the 1H coil (two loops, side by side, 20 cm \( \times \) 20 cm in size). Manual shimming on 1H was applied to get a better SNR and less spectrum distortion, after an auto-shimming by a prescan sequence [all subjects 1H full width half maximum (FWHM) mean \( \pm \) SD; 0.60 \( \pm \) 0.14 ppm]. A nonlocalized, single pulse-acquisition pulse sequence was applied to acquire the 31P spectra with the following scan parameters: repetition time (TR) 3 s, field of view = 8 cm, slice thickness = 8 cm, number of excitation = 1, RF pulse = hard. The field of view and slice thickness correspond to the shimming volume (33).

**Metabolic calculations.** Resting spectra were acquired every 3 s until 50 scans are taken. The resulting spectra were summed in a custom analysis program (Winspa, Ronald Meyer, Michigan State University). The summed spectrum was apodized using 5-Hz exponential line broadening, and zero-filled from 2,048 to 8,192 points. The area under the curve for each peak (P, PCr, \( \alpha \)-ATP, \( \beta \)-ATP, and \( \gamma \)-ATP) was determined using integration. Absolute concentrations were calculated using the assumed value of 8.2 mM for the \( \gamma \)-ATP peak. Saturation effects were corrected for using fully relaxed spectra collected from four individuals using the same pulse sequence above, except the TR was changed to 15 s. Muscle pH was calculated using the following equation:

\[ \text{pH} = 6.77 + \log\left(\frac{\text{P}_{\text{shift}} - 3.27}{5.68 - \text{P}_{\text{shift}}}\right) \]  

where \( \text{P}_{\text{shift}} \) is the chemical shift of P, relative to PCr in parts per million (ppm).

**Exercise Protocol**

Planar flexion exercise in a home-built nonmagnetic pneumatic ergometer was performed inside the bore of the MRI. Identical resistances (psi) were used for 31P-MRS and NIRS testing. The exercise protocol consisted of \( \sim \)45 s of rest, followed by \( \sim \)10 s of rapid plantar flexion, and \( \sim \)4 min for the measurement of the resynthesis of PCr. 31P spectra were obtained using the same pulse sequence characteristics described above. This short-duration exercise bout was designed to decrease PCr concentration ([PCr]) without causing significant acidosis.

**PCr recovery.** [PCr] values were determined from the peak heights from individual spectra (temporal resolution of 3 s) using custom-written routines in Matlab version 7.13.0.564 (The Mathworks, Natick, MA) (24). Individual spectra were apodized using 2-Hz exponential line broadening, followed by zero-filling to 8,192 points. Peak heights were determined using the magnitude of each spectrum. The assumption that changes in peak height represent changes in concentration is only valid when there is no change in the peak widths (FWHM). FWHM of each PCr peak was calculated to ensure no changes in magnetic field homogeneity occurred during the recovery period. PCr peak heights during recovery after exercise were fit to an exponential curve.

\[ \text{PCr} = \text{PCr}_{\text{end}} - \Delta\text{PCR} \times e^{-kt} \]  

where \( \text{PCr}_{\text{end}} \) is the percent PCr immediately after cessation of exercise, \( \Delta\text{PCR} \) is the change in PCr from rest to end exercise, and \( k \) is the fitting rate constant, \( t \) is the time. The maximal rate of ATP synthesis (\( Q_{\text{max}} \)) was calculated using the following equation:

\[ Q_{\text{max}} = \frac{\text{[PCr]}_{\text{resting}} \cdot k_{\text{PCr}}}{\text{PCr}_{\text{end}}} \]  

where \([\text{PCr}]_{\text{resting}}\) is the resting concentration of PCr, and \( k_{\text{PCr}} \) is the rate constant for the recovery of PCr after exercise.

**Calculation of SNR**

The quality of phosphorus data collected was determined by calculating a SNR for each individual spectra. The signal was calculated as the peak height of PCr, \( \text{PCr}_{\text{end}} \) without exercise, \( \Delta \text{PCR} \) is the percent PCr immediately after cessation of exercise, \( \text{PCr}_{\text{resting}} \) is the resting concentration of PCr, and \( k_{\text{PCr}} \) is the rate constant for the recovery of PCr after exercise.

**Comparison of Peak Heights Analysis and AMARES**

The peak heights analysis approach described above was directly compared with a commonly used time-domain fitting algorithm, Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES) with prior knowledge (37), using the freely available JMRUI software (28). The recovery of PCr after exercise was determined using both AMARES and the peak heights approach. The sum of squared residuals between the monoexponential curve fit and [PCr] was used to evaluate the quality of curve fitting. For resting phospho-

**Table 2. Resting and end-exercise [PCr] and pH**

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>End Exercise</th>
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<tbody>
<tr>
<td>[PCr]</td>
<td>pH</td>
<td>[PCr]</td>
</tr>
<tr>
<td>All participants</td>
<td>34.25 ± 2.86</td>
<td>7.05 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SD. [PCr], phosphocreatine concentration. End-exercise pH was calculated from the first phosphorus spectra during the recovery measurements. The minimum pH is the lowest pH recorded during the recovery measurements.
compared with NIRS time constants using a two-tailed Student’s
1,1). CV was expressed as a percentage. PCr time constants were
analyzed using CV and intraclass correlation coefficients (ICC model
for paired samples. Pearson’s correlation coefficients were calculated
Statistical Analysis
of variation (CV) was calculated for the [PCr] using both approaches. The coefficient
and all trials. Values are means ± SD.

rus scans, the [PCr] was calculated for individual spectra (N = 50
scans per participant) using both analytic approaches. The coefficient
of variation (CV) was calculated for the [PCr] using both approaches.

Statistical Analysis
Data are presented as means ± SD. Test-retest reliability was
analyzed using CV and intraclass correlation coefficients (ICC model
1,1). CV was expressed as a percentage. PCr time constants were
compared with NIRS time constants using a two-tailed Student’s t-test
for paired samples. Pearson’s correlation coefficients were calculated
to determine the relationship between two variables. Bland-Altman
limits of agreement analysis was performed to determine the level of
agreement between two variables (1). Statistical analyses were per-
formed using either SPSS 19.0 (IBM, Armonk, NY) or GraphPad
Prism (GraphPad Software, La Jolla, CA). Significance was accepted
when P < 0.05.

RESULTS
All participants completed testing without any adverse
events. The physical characteristics of the participants are
shown in Table 1. The group average recovery of mVO2 is
shown in Fig. 1, overlapped with the recovery of PCr (error
bars represent SD).

$^{31}$P-MRS
The average recovery time constant for PCr was 31.5 ± 8.5
s for all participants. The recovery rate was reproducible
between trials (CV = 7.6%, ICC = 0.90). Comparisons be-
tween trials for the PCr recovery time constant are shown in
Fig. 2A. The corresponding Bland-Altman plot is shown in Fig.
2B. During the 10 s of rapid plantar flexion exercise, [PCr]
decreased by ~24%.

The resting and end-exercise values for [PCr] and pH are
shown in Table 2. FWHM of PCr was calculated for each
spectrum, on all tests, and did not change throughout the recov-
ery from exercise (data not shown). The average SNR of
resting phosphorus spectra was 154 ± 47. During the recovery
of PCr, the SNR increased as PCr is resynthesized (Fig. 3). We
did not find a strong relationship between the variability of the
time constant (CV) and SNR for $^{31}$P-MRS ($R^2 < 0.07$).

NIRS
The resting value for mVO2 was 0.26 ± 0.10%/s for all
participants. The average time constant for the recovery of
mVO2 after exercise was 31.5 ± 8.9 s. The time constant was
reproducible between trials for both channels of NIRS device

Table 3. Metabolic parameters for NIRS and $^{31}$P-MRS

<table>
<thead>
<tr>
<th>Channel</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>CV (Range), %</th>
<th>ICC</th>
<th>Pearson’s r</th>
<th>P (Paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NIRS parameters</strong></td>
<td></td>
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<tr>
<td>End-exercise mVO2, %/s</td>
<td>5.1 ± 1.2</td>
<td>5.4 ± 2.0</td>
<td>9.8 (0.2–57.4)</td>
<td>0.85</td>
<td>0.827</td>
<td>0.311</td>
</tr>
<tr>
<td>Time constant, s</td>
<td>30.5 ± 9.5</td>
<td>32.2 ± 8.6</td>
<td>8.1 (0.2–15.7)</td>
<td>0.93</td>
<td>0.936</td>
<td>0.599</td>
</tr>
<tr>
<td>Time constant, 95% CI</td>
<td>1.6 ± 1.3</td>
<td>1.8 ± 1.8</td>
<td>8.1 (0.2–15.7)</td>
<td>0.93</td>
<td>0.838</td>
<td>0.513</td>
</tr>
<tr>
<td>$k_{mVO2}$, s⁻¹</td>
<td>0.0353 ± 0.0091</td>
<td>0.0329 ± 0.0078</td>
<td>8.1 (0.2–15.7)</td>
<td>0.93</td>
<td>0.838</td>
<td>0.513</td>
</tr>
<tr>
<td><strong>$^{31}$P-MRS parameters</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-exercise PCr, mM</td>
<td>25.7 ± 3.0</td>
<td>26.0 ± 3.0</td>
<td>3.8 (0.4–12.9)</td>
<td>0.87</td>
<td>0.848</td>
<td>0.463</td>
</tr>
<tr>
<td>End-exercise pH</td>
<td>7.00 ± 0.08</td>
<td>7.02 ± 0.06</td>
<td>0.3 (0.0–1.1)</td>
<td>0.86</td>
<td>0.896</td>
<td>0.150</td>
</tr>
<tr>
<td>Time constant, s</td>
<td>31.3 ± 8.2</td>
<td>31.8 ± 9.4</td>
<td>7.6 (1.3–19.7)</td>
<td>0.90</td>
<td>0.862</td>
<td>0.946</td>
</tr>
<tr>
<td>Time constant, 95% CI</td>
<td>3.2 ± 2.9</td>
<td>2.6 ± 1.5</td>
<td>7.6 (2.3–19.7)</td>
<td>0.89</td>
<td>0.883</td>
<td>0.999</td>
</tr>
<tr>
<td>$k_{PCr}$, s⁻¹</td>
<td>0.0338 ± 0.0078</td>
<td>0.0337 ± 0.0086</td>
<td>7.6 (1.3–19.7)</td>
<td>0.90</td>
<td>0.862</td>
<td>0.946</td>
</tr>
<tr>
<td>$Q_{max}$, mM ATP/s</td>
<td>1.16 ± 0.28</td>
<td>1.16 ± 0.32</td>
<td>7.6 (2.3–19.7)</td>
<td>0.89</td>
<td>0.883</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Values are means ± SD. $^{31}$P-MRS, phosphorus magnetic resonance spectroscopy; CV, coefficient of variation; ICC, intraclass correlation coefficient; mVO2, muscle oxygen consumption; $k_{mVO2}$, rate constant for the recovery of mVO2; $k_{PCr}$, rate constant for the recovery of PCr; $Q_{max}$, maximal rate of ATP synthesis (in mM ATP/s); 95% CI, 95% confidence interval for curve fitting in seconds. Student’s Paired t-test was performed between trials.
Constants show that the errors (differences between NIRS and average of all participant and all trials) and channel 2 (Pearson’s $r = 0.93$, $P < 0.0001$) and channel 2 (Pearson’s $r = 0.94$, $P < 0.0001$) (Fig. 2, C and E). Corresponding Bland-Altman plots for the trials of NIRS channel 1 and channel 2 are shown in Fig. 2, D and E, respectively. Statistical values regarding the repeatability of NIRS measurements are shown in Table 3. The SNR for NIRS measurements of $\text{mV}_2$ decreased as $\text{mV}_2$ returned to resting levels (Fig. 4). The average SNR for resting $\text{mV}_2$ was $7.38 \pm 7.06$ for channel 1 and $6.34 \pm 5.94$ for channel 2. We did not find a strong relationship between the variability of the time constant (CV) and SNR for NIRS ($R^2 < 0.1$).

**Relationship Between NIRS and $^{31}$P-MRS**

The rate of recovery of $\text{mV}_2$ measured by NIRS correlated well with the rate of recovery of PCr measured by $^{31}$P-MRS. Using the average time constant for both trials, $^{31}$P-MRS correlated well with NIRS for both channel 1 (Pearson’s $r = 0.88$, $P < 0.0001$) and channel 2 (Pearson’s $r = 0.95$, $P < 0.0001$) (Fig. 5, A and B). Bland-Altman plots (Fig. 5, B and C) of the time constants show that the errors (differences between NIRS and $^{31}$P-MRS time constants) were approximately symmetrically distributed around zero, with no indication of a systematic bias between measurement techniques.

**Comparison of Peak Heights and AMARES**

An example from one participant of PCr recovery kinetics analyzed using the peak heights approach and AMARES is shown in Fig. 6A. The sum of squared residuals between the measured [PCr] and the monoexponential curve fit was significantly smaller ($P < 0.001$) with the peak heights approach, indicating a better quality fit (Fig. 6B). The reproducibility of peak heights analysis was determined from the resting scan (50 spectra per participant). The mean CV of PCr peak heights was $1.3 \pm 0.7\%$ (range $= 0.6–2.9\%$) and $8.1 \pm 10.9\%$ (range $= 0.96–21.0\%$) for AMARES ($P = 0.02$) (Fig. 6C).

**DISCUSSION**

This study found that NIRS-measured recovery kinetics of $\text{mV}_2$ were not statistically different from and correlated well with $^{31}$P-MRS-measured recovery kinetics of PCr after short-duration exercise. Furthermore, both NIRS and $^{31}$P-MRS measurements exhibited excellent reproducibility in the current experimental conditions. The level of agreement between NIRS and $^{31}$P-MRS is similar to a previous study. Nagasawa et al. (27) made similar measurements in the forearm muscles of eight male participants, reporting a similar relationship between NIRS and $^{31}$P-MRS recovery kinetics ($r = 0.92$). In contrast to the present study, the study by Nagasawa and colleagues did not utilize a correction for blood volume shifts, which has been shown to reduce the variability of NIRS time/rate constants with continuous-wave NIRS devices (33). Nonetheless, our findings support those reported by Nagasawa et al. (27). The recovery time constants in the present study ($31.5 \pm 8.5$ s for PCr and $31.5 \pm 8.9$ s for NIRS) are also similar to those reported by previous studies using $^{31}$P-MRS in untrained individuals (9, 19, 39).

NIRS measurements of $\text{mV}_2$ have been quantitatively validated using $^{31}$P-MRS in both resting and exercising skeletal muscle (12, 35). Hamaoka et al. (12) utilized arterial occlusions to measure $\text{mV}_2$ with NIRS and quantified the NIRS $\text{mV}_2$ using the rate of PCr breakdown at rest (as a indication of ATP consumption). These authors also reported strong linear relationships between $\text{mV}_2$ and both [ADP] and [PCr], consistent with kinetic and thermodynamic models of respiration. Using a similar experimental design, Sako et al. (35) quantified NIRS measured $\text{mV}_2$ during various levels of exercise intensity using a similar “calibration” with resting metabolism measurements with NIRS and $^{31}$P-MRS. After quantification, Sako et al. (35) reported a strong correlation between NIRS and $^{31}$P-MRS measurements of muscle oxidative metabolism. While both of these studies have important findings and support NIRS as a valid tool for assessing muscle oxidative metabolism noninvasively, the methodology used is such that NIRS in combination with $^{31}$P-MRS provides valid measurements. In contrast, the present study demonstrates clearly that the NIRS-based recovery kinetics of $\text{mV}_2$, using repeated arterial occlusions, can provide an index of the maximal oxidative capacity of skeletal muscle (i.e., time constant or rate constant), which is measured independent of $^{31}$P-MRS, but is both similar and well correlated with $^{31}$P-MRS. These findings, in combination with those by Nagasawa et al. (27), suggest that NIRS could be used to study muscle metabolism.

Fig. 4. Signal-to-noise analysis of NIRS data. A: sample NIRS signal showing how the SNR was calculated. SNRs for each postexercise arterial occlusions are shown for channel 1 (B) and channel 2 (C). Values in B and C are the average of all participant and all trials $\pm$ SD.
in vivo, when $^3$P-MRS is not available. Importantly, the use of repeated arterial occlusions to measure the recovery kinetics of oxygen consumption provides a more direct measurement of oxidative metabolism compared with previous studies that measured the recovery kinetics of NIRS signals (mainly O$_2$Hb or oxygen saturation), which provides information about the balance of oxygen consumption and oxygen delivery (13, 23).

The direct comparisons between NIRS and $^3$P-MRS in this study were made using a convenience sample of young healthy, college-aged individuals. This sample of individuals was chosen to achieve the highest quality of data possible. For this reason, we have also calculated SNR measurements for both NIRS and $^3$P-MRS. Some potential factors that could influence the SNR of $^3$P-MRS data include the following: leg positioning compared with the isocenter of the magnet, coil positioning and loading, shim quality, muscle composition (intramuscular fat and subcutaneous fat levels), and movement during in-magnet exercise protocols. There are several approaches to calculating SNR of magnetic resonance spectra. Some approaches account for differences in magnetic resonance signal acquisition parameters (acquired flip angle, T$_1$ and T$_2$* relaxation rate, and partial saturations) (31). The approach used in this study is a rather simple method for calculating in vivo SNR that is easy to perform and similar to previous studies (2, 7, 40).

Fig. 5. Comparison between $^3$P-MRS and NIRS data. All comparisons were made by averaging the time constants for both trials. Correlations between $^3$P-MRS and NIRS channel 1 (A) and NIRS channel 2 (C) are shown, where dashed lines represent the 95% confidence intervals for the regression equation. Corresponding Bland-Altman plots for comparisons between $^3$P-MRS and NIRS channel 1 (B) and NIRS channel 2 (D) are also shown, with the dotted lines indicating the 95% limits of agreement.
data collection to ensure the best possible data quality was collected. It is difficult to make direct comparisons of SNR between NIRS and \(^{31}\)P-MRS, but using the approach described herein should allow for interlaboratory comparisons of data quality for both methodologies.

We utilized two approaches to analyzing \(^{31}\)P-MRS spectra: the peak heights and AMARES fitting routine. A direct comparison between analysis approaches showed a significantly better quality of curve fit for recovery kinetics of PCr and lower variation in the [PCr] in the resting scans using the peak heights analysis. The peak height analysis uses only the peak of the spectrum, which has the highest SNR of any point in the spectrum. After zero filling our spectra several times, we can accurately obtain the highest point. For a single resonance like PCr (in skeletal muscle), the peak height accurately reflects relative changes in peak area, assuming no overlapping peaks or changes in line shape (FWHM). Peak fitting algorithms like AMARES have advantages for analyzing MRS data that have complex and/or changing peak shapes, but requires extreme accuracy in phasing of the data. While the peak heights approach was superior to AMARES for the recovery kinetics, absolute concentrations (as done with our summed resting

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**Fig. 6.** A: comparison between peak heights and Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES) analysis approaches. B: the sum of squared residuals between the measured PCr concentration ([PCr]) and the monoexponential curve fit was significantly lower using the peak heights analysis. C: the variance of resting PCr peaks was also significantly lower using the peak height approach.

*P < 0.05.


REFERENCES


