Reliability of Near-Infrared Spectroscopy for Determining Muscle Oxygen Saturation During Exercise

Krista G. Austin, Karen A. Daigle, Patricia Patterson, Jason Cowman, Sara Chelland, and Emily M. Haymes

Near-infrared spectroscopy is currently used to assess changes in the oxygen saturation of the muscle during exercise. The primary purpose of this study was to assess the reliability of near-infrared spectroscopy in determining muscle oxygen saturation (S\textsubscript{tO\textsubscript{2}}) in the vastus lateralis during cycling and the gastrocnemius during running for exercise intensities at lactate threshold and maximal effort. Test-retest reliability was determined from an intraclass correlation coefficient obtained from a one-way analysis of variance. Reliability of muscle S\textsubscript{tO\textsubscript{2}} for the gastrocnemius at lactate threshold was R = .87, and R = .88 at maximal effort. Reliability of muscle S\textsubscript{tO\textsubscript{2}} for the vastus lateralis at lactate threshold was R = .94 and R = .99 at maximal effort.

Key words: lactate threshold, maximal oxygen consumption, muscle S\textsubscript{tO\textsubscript{2}}

Since the early work of A. V. Hill in the 1920s, oxygen transport and muscle oxygen use have been studied to understand how intracellular and intravascular interactions affect the ability of skeletal, smooth, and cardiac muscle to function at rest and during exercise. Richardson and colleagues (Richardson et al., 1999; Richardson, Leigh, Wagner, & Noyszewski, 1999) demonstrated the importance of oxygen supply on maximal oxygen consumption and suggested that this limits performance in trained skeletal muscle. Direct measures of muscle oxygen use as determined from arterial and venous blood samples have also been used to evaluate limitations in mitochondrial metabolism and determine peripheral contributions to changes in muscle bioenergetics and systemic oxygen uptake (Chance, Dait, Zhang, Hamaoka, & Hagerman, 1992; Chuang et al., 2002; Hamaoka et al., 1996; Neary, McKenzie, & Bhambhani, 2002). Limitations in the muscle’s ability to use oxygen and changes in muscle oxygen use resulting from physical training can provide insight into exercise tolerance and functional capacity (Chuang et al., 2002; Grassi, Quaresima, Marconi, Ferrari, & Cerretelli, 1999; Pamenter & Snyder, 2002). However, there are limited data on direct measurements of muscle oxygen use due to the invasive nature of the techniques and their associated technical difficulties. The measurement is also time consuming and can cause significant discomfort to participants. Alternative measurement by magnetic resonance spectroscopy, while highly accurate, can be costly and is not widely available for use by laboratories and exercise training facilities.

Given these difficulties, near-infrared spectroscopy (NIRS), a noninvasive technique, has been developed to measure changes in oxygenated and deoxygenated hemoglobin to determine the muscle’s use of oxygen (S\textsubscript{tO\textsubscript{2}}; Mancini et al., 1994). S\textsubscript{tO\textsubscript{2}} is considered to be a measure that incorporates both the venous and arterial saturation of oxygenated hemoglobin across a tissue bed. The NIRS quantification of S\textsubscript{tO\textsubscript{2}} is a relatively inexpensive measurement that provides no discomfort to the

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participant and can be easily assessed. This noninvasive sampling technique measures the changes in muscle oxygenation by determining the difference in the reflection of light at near-infrared wavelengths of 680–800 nm. The determination of \( \text{StO}_2 \) by NIRS is based on tissue absorbance values of oxygenated and deoxygenated hemoglobin, which provides a practical percentage of saturation when incorporated into the following formula (Garr, Gentilello, Cole, Mock, & Matsen, 1999):

\[
\text{StO}_2 = \frac{\text{HbO}_2}{(\text{HbO}_2 + \text{Hb})} \times 100
\]

where \( \text{StO}_2 \) = tissue oxygen saturation, \( \text{HbO}_2 \) = oxyhemoglobin, and \( \text{Hb} \) = hemoglobin. Thus, the absolute value of \( \text{StO}_2 \) indicates a measure of the blood hemoglobin oxygen saturation contained in the volume of tissue and is a result of the oxygen supply and tissue consumption being measured (Investigator’s Brochure, Hutchinson Technology, 2004).

Near-infrared spectroscopy has been used to examine muscle deoxygenation during exercise, peripheral adaptations resulting from training, and in diagnosing compartmental syndrome (Belardinelli, Barstow, Forszpan, & Wasserman, 1994; Garr et al., 1999; Grassi et al., 1999; Neary et al., 2002). More recently, NIRS has been used to assess changes in muscle oxygen use to determine the work level at the lactate threshold. A breakpoint in \( \text{StO}_2 \) has been considered reflective of the breakpoint seen in lactate accumulation during exercise above the lactate threshold (Grassi et al., 1999; Parmenter & Snyder, 2002). It was, therefore, suggested that NIRS measurement of \( \text{StO}_2 \) might also provide insight into peripheral changes of cardiorespiratory fitness. Some evidence suggests that lactate production depends on oxygen supply during exercise and increases with an inadequate oxygen availability (Wasserman, 1984); conversely, other studies have shown that intracellular \( \text{PO}_2 \) remains constant during incremental exercise to exhaustion, and, thus, it would be concluded that oxygen supply does not cause elevated net muscle lactate efflux (Richardson, Noyszewski, Leigh, & Wagner, 1998). Richardson et al. demonstrated that net muscle lactate efflux is independent of intracellular \( \text{PO}_2 \). However, in hypoxic conditions, where intracellular \( \text{PO}_2 \) is lower than in normoxia, the rate of lactate efflux increases, indicating that oxygen supply is a critical determinant of lactate production. NIRS measurements depend on oxygen supply and demand and, thus, may provide further insight and evidence for the role of oxygen supply in determining the rate of net muscle lactate efflux despite maintenance of intracellular \( \text{PO}_2 \).

To draw meaningful conclusions about potential changes in muscle \( \text{StO}_2 \), both the validity and reliability of the instrument used to assess \( \text{StO}_2 \) must be known. Mancini et al. (1994) demonstrated that NIRS is a valid tool for determining muscle oxygen use. Mancini and associates found NIRS to be highly correlated \((r = .92)\) with direct measurements of venous oxygen saturation and minimally altered by changes in skin blood flow. This group also demonstrated that NIRS reflects alterations in limb perfusion and is derived from deoxygenated hemoglobin rather than myoglobin. To date, no one has examined the reliability of NIRS as a measure of \( \text{StO}_2 \) or have any studies used a large sample size to examine the relationship of NIRS to other cardiorespiratory variables and its use for determining maximal steady state. Therefore, the primary purposes of this study were to: (a) determine the test-retest reliability of NIRS measurements of venous oxygen saturation of hemoglobin in the gastrocnemius of trained runners and vastus lateralis of trained cyclists during an incremental exercise test to exhaustion, (b) compare the reliability of \( \text{StO}_2 \) to the reliability of oxygen consumption, heart rate, and velocity at lactate threshold (Pfitzinger & Freedson, 1998; Weltman et al., 1990), (c) examine the relationships among oxygen consumption, heart rate, blood lactate, work intensity, and \( \text{StO}_2 \); and (d) examine whether a breakpoint in \( \text{StO}_2 \) predicted the same lactate threshold work intensity as that determined by the breakpoint in blood lactate (HLa) or the individual anaerobic threshold (IAT), as defined by Stegmann, Kindermann, and Schnabel (1981).

**Method**

**Participants**

This study was approved by the Human Subjects Committee at a large southeastern university. Twenty-five runners (19 men and 6 women) and 21 cyclists (11 men and 10 women) volunteered for this study. Two male runners were excluded from the study due to an error in the NIRS signal.

Participants were recruited from National Collegiate Athletic Association Division I athletic teams and competitive track and cycling clubs throughout the local community. All participants were involved in their mode of activity at least 5 days per week for 30 min or more per day. Prior to beginning the study, all participants provided written informed consent and completed health history forms. Participants were excluded from the study if there were any contraindications to exercise noted by the laboratory staff and if they did not meet the activity level requirements. None of the participants recruited were excluded for these reasons. Each participant completed two incremental lactate threshold (LT) and maximal oxygen consumption (VO\(_{2\text{max}}\)) tests to exhaustion, with 5–7 days separating the first and second tests. Participants were blinded to the results of their tests until after completing the second trial.
Testing Protocol

Lactate Threshold/VO₂ max Protocol (Running). Prior to testing, each participant was asked to observe a day of rest from all physical activity. Participants were instructed to consume the same meal (at least 50 g of carbohydrate) 2 hr prior to the test and report this to the laboratory staff. On arrival at the laboratory, a pre-exercise blood lactate sample was taken following a 10-min seated resting period. Participants were then fitted with a heart rate monitor and measured for height and weight in their exercise attire. They wore the same exercise attire for both tests. The treadmill protocol was initiated at a treadmill velocity corresponding to 2.4 km/h below the participant’s known race pace for a 10 km run with a speed increase of 0.8 km/h at each 3-min stage. Measurements for oxygen uptake (ml·kg⁻¹·min⁻¹), heart rate (HR), blood lactate (mmol·L⁻¹), and muscle oxygen saturation (StO₂) were recorded at each stage. LT was identified by the method of Stegmann et al. (1981), which has been demonstrated to be objective and reliable for determining the IAT from the blood lactate curve obtained during exercise and recovery. A horizontal line from the lactate value corresponding to the last stage of exercise intersects the recovery curve. From this intersection, a tangent is drawn from the recovery curve to the point at which it intersects the exercise curve. This is identified as the IAT. The highest VO₂ observed, averaged over 1 min (four consecutive 15-s intervals) was designated as VO₂ max. Criteria for achieving VO₂ max were three of the following: (a) a plateau in oxygen consumption with an increase in exercise intensity (as defined by a 2 ml·kg⁻¹·min⁻¹ increase), (b) a respiratory exchange ratio ≥1.05, (c) a heart rate ≥85% of an age-predicted maximum (as determined by 220 - participant’s age), (d) a blood lactate concentration ≥8 mmol·L⁻¹, and (e) voluntary cessation of the test by the participant (Davis, 1995; Howley, Basset, & Welch, 1995). Muscle oxygen use was determined by a 25-mm NIRS probe (Hutchinson Technology Inc., Hutchinson, MN) placed on the participant’s left lateral gastrocnemius and secured by adhesive tape. The depth of tissue measured is directly related to the distance between the illumination and detection fibers; thus, the spacing between the illumination and detection fibers with a 25-mm probe indicates that 95% of the detected signal is from a depth of 0–23 mm. The NIRS signal was collected every 3 s throughout the test, and StO₂ was determined based on a ratio of the second derivative of the changes in oxyhemoglobin and deoxyhemoglobin concentrations measured at 720 and 760 nm. The ratio of this second derivative is empirically scaled to hemoglobin saturation, which the instrument uses to calculate the reported StO₂ values (Investigator’s Brochure, Hutchinson Technology, 2004). Before each test, the NIRS probe was calibrated to a known wavelength equivalent to an StO₂ measurement of 49 ± 2%.

Lactate Threshold/VO₂ max Protocol (Cycling). Preparation for testing was the same as for runners. Participants cycled on a Lode cycle ergometer (Excaliber Sport, Groningen, The Netherlands). The protocol was initiated at 100 W and 90–100 rpm for men and 75 W at 80–90 rpm for women. Workload was increased 8 W every 3 min for men and 5 W every 3 min for women until participants reached exhaustion. Criteria for determining LT and VO₂ max were the same as for the treadmill tests. Testing also ended if participants did not maintain cadence, which was defined as 10 rpm below the set lower limits for the participant’s sex. StO₂ was determined during cycling by securely placing the NIRS probe on the lateral portion of the vastus lateralis midway between the participant’s knee joint and major axis of the thigh. These muscles were chosen in the runners and cyclists due to the predominance of their involvement during these activities (Bijker, de Groot, & Hollander, 2002; Miyashita, Kanemiza, & Nemoto, 1981).

Metabolic Measurements. Oxygen consumption was determined by an automated metabolic analysis system (TrueMax® 2400; ParvoMedics, Sandy, UT) and recorded every 15 s. Prior to each test, gas analyzers were calibrated with known oxygen and carbon dioxide concentrations (16% O₂, 4% CO₂), and flow volume was calibrated with a known 3-L syringe. Blood lactate samples were obtained from finger-sticks of the left hand. Blood samples were collected in heparinized tubes and immediately analyzed by an automated lactate analyzer (YSI 2400 Stat; YSI, Inc., Yellow Springs, OH). Heart rate was determined by telemetry (Polar Electro Inc., Oulu, Finland).

Statistical Analysis

A one-way repeated measures analysis of variance (ANOVA) was used to determine whether Trials 1 and 2 were significantly different from one another (p < .05) for each dependent variable. An intraclass correlation coefficient (and 95% CI) obtained from a one-way ANOVA was used to determine the reliability of all dependent variables. The root mean square error (RMSE) was also reported to indicate the level of measurement precision.

Participants were divided into quartiles based on the runners’ velocity or cyclists’ power output at lactate threshold to examine whether a breakpoint in muscle StO₂ could determine the work intensity at which lactate threshold occurred. A breakpoint was defined as a change in linearity ≥2 standard errors when plotting StO₂ versus velocity or power output. For those demonstrating a breakpoint in StO₂, this exercise intensity was compared to that determined by HLa breakpoint and LT as defined by Stegmann et al. (1981). A one-way repeated measures ANOVA was used to reveal differences between the methods for determining work intensity at the lactate thresh-
old. Statistical power was determined for detecting important practical differences. Power was used to assess practical differences based on the apparent large differences in means and standard deviations. Pearson product-moment correlations were used to examine the relationships at lactate threshold and maximal effort between $\text{StO}_2$, $\text{VO}_2$, and HR for each group. Pearson product-moment correlations were also used to compare the relationship between the work intensities corresponding to the lactate threshold obtained by HLa breakpoint, the IAT, and the breakpoint in $\text{StO}_2$. This was done to examine the validity of using the $\text{StO}_2$ breakpoint to measure work intensity corresponding to the lactate threshold. The associated coefficient of determination ($r^2$) was used to determine the shared variance of the three methods for determining the work intensity corresponding to the lactate threshold. Statistical analyses were conducted using SPSS Version 10.0. Due to the number of correlations tested, the significance level was set at $p < .005$ to correct for Type I errors.

**Results**

Participants’ descriptive characteristics are reported in Table 1, while the means and standard deviations for $\text{VO}_2$, HR, HLa, and $\text{StO}_2$, at LT and $\text{VO}_2$ max are reported in Table 2. Results of a one-way repeated measures ANOVA for each variable at LT and $\text{VO}_2$ max indicated nonsignificant differences between Trials 1 and 2 for all tests. Single trial reliability coefficients ($R$), 95% confidence intervals (CI), and RMSE for all measures at LT and $\text{VO}_2$ max are presented in Table 3 for runners and cyclists. For runners, reliability ranged from $R = .71$ for lactate at LT to $R = .99$ for speed at LT. Reliability was lower for cyclists, ranging from $R = .51$ for lactate at LT to $R = .99$ for $\text{StO}_2$ at $\text{VO}_2$ max.

Breakpoints for $\text{StO}_2$ were identified in 11 of 23 runners and 18 of 21 cyclists. When the groups were divided into quartiles based on velocity for runners and power output among cyclists at LT, the presence or absence of a $\text{StO}_2$ breakpoint was not dependent on velocity or power output at LT. ANOVA revealed no differences among HLa, IAT, and $\text{StO}_2$ breakpoint for determining the work intensity corresponding to the lactate threshold for either runners ($p = .245$) or cyclists ($p = .064$). However, the power of these data is quite low (runners: HLa vs. IAT = .10, HLa vs. $\text{StO}_2 = .35$, IAT vs. $\text{StO}_2 = .38$; cyclists: HLa vs. IAT = .20, HLa vs. $\text{StO}_2 = .40$), with the exception being IAT and $\text{StO}_2$ for determining LT in cyclists (.90). Thus, it may be possible that a Type II error occurred. Means and standard deviations for each group and method are provided in Table 4.

Pearson product-moment correlations and coefficients of determination were used to examine relationships between HLa, IAT, and $\text{StO}_2$ breakpoint in runners

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### Table 1. Descriptive characteristics of the participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Female runners ($n = 6$)</th>
<th>Male runners ($n = 11$)</th>
<th>Female cyclists ($n = 10$)</th>
<th>Male cyclists ($n = 11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.3 ± 5.2</td>
<td>22.4 ± 3.6</td>
<td>25.3 ± 6.2</td>
<td>25.5 ± 3.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.3 ± 7.4</td>
<td>178.1 ± 9.4</td>
<td>165.6 ± 6.9</td>
<td>178.1 ± 5.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.7 ± 4.4</td>
<td>72.7 ± 8.8</td>
<td>61.5 ± 8.0</td>
<td>78.7 ± 11.4</td>
</tr>
</tbody>
</table>

**Note.** $M$ = mean; $SD$ = standard deviation.

### Table 2. Means and standard deviations for cardiovascular measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Runners’ LT</th>
<th>Runners’ $\text{VO}_2$ max</th>
<th>Cyclists’ LT</th>
<th>Cyclists’ $\text{VO}_2$ max</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{VO}_2$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>45.1 ± 11.4</td>
<td>53.4 ± 8.6</td>
<td>38.5 ± 11.3</td>
<td>47.1 ± 11.4</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>182 ± 11</td>
<td>195 ± 10</td>
<td>166 ± 12</td>
<td>186 ± 8</td>
</tr>
<tr>
<td>HLa (mmol·L$^{-1}$)</td>
<td>3.5 ± 0.4</td>
<td>6.3 ± 1.9</td>
<td>3.6 ± 0.4</td>
<td>7.6 ± 1.8</td>
</tr>
<tr>
<td>$\text{StO}_2$ (%)</td>
<td>43.8 ± 14.7</td>
<td>39.7 ± 14.0</td>
<td>39.6 ± 17.1</td>
<td>26.2 ± 16.0</td>
</tr>
<tr>
<td>Velocity (km/h/W)</td>
<td>14 ± 1.0</td>
<td>193.7 ± 60.0</td>
<td>261.2 ± 63.5</td>
<td></td>
</tr>
</tbody>
</table>

**Note.** LT = lactate threshold; $\text{VO}_2$ max = maximal oxygen consumption; $M$ = mean; $SD$ = standard deviation; $\text{VO}_2$ = oxygen consumption; HR = heart rate; HLa = blood lactate; $\text{StO}_2$ = muscle oxygen saturation.

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for the work intensity corresponding to the lactate threshold. There was a significant relationship in the running group between HLa and IAT (r = .99, r² = .98, respectively), HLa and StO₂ (r = .91, r² = .83, respectively) and IAT and StO₂ (r = .90, r² = .81, respectively), indicating that the shared variance was 98%, 89%, and 81%, respectively. For the cycling group, there was a significant relationship between HLa and IAT (r = .99, r² = .98, respectively), indicating a shared variance of 98%, while a nonsignificant relationship was found between HLa and StO₂ (r = .44, r² = .19, respectively) and IAT and StO₂ (r = .44, r² = .19, respectively), indicating a low shared variance of 19% between measures of blood lactate and muscle StO₂ in cyclists.

Pearson product-moment correlations were used to examine the relationships at lactate threshold and maximal effort between StO₂, VO₂ max, HR, and velocity among runners and power output among cyclists. For runners, there was a significant positive correlation between velocity at LT and VO₂ at LT (r = .70) and velocity at LT and VO₂ max (r = .72). For the cycling group, there was a significant positive correlation between power output at LT and VO₂ at LT (r = .96) and power output at LT and VO₂ max (r = .89). Maximal power output and VO₂ at LT and VO₂ max also demonstrated positive correlations of r = .94 and r = .92, respectively.

### Table 3. Reliability, 95% confidence intervals, and correlation coefficients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Runners (R)</th>
<th>CI</th>
<th>RMSE</th>
<th>Cyclists (R)</th>
<th>CI</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>StO₂ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>.87</td>
<td>.72–.94</td>
<td>5.3</td>
<td>.94</td>
<td>.87–.98</td>
<td>4.1</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>.88</td>
<td>.73–.95</td>
<td>5.0</td>
<td>.99</td>
<td>.98–.99</td>
<td>1.6</td>
</tr>
<tr>
<td>VO₂ (mL·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>.97</td>
<td>.93–.99</td>
<td>2.0</td>
<td>.98</td>
<td>.95–.99</td>
<td>0.1</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>.98</td>
<td>.95–.99</td>
<td>1.0</td>
<td>.99</td>
<td>.97–.99</td>
<td>0.1</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>.94</td>
<td>.88–.97</td>
<td>2.7</td>
<td>.95</td>
<td>.87–.98</td>
<td>2.8</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>.92</td>
<td>.83–.97</td>
<td>2.7</td>
<td>.93</td>
<td>.84–.97</td>
<td>2.0</td>
</tr>
<tr>
<td>Lactate (mmol·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>.71</td>
<td>.43–.86</td>
<td>0.2</td>
<td>.51</td>
<td>.12–.76</td>
<td>0.2</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>.97</td>
<td>.94–.99</td>
<td>0.3</td>
<td>.97</td>
<td>.92–.99</td>
<td>0.3</td>
</tr>
<tr>
<td>Velocity (mph) /W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>.99</td>
<td>.98–.99</td>
<td>0.13</td>
<td>.98</td>
<td>.96–.99</td>
<td>7.7</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>.99</td>
<td>.98–.99</td>
<td>5.5</td>
<td>.99</td>
<td>.98–.99</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Note: CI = confidence interval; RMSE = root mean square error; StO₂ = muscle oxygen saturation; LT = lactate threshold; VO₂ max = maximal oxygen consumption; HR = heart rate.

### Table 4. Comparison of the velocity among runners and power output among cyclists as determined by StO₂ breakpoint, HLa breakpoint, and IAT

<table>
<thead>
<tr>
<th>Method</th>
<th>Runners (km/h) M (SD)</th>
<th>Cyclists (W) M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>StO₂</td>
<td>12.0 (2.4)</td>
<td>152 (30)</td>
</tr>
<tr>
<td>HLa</td>
<td>13.3 (2.2)</td>
<td>175 (51)</td>
</tr>
<tr>
<td>IAT</td>
<td>13.9 (3.5)</td>
<td>191 (47)</td>
</tr>
</tbody>
</table>

Note: StO₂ = muscle oxygen saturation; HLa = breakpoint in blood lactate; IAT = individual anaerobic threshold; M = mean; SD = standard deviation; no significant differences were found between the methods in runners or cyclists.

### Discussion

The reliability of NIRS as a measure of StO₂ in the runners' gastrocnemius was high (R = .87 at LT and .88 at VO₂ max) but the lower limit of the CI is slightly lower than desirable (≥ .80). The reliability of NIRS as a measure of StO₂ in the cyclists' vastus lateralis during exercise was high, even considering the lower limit of the 95% CI. These results indicate that using a single trial protocol to determine StO₂ is sufficient. The RMSEs of these measurements are also acceptable and should be used to place an error band around an individual score, particularly when examining the treatment effects of an intervention.

In agreement with prior studies (Fitzinger & Freedson, 1998; Weltman et al., 1990), the reliability coefficients for VO₂, HR, maximal HLa values, and work performed were also high at both LT and VO₂ max for runners and cyclists. However, reliability of blood lactate at LT for both runners and cyclists was unacceptable, particularly given the lower limit of the 95% CI. While results from this study and that of Coen, Urhausen, and Kindermann (2001) have found the reliability of blood lactate at LT to be low when using IAT, the reliability for velocity and power output at LT and HR at LT was high. When using IAT for training purposes, the heart rate corresponding to the exercise intensity associated with the lactate threshold is important, not a specific lactate concentration (Coen et al., 2001; Stegmann et al., 1981).

Correlation analyses did not reveal any significant relationships between StO₂ and the other cardiorespiratory fitness measures. However, there was a significant relationship (r = .70–.96) between VO₂ at (LT and VO₂ max) and velocity among runners and power output among cyclists at LT. This agrees with the studies by Weltman et al. (1990) and Yoshida, Suda, and Takeuchi (1982) who also demonstrated a strong relationship between these variables. The significant correlations be-
between velocity and power output at the LT and VO₂ at LT and maximal effort can also be attributed to the heterogeneity of the runners and cyclists tested. Some participants were recreational club athletes, some competed at the collegiate level, and some were competitive amateur athletes. Ranges of physical abilities were intentionally recruited to infer results for various ability levels. However, because heterogeneity of a sample can inflate both reliability and correlations, caution should be used when applying these findings to a more homogenous group.

VO₂ kinetics has been studied extensively to understand the muscle's response to the supply and demand for oxygen during exercise (Barstow, Buchthal, Zanconato, & Cooper, 1994; Chuang et al., 2002; Grassi et al., 1996; Xu & Rhodes, 1999). A prior study by Kawaguchi, Tabusadani, Sekikawa, Hayashi, and Onari (2001) found that the peripheral muscle oxygenation of the vastus lateralis measured by NIRS was reflective of systemic VO₂ during an exercise test to exhaustion, participants demonstrated a significant negative correlation between oxygenated hemoglobin and VO₂ (from r = -0.73 to -0.98). While this has also been found by others, (Belardinelli et al., 1994; Grassi et al., 1999), the current study did not. One reason may be that the studies by Belardinelli et al. (1994), Grassi et al. (1999), and Kawaguchi et al. (2001) used a correlation in a design with multiple testing stages; thus, each participant would have multiple scores for the independent variable and multiple scores for the dependent variable. This use of a correlation violates the assumption of independence and may result in a spurious correlation due to the relationship of increased workloads with the variables being examined.

When groups were divided into quartiles based on velocity among runners and power output among cyclists at HLA breakpoint and LT, the lack of an STO₂ breakpoint in at least one individual was evident for each quartile, indicating that physical capabilities were not a determinant for a breakpoint in STO₂. The only exception was the 50th percentile in the cycling group. The relationship of the variables VO₂, HR and STO₂ are displayed in Figures 1–3 and demonstrate the varied response seen in STO₂ during the incremental test to exhaustion. STO₂ was compared to VO₂ and HR, because these two variables display a linear relationship to one another and are the most common measures for examining changes in cardiorespiratory fitness (Spurr et al., 1988). Eight runners and 18 cyclists who demonstrated a breakpoint in STO₂ had a steady decline in venous oxygenated hemoglobin as work intensity, VO₂, and HR increased (see Figure 1); other reports examining the O₂ kinetics of the vastus lateralis and gastrocnemius during incremental exercise testing also observed the steady decline in STO₂ with increased exercise intensity (Belardinelli et al., 1994; Bhambhani et al., 1997; Grassi et al., 1999; Kawaguchi et al., 2001).

In contrast to this, the relationship of STO₂, VO₂, and HR was not found in 3 of the 11 runners who demonstrated a breakpoint (see Figure 2). At exercise intensities above the LT, the NIRS response is reported to vary between participants; either reoxygenation or continued deoxygenation is seen (Chuang et al., 2002). This

![Figure 1](image-url)

**Figure 1.** A participant for whom a muscle oxygen saturation (STO₂) breakpoint was identified and an inverse relationship to oxygen consumption (VO₂) and heart rate was found.
may reflect the participants' training. When Chance et al. (1992) examined individual participants, it was suggested that those who showed little desaturation and rapid resaturation with low lactate values could increase oxygen extraction and performance through training (see Figure 3). This may explain the varied response observed, as the training phase was different between participants. Some participants had just completed a competitive season (European outdoor track and cycling circuit), while others were performing base training (cross

![Figure 2](image1.png)

**Figure 2.** A participant for whom a muscle oxygen saturation ($\text{StO}_2$) breakpoint was identified, but the inverse relationship to oxygen consumption ($\text{VO}_2$) and heart rate was not found.

![Figure 3](image2.png)

**Figure 3.** A participant for whom a muscle oxygen saturation ($\text{StO}_2$) breakpoint could not be identified.
country, indoor track, road cyclist). However, this still does not explain why some participants did not demonstrate a 
$\text{StO}_2$ breakpoint. Although muscle biopsies were not taken in the present study, another possibility for the differences may be the proportion of oxidative, slow-twitch Type I fibers and glycolytic fast-twitch type IIb fibers in the participants' respective muscles and the muscles chosen for measurement. Type I fibers have a high oxidative capacity due to a high mitochondrial and capillary density and, therefore, have a greater ability to use oxygen. Conversely, fast-twitch muscle fibers rely on anaerobic processes and are associated with a lower oxidative capacity and capillary density. While this would not affect the reliability of the measurements taken, the relationship between 
$\text{StO}_2$ and oxygen consumption may have been affected in those individuals with a greater proportion of fast-twitch muscle fibers and may provide an additional reason for the differences seen.

Another confounding factor that may explain why some participants did not demonstrate a breakpoint in 
$\text{StO}_2$ is the effect of adipose tissue thickness on the NIRS signal. The vanBeekvliet, Borghuis, vanEngelen, Wevers, and Colier (2001) study reported that muscle oxygen consumption, as measured by NIRS, is negatively correlated ($r = -.70$, $p < .01$) to adipose tissue thickness, indicating that adipose tissue is a confounding factor in obtaining valid NIRS measurements. Because we did not measure skinfold thickness at the measurement sites, it is possible some participants did not obtain breakpoints in 
$\text{StO}_2$ because of this factor. However, it must be noted that all of our participants were regularly active in their sport and did not appear to display large amounts of body adiposity at the measurement sites. Other reasons we believe body adiposity was not the only factor accounting for participants not obtaining a breakpoint in 
$\text{StO}_2$, was that in almost every quartile at least one individual did not display a breakpoint in 
$\text{StO}_2$. In distance running and road cycling, it is well known that low body adiposity levels are associated with a high velocity and power output, and, thus, in these quartiles, it would be expected that, if adipose tissue thickness was the confounding factor, all participants in the top quartile would have demonstrated a breakpoint in 
$\text{StO}_2$. This was not the case and suggests that other factors, such as muscle fiber composition, blood hemoglobin concentrations, skin temperature, and the ratio of capillary density to muscle fiber cross-sectional area, may also influence the NIRS values obtained and warrant further investigation.

The present study has several assumptions that must also be addressed and may affect the results obtained by NIRS. The first is that the measurements obtained are derived from hemoglobin and not myoglobin. There has been significant debate regarding the role of hemoglobin and myoglobin in the oxygen saturation values obtained through NIRS. However, research by Mancini et al. (1994) demonstrated, through $^1$H-proton spectroscopy, that the measurement obtained through NIRS resulted from continued hemoglobin deoxygenation without any concomitant deoxygenation of myoglobin. Additionally, the values obtained through NIRS were highly correlated to venous hemoglobin oxygen saturation. Thus, it can be assumed that the measurements obtained in this study were derived from hemoglobin and not myoglobin. Second, an assumption of the current study was that the respective muscles measured for the runners and cyclists were recruited throughout the incremental exercise test. Miyashita et al. (1981) and Bijker et al. (2002) reported that the integrated electromyographic response of the vastus lateralis during an incremental cycling exercise was linear from rest to exhaustion. Chance et al. (1992) reported a plateau in deoxygenation levels of the vastus lateralis during maximal exercise. Bijker et al. (2002) additionally reported that during running (especially at an incline) the gastrocnemius displayed the greatest electromyographic activity to workload relationship. Together, these observations indicate that using the gastrocnemius in runners and the vastus lateralis in cyclists to assess 
$\text{StO}_2$ are appropriate measurements.

In both the field and lab setting, lactate threshold has traditionally been identified by analyzing blood lactate measurements made at the end of each stage during an incremental exercise test to exhaustion. The lactate threshold is the workload at which the production and clearance of lactate are equal. Several groups have demonstrated that this work intensity can be used to control training in endurance athletes and provides adaptations that improve the work intensity at which LT occurs (Coen, Scharz, Urhausen, & Kindermann, 1991; Keith, Jacobs, & McLellan, 1992). Grassi et al. (1999) defined LT as an increase in blood lactate concentration $>$ 0.5 mmol-L$^{-1}$ and found the breakpoint in 
$\text{StO}_2$ to coincide with the “point of lactate inflection.” Recent work by Snyder and Parmenter (2002) suggested that NIRS determination of an 
$\text{StO}_2$ breakpoint appeared to be a noninvasive technique comparable to HLa breakpoint in determining maximal steady-state exercise intensity. Snyder and Parmenter determined an 
$\text{StO}_2$ breakpoint in 14 of 16 athletes during a treadmill test to exhaustion. In the current study, 11 of 23 runners and 18 of 21 cyclists demonstrated a 
$\text{StO}_2$ breakpoint. Why a breakpoint was not seen in the other 13 runners and 3 cyclists is not clear. In the present study, a breakpoint in 
$\text{StO}_2$ was defined as a change in percentage of saturation $\geq$ 2 standard errors, which is an approximate change of 10.6% in the runners and 8.2% in the cyclists, thus providing a 95% confidence interval around the values seen. In prior studies, the first change in linearity when plotting 
$\text{StO}_2$ versus exercise intensity was defined as a breakpoint and may have taken into account the standard error of the equipment being used.
(Belardinelli et al., 1994; Bhambani, Buckley, & Susaki, 1997; Grassi et al., 1999; Kawaguchi et al., 2001; Snyder & Parmenter, 2002). In the present study, these participants displayed the same response on two different occasions, indicating a true absence of an ST\textsubscript{O2} breakpoint.

In both runners and cyclists for whom a breakpoint was identified, ST\textsubscript{O2} consistently indicated a lower work intensity for maximal steady state when compared to that determined by HLA and LAT (see Table 4). Prior work by Belardinelli et al. (1994) and Bhambani et al. (1997) have also provided evidence that the LT and ventilatory threshold provided by NIRS occurs earlier than that provided by the V-slope method for determining LT and HLA breakpoint. Because NIRS evaluates changes in oxygenation at the muscle, whereas determining LT or VT depends on measures of diffusion time and gas exchange, it makes sense that the changes seen in muscle oxygenation may occur prior to this because of the time required for lactate to diffuse from the cell into the blood and produce an excess of CO\textsubscript{2}. Further work is necessary to determine the relationship between muscle oxygenation and blood lactate concentration during exercise at and above maximal steady state, as only the study by Snyder and Parameter (2002) have compared these measures.

Conclusion

The findings of this study indicate that NIRS is a reliable instrument for measuring the oxygen saturation of venous hemoglobin during treadmill running and cycling. While a strong correlation was not found with other cardiorespiratory fitness measures, it can be used to monitor changes over time in the muscle’s ability to use oxygen. A breakpoint in ST\textsubscript{O2} was not found in all participants, which may be due to the proportion of oxidative slow-twitch Type-I muscle fibers in the participants’ respective muscles. This deserves further investigation with more homogenous groups and repeated tests for determining the work intensity corresponding to the lactate threshold.

Near-infrared spectroscopy provides a noninvasive tool that can assess changes in the oxygen saturation of exercising muscles. This can be useful for providing insight to peripheral and central changes possibly resulting from training, injury, nutritional status, or the environment that can affect the performance of the cardiorespiratory system. It is concluded that near-infrared spectroscopy is a reliable instrument for measuring the oxygen saturation of venous hemoglobin at LT and VO\textsubscript{2}max during running and cycling, with standard errors ranging from 1.6 to 5.3%. Near-infrared spectroscopy appears to be an additional tool that can be used when examining exercise intensity and the kinetics of peripheral muscle oxygenation.

References


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