Noninvasive determination of exercise-induced hydrodgen ion threshold through direct optical measurement

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Soller BR, Yang Y, Lee SM, Wilson C, Hagan RD. Noninvasive determination of exercise-induced hydrodgen ion threshold through direct optical measurement. J Appl Physiol 104: 837–844, 2008. First published December 20, 2007; doi:10.1152/japplphysiol.00849.2007.—The intensity of exercise above which oxygen uptake (\(\dot{V}\)O\(_2\)) does not account for all of the required energy to perform work has been associated with lactate accumulation in the blood (lactate threshold, LT) and elevated carbon dioxide output (gas exchange threshold). An increase in hydrogen ion concentration ([H\(^+\)]) is approximately concurrent with elevation of blood lactate and CO\(_2\) output during exercise. Near-infrared spectra (NIRS) and invasive interstitial fluid pH (pH\(_m\)) were measured in the flexor digitorum profundus during handgrip exercise to produce a mathematical model relating the two measures with an estimated error of 0.035 pH units. This NIRS pH\(_m\) was subsequently applied to spectra collected from the vastus lateralis of 10 subjects performing an incremental-intensity cycle protocol. Muscle oxygen saturation (SmO\(_2\)) was also calculated from spectra. We hypothesized that a H\(^+\) threshold could be identified for these subjects and that it would be different from but correlated with the LT. Lactate, gas exchange, SmO\(_2\), and H\(^+\) thresholds were determined as a function of VO\(_2\) using bilinear regression. LT was significantly different from both the gas exchange threshold (\(\Delta = 0.27 \pm 0.29\) l/min) and H\(^+\) threshold (\(\Delta = 0.29 \pm 0.23\) l/min), but the gas exchange threshold was not significantly different from the H\(^+\) threshold (\(\Delta = 0.00 \pm 0.38\) l/min). The H\(^+\) threshold was strongly correlated with LT (\(R^2 = 0.95\)) and the gas exchange threshold (\(R^2 = 0.85\)). This initial study demonstrates the feasibility of noninvasive pH\(_m\) estimations, the determination of H\(^+\) threshold, and the relationship between H\(^+\) and classical metabolic thresholds during incremental exercise.

pH; anaerobic threshold; near-infrared spectroscopy; cycling; lactate; carbon dioxide

Historically, the term anaerobic threshold (AT) has been used to describe a shift in muscle metabolism during incremental exercise from predominantly aerobic energy pathways to a greater reliance on anaerobic exercise sources, but the central concepts of this threshold have been the subject of debate over the last 20 years (5, 9). Recently, AT has been defined by Svedahl and MacIntosh (32) as an intensity of exercise above which oxygen uptake does not account for all of the required energy to perform work and has been associated with lactate accumulation in the blood, elevated carbon dioxide output, and increased ventilatory rate. The lactate threshold (LT) is identified as the level of exercise where there is an abrupt increase in blood lactate concentration as exercise intensity increases. Corrective respiratory changes can also be identified through the measurement of pulmonary CO\(_2\), by determining either the ventilatory threshold (VT) or a change in slope of the rate of CO\(_2\) output through the V-slope method (gas exchange threshold) (3, 8, 35). These methods have been successfully used in clinical exercise testing (36), are widely used as predictors of aerobic endurance performance, and are considered indexes of the level of exercise capacity (10, 22, 24).

A comparison of the measurement techniques has shown that under some conditions the gas exchange threshold is different from LT (13, 14, 22, 42). Underlying this difference are disparities in the biochemical mechanisms that result in lactate accumulation and CO\(_2\) output during exercise. Lactate accumulates in the blood when its rate of consumption is overshadowed by its rate of production (6). Wasserman et al. describe the threshold in the rate of CO\(_2\) output identified with the V-slope method as the result of bicarbonate buffering of hydrogen ions produced with the lactate (35), although the concept of bicarbonate buffering has been questioned (18). H\(^+\) is transported from the muscle cell stoichiometrically with lactate through the monocarboxylate transporter. Moderate and intense exercise are marked by a significant reduction in cellular and blood pH (31, 33).

Near-infrared light passes through skin and subcutaneous fat and can be used to noninvasively measure metabolic parameters in muscle. Near-infrared spectroscopy (NIRS) is most commonly used to measure capillary oxygen saturation in muscle (SmO\(_2\)) (20); however, we have shown that in the setting of local hypovolemia and ischemia, NIRS also can be used to noninvasively and continuously measure muscle, or interstitial fluid, pH (pH\(_m\)) (26, 43). We hypothesize that this methodology can be extended to determine a threshold based on the accumulation of hydrogen ions in the muscle interstitial fluid during graded exercise, the H\(^+\) threshold. We also hypothesize that the H\(^+\) threshold will be different from, but correlated with, the LT. In this paper we describe a method for determining pH\(_m\) from spectra acquired noninvasively during handgrip exercise and the application of the derived mathematical model to noninvasively determine H\(^+\) threshold during graded cycle ergometry. We also present comparisons between H\(^+\) threshold and classical metabolic thresholds (lactate and gas exchange), as well as NIRS-determined SmO\(_2\) thresholds in 10 exercising subjects.

MATERIALS AND METHODS

Overall protocol. This project had two components, with human studies conducted at two different centers. Handgrip dynamometry

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Innovative Methodology

NONINVASIVE H⁺ THRESHOLD FROM NIRS

tests conducted at the University of Massachusetts Medical School in Worcester, MA, were intended to develop and validate the pHm model for the NIRS-derived measurements. Cycle ergometry tests conducted at National Aeronautic and Space Administration-Johnson Space Center (NASA-JSC) in Houston, TX, were intended to determine whether these pHm measures could be used as a noninvasive method to determine the H⁺ threshold. In each case, study volunteers received a verbal and written explanation of the procedures to be performed and provided written informed consent before participation. Testing performed at the University of Massachusetts Medical School was reviewed and approved by the University of Massachusetts Medical School Institutional Review Board for Human Studies; testing performed at the NASA-JSC was reviewed and approved by the NASA-JSC Committee for the Protection of Human Subjects.

Noninvasive determination of muscle pH: measurements to derive calibration model. In this study NIR spectra were continuously collected from eight adult subjects (4 men/4 women) during rhythmic handgrip exercise using the right hand. Simultaneously, pHm was measured by a small fiber-optic sensor (Paratrend 7 Plus, Diametrics Medical, St. Paul, MN) previously inserted under sterile conditions into the right flexor digitorum profundus (FDP) muscle. This sensor measures pH, PO₂, and PCO₂ of the fluid in which it rests with three separate fiber sensors. Measurements with this sensor during handgrip exercise have been described previously (28), and identical methods were used. Briefly, the sensors were calibrated before use in a separate calibration unit (Diametrics Medical) using three different calibration gases (CO₂– O₂–N₂ = 2%-0%-98%, 7%-15%-78%, and 22.5%-50%-27.5%). The right FDP muscle was located with ultrasound (SonoSite 180, SonoSite Corp, Bothell, WA). A 20-gauge catheter was placed under sterile conditions into the FDP at an angle of ~20° and ~3 cm deep into the muscle. The needle was then removed from the catheter, and the sensor was threaded into the catheter lumen. Finally, the catheter was withdrawn to the skin level, leaving only the sensor in the muscle. Location of the sensor in the FDP was confirmed with ultrasound after placement. Intramuscular measurements of pH, PCO₂, and PO₂ were recorded continuously at 2-s intervals throughout the rest, exercise, and recovery periods.

After placement of the Paratrend sensor, a custom-fabricated spectroscopic sensor (Luxtec, West Boylston, MA) was placed on the skin surface overlying the FDP. The spectroscopic sensor head contained two fiber bundles, spaced 30 mm apart. A 4-mm-diameter fiber bundle transmitted white light to the skin surface, and a 1-mm-diameter fiber bundle transmitted the received light to the spectrometer (USB2000, Ocean Optics, Dunedin, FL). The white light source was a custom-built fixture using an 8-W tungsten-halogen bulb (model 8106-001, Welch Allyn, Skaneateles, NY). OOIBase32 software (Ocean Optics) was used to acquire the reflected photon count spectra, which were saved every 3 s.

For each subject in the study, the integration time was selected to maximize the sample count of the light reflected from the tissue to the spectrometer. Since the optimum integration time varied across the different subjects because of variations in the sampled tissue, the measured spectra from the tissue were normalized through division by the integration time across all the measurement wavelengths. Multiwavelength tissue reflectance spectra in absorbance units (A) were calculated by comparing the count rate (counts/integration time) of the detected radiation to the count rate from a 99% reference standard (model SRT-99–050, Labsphere, North Sutton, NH) and taking the log as shown in Eq. 1:

\[
A = \log 10 \left( \frac{I}{I_r} \right)
\]

where A is the absorbance and \(I_r\) and \(I\) are the count rate spectra for the reference and sample measurements, respectively. Only data in the wavelength range 725–880 nm were used for determination of pHm.

After the placement of both the Paratrend and noninvasive NIR sensor, each study subject performed four 5-min bouts of repetitive handgrip exercises with a hand dynamometer (MIE Medical Research, Leeds, UK). Each exercise bout entailed repeated cycles of 2-s contractions with intervening 1 s of relaxation. The exercise induces rapid pH changes in the FDP muscle (28). The force of contraction was prescribed according to a specified percentage of maximal voluntary contraction (MVC), which was measured before data collection. Each subject was prompted through one bout of exercise at 15% MVC followed by three rounds of exercise at 30% MVC, 45% MVC, and 30% MVC, respectively. Subjects received visual and audio feedback through a laptop computer to instruct them in the proper timing and level of effort. As exercise intensity increased, pH fell to a lower level by the end of the exercise bout. The subject rested for approximately 30–45 min between bouts, during which time the pH recovered to baseline values.

Noninvasive determination of muscle pH: calculations to derive and evaluate calibration model. Partial least-squares (PLS) regression (21) was used to develop the multisubject calibration equation (model), which correlates NIR spectra to pH values from the invasive sensor placed in the muscle of the calibration subjects. Once the model is developed, it can be applied to NIR spectra acquired from future independent subjects to predict pHm without the invasive reference pH sensor. Additional details on the PLS-determined calibration equation are contained in Appendix A.

Before the calibration model development, several preprocessing steps were performed on the spectral and/or reference pH data. Principal component analysis loading correction (PCALC) was used to correct for the subject-to-subject spectral variations, such as skin color, fat thickness, and muscle scattering. Details of the PCALC method have been described previously (39, 40), and further information is contained in Appendix A. The NIR spectral response to a step change in pHm is considerably faster (~5 s) than the corresponding response of the invasive reference sensor to the same change in pH (4 min). To correct for this, we have developed a “weighted averaging filter” from time profiles of the step changes in invasive sensor pH values that is used to match each subject’s NIRS sensor response to the corresponding invasive sensor response (25). This filter was applied, and then each subject’s NIR spectral time was matched with its invasive sensor time. For each subject, 300 spectral/reference pH pairs were selected based on spectral Euclidian distance (DISTSLCT function in PLS_Toolbox version 3.5, Eigenvector Research, Manson, WA) for the development of the multisubject tissue pH calibration model.

PLS “subject-out” cross-validation (21) analysis was performed on the preprocessed NIR tissue spectra and the reference pHm measurements to develop and validate the PLS model. During the subject-out cross-validation, one subject was selected as the test subject, and the remaining subjects were used as calibration subjects. The spectra and reference pHm from the calibration subjects were used to build a calibration model, and the model was used to predict pHm from the test subject’s spectra. This process was repeated as each subject was rotated out until every subject had been used as a test subject.

Both concentration and spectral outliers were removed if the corresponding concentration or spectral F-ratio was ≥3 (17). The prediction accuracy was described by \(R^2\) (coefficient of determination) between the estimated and actual pHm, and the estimated measurement error, which was calculated as the root mean squared error of prediction, RMSEP, described in Eq. 2:

\[
RMSEP = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (\hat{y}_i - y_i)^2}
\]

where \(N\) is the number of test samples, and \(\hat{y}_i\) and \(y_i\) are NIRS estimated and reference (invasive) pHm values, respectively. A high \(R^2\) and low RMSEP indicate good model prediction ability.
The optimal number of model factors was chosen to be the one with the fewest number of factors such that root mean square error of cross-validation (RMSECV) for that model was not significantly greater than the RMSECV for the model with the smallest RMSECV (17). The $F$-statistic was used to make the significance determination with a significance level of 0.05 (17). RMSECV was computed in the same way as RMSEP by Eq. 2 but with the number of test samples replaced by the number of calibration samples. The model accuracy was described by the average $R^2$ between the estimated and actual pHm for each left-out subject, and the RMSECV. A high average $R^2$ and low RMSECV indicate good multisubject model prediction ability.

Data analysis was performed using customized programs written in Matlab version 7.0 (Mathworks, Natick, MA) and PLS_Toolbox version 3.5 (Eigenvector Research, Manson, WA).

Leg exercise protocol and measurements. Ten healthy men and women (5/5) volunteered to participate in this study. Subjects reported to the Exercise Physiology Laboratory at NASA-JSC well rested without performing strenuous exercise within the last 24 h and without performing any exercise on the day of testing. Subjects dressed in comfortable exercise clothing that was not constrictive, permitted ease of movement while performing cycle ergometry exercise, and allowed easy access to the thigh for NIRS measurements.

Subjects performed a graded exercise test on an upright cycle ergometer (Excalibur Sport, LODE BV, The Netherlands) to maximal exertion. Before exercise, seated resting blood pressure was measured by auscultation, resting heart rate was recorded from a heart rate monitor (Polar S-810i, Polar Electro, Lake Success, NY), and two small blood samples (25 μl) were obtained from a fingertip to determine resting blood lactate concentration (BLa). BLa was determined using a standard analyzer (1500 Sport, YSI, Yellow Springs, OH). Universal precautions were followed during the collection and handling of the sample. The sample was drawn into a capillary tube and then processed according to the vendor’s protocol. Before lactate analysis in the instrument, the erythrocytes were lysed with YSI 1515 lysing agent.

A NIRS sensor of the same design as used in the handgrip dynamometry protocol was placed over the vastus lateralis muscle at the palpated midpoint between the greater trochanter (hip) and knee joint at least 10 min before the beginning of exercise. The sensor was held in place with a special mounting pad that conformed to the shape of the thigh and contained a plastic restraint that prevented the sensor from moving on the skin during exercise. Spectra were collected approximately every 10 s during the exercise protocol and processed with the pH calibration equation derived from data in the handgrip dynamometry study. In addition, the same spectra were used to calculate muscle oxygen saturation (Smo2) using methods previously published by our group (41) and described briefly in APPENDIX B.

After the initial seated rest period, subjects performed 3-min exercise stages of increasing intensity on the cycle ergometer, beginning at 50 W. The exercise intensity was increased 50 W per stage until the subject could no longer maintain a pedaling cadence of 75 rpm. Additional blood samples were drawn from the finger at the end of each stage for the measurement of BLa. After reaching their peak effort, subjects completed 5 min of exercise at 50 W as an active recovery.

Heart rate (HR) and rhythm were measured using 12-lead electrocardiography (Q-Stress, Quinton Cardiology, Bothell, WA), and oxygen uptake ($V_O_2$) was measured using a metabolic cart (TrueOne 2400, Parvo-Medics, Salt Lake City, UT). Peak $V_O_2$ ($V_O_2$ peak) was determined as the highest level of $V_O_2$ measured during a 1-min period in which at least two of the following criteria were met: respiratory exchange ratio (RER) exceeded 1.1, maximal HR was greater than 85% of the age-predicted HR maximum, and/or a plateau of the $V_O_2$ curve occurred. Subjects achieved a mean $V_O_2$ peak of 47.7 ± 10.5 ml·kg$^{-1}$·min$^{-1}$ (mean ± SD) with a mean peak HR of 185 ± 9 beats/min (98 ± 3% of age-predicted maximum) and a peak RER of 1.18 ± 0.03.

Threshold calculations and statistics. Hydrogen ion concentration was calculated from the NIRS-measured pHm with Eq. 3:

$$[H^+] = 10^{-pH} \quad (3)$$

Metabolic thresholds were defined as the $V_O_2$ at which the slope in lactate (LT), carbon dioxide production ($V_CO_2$; gas exchange threshold), and $[H^+]$ ($H^+$ threshold) increased and SmO2 decreased with incrementally greater work. Thresholds were determined with a simultaneous bilinear regression using the fitting equation

$$x = \begin{cases} y_0 + s_1(x - x_0), & x < x_0 \\ y_0 + s_2(x - x_0), & x > x_0 \end{cases} \quad (4)$$

where $x_0$ is $V_O_2$ at the threshold and $y_0$ is the corresponding value for $V_CO_2$, lactate, or $[H^+]$, and $s_1$ and $s_2$ are slopes for the linear segments. The bilinear regression for $V_O_2$ was further constrained such that $s_1$ was required to be less than 1.0 and $s_2$ was required to be greater than 1.0 as defined by the V-slope method (3).

The average values for LT, gas exchange, and $H^+$ thresholds for all subjects are reported as means ± average SE of the bilinear regression. Comparison of $H^+$ thresholds with the other two methods was assessed with a paired $t$-test with Bonferroni adjustment, with $P < 0.05$ considered significant.

RESULTS

Validation of noninvasive pH measurement during handgrip exercise. NIRS-derived pHm is a good predictor of the invasive (Paratrend) pHm measurement (Fig. 1, Table 1). Four PLS factors were used in the model (see APPENDIX A); the average coefficient of determination ($R^2$) was 0.722; three subjects had $R^2$ below 0.70, indicating that their spectra were not completely described by the model developed from the other subjects. The estimated error of measurement (RMSECV) was 0.035 pH units. The calibration equation determined with these data was used to determine pHm during the cycle exercise to calculate the $H^+$ threshold.

Metabolic threshold measurements. Subjects were 30.4 ± 4.7 yr old and 172.7 ± 9.7 cm tall, and their body mass was 68.4 ± 12.2 kg (mean ± SD). The average $V_O_2$ peak was 3.29 ± 1.04 l/min with a range of 2.26–5.04 l/min. Although metabolic gas analysis data and NIRS spectra were collected from all 10 subjects, complete blood lactate measurements were...
obtained for only 9 of the 10 subjects. Figure 2 shows data used to calculate the respective thresholds from each of the parameters for one subject.

LT (1.95 ± 0.27 l/min, n = 10) was statistically different from both the gas exchange (1.64 ± 0.08 l/min, n = 10) and H+ (1.64 ± 0.15 l/min, n = 10) thresholds. The average difference between LT and H+ threshold was 0.29 ± 0.23 l/min; LT was greater than the H+ threshold for all but one of the subjects (average absolute difference: 0.31 ± 0.20 l/min). Expressed as %VO2_peak, this average difference was 16.2 ± 9.6%. The gas exchange threshold was not statistically different from the H+ threshold. The average difference between the gas exchange and H+ thresholds was 0.00 ± 0.38 l/min; the gas exchange threshold was greater than the H+ thresholds for 4 of the 10 subjects (average absolute difference: 0.27 ± 0.25 l/min). Although the average difference was zero, our results do not indicate that the gas exchange and H+ thresholds are necessarily equivalent.

The H+ threshold and LT were highly correlated ($R^2 = 0.946$) with a slope very close to 1 (slope = 1.06, Fig. 3A). The H+ threshold was also well correlated with the gas exchange threshold ($R^2 = 0.849$) with a slope near 1 (slope = 1.17, Fig. 3B).

Thresholds were found for all the available lactate, VCO2, and H+ data; however, no clear SmO2 thresholds could be determined for 3 of the 10 subjects. For subjects where an SmO2 threshold could be determined it was, on average, greater than the H+ threshold (average difference 0.11 ± 0.11 l/min, n = 7). Visual comparison of time trends for pH and SmO2 showed that for all 10 subjects the decrease in pH either began earlier than the decrease in SmO2 (Fig. 4A) or reached a minimum value at an earlier time than the minimum in SmO2 (Fig. 4B).

**DISCUSSION**

The primary findings of this study were that an accurate mathematical model was established relating pHm to near-infrared spectra of exercising muscle during handgrip dynamometry and that the resultant model can be used during graded cycle exercise to measure [H+]. H+ threshold was highly correlated with classic metabolic thresholds during incremental exercise, LT, and the gas exchange threshold. For the subjects in this study, the average VO2 at which the NIRS-determined H+ threshold occurred was significantly lower than the average VO2 at which LT was observed.

NIRS has previously been used to estimate LT or VT during incremental cycling by analyzing trends in the deoxyhemoglobin concentration (HHb) (12, 16), multiple calculated parameters (34), and raw absorbance spectra (4). Depending on the method used to identify the threshold and the NIRS parameter chosen, these authors were able to demonstrate a relationship between LT, VT, and NIRS-determined parameters. Wang et al. (34) used the same method employed in this study to identify LT and found that change from baseline HHb was the best predictor of LT and VT compared with change from baseline oxygenated hemoglobin (O2Hb), total hemoglobin index, and the tissue oxygenation index. Except for Ferreira et al. (12), who used an instrument that calculated the absolute value for the concentration of HHb, these other methods determined relative changes in HHb.

Our methodology allows us to calculate absolute values for pHm and SmO2, and trends in these parameters during the
cycling protocol are compared in Fig. 4. Figure 4 demonstrates that there were significant portions of the experiment where pHm and SmO2 are uncorrelated, indicating that the pH calibration equation is not modeling microvascular SmO2 but is an indication of the interstitial fluid pH. One of the unique aspects of NIRS is that collected spectra result from absorption of light by blood in only the small blood vessels (arterioles, capillaries, and venules) (19). Light that illuminates the larger blood vessels is so strongly absorbed that any light which might be reflected back out of the skin from those vessels is too weak to reach the spectrometer for analysis. Only light that is scattered back to the spectrometer by the muscle fibers (and skin and fat) is analyzed. Our mathematical models calculate SmO2 and pHm from these absorbance spectra. The absorbance of myoglobin and hemoglobin cannot be easily distinguished, so SmO2 is considered to reflect both hemoglobin and myoglobin oxygen saturation. However, the mathematical methods used to develop the pHm model relate the absorbance spectra with direct pH measurements in the interstitial fluid; therefore the NIRS pHm measurement reflects pH of the interstitial fluid, rather than either venous or intracellular pH.

One possible limitation of our study is that the H+ threshold was measured locally in the interstitial fluid of the exercising muscle, yet both LT and the gas exchange thresholds are determined from systemic parameters. The fundamental assumption in cardiopulmonary exercise testing is that pulmonary measurements are a surrogate for VO2 in the exercising muscle (36); this has been previously demonstrated for cycling exercise (15, 23).

Another limitation of the regional spectroscopic measurement was our use of handgrip exercise to derive the mathematical model used with spectra collected from the leg for H+ threshold determination. The mathematical methods used to correct spectra for subject-to-subject differences in the scattering properties of the muscle were derived from spectra collected from the forearm (39). Light scattering in muscle varies with fiber type and distribution, so these correction factors should be derived for the leg if absolute values of pH or [H+] are required. However, since we assessed changes in [H+] during exercise to identify VO2 where there was a significant, uncompensated increase in [H+], the absolute value of [H+] is not required.

The cycling exercise protocol was not designed specifically for the purpose of determining LT, and the step increases were somewhat large (50 W), resulting in a very small number of lactate samples for subjects with low VO2 peak. This is seen in the large SE for LT determination. Yet, despite this large error, LT was statistically greater than HT. Step intervals of 3 min may be considered long, for a graded intensity protocol; however, Whipp et al. (37) have previously shown that threshold determination is independent of step duration under 5 min in graded exercise protocols.

Despite these limitations, we have demonstrated the feasibility of determining a metabolic threshold using a noninvasive, optical determination of [H+] in 10 normal subjects. The
instrumentation needed for this method is small and could be miniaturized to be worn by a subject outside the exercise laboratory or by athletes training or participating in their activity of choice. The technology has application in occupational hazard monitoring by allowing continuous determination of work effort in situations where it is difficult to measure pulmonary gas exchange, such as for astronauts, firefighters and hazardous material crews who perform strenuous work in protective garments. This initial study demonstrates the feasibility of noninvasive [H^+] measurement to assess metabolic threshold. Its application in assessing cardiopulmonary performance and its use in predicting aerobic performance and training endurance athletes remain to be tested in subsequent studies.

APPENDIX A

**PLS regression to determine muscle pH.** The binding of oxygen to hemoglobin is mediated in part by electrostatic interactions between individual amino acid residues and external factors such as hydrogen ion, chloride, inorganic phosphate and 2,3-diphosphoglycerate (30). The Bohr effect is described as the change in oxygen affinity as a function of pH. Recently Fang et al. (11) have shown that 24 different histidyl residues at the surface of the hemoglobin molecule can explain 86% of the Bohr effect at pH 7.4. The various histidyl residues protonate and deprotonate in different pH ranges so as the pH changes, the oxygen affinity changes, resulting in spectroscopic variations. When oxygen binds to the hemoglobin molecule, there are configurational changes in the protein structure. These configurational changes result in a distortion of the symmetry of the porphin ring, which changes the oscillator strength of the electronic transition, which is subsequently reflected as a small change in the absorption spectrum. The distortion varies as the pH changes because different histidine residues on the hemoglobin molecule are affected. pH-induced changes have been observed for hemoglobin absorption spectra in both the visible (7) and near-infrared regions (1) of the spectrum. PLS regression has previously been shown to be successful for developing calibration equations for determining pH from NIRS of both blood (1, 2) and tissue (26, 27).

PLS calibration equations are determined by simultaneously collecting spectra from the in vivo component along with a gold standard method. Alam et al. (2) demonstrated that NIRS pH calibration equations can be determined independently of the blood oxygen saturation using PLS when the calibration data set is constructed in a way that pH and oxygen saturation are uncorrelated. In the handgrip study used to develop our calibration equation, the invasive sensor pH and venous oxygen saturation measurements were decoupled, and the \( R^2 \) for this relationship was near zero, so the NIRS pHm determination is expected to be independent of oxygen saturation. In our previous work comparing intramuscular pH and \( P_O_2 \) during handgrip exercise, it is clear that the time course for changes in these two parameters are different (28).

The PLS method deconvolves the spectra into a set of common factors and weights. The factors describe the wavelength regions of the spectrum that are most correlated with pH. The sum of the weighted factors should describe each measured spectrum. Various pH values correspond to different weights for each of the factors. The process of developing a PLS model involves determining the minimum number of factors that produces the smallest error in estimating pH from the calibration data set. For our pH calibration model there are four factors.

The more complex the data, the larger is the number of factors. We were able to derive a pH model with a relatively small number of factors because we removed subject-to-subject and non-pH-related exercise-induced variability from the spectra using the PCA loading correction (PCALC) method (39). PLS is employed on the corrected spectra to derive the model. To calculate pH from newly acquired spectra, those spectra are first corrected with the PCALC method, and then the calibration model is applied.

**APPENDIX B**

**Method for determining SmO2 from NIRS.** The method for determining SmO2 is reported in detail by Yang et al. (41) but is described briefly here. SmO2 is defined by Eq. 5:

\[
SmO_2 = \frac{c_{HBO_2} \cdot c_{MbO_2} <L>}{c_{HBO_2} \cdot c_{MbO_2} <L> + c_{Hb} \cdot c_{Mb} <L>} = \frac{c_{HBO_2} \cdot c_{MbO_2}}{c_{HBO_2} \cdot c_{MbO_2} + c_{Hb} \cdot c_{Mb}}
\]

where \(<L>\) is the average photon pathlength through the tissue, \(c_{HBO_2} \cdot c_{MbO_2}\) is the oxygenated heme concentration, \(c_{Hb} \cdot c_{Mb}\) is the deoxygenated heme concentration, Hb represents hemoglobin, and Mb represents myoglobin.

Attenuation of light by the subject’s tissue, \(A_{\text{exp}}(\lambda)\) at wavelength \(\lambda\) is defined in Eq. 6:

\[
A_{\text{exp}}(\lambda) = \ln \left( \frac{I_0(\lambda)}{I(\lambda)} \right)
\]

where \(I_{\text{ref}}(\lambda)\) is the measured diffuse reflectance intensity from a 99% diffuse reflectance reference standard at wavelength \(\lambda\), and \(I(\lambda)\) is the measured diffuse reflectance intensity from the subject at wavelength \(\lambda\). Incident light attenuation by tissue is caused by both absorption and scattering events. Light is absorbed by hemoglobin in the small blood vessels and myoglobin in the cells, as well as both intravascular and extravascular water and melanin pigment in the skin and other chromophores in the tissue. Light is scattered away from physical structures in the tissue such as blood vessels and muscle fibers, as well as fat that lies over the muscle.

To calculate SmO2, we first remove the components of the spectrum that result from skin pigment absorption and fat scattering by using a two-source fiber-optic probe. One source is placed close (2.5 mm) to the detector fiber-optic bundle, which transmits light to the spectrometer. This captures light from only the skin and fat layers. The second source, a farther distance from the detector bundle (30 mm), captures light from the skin, fat, and muscle layers. Light collected from the short-distance pair is orthogonalized with the light from the long-distance pair to generate a spectrum that describes attenuation from only the muscle layer. The details of this method are described in a prior publication (38). The corrected spectrum is then used for the calculation of SmO2 based on an adaptation of a method first proposed by Stratonnikov and Loschenov (29). In this method, a Taylor expansion attenuation model is used to model light that penetrates through the tissue. The light absorption is modeled by Beer’s law, and that of the scattering is modeled as a Taylor expansion term.

The skin color- and fat-corrected attenuation spectrum is then described with the Taylor series expansion model \(A_{\text{model}}\) detailed by Stratonnikov and Loschenov (29) in Eq. 7:

\[
A_{\text{model}}(\lambda) = \ln \left( \frac{I_0(\lambda)}{I(\lambda)} \right) = (c_0 + c_1 \lambda) + <L>[c_{\text{Hb}} \cdot c_{\text{Mb}} + c_{\text{HbO}_2} \cdot c_{\text{MbO}_2} R_{\text{HbO}_2}(\lambda) + c_{\text{water}} A_{\text{water}}(\lambda)] \ln(10)
\]

where \(I_0\) is the light source intensity, \(c_0\) and \(c_1\) are constants, \(c_{\text{water}}\) is the concentration of water; and \(c_{\text{Hb}}, c_{\text{Mb}}, c_{\text{HbO}_2}, c_{\text{MbO}_2}, c_{\text{water}}\) are known extinction coefficients of Hb, HbO2, and water, respectively. Since hemoglobin and myoglobin have nearly identical extinction coefficients, only the extinction coefficients of hemoglobin are required. The function \((c_0 + c_1 \lambda)\) describes the portion of the spectrum resulting from light that is scattered, as well as the wavelength-
independent absorption caused by chromophores other than heme and water, and the experimental difference caused by using $I_{0\text{c}}$ in Eq. 6 as the incident light intensity ($I_0$) when calculating the attenuation spectra.

$\text{cHb}_r - \text{cHb}_s$, as well as $\text{cHbO}_2 - \text{cHb}_r$, $\text{cHb}_s$, and $\langle L \rangle$ are obtained by nonlinear least-squares fitting of the measured attenuation spectrum $A_{\text{meas}}$ to the modeled spectrum $A_{\text{model}}$ described by Eq. 7. Once $\text{cHb}_r$ and $\text{cHbO}_2 - \text{cHb}_r$ are obtained, $\text{SmO}_2$ is calculated using Eq. 5. One of the limitations of this technique is the inability to separate the deoxygenation of myoglobin from hemoglobin, hence the general name muscle oxygen saturation.

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DISCLOSURE

B. R. Soller and Y. Yang are coinventors of the NIRS technology described in this publication and could gain financially if commercially developed, in agreement with the University of Massachusetts’ policy of sharing its licensing income with inventors.

REFERENCES


