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Quantification of ischemic muscle deoxygenation by near infrared time-resolved spectroscopy

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Abstract. The purpose of this study was to quantify muscle deoxygenation in human skeletal muscles using near infrared time-resolved spectroscopy (NIR_{TRS}) and compare NIR_{TRS} indicators and blood saturation. The forearm muscles of five healthy males (aged 27–32 yrs.) were monitored for changes in hemoglobin saturation (SO_2) during 12 min of arterial occlusion and recovery. SO_2 was determined by measuring the temporal profile of photon diffusion at 780 and 830 nm using NIR_{TRS}, and was defined as $SO_{2\text{-}TRS}$. Venous blood samples were also obtained for measurements of S_vO_2, and P_vO_2. Interstitial PO_2($P_{int}O_2$) was monitored by placing an O_2 electrode directly into the muscle tissue. Upon the initiation of occlusion, all parameters fell progressively until reaching a plateau in the latter half of occlusion. It was observed at the end of occlusion that $SO_{2\text{-}TRS}$ ($24.1 \pm 5.6\%$) agreed with S_vO_2 (26.2 ± 6.4) and that $P_{int}O_2$ (14.7 ± 1.0 Torr) agreed with P_vO_2 (17.3 ± 2.2 Torr). The resting O_2 store (oxygenated hemoglobin) and O_2 consumption rate were 290 μM and 0.82 $\mu M\,s^{-1}$, respectively, values which reasonably agree with the reported results. These results indicate that there was no O_2 gradient between vessels and interstisium at the end of occlusion. *© 2000 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(00)01301-0]

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1 Introduction

Until now, muscle oxygenation has been evaluated by near infrared continuous wave spectroscopy (NIR_{CWS}).[1–7] However, since photons travel randomly in biological tissue, optical pathlength cannot be determined by NIR_{CWS} measurements. Therefore, NIR_{CWS} measurements give only relative values of tissue oxygenation according to the Beer–Lambert law—an equation which includes optical pathlength.

Near infrared time-resolved spectroscopy (NIR_{TRS}) using picosecond light pulses determines the optical pathlength by measuring the time of photon flight.[8] NIR_{TRS} has been applied to the study of both *in vitro*[5,9,10] and *in vivo*[5,11,12] quantification of hemoglobin O_2 saturation (SO_2). *In vitro* experiments have confirmed that medium optical properties and SO_2 can be determined precisely by NIR_{TRS}.[5,9,10] Although several investigators have reported oxygenation changes in ischemic and/or contracting muscles,[5,11,12] none of the studies have compared the values obtained by NIR_{TRS} to intravascular SO_2. Thus, the purpose of this study was to determine the quantitative correlation between microvascular SO_2 measured by NIR_{TRS}, blood SO_2 and interstitial oxygen tension ($P_{int}O_2$) determined using an O_2 electrode in human subjects.

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2 Methods

2.1 NIR_{TRS}

Optical propagation in a highly scattering medium can be described by the optical diffusion approximation.[9] Using semi-infinite geometry as a realistic measurement of tissue, the solution of the optical diffusion equation is expressed as follows:[9]

$$R(\rho,t) = (4\pi Dc)^{-3/2} z_0 t^{-5/2} \exp(-\mu_a c t) \times \exp[-(\rho^{2+} z_0^2)/4Dct], \quad (1)$$

where $R(\rho,t)$ is the light intensity on a tissue surface at time (t) and separation (ρ) between source and detector; μ_a and μ_s' are the absorption coefficient and reduced scattering coefficient, respectively; D is the diffusion coefficient and is defined as $1/3(\mu_a + \mu_s')$; c is the speed of light (20 cm ns^{-1}) in the medium and z_0 is the mean scattering length defined by $1/\mu_s'$. Mean optical pathlength $\langle L \rangle$ is defined as $\int[R(\rho,t)t \cdot dt] \cdot c / \int[R(\rho,t)dt]$. When ρ is much greater than $1/\mu_s'$, the logarithm of Eq. (1) gives

$$\log R(\rho,t) = -5/2 \log t - \mu_a c t - (\rho^2/4Dct). \quad (2)$$

Therefore, the absolute concentrations of deoxyhemoglobin ([HbR]), oxyhemoglobin ([HbO_2]), total hemoglobin ([T−Hb]), and SO_2($SO_{2\text{-}TRS}$) can be determined using two wavelengths[13] as follows:

$$[\mathrm{HbR}]=(\epsilon_1^{\lambda 2}\mu_a^{\lambda 1}-\epsilon_1^{\lambda 1}\mu_a^{\lambda 2})/(\epsilon_2^{\lambda 1}\epsilon_1^{\lambda 2}-\epsilon_2^{\lambda 2}\epsilon_1^{\lambda 1}), \quad (3)$$

$$[\mathrm{HbO_2}]=(\epsilon_2^{\lambda 1}\mu_a^{\lambda 2}-\epsilon_2^{\lambda 2}\mu_a^{\lambda 1})/(\epsilon_2^{\lambda 1}\epsilon_1^{\lambda 2}-\epsilon_2^{\lambda 2}\epsilon_1^{\lambda 1}), \quad (4)$$

$$[\mathrm{T\text{–}Hb}]=[\mathrm{HbR}]+[\mathrm{HbO_2}], \quad (5)$$

$$\mathrm{SO_{2\text{-}TRS}}(\%)=[\mathrm{HbO_2}]\times 100/[\mathrm{T\text{–}Hb}], \quad (6)$$

where $\epsilon_1^{\lambda 1}(\epsilon_1^{\lambda 2})$ and $\epsilon_2^{\lambda 1}(\epsilon_2^{\lambda 2})$ are the extinction coefficient of HbO_2 at wavelength λ1 (λ2) and the extinction coefficient of HbR at wavelength λ1 (λ2), respectively. Values for μ_a and μ_s' are expressed in units of common logarithm.

An *in vitro* sample or tissue is illuminated through a 200 μm diameter optical fiber by 780 and 830 nm laser diodes (Hamamatsu, PLP) with 50 ps half width, 5 MHz repetition rate, and 100 mW peak power. The emitted photons penetrate the tissue and are reflected to a 5 mm diameter optical bundle fiber where they are sent to a time correlated single photon counting (TCPC) system. The digitized temporal profile data from an *in vitro* sample or tissue is deconvoluted with the instrumental response function so that the time response of the instrument itself is compensated. Following deconvolution, the temporal profile data is fitted with Eq. (2) and the values for μ_a and μ_s' at 780 and 830 nm are obtained. HbR, HbO_2, T−Hb, and $SO_{2\text{-}TRS}$ are calculated by Eqs. (3)–(6), respectively. The NIR_{TRS} system provides data on HbR, HbO_2, T−Hb, and $SO_{2\text{-}TRS}$ every 30 s.

2.2 In vitro Validation of the NIR_{TRS} System

We examined oxygenation changes in a purified-hemoglobin solution (200 μM) using a phantom consisting of 2500 ml water and a 1% intralipid suspension (Kabi Pharmacia) as a photon scatterer in order to simulate the *in vivo* μ_s' of 4.0 cm^{-1}. The separation between input and output fibers was set at 3 cm. Yeast was added to the solution in order to lower oxygenation levels as a result of yeast respiration. We compared the changes in solution SO_2 by NIR_{TRS} and by blood gas analyzer (B. G. A. Inc.) as a reference. A significant correlation ($r^2=0.98$, $p<0.001$) was found between changes in SO_2 measured by NIR_{TRS} and those determined by blood gas analysis. These results indicate that NIR_{TRS} can quantitatively determine SO_2 *in vitro*.

2.3 In vivo Experiments

2.3.1 *Subjects*

Five healthy male volunteers (27–32 years old) were recruited for the experiment. Informed consent was obtained from all the subjects prior to the experiment.

2.3.2 *Experimental setup*

The optodes of the NIR_{TRS} (Hamamatsu Photonics K. K.) were securely placed on the skin surface over the radial digitorum extensor muscle. The optode separation was set at 3 cm to monitor changes in muscle oxygenation at a depth of ~1.5 cm. The PO_2 electrode (Unique Medical Inc., Japan) was used for simultaneous measurements of interstitial PO_2 ($P_{int}O_2$). The catheters for the blood sampling were placed in the brachial vein, and filled with 2% heparin solution to prevent intravascular coagulation.

2.3.3 *Protocol*

Arterial occlusion of the forearm was conducted by inflating the cuff tourniquet to a pressure of 280 mm Hg. The forearm muscle was rhythmically compressed (milking) on the skin surface in an attempt to drain the blood near the microvasculature into the catheter in order to obtain data from regions near the ischemic muscle. The size of the catheter used for blood sampling was 22 gage, inner diameter was 0.6 mm, and the volume of the inner space was 0.03 ml. Blood sampling during arterial occlusion was conducted once between 2 and 4 min, once between 5 and 7 min, three times between 7 and 10 min, and once between 11 and 13 min. The blood volume sampled was 1 ml each time with a total of 6 ml during arterial occlusion. The duration of cuff ischemia was 12 min. on average. NIR_{TRS} and $P_{int}O_2$ measurements were continued for 10 min after liberation of cuff ischemia. Blood sampling was also continued every 2 min. following the release of cuff ischemia.

2.3.4 *Blood gas analysis*

Blood samples were analyzed using a blood gas analyzer (Ciba Corning 148). The venous blood sample was analyzed for venous PO_2(PvO_2) and venous hemoglobin saturation (SvO_2).

2.3.5 *Statistics*

All data were expressed as mean±SE. Statistical differences were analyzed between $SO_{2\text{-}TRS}$ and SvO_2 and between $P_{int}O_2$ and PvO_2 using paired Student *t* test. Significance was defined as $p<0.05$.

3 Results

Figure 1 shows the average change in μ_a, μ_s', HbO_2, HbR, T−Hb, and $SO_{2\text{-}TRS}$ during rest, arterial occlusion, and after release of occlusion. HbO_2 decreased and HbR concomitantly increased in a linear fashion following the onset of occlusion. Thereafter, the rate of HbO_2 decline was attenuated and reached a plateau after approximately 360 s of occlusion, at which point, the stable minimum value was 0.030 ±0.005 mM for HbO_2 and 24.1±5.6% for $SO_{2\text{-}TRS}$ on average. T−Hb showed little change throughout arterial occlusion. The initial rates of HbO_2 and $SO_{2\text{-}TRS}$ decline calculated by linear regression in the five subjects were 0.82 ±0.09 μ MO_2 s^{-1} and 0.13±0.015 % s^{-1}, respectively.

Figure 2 shows the average change in $SO_{2\text{-}TRS}$ and SvO_2 during arterial occlusion and recovery. Figure 3 shows the average change in PvO_2 and $P_{mus}O_2$. No significant differences were found between $SO_{2\text{-}TRS}$ (24.1±5.6%) and SvO_2 (26.2±6.4) or between $P_{int}O_2$ (14.7±1.0 Torr) and PvO_2 (17.3±2.2 Torr) at the end of arterial occlusion.

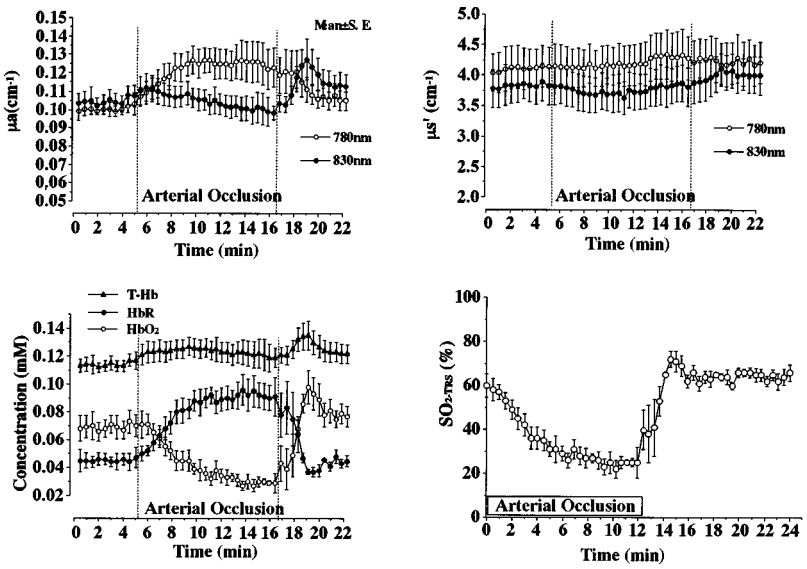

Fig. 1 Changes of NIR_{TRS} parameters in human muscles under arrested arterial circulation. μa, absorption coefficient; $\mu s'$, reduced scattering coefficient; HbR, deoxygenated hemoglobin; HbO_2, oxygenated hemoglobin; T–Hb, total hemoglobin. The μa and $\mu s'$ are plotted in units of common logarithm.

4 Discussion

Although recent studies have investigated the optical properties of highly scattering medium or tissue oxygenation using NIR_{TRS},[5,13,14] none have compared the *in vivo* $SO_{2\text{-}TRS}$ indicators and blood saturation. This study is original in two ways. First, it compares microvasculature SO_2 determined by NIR_{TRS} with that of blood samples obtained during arrested arterial blood flow in the human forearm, and second, it demonstrates an agreement between values of $SO_{2\text{-}TRS}$ and SaO_2 (SvO_2) at the end of occlusion.

During arterial occlusion, $SO_{2\text{-}TRS}$ and SvO_2 declined progressively and $SO_{2\text{-}TRS}$ was finally reaching a plateau. SvO_2 from the first half of occlusion represents the O_2 saturation of the conducting vessels which gradually becomes equilibrated with lower O_2-saturated blood derived from the exchange vessels near the muscle tissue in the latter half of occlusion. Therefore, $SO_{2\text{-}TRS}$ at the end of arterial occlusion agreed with SvO_2. In the study reported on by Boushel et al.,[15] they used continuous wave spectroscopy which provides only a relative value of O_2 saturation. So it is not possible to make an accurate comparison of the NIRS values obtained by Boushel et al. and those which we obtained with NIR_{TRS} that provides the absolute value. The SvO_2 values they obtained[15] are around 60% from 6 to 9 min., which is similar to our values (50%–60%) at that time. The SvO_2 further declined during the last minute of arterial occlusion and reached 26.2% in this study.

During the first half of arterial occlusion $P_{int}O_2$ decreased progressively and reached a plateau. PvO_2 also declined and the values at the end of occlusion agreed with those of $P_{int}O_2$, indicating that there was no O_2 gradient between muscle tissue and the vessels, and there was actually a decreased availability of mitochondrial O_2. In this case, mitochondrial respiration might have been attenuated resulting in anaerobic, as opposed to aerobic, ATP production. In fact, we have previ-

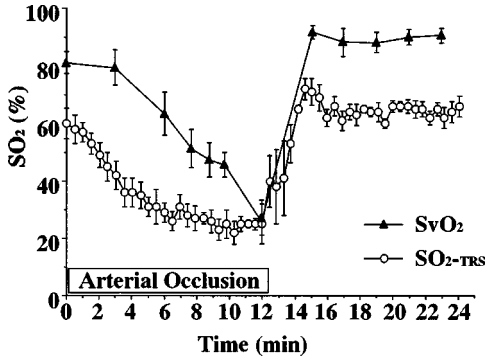

Fig. 2 Changes in hemoglobin saturation (SO_2) in venous blood and muscle tissue measured by near infrared time-resolved spectroscopy (NIR_{TRS}) during resting arterial occlusion. $SO_{2\text{-}TRS}$, hemoglobin saturation in muscle tissue measured by NIR_{TRS}; SvO_2, venous hemoglobin saturation.

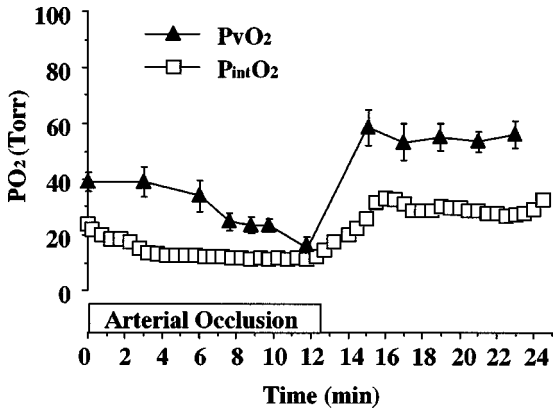

Fig. 3 Changes in muscle interstitial PO_2($P_{int}O_2$) and venous PO_2(P_vO_2) during resting arterial occlusion.

ously reported that phosphocreatine (PCr) breakdown occurs after the decrease in the rate of muscle deoxygenation seen during the latter half of arterial occlusion.[3] This evidence confirms that stabilized $P_{int}O_2$ value is no different from PO_2 values in blood samples at the end of occlusion in this study. This testifies that blood samples obtained at the end of occlusion can provide information on O_2 saturation of the exchange vessels.

The minimum $SO_{2\text{-}TRS}$ value at the end of resting occlusion was 24.1% on average, a value much greater than zero. Although the mechanism remains unknown, the initiation of phosphocreatine (PCr) breakdown reported in the previous study[3] in the latter half of occlusion suggests that mitochondrial O_2 availability has been attenuated. Further studies are needed to clarify this mechanism.

After release of occlusion, $SO_{2\text{-}TRS}$ increased progressively and reached a peak value after approximately 2 min, as a result of postischemic hyperemia. Bosman et al.[14] reported that during this period, capillary diameter increased by 12% compared with the control period. Although their model was rabbit skeletal muscle, the magnitude of vasodilatation in our results (15% increase in T–Hb) is comparable to the results reported by Bosman et al.

We calculated resting O_2 store from the resting value of HbO_2 (72.5 μM). The HbO_2 value represents the concentration of hemoglobin fully saturated with the oxygen molecules. In this case, each hemoglobin obtains four moles of oxygen molecule. Thus, the resting O_2 stored in this study was 0.29 mM on average. This value agrees with our previously reported result of 0.30 mM[3] determined using both ^{31}P-MRS and NIR_{CWS}. The basal oxygen consumption rate calculated from the initial rate of decline of HbO_2 during resting arterial occlusion was $0.82 \pm 0.09\ \mu M\, O_2\, s^{-1}$. This value reasonably agrees with our previous data of $1.2\ \mu M\, O_2\, s^{-1}$ determined using both ^{31}P-MRS and NIR_{CWS},[3] and with the value of $1.4\ \mu M\, O_2\, s^{-1}$ obtained by Harris.[16]

4.1 Changes in Tissue Optical Properties

During occlusion, μa at 780 nm increased due to a rise in absorption by HbR, while $\mu a'$ at 830 nm decreased slightly due to a decrease in absorption. The $\mu s'$ showed little change ($<5\%$) at either wavelength. Although factors which alter $\mu s'$ in living tissue are not fully understood, Beauvoit et al.[10] demonstrated that the *in vitro* $\mu s'$ value depends on mitochondria content and succinate dehydrogenase (SDH) activity. However, further investigations are needed to clarify factors which influence the changes in $\mu s'$ *in vivo*. The change in $\langle L \rangle$ relative to the preocclusion level throughout arterial occlusion was rather small, ranging from -8% to $+1\%$.

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