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Quantitative Evaluation of Oxygenation and Metabolism in the Human Skeletal Muscle

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The forearm muscles of five healthy males were monitored for changes in microvessel hemoglobin saturation ($SO_{2\text{-}TRS}$) by near infrared time-resolved spectroscopy (NIR_{TRS}) and changes in phosphorus metabolites by magnetic resonance spectroscopy (^{31}P-MRS) during 12 min of resting arterial occlusion. Muscle oxygenation and phosphorus metabolites were also measured during grip exercises at varying intensities. Upon the initiation of occlusion, $SO_{2\text{-}TRS}$ fell progressively until it reached a plateau in the latter half of the occlusion. Phosphocreatine (PCr) began to decrease around 6 min after the initiation of arterial occlusion. The resting O_2 store and O_2 consumption were 295 μM and 0.95 μM/sec, respectively-values which reasonably agree with the reported results. A significant correlation was observed between the changes in $SO_{2\text{-}TRS}$ and PCr during exercise ($r^2 = 0.80$, $p < 0.001$). These results indicate that NIR_{TRS} is able to provide reliable information about resting metabolism and oxidative rate during exercise. NIR_{TRS} and MRS are useful to monitor oxygenation and energetics noninvasively in the human muscle.

Key words: muscle oxygenation; O_2 gradient; basal O_2 consumption; phosphocreatine (PCr); near infrared

1. Introduction

Muscle oxygenation has been evaluated by near infrared continuous wave spectroscopy (NIR_{CWS}) in subjects with physiological[1–9] and pathological[10,11] conditions. However, since photons randomly travel in biological tissue, optical path length cannot be determined by NIR_{CWS} measurements. Such measurements provide only relative values of tissue oxygenation according to the Beer-Lambert law - an equation which includes optical path length. Thus, a method for a quantitative determination of tissue oxygenation has been greatly desired for many years.

Near infrared time-resolved spectroscopy (NIR_{TRS}) uses picosecond light pulses to determine the optical path length by measuring the time of photon flight.[4,12–14] NIR_{TRS} has been applied to the study of both *in vitro* and *in vivo* quantification of hemoglobin O_2 saturation ($SO_{2\text{-}TRS}$). *In vitro* experiments have confirmed that medium optical properties and SO_2 can be precisely determined by NIR_{TRS}.[4,15,16] Although several investigators have reported oxygenation changes in ischemic and/or contracting muscles,[4,13,14,17] few of the studies have ever focused on the physiological significance of the values obtained by NIR_{TRS}.[14] In this study, we also used ^{31}phosphorus magnetic resonance spectroscopy (^{31}P-MRS) to detect changes in muscle energetics and compared indicators determined by the two measurements. Thus, the purpose of this study was to investigate optical characteristics of the ischemic and exercising muscles and the possibility of a quantitative evaluation of muscle metabolism using NIR_{TRS}.

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2. Methods

2.1 NIR_{TRS}

Optical propagation in a high-scattering medium can be described by optical diffusion approximation.[18] Using semi-infinite geometry as a realistic measurement of tissue, the optical diffusion equation is expressed as eq. (1):[18]

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

where $R(\rho, t)$ is the light intensity on a tissue surface at time (t) and separation (ρ) between the source and the detector; μ_a and μ_s' are the absorption and reduced scattering coefficients, respectively; D is the diffusion coefficient and is defined as $1/3(\mu_a + \mu_s')$; c is the speed of light (20 cm·nsec^{-1}) in the medium; and z_0 is the mean scattering length defined by $1/\mu_s'$. Mean optical path length $\langle L \rangle$ is defined as $\int(R(\rho, t) \cdot t \cdot dt) \cdot c / \int(R(\rho, t) \cdot dt)$. When ρ is much greater than $1/\mu_s'$, the logarithm ($\log_{10}$) of eq. (1) gives eq. (2):

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

Therefore, the absolute concentrations of deoxyhemoglobin (HbR), oxyhemoglobin (HbO_2), total hemoglobin (T-Hb), and $SO_{2\text{-}TRS}$ can be determined using the following two wavelength equations:

$$[HbR] = (\varepsilon_1^{\lambda 2}\mu_a^{\lambda 1} - \varepsilon_1^{\lambda 1}\mu_a^{\lambda 2})/(\varepsilon_2^{\lambda 1}\varepsilon_1^{\lambda 2} - \varepsilon_2^{\lambda 2}\varepsilon_1^{\lambda 1}) \quad (3)$$

$$[HbO_2] = (\varepsilon_2^{\lambda 1}\mu_a^{\lambda 2} - \varepsilon_2^{\lambda 2}\mu_a^{\lambda 1})/(\varepsilon_2^{\lambda 1}\varepsilon_1^{\lambda 2} - \varepsilon_2^{\lambda 2}\varepsilon_1^{\lambda 1}) \quad (4)$$

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

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

where $\varepsilon_1^{\lambda 1}(\varepsilon_1^{\lambda 2})$ and $\varepsilon_2^{\lambda 1}(\varepsilon_2^{\lambda 2})$ are the extinction coefficients of HbO_2 at wavelength $\lambda_1(\lambda_2)$ and the extinction coefficients

of HbR at wavelength $\lambda_1(\lambda_2)$, respectively. Values for μ_a and μ_s' are expressed in units of common logarithm.

Tissue is illuminated through a 200 μm diameter optical fiber by 780 nm and 830 nm laser diodes (Hamamatsu, PLP) with a 50 psec half-width, a 5 MHz repetition rate and 100 mwatts of 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 TCPC system consisted of a micro-channel plate photomultiplier tube (Hamamatsu, R3809U-01) with a 50 picosecond transit time spread (TTS), a constant fraction discriminator (Oxford/Tennelec, TC454) and a time to amplitude converter (Oxford/Tennelec, TC864). The analog signal from the TCPC system was digitized by an A/D converter (Oxford/Tennelec, PCA II) and was stored in the memory of a personal computer. The digitized temporal profile data from tissue was deconvoluted with the instrumental response function so that the time response of the instrument itself was compensated. Following deconvolution, the temporal profile data was fitted into eq. (2) and the values for μ_a and μ_s' at 780 nm and 830 nm were obtained. HbR, HbO_2, T-Hb and $SO_{2\text{-}TRS}$ were calculated by eqs. (3)–(6), respectively. Twenty-five seconds was required to obtain a reasonable signal to noise (S/N) ratio for data integration of 780 and 830 nm signals; 5 s was required to analyze the data for μ_a and μ_s'. Therefore, the NIR_{TRS} system provides data on HbR, HbO_2, T-Hb, and $SO_{2\text{-}TRS}$ every 30 s.

2.2 *^{31}P-MRS*

An NMR spectrometer (Otsuka Electronics Inc.) was used with a 2.0-T superconducting 26-cm-bore magnet. An inductively coupled 3 cm diameter circular surface coil was used for studies on the human forearm muscle. The magnetic field homogeneity was optimized by using proton NMR, decreasing the half-line width of the water peak to <0.3 ppm. A radio frequency pulse (60 μsec pulse width at 43.58 Hz) was used for phosphorus signal acquisition and phosphocreatine (PCr) signal line-broadening was adjusted to 5 Hz to improve S/N ratio. The scan repetition time for the resting cuff ischemia was 2 sec and the free-induction decays (FIDs) were averaged at 60 sec intervals. The scan repetition time for exercise was 2 sec and the mean FID values were obtained for the final 1 min of each exercise.

The FIDs were obtained and Fourier-transformed into spectra; the peak areas were fitted by a non-linear curve-fitting method to calculate the areas under the β-ATP, PCr, and inorganic phosphate (Pi) peaks. To acquire saturation factors for each phosphate compound, a ratio was calculated between each area, obtained at 20 sec repetitions (fully relaxed spectra), and those obtained at 2 sec pulse intervals. Thereafter, PCr and Pi peak areas were quantified from the ratio of PCr and Pi areas to the β-ATP area with corrections for the saturation factor. Since there was no data available for direct chemical analysis of forearm muscles, the ATP concentration reported from muscle biopsies of human vastus lateralis (ATP=8.2 mM) was used for PCr and Pi quantification.[19] Muscular pH was calculated from the median chemical shift of the Pi peak relative to PCr.[20]

2.3 *Subjects*

Five healthy male volunteers (27 to 32 y.o.) were recruited for the experiment. Informed consent was obtained from all the subjects prior to the experiment. The procedures followed in this study were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

2.4 *Experimental design*

To examine changes in energetics and oxygenation kinetics in muscles, the subjects performed the same procedure twice: once with the ^{31}P-MRS measurement in magnet and once with the NIR_{TRS}. This dual measurement was made because of the strong magnetic fields in the NIR_{TRS} device that influenced its operation. The specific location of measurement was marked each time for higher precision and reproducibility.

2.5 *Experimental protocol*

The subjects sat on a chair with their right forearm positioned horizontally. The elbow was naturally extended, and the handgrip on the ergometer handle was adjusted so that the subjects could grip the handle comfortably. The measurement site was the upper part of the forearm over the finger flexor muscles.

For the NIR_{TRS} measurement, the optodes of the NIR_{TRS} (Hamamatsu Photonics K. K.) were securely placed on the skin surface of the forearm muscle with a velcro strap. The thickness of the subcutaneous adipose tissue was measured by ultrasound (SONOS 1500, Hewlett Packard). The thickness of the subcutaneous adipose tissue was less than 2 mm in all subjects. The optode separation was set at 3 cm to monitor changes in muscle oxygenation at ~1.5 cm depth.[4] For the ^{31}P-MRS measurement, the forearm was placed on the 3 cm. diameter surface coil and the coil and arm were held in a fixed position in the magnet by a cradle. Therefore, both devices could simultaneously take measurements at nearly identical sites.

2.5.1 *Arterial occlusion protocol*

A cuff tourniquet was placed on the upper arm to occlude arterial blood flow to the forearm. After 5 min of baseline monitoring, arterial occlusion was conducted. Arterial blood flow to the forearm was completely interrupted by inflating the cuff tourniquet to a pressure of 280 mmHg for 12 min.

2.5.2 *Exercise protocol*

The subjects performed dynamic handgrip contractions in the upright position using a handgrip ergometer equipped with a weight-loaded system made of non-magnetic materials.[2] Subjects lifted the mass a distance of 2 cm, adjusted to a percentage fraction of maximum voluntary contraction (MVC). The tests on each subject were done at 10%, 15%, and 20% of MVC for 2 min (one contraction every 2 s).

2.6 *Data analysis*

The relationship between PCr and $SO_{2\text{-}TRS}$ during exercise was determined using Pearson's correlation coefficient. The values are means ± SD unless otherwise indicated.

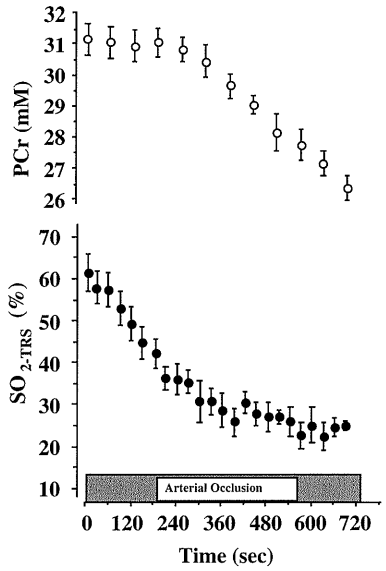

Fig. 1. Changes in muscle oxygen saturation ($SO_{2\text{-TRS}}$) and phosphocreatine (PCr) during resting arterial occlusion in the forearm.

3. Results

The resting HbO_2, HbR, and T-Hb were 0.069 ± 0.013 mM, 0.044 ± 0.010 mM, and 0.113 ± 0.009 mM, respectively. HbO_2 decreased and HbR concomitantly increased in a linear fashion following the onset of the occlusion. Thereafter, the rate of HbO_2 decline was attenuated, reaching a plateau at approximately 6 min. At that point, the stable minimum value was 0.029 ± 0.005 mM for HbO_2. T-Hb showed little change throughout the arterial occlusion. The initial rates of HbO_2 and $SO_{2\text{-TRS}}$ decline, calculated by linear regression in the 5 subjects, were 0.95 ± 0.09 μM $O_2 \cdot sec^{-1}$ and $0.16 \pm 0.015\% \cdot sec^{-1}$, respectively. The changes in PCr and $SO_{2\text{-TRS}}$ during arterial occlusion are presented in Fig. 1. The resting $SO_{2\text{-TRS}}$ was $61.1 \pm 4.4\%$ and the $SO_{2\text{-TRS}}$ value at the end of occlusion was $26.1 \pm 4.9\%$. No significant changes in ATP or pH were recorded throughout the arterial occlusion. The resting metabolic rate, calculated from the decline rate of PCr after the O_2 depleted condition, was 6.8 ± 1.2 μM $PCr \cdot sec^{-1}$ on average.

During exercise, $SO_{2\text{-TRS}}$ showed a rapid decline at the onset of muscle contraction, reaching $31.6 \pm 3.8\%$ at 10%MVC, $20.2 \pm 1.2\%$ at 15%MVC, and $22.5 \pm 2.3\%$ at 20%MVC at the end of exercise (Fig. 2). Changes in pH and PCr are shown in Fig. 3. A significant correlation was found (Fig. 4) between the changes in $SO_{2\text{-TRS}}$ and PCr ($r^2 = 0.80$, $p < 0.001$).

4. Discussion

The resting O_2 store in this study was 0.28 mM on average when taking the molecular ratio between Hb and O_2 (1:4) into account. This value agrees with our previously reported results.[2,14] The basal oxygen consumption rate calculated from the initial decline rate of HbO_2 during resting arterial occlusion was 0.95 μM $O_2 \cdot sec^{-1}$. This value reasonably agrees with our previous data. We observed a significant correlation between $SO_{2\text{-TRS}}$ and PCr during exercise, indicating that oxidative metabolism and bioenergetics are closely related in human muscles.

During the first half of arterial occlusion $SO_{2\text{-TRS}}$ decreased progressively and reached a plateau around 6 min after the onset of occlusion. There was a decreased availability of mitochondrial O_2. In this case, mitochondrial

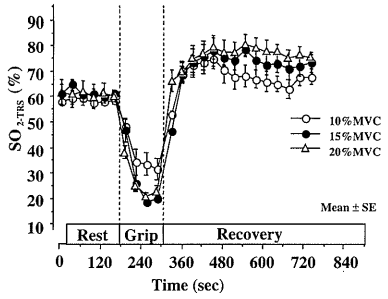

Fig. 2. Changes in muscle oxygen saturation ($SO_{2\text{-TRS}}$) during grip exercises at 10% maximum voluntary contraction (MVC), 15% MVC, and 20% MVC and recovery in the forearm.

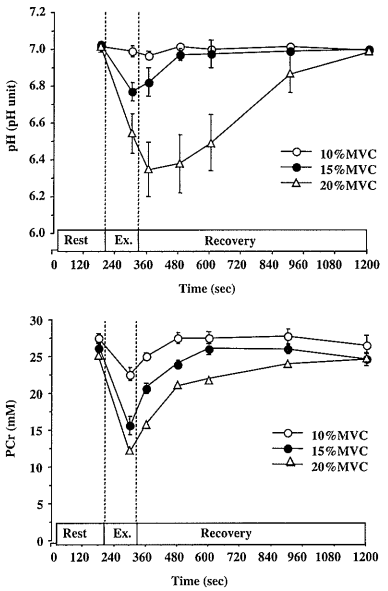

Fig. 3. Changes in muscle phosphocreatine (PCr) and pH during grip exercises at 10% maximum voluntary contraction (MVC), 15% MVC, and 20% MVC and recovery.

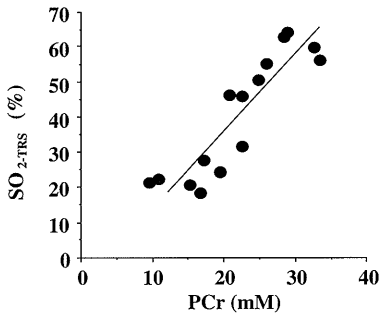

Fig. 4. Correlation between phosphocreatine (PCr) and oxygen saturation ($SO_{2\text{-TRS}}$) during grip exercises in the forearm muscle.

respiration might have been attenuated resulting in anaerobic, as opposed to aerobic, ATP production. In this study, we observed PCr breakdown after the plateau of the rate of muscle deoxygenation during the latter half of arterial occlusion. The minimum $SO_{2\text{-}TRS}$ value at the end of resting occlusion was 26.1% on average, - a value which agreed with our previous data determined by NIR_{TRS}.[14] Although the mechanism of remaining oxygen is unknown, the initiation of PCr breakdown in the latter half of occlusion suggests that mitochondrial O_2 availability has been attenuated and the anaerobic metabolism begins to take over the aerobic. Oxygen would remain in the venous side where it would not be easily exchangeable with the mitochondria. Further studies are definitely 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 post-ischemic hyperemia. Bosman *et al.*[21] 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 (69 μM). The HbO_2 value represents the concentration of hemoglobin fully saturated with the oxygen molecules. In this case, each hemoglobin obtains 4 moles of oxygen molecule. Thus, the resting O_2 store in this study was 0.28 mM on average. This value agrees with our previously reported result, 0.28 mM determined by NIR_{TRS}[14] and 0.30 mM[2] determined using both ^{31}P-MRS and NIR_{CWS}. The basal O_2 consumption rate calculated from the initial rate of decline of HbO_2 during resting arterial occlusion was 0.95 ± 0.09 μM $O_2 \cdot sec^{-1}$. This value is reasonably comparable to our previous data of 1.2 μM $O_2 \cdot sec^{-1}$ determined using both ^{31}P-MRS and NIR_{CWS},[2] and with the value of 1.4 μM$O_2 \cdot sec^{-1}$ obtained by Harris *et al.*[19]

We measured the changes in PCr, pH, and $SO_{2\text{-}TRS}$ during grip exercises at different energy demands in order to examine the correlation between oxygenation and energetics in the human skeletal muscle. PCr, pH, and $SO_{2\text{-}TRS}$ decreased with an increase in intensity from 10 to 15%MVC. In contrast, little more deoxygenation and PCr breakdown occurred at higher intensity (from 15 to 20%MVC), while pH continuously decreased with increasing exercise intensity. These observations indicate that anaerobic glycolytic energy production becomes prominent during exercise at higher intensities. We observed a significant correlation between $SO_{2\text{-}TRS}$ and PCr during exercise. This result indicates that oxidative metabolism and bioenergetics are closely related in human muscles. Thus, $SO_{2\text{-}TRS}$ may be a good indicator of oxidative rates in muscles, as the PCr levels have a linear relation to the oxidative rate.[22] Furthermore, we previously reported a significant correlation between muscle PCr level and muscle oxygen consumption[2] during steady-state exercise that is in accordance with the thermodynamic control model of mitochondrial respiration.[22] Therefore, it is speculated that PCr, oxygen consumption (mitochondrial respiration), and muscle oxygen saturation would be closely related and the homeostasis of the phosphorus energetics and oxidative metabolism (oxygen supply and consumption) are well controlled during steady-state exercise.

McCully *et al.*[23] examined the kinetics of PCr and reoxygenation recovery after low intensity exercise in the calf muscle. They observed that the kinetics (time constant) of these two indicators is similar at low intensity exercise, but dissociated at higher intensity exercise with an accompanying large pH drop (pH<6.9). Since we did not focus on the recovery kinetics of these indicators in this study and NIR_{TRS} measurement used in this study did not allow a higher time resolution than 30 seconds, we cannot compare the detailed recovery time constant of these indicators. However, it seems that PCr recovery becomes slower with a decrease in pH, while $SO_{2\text{-}TRS}$ recovery is not prolonged. This observation agrees with the reported data by McCully *et al.*[23] A methodology with higher temporal resolution is needed for more precise analysis of $SO_{2\text{-}TRS}$ recovery kinetics.

4.1 *Contribution of myoglobin signals to the NIR Measurements*

In this study, since oxygen concentration of the muscle would be well above the oxygen affinity of myoglobin, myoglobin is completely oxygenated and therefore should not affect the absorption change. It has also been postulated, however, that the gradient between the blood vessels and the cytosol is high, especially in ischemia/hypoxia. Thus, myoglobin should be completely deoxygenated. There is a report indicating that myoglobin deoxygenation begins at the onset of muscle contraction.[24] Using only optical methodology, it is difficult to differentiate between these two possibilities because of an overlap of hemoglobin and myoglobin bands in the NIR region.

4.2 *Changes in tissue optical properties*

During occlusion, μ_a at 780 nm increased due to a rise in absorption by HbR, while μ_a at 830 nm slightly decreased due to a decrease in absorption. The μ_s' showed little change (<5%) at either wavelength during occlusion. Although factors which alter μ_s' in living tissue are not fully understood, Beauvoit *et al.*[16] demonstrated that the *in vitro* μ_s' value depends on mitochondria content and succinate dehydrogenase (SDH) activity. However, further investigations are needed to clarify factors which influence the changes in μ_s' *in vivo*. The change in $\langle L \rangle$ relative to the pre-occlusion level throughout arterial occlusion was rather small, ranging from −8% to +1%. The change in $\langle L \rangle$ at the peak hyperemia after exercise relative to the pre-occlusion level ranged from −0.3 to −3.8% at 780 nm and −2.0 to −5.5% at 830 nm. As the change in $\langle L \rangle$ relative to pre-occlusion levels during arterial occlusion and exercise was rather small (<8%), it is speculated that muscle oxygenation measurements using a commercial available NIR_{CWS} are valid when a similar protocol to this study is used. More research is needed to determine optical properties in varying subjects and protocols (and interventions).

In conclusion, $SO_{2\text{-}TRS}$ may be a good indicator of oxidative rates in muscles as the PCr levels have a linear relation to the oxidative rate. NIR_{TRS} and MRS are useful to noninvasively monitor oxygenation and energetics in the

human muscle.

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