Active Muscle Oxygenation Dynamics Measured During High-Intensity Exercise by Using Two Near-Infrared Spectroscopy Methods

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Abstract Near-infrared spectroscopy is a noninvasive optical technique used to monitor tissue oxygenation. Generally, the modified Beer-Lambert's law (MBL) using continuous-wave light has been used to measure active muscle oxygenation during exercise; however, it cannot measure absolute changes in the oxy- (oxy-[Hb + Mb]), deoxy- (deoxy-[Hb + Mb]), and total hemoglobin/myoglobin concentrations (total-[Hb + Mb]) because the pathlength and scattering coefficient are not measured. In contrast, the time-resolved spectroscopy (TRS) using a ultra short pulsed laser can be used to determine absolute changes in the concentration, although the temporal resolution is inferior to that in MBL. This study evaluated the absolute changes in active muscle oxygenation and the optical mean pathlength and scattering and absorption coefficient during high-intensity exercise by using the TRS system. In addition, the difference between the changes determined using TRS and MBL measurements was assessed. When the TRS and MBL measurements obtained during high-intensity exercise were compared, the total-[Hb + Mb] and oxy-[Hb + Mb] dynamics differed markedly during high-intensity exercise, while the deoxy-[Hb + Mb] dynamics and kinetics did not differ.

1 Introduction

Near-infrared spectroscopy (NIRS), which is the spectrum measurement method based on the absorption of light in the near-infrared radiation wave length region that is generally from 0.7 to 2.5 μm, is widely used as a noninvasive measurement method of oxygenation in biological tissues, which are an optical scatterer [8, 10]. The modified Beer-Lambert law (MBL) system that is a continuous wave NIRS system and assumes that scattering and mean optical

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pathlength are constant [2, 8] estimates simultaneously the relative changes in the concentrations of oxyhemoglobin/myoglobin (oxy-[Hb + Mb]) and deoxyhemoglobin/myoglobin (deoxy-[Hb + Mb]) but not absolute changes in the concentrations of oxy- and deoxy-[Hb + Mb]. On the other hand, although temporal resolution is inferior to that in the MBL system, the time-resolved spectroscopy (TRS) system that is the ultra short pulse NIRS system can estimate the absolute values of oxy- and deoxy-[Hb + Mb] because the mean optical pathlength is measured from the center of gravity of the temporal response characteristic and scattering and absorption coefficient are estimated using the optical diffusion equation [9].

Several interesting studies showing dynamic changes in the active muscle oxygenation measured using NIRS have been reported [1, 4–7]. Most of these studies have also used the MBL system for estimating tissue oxygenation [1, 5–7]. However, it is possible that a contraction of active skeletal muscle and an environmental change in the cellular tissue by exercise influence the mean optical pathlength and scattering and absorption coefficient during exercise, particularly high-intensity exercise. If the mean optical pathlength and/or scattering and absorption coefficient change at the work-rate transition or during exercise, it may influence the estimation of tissue oxygenation by NIRS. This influence can be elucidated by TRS measurement which can estimate the mean optical pathlength and scattering and absorption coefficient. In this study, we aimed to evaluate the mean optical pathlength and scattering and absorption coefficient by using the TRS system and investigate the difference between MBL measurement and TRS measurement during high-intensity exercise.

2 Methods

Seven healthy men (mean ± SD: age, 22.7 ± 4.7 years; height, 168.4 ± 7.0 cm; body mass, 56.7 ± 6.3 kg) who exercise regularly participated in this study, which was approved by the Human Subjects Committee of Kobe Design University. All exercise tests were performed on an electronically braked cycle ergometer in a sitting position. In each subject's first visit to the laboratory, a ramp incremental cycling exercise test was performed to determine his individual peak of pulmonary oxygen uptake (pVO_2) for each exercise mode. In the second visit to the laboratory after 3 days from the first visit, each subject performed a high-intensity cycling exercise test. The protocol consisted of 1 min rest and 4 min of unloaded exercise, followed by 6 min of heavy exercise (work-rate of 80% peak pVO_2 intensity).

Throughout the experiment, gas exchange and heart rate (HR) measurements were performed similar to those described previously [7]. Muscle oxygenation profiles of the quadriceps vastus lateralis muscle were evaluated by the MBL (NIRO-200, Hamamatsu Photonics KK, Japan) and TRS systems

(TRS-20, Hamamatsu Photonics KK). In both systems, the distal probes were set on an optically dense plastic board, thus ensuring that the position of the probes, with a distance of 3 cm from each other, was fixed and invariant. The probes of NIRO-200 were attached to the vastus lateralis of the left leg and the probes of TRS-20 were attached to the vastus lateralis of the right leg by using double-sided tape and surgical tape. The NIRS data were collected continuously throughout the experiment. The relative and absolute changes in the concentrations of total hemoglobin/myoglobin (total-[Hb + Mb]) were calculated from oxy-$[Hb + Mb]_{MBL}$ + deoxy-$[Hb + Mb]_{MBL}$ and oxy-$[Hb + Mb]_{TRS}$ + deoxy-$[Hb + Mb]_{TRS}$, respectively.

To evaluate the adjustment deoxy- and total-[Hb + Mb] kinetics prior to the slow component, each data was respectively fitted using a nonlinear least squares regression technique and a single exponential function of form:

$$Y(t) = BL + Amp \cdot [1 - e^{-(t-TD)/\tau}]$$

where $Y(t)$ represents each response at a given time t; BL represents the baseline data before starting the heavy exercise; and Amp, TD, and τ represent the amplitude, time delay, and time constant of the primary component, respectively. For the deoxy-[Hb + Mb] kinetics, the analyses were conducted on data from 30-s baseline cycling to the first 60 s following the increase in work-rate. For the total-[Hb + Mb] kinetics, the analyses were conducted on data from 30-s baseline cycling to the first 180 s following the increase in work-rate.

The amplitude of deoxy- and total-[Hb + Mb] in the slow component was evaluated for each data from 3 to 6 min after the onset of the high-intensity exercise.

Significant statistical differences between the MBL and TRS measurements were analyzed by a paired t-test. Statistical significance was accepted at $P < 0.05$.

3 Results

All the subjects participated in daily exercise, and the subjects' peak pVO_2 was 3069.5 ± 551.4 ml/min and peak pVO_2/body mass was 53.8 ± 4.2 ml/(min·kg). At rest, the subjects' pVO_2, $pVCO_2$, and HR were 299.6 ± 44.1 ml/min, 247.5 ± 45.0 ml/min, and 70.0 ± 13.4 beats/min, respectively.

The dynamics of oxy-, deoxy-, and total-[Hb + Mb] measured using the MBL and TRS systems during the exercise test are shown in Fig. 1, after the initial value in the relative changes evaluated by the MBL measurement was replaced with the value by the TRS measurement. The oxy-[Hb + Mb] decreased rapidly at the start of high-intensity exercise, and the oxy-$[Hb + Mb]_{MBL}$ then increased gradually, while the oxy-$[Hb + Mb]_{TRS}$ remained constant. The deoxy-[Hb + Mb] increased exponentially at the start of high-intensity exercise and then had gradual slow component (Table 1). After the start of high-intensity exercise, the total-$[Hb + Mb]_{TRS}$ increased exponentially, while

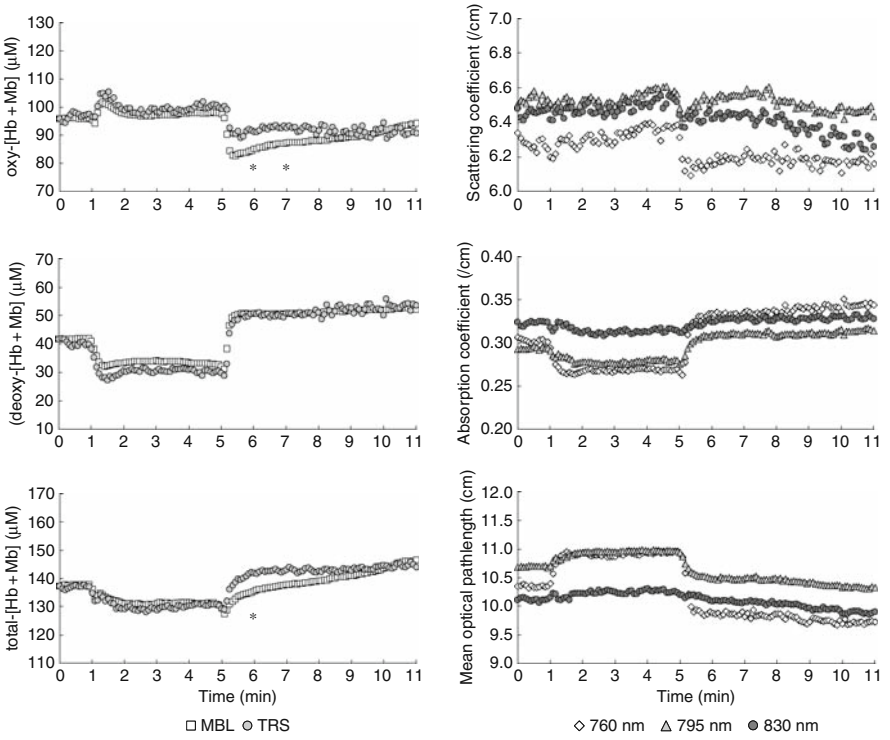

Fig. 1 Oxy-, deoxy-, and total-[Hb + Mb] responses (*left*) and scattering and absorption coefficient and mean optical pathlength (*right*) during the experiment. ∗ significant different between the MBL and TRS measurement ($P < 0.05$)

the total-$[Hb + Mb]_{MBL}$ kept increasing progressively until the end of high-intensity exercise. In the kinetics of deoxy-[Hb + Mb] response, the difference between the MBL and TRS measurement was not significant (Table 1). In the kinetics of total-[Hb + Mb] response, the time constant and amplitude in the slow component of MBL measurement were significantly greater than those of TRS measurement (Table 1; $P < 0.05$)

The mean optical pathlength and scattering and absorption coefficient are shown in Fig. 1. The scattering coefficients of the 3 wavelengths lasers rapidly

Table 1 Kinetic parameters of deoxy- and total-[Hb + Mb] measured using the MBL and TRS systems

	deoxy-[Hb + Mb]		Total-[Hb + Mb]	
	MBL	TRS	MBL	TRS
Amp (μM)	17.6 ± 7.2	19.6 ± 7.7	8.8 ± 4.2	12.5 ± 6.1
TD (s)	8.0 ± 1.5	9.5 ± 2.8	14.8 ± 13.4	6.4 ± 3.5
τ (s)	5.0 ± 1.4	4.9 ± 1.9	54.2 ± 36.3	18.8 ± 12.4 ∗
SC (μM)	1.5 ± 1.2	2.8 ± 3.0	6.9 ± 4.8	2.0 ± 2.0 ∗

∗: significant difference between the MBL and TRS measurements ($P < 0.05$).

decreased after the start of high-intensity exercise. The absorption coefficients of 760- and 795-nm wavelength laser increased exponentially at the start of high-intensity exercise. The absorption coefficient of 830-nm wavelength laser was almost constant until the end of high-intensity exercise. The mean optical pathlengths of the 3 wavelengths lasers showed a pattern completely reverse of that of absorption coefficients.

4 Discussion

In the present study, we first showed simultaneous changes in the mean optical pathlength and scattering and absorption coefficient during high-intensity exercise. In addition, the difference between the 2 muscle oxygenation response that were simultaneously evaluated by the MBL and TRS systems during high-intensity exercise was investigated.

During high-intensity exercise, the scattering coefficients tended to be a little low; the mean optical pathlengths tended to be a little short; and the absorption coefficients tended to be a little high when compared with baseline exercise. These results might suggest that some factors of optical absorption were increased because of the environmental change in the tissue by high-intensity exercise; as a result, it might influence the evaluation of oxy-[Hb + Mb] during high-intensity exercise, particularly immediately after the start of high-intensity exercise.

In a previous study using the MBL system [1, 5], although total-[Hb + Mb] after the start of high-intensity exercise tended to increase progressively as the MBL measurement in the present study, the result of the present study using the TRS system suggests that the total-[Hb + Mb] comparatively rapidly increased, and then achieved a steady state.

Since the total-[Hb + Mb] is the concentration of total hemoglobin and myoglobin per unit volume, there is a possibility that the rapid increase in total-[Hb + Mb] indicate the rapid distribution of capillary blood flow to the active muscle. Ferreira et al. [3] reported the following equation:

$$\text{capillary blood flow} \approx \text{pVO}_2(\text{primary component})/\text{deoxy} - [\text{Hb} + \text{Mb}]$$

However, the kinetics of total-[Hb + Mb] × deoxy-[Hb + Mb] did not coincide with the kinetics of pVO_2 in the primary component in this study (data not shown). Therefore, there is a possibility that the total-[Hb + Mb] response is not approximate to the capillary blood flow response. Further studies are required to clarify this issue.

In conclusion, the present study showed changes in the optical characteristics and dynamics of active muscle oxygenation measured using the MBL and TRS systems simultaneously during high-intensity exercise. It was suggested that the evaluation of active muscle oxygenation was influenced by the changes in

optical characteristics. It was also confirmed that the kinetics of deoxy-[Hb + Mb] response was not significantly different between the MBL and TRS measurements. Furthermore, it was clarified that total-$[Hb + Mb]_{TRS}$ showed a faster exponential increase compared with MBL, and then achieved a steady state for a while.

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