New calibration method for muscle oxygenation signals measured using NIRS

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近赤外分光法により測定された筋酸素化レベルの新しい定量法

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Abstract

Although continuous-wave near infrared spectroscopy (NIRS) can be used to noninvasively evaluate the kinetics of oxygenated hemoglobin/myoglobin [oxy(Hb+Mb)] in skeletal muscles, the signals only indicate relative changes. Therefore, we are unable to quantify the actual muscle oxygenation level or compare it among individuals. The purpose of this study was to propose a new calibration method for oxy (Hb+Mb). Five male subjects performed repeated dorsiflexion with ischemia for 2 min followed by aerobic recovery (Exercise with Ischemia Protocol, EIP). On different day, the subjects participated in a resting ischemia experiment, that consisted of 10-min of rest and, 20-min of muscle ischemia followed by 12-min of recovery (Resting Ischemia Protocol, RIP). Muscle oxygenation levels were measured in the tibialis anterior muscle. Oxy(Hb+Mb) reached a plateau during both protocols. However, the rate of oxy (Hb+Mb) changes during the plateau calculated by linear regression was $-0.11\ \%\cdot min^{-1}$ and $-0.83\ \%\cdot min^{-1}$ in EIP and RIP, respectively, thereby suggesting that EIP led to an almost complete plateau despite the short ischemic time. It is therefore concluded that EIP is an effective calibration method for oxy(Hb+Mb) signals uesd to quantify muscle oxygenation levels.

Key words； NIRS, oxy(Hb+Mb), calibration, plateau, dorsiflexion, tibialis anterior

Introduction

Continuous-wave near infrared spectroscopy (NIRS) is based on the principle of differential absorption properties of oxygenated and deoxygenated forms of hemoglobin/myoglobin in the near infrared range at wavelengths between 760 nm and 850 nm of the absorption spectrum. Therefore, one can noninvasively evaluate the trend in muscle oxygenation (balance of oxygen supply and utilization) by monitoring the difference in tissue absorbance at these two wavelengths. However, measured NIRS signals only indicate relative changes in muscle oxygenation levels. Therefore we are unable to quantify the actual muscle oxygenation level during rest and exercise. Consequently, it has been difficult to compare muscle oxygenation levels among individuals.

Recently, resting ischemia induced by arterial occlusion has been used as a calibration method for NIRS signals (Hamaoka et al. 1996, Shiozaki et al. 1998, Costes et al. 1999, Higuchi et al. 1999, Wadazumi et al. 1999, Kambayashi et al. 2001 and 2003a). This is based on previous studies, in which it was reported that oxygenated hemoglobin/myoglobin [oxy(Hb+Mb)] began to decrease immediately after completion of cuff inflation at rest. Afterwards, attenuated oxy(Hb+Mb) signals reached a plateau (Hamaoka et al. 1992). This plateau represents the almost complete depletion of intramuscular oxygen store(Hamaoka et al. 1996). If oxy(Hb+Mb) kinetics indicate a plateau, it is possible that oxy(Hb+Mb) signals were converted as a percentage of oxygenation relative to the overall change from rest (100 %) to the minimum level observed during ischemia(0 %).

The most important factor in calibration for oxy(Hb+Mb) signals is whether or not a complete plateau appears. A resting ischemic period of approximately 10 to 15 min required to confirm the plateau in oxy(Hb+Mb) since it takes 4 to 6 min for its tailing off (Hamaoka et al. 1996, Bae et al. 2000, Sako et al. 2001). However, cuff inflation itself and long lasting arterial occlusion cause pain and discomfort in subjects. It could therefore lead to perilous condition if older subjects were to be recruited for NIRS experiments. In addition, resting ischemia does not necessarily lead to a plateau as previously reported (Costes et al. 1999, Kambayashi et al. 2003b).

The purpose of this study was to propose a new calibration method for oxy (Hb+Mb) signals that consists of dynamic muscle contractions at a low intensity with a short period of ischemia induced by arterial occlusion.

Materials and Methods

Subjects. Five healthy male subjects (age 23 − 38 yr, height 170−191 cm and weight 62−86 kg) gave their informed consent to take part in this experiment. None of the subjects participated in any specific training program.

Experimental Design. Experiments were conducted at a room temperature of 23−24 ℃. Subjects always rested for approximately 30 min in the sitting position before the commencement of the experiments. They performed dynamic (isotonic) dorsiflexion exercises with ischemia followed by aerobic recovery (Exercise with Ischemia Protocol : EIP, Fig. 1). The measurement site for NIRS was the mid portion of the tibialis anterior muscle (TA) in the right leg, which is the most active site during dorsiflexion. The site was identified during isometric dorsiflexion and was marked each time for higher precision and reproducibility.

On different day, all of the subjects participated in a resting ischemia experiment. This experiment consisted of 10-min of rest and, 20-min of muscle ischemia followed by 12-min of recovery with NIRS measurements on TA (Resting Ischemia Protocol : RIP, Fig. 1). Ischemia in TA was induced by inflating a pneumatic 16cm-wide cuff on the thigh to a pressure of 300 mmHg.

Dynamic Dorsiflexion. Subjects sat upright in a chair with a vertical back support. The knee and ankle of their right leg were at an angle of approximately 170° and 110°, respectively.

The foot was strapped to plastic pedal, which was in close contact with a strain gauge, in an ankle-flexion ergometer with a weigh-loaded system. Maximum volitional isometric contraction (MVIC) was determined to be the best of the three maximum dorsiflexion. After taking a short rest, subjects performed exercises consisting of repetitive dorsiflexion at a rate of 12 times per minute for 2 min. The cuff was inflated 10 sec before the first muscle contraction started. To normalize the intensity of the exercise, the weight of mass was adjusted to 15 % of MVIC. Subjects lifted a mass of 4.5 cm for each dorsiflexion.

NIRS Measurement. NIRS (HEO-200, Omron, Tokyo, Japan), consisting of an NIRS probe and computerized control system, was used to monitor changes in oxy(Hb+Mb) and total hemoglobin (tHb) reflecting muscle oxygenation and blood volume, respectively. The probe con-

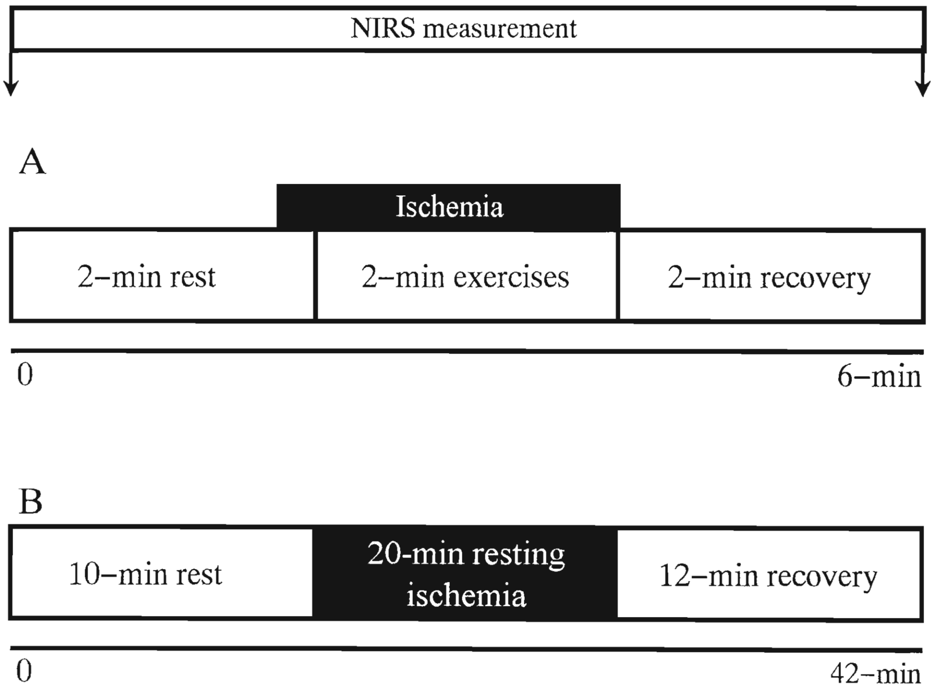

Fig. 1 Experimental protocols used in this study. Bars A and B show exercises with ischemia protocol (EIP) and resting ischemia protocol (RIP), respectively. Muscle oxygenation in the tibialis anterior muscle was measured using near infrared spectroscopy throughout both protocols. Exercises consisted of dynamic (isotonic) dorsiflexion (every 5 sec) at an intensity of 15% maximum voluntary isometric contraction. Ischemia was induced by arterial occlusion with cuff inflation

tained a light source (760 nm and 840 nm) and an optical detector (photodiode), with a 3.5 cm source-detector distance. NIRS signals were recorded every 0.5 sec throughout the protocol and averaged over 5 and 10 sec intervals in EIP and RIP, respectively. The averaged signals were expressed as a percentage of oxygenation relative to the overall change from rest (100 %) to the minimum level (0 %) observed during the protocol.

Results

Fig. 2 presents the mean oxy(Hb+Mb) kinetics during EIP. Oxy(Hb+Mb) rapidly began to decrease when muscle contractions started and reached a bottom level after 30 sec, i. e. after the six initial contractions. Afterwards, oxy-(Hb+Mb) was followed by a plateau that served as its minimum level until the end of the ischemic period although subjects continued to perform dorsiflexion exercises. The slope of oxy(Hb+Mb) during the plateau calculated by linear regression was $-0.11\% \cdot min^{-1}$.

Data on oxy(Hb+Mb) during RIP are presented in Fig. 3. Oxy(Hb+Mb) began to decrease immediately after cuff inflation, initially with linear kinetics and then tailing off after 6 min. A plateau was also observed from approximately 6 min to the end of ischemia (cuff release). The slope of oxy(Hb+Mb) ($-0.83\% \cdot min^{-1}$), however, was higher than that of EIP.

The tHb did not change during either of the protocols (data not shown).

Discussion

Oxy(Hb+Mb) reached the plateau during both protocols (Fig. 2 and 3). The slope of oxy-(Hb+Mb) during the plateau was $-0.11\% \cdot min^{-1}$ and $-0.83\% \cdot min^{-1}$ in EIP and RIP, respectively. Thus, EIP would be superior to RIP due to the much slower rate and shorter ischemic time. We suggest that the protocol presented here (dynamic exercises at an intensity of 15 % MVIC with 2- min ischemia) is an

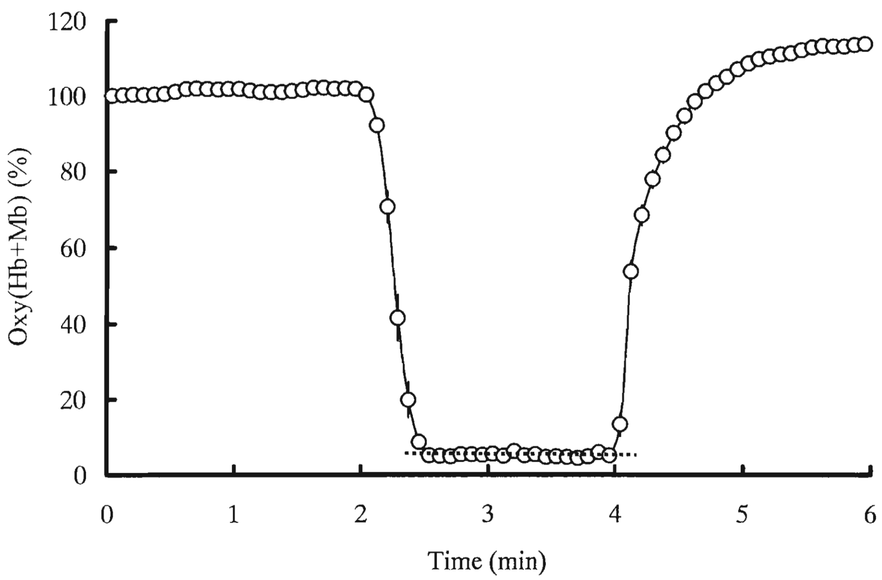

Fig.2 Mean oxy(Hb+Mb) kinetics in the tibialis anterior muscle during Exercise with Ischemia Protocol (EIP). The linear regression equation (dotted line) describing between time and oxy(Hb+Mb) is $y=-0.11x+5.4$.

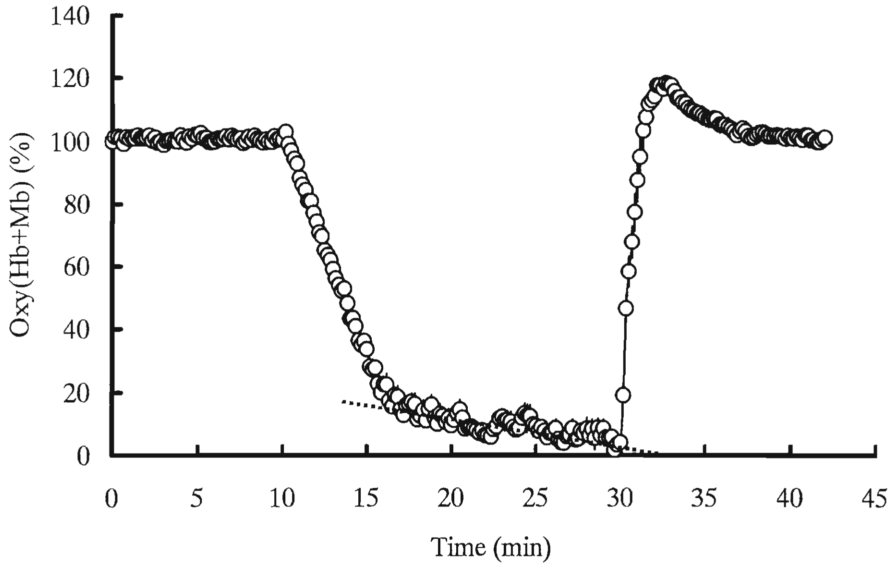

Fig. 3 Mean oxy(Hb+Mb) kinetics in the tibialis anterior muscle during Resting Ischemia Protocol (RIP). The linear regression equation (dotted line) describing between time and oxy(Hb+Mb) is $y=-0.83x+29.1$.

effective and useful calibration method for using oxy(Hb+Mb) signals to quantify muscle oxygenation levels. After conducting the experiments, subjects stated that there was almost no pain or discomfort during EIP in comparison with RIP.

In this study, exercise intensity was set at a low level. Muscle oxygen consumption increases together with exercise intensity of %MVIC (Hamaoka et al. 1996, Homma et al. 1996, van Beekvelt et al. 2002). These results may indiate that high intensity exercises should be used to deplete intramuscular oxygen as soon as possible. However, muscle oxygen consumption was linear up to approximately 20% MVIC but became less at higher intensities during dynamic exercises (Hamaoka et al. 1996, Homma et al. 1996). Therefore, anaerobic energy supply, which induces metabolic acidosis, is thought to begin at a fairly low intensity. If metabolic acidosis occurs in an ischemic muscle, the subject feels pain, resulting in the cessation of exercises. As shown in Fig. 2, oxy(Hb+Mb) reached a bottom during the first 6 muscle contractions. Intramuscular oxygen store, which was reported to be 0.3−0.4 mmol・(kg wet wt)$^{-1}$ at rest (Hamaoka et al. 1996, Kambayashi et al. 2003b), was consumed early on the protocol.

The application of 20-min resting ischemia also induced the plateau level approximately 6 min into the ischemic period (Fig. 3). However, it continued to decrease until the end of ischemia at a much slower rate. It was reported that resting ischemia does not necessarily lead to a plateau in vastus lateralis muscle (Costes et al. 1999), and gastrocnemius and soleus muscle (Kambayashi et al. 2003b). We may be mislead by muscle oxygen levels among individuals if resting ischemia is used for calibration.

Although some studies (Hamaoka et al. 1996, Bae et al. 2000, Sako et al. 2001) have shown the complete plateau of oxy(Hb+Mb) by resting ischemia, it is dubious whether the plateau reflects the depletion of intramuscular oxygen store. Santravirta et al. (1978) demonstrated that a decrease in oxygen tension to the minimal value of 9 −11 mmHg was achieved in approximately 20 min and remained constant during remaining ischemic time, but never reached zero. Hamaoka et al. (2000) also showed that the minimum oxygen saturation in muscles at the end of 12-min resting ischemia was 24.1% as measured using time-resolved NIRS, a value much greater than zero. Thus, intramuscular oxygen store may still remain despite the plateau. During EIP, oxy (Hb+Mb) was followed by a plateau even though the subjects continued to perform dorsiflexion exercises, suggesting that intramuscular oxygen store was probably depleted. Further studies are necessary to clarify the plateau of oxy-(Hb+Mb) during resting ischemia since it is far from satisfactory.

Conclusion

The slope of oxy(Hb+Mb) during the plateau in EIP was $-0.11\ \%\cdot min^{-1}$, which was lower than that of RIP ($-0.83\%\cdot min^{-1}$). Moreover, subjects did not feel any pain or discomfort due to the fact that ischemic time was shorter in EIP than in RIP. Accordingly, it is concluded that this proposed protocol for calibration of oxy-(Hb+Mb) is an effective method.

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