Comparison of oxygenation kinetics measured by different placements of the NIRS probe during sustained isometric gripping

【Original Article】

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Running head: Oxygenation by different probe placement

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Abstract

The purpose of this study was to compare oxygenation kinetics measured by slightly different placements of the NIRS probe during sustained isometric gripping. Oxygenation kinetics of sixteen young adult males was measured by two NIRS probes attached to the flexor carpiradialis muscle during gripping for 3 min. One probe (Channel 1) attached at the one third length of the line from medial epicondyle of the humerus to the styloid process of radius. Another probe (Channel 2) attached at near side (2cm) from channel 1. Although the cross correlation coefficients between both probe placements regarding oxygenation hemoglobin (Oxy-Hb/Mb), deoxygenation hemoglobin (Deoxy-Hb/Mb), tissue oxygenation saturation (StO_2) were low (r_{xy} = 0.119-0.405), the Pearson correlation coefficients between the times of reaching almost steady state for these parameters were very high (Oxy-Hb/Mb: r = 0.878, Deoxy-Hb/Mb: r = 0.769, StO_2: r = 0.843, $p < 0.05$). The difference of oxygenation kinetics between both probe placements may reflect the difference of fiber recruitment characteristics in the flexion muscle group. In conclusion, to obtain a stable measurement value, it is important that the NIRS probe is placed at the same anatomical point.

Key Words: Oxygenation Hemoglobin, Deoxygenation Hemoglobin, Cross correlation

Introduction

From the latter 1970s to the latter 1980s, near-infrared spectroscopy (NIRS) has been widely used in an attempt to measure the changes in intravascular (hemoglobin) oxygenation in the cerebrum and muscles [1, 2]. The measurement principle of oxygenation kinetics by NIRS depends on the high transmission rate of tissue and the wavelength-specific extinction of hemoglobin/myoglobin [3]. The NIRS illuminates two to four near-infrared lights with wavelengths of 700-900 nm, which easily penetrate biological tissue [1, 4]. It is possible to approximately calculate the change of oxygenation hemoglobin/myoglobin with a modification of the Beer-Lambert law due to the fact that the near-infrared light-absorption differs in response to the hemoglobin/myoglobin oxygenation [4, 5]. This has recently been used not only to measure oxygenation kinetics, but also to predict the oxygen consumption of an active muscle with an arterial/veinous occlusion method [5-8]. Of all the various NIRS instruments, the NIRO-300 (Hamamatsu Photonics, Japan) has confirmed the validity of oxygen saturation measurements and their reliability *in vivo* by a simulation experiment using a phantom tissue [5, 9]. The instrument has now become commercially popular. This instrument emits light from near-infrared laser diodes with four near-range wavelengths via fiber optics, and measures the light attenuation at three receiving optodes placed in almost the same location. Based on these light volumes, the O_2 saturation in the tissue (StO_2) and the total hemoglobin (Total Hb/Mb) volume were analysed with an algorithm incorporating the modified Beer-Lambert law [1, 4, 5].

Because the NIRS predicts hemoglobin/myoglobin volume and the ratio of the reduction based on the light volume not absorbed in tissue, the absorption of light may change by the placement or direction of the NIRS probe. Therefore, it is very important that the tester fits the probe to the same anatomical point on all subjects. However, the degree of change of

NIRS measurements by different placements has not been examined.

The flexor muscles in the forearm during maximal isometric gripping relate to performance, and maximal strength was obtained by flexing each muscle at the same time [10, 11]. In addition, when maximal isometric contraction was sustained, in the initial phase (about 10 s after onset of gripping) ischemia occurred with an increase of an intramuscular pressure [11-13]. Therefore, even if the NIRS probe is fitted to each flexor muscle, the change of measurement values will show a similar pattern. The oxygenation kinetics appears to change according to a decrease of the strength value. For example, Yamaji et al. [12] reported that Deoxy-Hb/Mb reaches the highest value when grip strength decreases to 50% MVC during sustained maximal isometric gripping, and all oxygenation parameters also become an almost steady state when grip strength becomes almost constant. However, with a change in measurement placement, whether the oxygenation pattern is the same with different probe placements has been not examined.

The purpose of this study was to compare oxygenation kinetics measured by slightly different placements of the NIRS probe during sustained isometric gripping.

Methods

Subject

The subjects were 16 young adult males [mean ± SD age 24.0 ± 5.0 yr, height 171.4 ± 3.7 cm, body mass 63.2 ± 7.9 kg]. All subjects were healthy with no upper extremity impairments. Their physical characteristics approximated the standard values for Japanese males within that age range. Written informed consent was obtained from all subjects and their parents after a full explanation of the experimental purpose and protocol.

Materials

The grip strength was measured using a digital hand dynamometer with a load-cell sensor (EG-290, Sakai, Japan). Each signal during sustained static gripping (SSG) was sampled at 20 Hz by an analog-to-digital interface, and then relayed to a personal computer. To increase the motivation of the subjects during SSG in terms of feedback, the recorded digital data was immediately displayed on a screen as a sustained force curve.

NIRS instrument

Near infrared (NIR) light easily penetrates biological tissue and allows for the detection of changes in the light-absorbing specificity in humans *in vivo* [1, 5]. The light-absorbing specificity of NIR light is changed by the oxidation and de-oxidation state of hemoglobin in the blood or in cytochrome aa_3 in cells. The oxygenation kinetics in the tissue is assessed using this specificity. NIR spectroscopic instruments measured the muscle oxygenation in the forearm during the SSG.

The probe of the NIRO-300 contains a light source filtered at 775, 813, 850, and 913 nm and three optical detectors placed 5 cm from the light source. The changing volume of Oxy-/Deoxy-hemoglobin and oxidization/reduction-cytochrome aa_3 is determined by measuring the light attenuation at wavelengths of 775, 813, 850, and 913 nm and analyzed with an algorithm incorporating the modified Beer-Lambert law. The slope of light attenuation versus distance is determined in estimating the relative absorption coefficients of tissue, i.e., the non-scattered light. Scattered light is delivered via two fiber-optic light detectors to a photomultiplier at 0.5 sec intervals. It is hypothesized that oxygenation kinetics determined by NIRO-300 is measured at a depth of about 25 mm from the skin because the mean path length is half the distance between the light source and the detector.

Experimental procedure

An NIRS probes was attached to the flexor carpiradialis muscle during sustained

isometric gripping for 3 min. The placement of NIRS probe decided carefully in consideration of the anatomical structure of flexor carpiradialis muscle. One probe (Channel 1) attached at the one third length of the line from medial epicondyle of the humerus to the styloid process of radius. Another probe (Channel 2) attached at near side (2cm) from channel 1.

The subjects entered the experimental room that was kept at 22°C and remained sitting for a rest period. They were instructed not to carry out vigorous exercise and not eat in the two hours before the experiment. The dominant hand of each subject was judged using Oldfield's handedness inventory [14]. All subjects performed the handgrip test with the dominant hand, and the grip width was individually adjusted to achieve a 90-degree angle with the proximal-middle phalanges. The arm, supported by an armrest, was in a sagittal and horizontal position, the forearm being vertical with the hand in a semi-prone position. The subjects performed the maximal grip test twice before the sustained handgrip test, and the higher exertion value was used as the target value of the test. Before the test, they rehearsed sustained maximal gripping, and were instructed not to change the grip and to maintain a natural, straight posture during the handgrip measurement. Furthermore, they were instructed to maintain a maximal force for 3 min, during which time the line of the maximal value was displayed on the screen for encouragement. However, no verbal encouragement was given during the test.

Muscle oxygenation kinetics

Muscle oxygenation kinetics during SSG were assessed using oxygenated and deoxygenated hemoglobin and tissue oxygen saturation (StO_2). The parameters for the change in Oxy-Hb/Mb were used for the time to reach the lowest value during the SSG. The parameters for the change in Deoxy-Hb/Mb were used for the time to reach the highest value during the SSG.

Data analysis

The inter-probe placement relationships of the muscle oxygenation kinetics were evaluated by calculating the cross correlation coefficient between the parameters on the two probe placements. The relationships among the parameters were examined by Pearson's correlation coefficients. A probability level of 0.05 was considered to be an indication of a statistical significance.

Results

Figure 1 shows the average curve of these parameters measured by both probe placements. The discrete data for these average curves were used to calculate the average for each sampling time (2 Hz) of each subject. The changing curves for these parameters in all subjects were similar to the average curve. In both probe placements, the changing range of oxygenation kinetics in the palmaris longus muscle was smaller than that in the flexor carpiradialis muscle. In the flexor carpiradialis muscle, the Oxy-Hb/Mb tended to decrease markedly and linearly for about 20 s after the onset of gripping. After that, the Oxy-Hb/Mb increased markedly until about 120 s, and then reached an almost steady level.

In addition, the Deoxy-Hb/Mb tended to increase markedly for the first 40 s after the onset of gripping, and to decrease rapidly from 60 s to 120 s. The change of StO_2 was similar to that of Oxy-Hb/Mb, but the time at which a minimum value of StO_2 was reached was delayed by about 10 s compared with that of Oxy-Hb/Mb.

Following this decrease, the Deoxy-Hb/Mb reached an almost steady state with a higher level compared to that at rest. The changing pattern of StO_2 after reaching a minimum value almost traced that of the Oxy-Hb/Mb, and their time when reaching an almost steady state almost agreed. The steady state value of StO_2 was lower than that of the resting state

level.

On the other hand, the changing oxygenation kinetics in the palmaris longus muscle decreased linearly (Oxy-Hb/Mb and StO_2) or increased (Deoxy-Hb/Mb) until reaching an almost steady state. The times to reach an almost steady state in both probe placements approximately agreed.

Although the cross-correlation coefficients in both probe placements regarding each parameter were not high (r_{xy} = 0.119-0.405), the correlation coefficients of time to reach an almost steady state were very high (Oxy-Hb/Mb: r = 0.878, Deoxy-Hb/Mb: r = 0.769, StO_2: r = 0.843, $P < 0.05$).

***** Figure 1 near here *****

Discussion

NIRS measurements can assess changes in oxygen consumption and oxygen supply during exercise by non-invasively and continuously measuring the hemoglobin/myoglobin (Hb/Mb) oxygenation kinetics in the tissue [1, 4, 5]. Studies regarding oxygenation kinetics or oxygen consumption in local regions have progressed exponentially after the development of the NIRS instrument. The accuracy of NIRS measurements is somewhat affected by the subcutaneous fat thickness which differs among individuals [1, 15]. Because the forearm skinfold thickness of young males selected in this study was relatively thin (2-4 mm), individual differences are considered to be small. It is generally assumed that NIRS light penetrates approximately half the distance between the light sources and the detector. Based this assumption, NIRO-300 can assess the oxygenation kinetics for forearm muscles in each probe placement since the distance with this instrument is 25 mm [1, 5].

NIRO-300 has been used by many researchers to examine the oxygenation kinetics

or oxygen consumption in local regions [6-8, 10, 11]. It is impossible to examine the validity of the NIRS *in vivo* in spite of a common problem in any NIRS instrument [1]. Therefore, the examination of the theoretical validity regarding each optical method for oxidative metabolism or the statistical validity and reliability of the measurement value by a simulation experiment using phantom tissue is very important. The NIRO-300 has been sufficiently examined on the above in previous studies. Because NIRO-300 measures the changing volume of Oxy- and Deoxy-Hb/Mb kinetics from the onset of some point, it makes little sense to compare their absolute values. Therefore, in a comparison of the difference between probe placements, the similarity of their changing pattern is important.

The changing property of the oxygen kinetics measured in the flexor carpiradialis muscle during SSG may be summarized as follows: The Oxy-Hb declines temporarily at the beginning of SSG for 20 s, while the Deoxy-Hb increases for the 40 s after the onset of gripping. The change of StO_2 is a similar to that of Oxy-Hb, but the time of reaching the minimum value of StO_2 is delayed by about 10 s compared with that of Oxy-Hb. These change patterns occurred in all of the subjects. Perhaps these change patterns reflect the changes of physiological mechanisms with sustained gripping exercise [12].

For example, the decline in the Oxy-Hb and StO_2 at the beginning of SSG may reflect the blood flow obstruction caused by an increase in intramuscular pressure. Muscle blood flow decreases temporarily at the beginning of sustained static muscle contraction at a high level (about 50% MVC) [7, 10, 11, 16, 17]. In the present study, when the grip force at about 60 s after the onset of gripping decreased to almost 40% MVC, the Total-Hb/Mb, Deoxy-Hb/Mb, and Oxy-Hb/Mb increased. This phase, which shows a high level of Deoxy-Hb/Mb, is considered to be a state of still high oxygen demands, and a lack of oxygen supply.

De Blasi et al. [6] examined the oxygen consumption in the forearm in the initial phase (1 min) during sustained static maximal forearm flexion, measured by NIR, and reported that it was very similar, irrespective of the presence or absence of a blood flow limitation. Kimura et al. [7] examined muscle oxygenation levels during isometric gripping at 10%, 30%, and 50% MVC, and reported that the oxygenation level significantly decreases during the initial phase of force exertion at any intensity.

It is inferred that the decrease in oxygen supply caused by the obstruction of muscle blood flow and the increase in oxygen consumption during gripping influenced the marked decrease in Oxy-Hb and Total Hb until 20 s after the onset of gripping. Moreover, because the lowest Oxy-Hb appeared at the time when the exertion values decreased until 60-70% MVC, the muscle blood flow might have been obstructed until that time, considering also the results of previous studies [12].

It is assumed that the increase of Deoxy-Hb in the initial phase (0-30 s) during SSG was caused by a blood flow obstruction, as stated above. The obstruction of the blood flow by an increase of intramuscular pressure restricts the oxygen supply to the active muscle, and the oxygen leaving the muscle tissue and the blood are considered to be consumed.

On the other hand, the change pattern of oxygenation kinetics in the palmaris longus muscle was not the same as that in the flexor carpiradialis muscle. Although the NIRS probe to the palmaris longus muscle is attached just 1 cm from the ulnae side of the flexor carpiradialis muscle, the change pattern disagreed. Therefore, it is suggested that anatomical accuracy of the NIRS probe placement is very important in the NIRS measurement. In both NIRS probe placements, the Deoxy-Hb/Mb following the onset of gripping decreased to a minimal value in almost the same time, but after this phase the increase tendency in the palmaris longus muscle was gradual as compared with that in the flexor carpiradialis muscle.

In addition, although the Oxy-Hb/Mb and StO_2 decreased after the onset of gripping in both placements, those in the palmaris longus muscle did not increase with the resumption of blood flow like those in the flexor carpiradialis muscle.

In other words, the difference of change pattern between both placements was found in the phase of the blood flow resumption after the ischemia. The oxygenation kinetics in the palmaris longus muscle did not show the clearing Deoxy-Hb/Mb, and the increasing Oxy-Hb/Mb and StO_2 caused by the blood flow resumption as stated above. However, the relationships between both probe placements for the time of reaching the steady state in the latter phase were high in each parameter. The difference of oxygenation kinetics between both probe placements may reflect the difference of the contribution of each muscle for sustained isometric gripping. In the phase after the onset of the gripping, because the forearm muscle groups were ischemic caused by the increasing intramuscular pressure and were recruited maximally to exert maximal strength, there may be little difference of oxygenation kinetics between both placements. However, in the following phase of resuming the blood flow, muscle fiber recruitment occurred with muscle fatigue. Since this phenomenon differs by the fatigue resistance of each muscle, the contribution of each muscle for sustained isometric gripping may differ. This study did not observe the recruitment of muscle fiber because the EMG electrode cannot attach to the muscle at the same time. However, the difference of oxygenation kinetics between both placements is considered to receive the above stated influence because the ratios of ST fiber for the flexor carpiradialis muscle and the palmaris longus muscle differ.

In conclusion, it is very important that the tester attaches the NIRS probe in the same anatomical place when observing the oxygenation kinetics during sustained exercise or predicting the oxygen consumption using the arterial/veinous or veinous occlusion method in

NIRS. Since the development level of muscles differs with the subjects, it is difficult to accurately decide the placement of the NIRS probe on the surface. Therefore, when we observe an abnormal pattern of oxygenation kinetics after attaching the probe, it may be necessary to change the position of the probe.

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Table and Figure captions

Figure 1 the average curve of these parameters measured by both probe placements.

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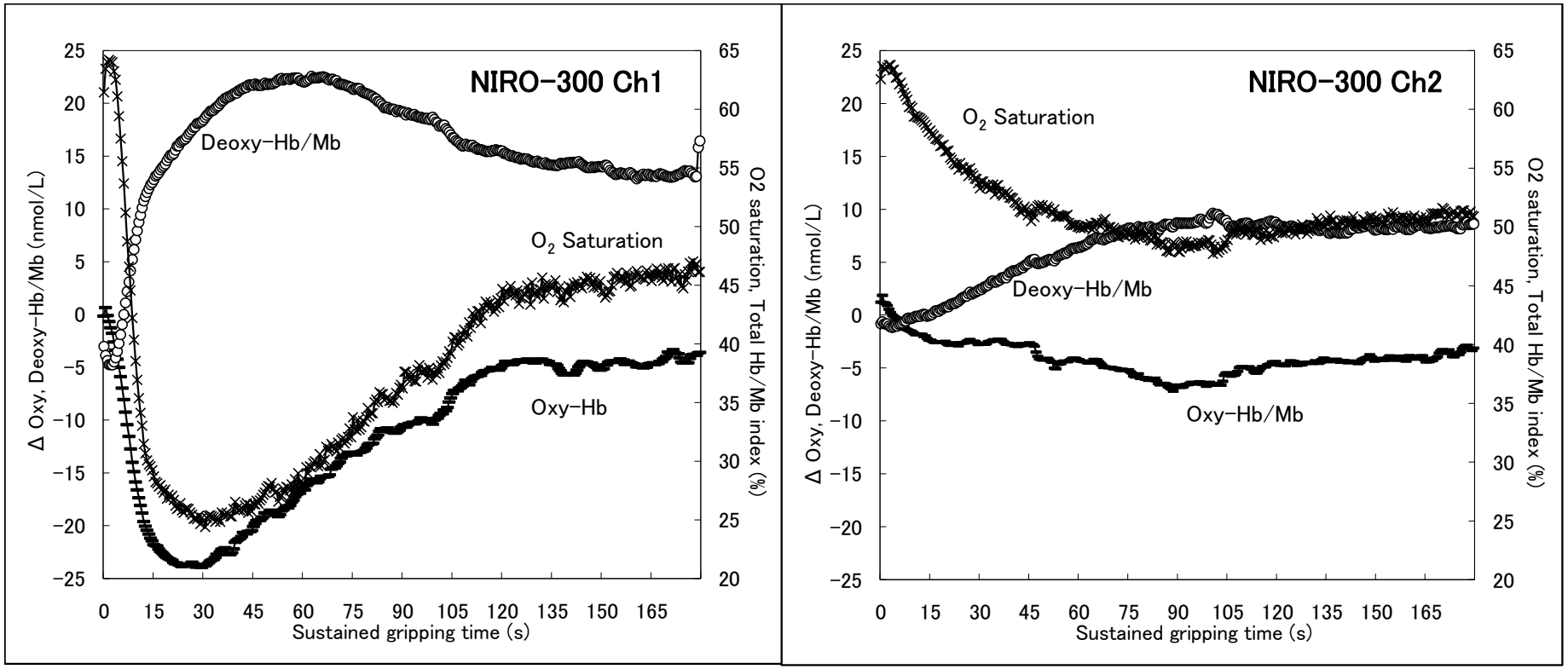

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