Assessing Muscular Oxygenation During Incremental Exercise Using Near-Infrared Spectroscopy: Comparison of Three Different Methods

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Summary

Using continuous-wave near-infrared spectroscopy (NIRS), this study compared three different methods, namely the slope method (SM), the amplitude method (AM), and the area under the curve (AUC) method to determine the variations of intramuscular oxygenation level as a function of workload. Ten right-handed subjects (22±4 years) performed one isometric contraction at each of three different workloads (30 %, 50 % and 90 % of maximal voluntary strength) during a period of twenty seconds. Changes in oxyhemoglobin ($\Delta[HbO_2]$) and deoxyhemoglobin ($\Delta[HHb]$) concentrations in the superficial flexor of fingers were recorded using continuous-wave NIRS. The results showed a strong consistency between the three methods, with standardized Cronbach alphas of 0.87 for $\Delta[HHb]$ and 0.95 for $\Delta[HbO_2]$. No significant differences between the three methods were observed concerning $\Delta[HHb]$ as a function of workload. However, only the SM showed sufficient sensitivity to detect a significant decrease in $\Delta[HbO_2]$ between 30 % and 50 % of workload ($p<0.01$). Among these three methods, the SM appeared to be the only method that was well adapted and sensitive enough to determine slight changes in $\Delta[HbO_2]$. Theoretical and methodological implications of these results are discussed.

Key words

NIRS • Muscle hemodynamics • Linear slope • Amplitude • Area under the curve

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Introduction

Since the end of the 1980s, continuous-wave near-infrared spectroscopy (NIRS) has been increasingly used as a noninvasive technique for investigating local muscle oxygenation changes during exercise or rest (Ferrari *et al.* 1997, Ferrari *et al.* 2011). The validity of NIRS has been examined by different studies (Sako *et al.* 2001, Van Beekvelt *et al.* 2001), and the results obtained using this technique are in agreement with those obtained by other imaging techniques such as phosphorus magnetic resonance spectroscopy (P-MRS) (Sako *et al.* 2001) and blood gas analysis (Van Beekvelt *et al.* 2001). Several studies have also examined the reproducibility of this optical imaging technique (Van Beekvelt *et al.* 2002, Celie *et al.* 2012, Lacroix *et al.* 2012). In this way, agreement was observed between measurements of oxygen consumption of the superficial flexor of fingers performed on three different days (Van Beekvelt *et al.* 2002). Based on intra-class correlation coefficients (ICC), a high reproducibility of data derived from NIRS was shown (ICC = 0.85-1, Lacroix *et al.* 2012). Furthermore, Celie *et al.* (2012) also confirmed the reproducibility of this optical technique, particularly when the intensity of the muscular contraction was increased. Beside its reproducibility, there has been shown a strong relationship between NIRS signals and electromyography data during static and sinusoidal isometric exercises of the biceps brachii muscle (Felici *et al.* 2009). However, few studies have specifically examined the sensitivity of NIRS to measure muscle oxygen consumption as

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a function of workload.

At the muscle level, near-infrared light is absorbed by hemoglobin and myoglobin (Ferrari *et al.* 2011). Because these two chromophores have identical spectral characteristics, it is impossible to distinguish their respective light absorption (Binzoni *et al.* 1999, Van Beekvelt *et al.* 2001, Bhambhani 2004). As the investigation of muscular oxidative metabolism is independent of the oxygen source, hemoglobin is then the term used to name the two chromophores (Lacroix *et al.* 2012). In general, the main recorded parameters using NIRS to study muscular oxidative metabolism are the following: 1) changes in oxyhemoglobin concentrations ($\Delta[HbO_2]$) and deoxyhemoglobin concentrations (Δ[HHb]); 2) changes in total hemoglobin concentrations (Δ[HbT]); and 3) muscle oxygen saturation (SmO_2) (Ferrari *et al.* 2011).

To determine the level of change in hemodynamic parameters, previous studies have used different methods such as the slope method (SM) (Quaresima *et al.* 2001), the amplitude method (AM) (Celie *et al.* 2012) and the area under the curve (AUC) method (Manfredini *et al.* 2009). The SM consists of calculating, in the entire recorded signals window, a linear regression to obtain the slope coefficient, which indicates the magnitude and direction of the hemodynamic parameters (e.g. $\Delta[HbO_2]$, Δ[HHb]). The AM consists of subtracting the level obtained during a resting state (typically the last 10 s of the rest period) from an activation period (typically the last 10 s of the contraction) after reaching a plateau for each trial. To determine the amplitude of changes of the hemodynamic parameters, the AUC method integrates the surface obtained under the curve of the hemodynamic parameter changes during the entire recorded signal window.

As stated above, few studies have used NIRS to investigate muscle oxygen consumption during active contraction, and those studies have used different quantification methods (Quaresima *et al.* 2001, Celie *et al.* 2012, Manfredini *et al.* 2009). Among these studies, only Celie *et al.* (2012) used different workloads in their protocol. Their results indicate that the muscle's hemodynamic response (increase in Δ[HHb] and decrease in $\Delta[HbO_2]$) appears somewhat proportional to workload. However, these authors did not specifically address this issue in their study. To our knowledge, no study has yet examined the concurrent validity of these three methods (SM, AM, AUC) or examined whether they show the same sensitivity to changes in workload. Accordingly, the aim of the present study was to examine, in the same protocol involving the same participants, the sensitivity of these three methods to determine slight changes in hemodynamic parameters during an incremental isometric handgrip exercise.

Material and Methods

Participants

Ten right-handed, healthy Caucasian adults (5 men and 5 women) participated in our study. Their mean ± SD age and body mass index (BMI) were 22±4 years and 21.11±2.4 kg/m^2, respectively. Because of potential effects of subcutaneous fat on NIRS signals (McCully and Hamaoka 2000), we recruited non-obese subjects whose BMI was no more than 25 kg/m^2 (range 17-24 kg/m^2). Indeed, subcutaneous fat greatly influences the NIRS signal intensity which must pass through the muscle (Hamaoka *et al.* 2011). All the participants gave their written informed consent to participate in the study, which complied with the declaration of Helsinki for human experimentation.

Experimental design

Handgrip strength was measured using a digital strain-gauge dynamometer (Takei TK 200, Takei Scientific Instruments, Tokyo, Japan) with an accuracy of ±2 kg. To standardize the muscle location for all participants, the distance (D) between the medial epicondylus humerus and the processus coronoideus ulnae was measured. The transmitter optode was then positioned at a distance equal to 1/3 D from the medial epicondylus. The receiving optode was positioned laterally, 4 cm from the transmitter optode, allowing measurement of muscle oxygenation of the flexor digitorum superficialis. The participants were seated in front of a table with their non-dominant upper limb along the body and their right hand in supination so that their forearm formed an angle of approximately 130° with their arm. The width of the handle was adjusted to the size of the hand to ensure that the middle phalanx rested on the inner handle. The participants were allowed to perform one test trial. Then, after a complete 30-s period of rest, the participants performed one isometric contraction at each of three different workloads: 30 %, 50 % and 90 % of maximal voluntary strength (MVS). Isometric contraction was chosen because this form of contraction is more prevalent in the studies (Quaresima *et al.* 2001, Muthalib *et al.* 2010, Celie *et al.* 2012) and

minimizes noise due to movements. The duration of each isometric contraction was 20 s with a rest period equal to 60 s between each contraction.

A continuous-wave NIRS (Oxymon Mk III, Artinis Medical Systems, Zetten, The Netherlands) was used to measure the changes in concentration of oxygenated hemoglobin ($\Delta[HbO_2]$), deoxygenated hemoglobin (Δ[HHb]) and total hemoglobin (Δ[HbT]), with a sampling rate set at 10 Hz (Quaresima *et al.* 2001, Muthalib *et al.* 2010). This device measures only hemodynamic relative changes and may be relatively sensitive to movement artifacts (Wolf *et al.* 2007). This constraint was limited by using isometric contractions. The measurement of changes in concentration involves the determination of optical densities of two wavelengths (857 nm and 764 nm) in the near-infrared range once they have passed through the muscle. The optical density of these two wavelengths, which are absorbed by oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (HHb), respectively, are converted to concentrations of oxygenated hemoglobin [HbO_2] and deoxygenated hemoglobin [HHb] by the modified Beer-Lambert law (Villringer and Chance 1997, see Eq. [1]):

$$A = \varepsilon \times c \times d \times DPF + G \quad [1]$$

where A is the absorption of light expressed as optical density, ε is a specific extinction coefficient, c is the chromophore concentration, d is the traveled distance, DPF is the differential path length factor and G is the loss of signal due to light scattering.

Processing of NIRS data

NIRS data were analyzed using commercially available software (Oxysoft, Artinis Medical Systems, Zetten, The Netherlands). A low-pass filter with a cutoff frequency of 0.7 Hz was used to remove the heart rate signal. Then, the changes in hemodynamic parameters at each workload (30 %, 50 %, and 90 % of MVS) were calculated using the SM, AM and AUC methods. Concerning the SM, a linear regression technique was applied to the 20-s time-series data to obtain the slope coefficient. For the AM, we subtracted the level obtained at the resting state (mean of the last 10 s of the rest period) from the one of the activation period (mean of the last 10 s of the isometric contraction). Finally, for the AUC, we determined the area under the curve of each hemodynamic parameter for the 20-s isometric contraction.

Statistical analysis

Statistical analyses were performed using STATISTICA software version 7. The assumption of data normality and homogeneity of distributions was assessed using Kolmogorov-Smirnov and Levene tests, respectively. A standardized Cronbach alpha was computed to measure the consistency between the three methods. To examine the sensitivity of each method as a function of workload, separate ANOVAs with 3 repeated measures (30 % vs. 50 % vs. 90 %) on $\Delta[HbO_2]$ and Δ[HHb] were first conducted. Second, to compare the three different methods, all $\Delta[HbO_2]$ and Δ[HHb] raw data were transformed into *z*-scores (using means and standard deviations). Separate 3 (workload: 30 % vs. 50 % vs. 90 %) × 3 (method: SM vs. AM vs. AUC) MANOVAs with repeated measures were performed on the z-scores of $\Delta[HbO_2]$ and Δ[HHb] data. For significant results, *post hoc* mean comparisons were performed using Bonferroni corrections for multiple comparisons. The level of significance was set at $p<0.05$ and partial estimated effect sizes (η^2_p) were reported for significant results.

Results

Figure 1 shows an illustration of the typical hemodynamic changes observed in one participant; Δ[HHb] increased and $\Delta[HbO_2]$ decreased as a function of workload. Globally, the standardized Cronbach alphas were 0.87 for Δ[HHb] and 0.95 for $\Delta[HbO_2]$, indicating a strong consistency between the three methods. The bivariate correlation coefficients between the three methods at each workload were all significant (between 0.66 and 0.98) with the exception of the AUC method and the SM at 30 % of workload for both $\Delta[HbO_2]$ and Δ[HHb] (all r=0.5, ns) and the AUC method and the AM for Δ[HHb] at 30 % of workload (r=0.57, ns).

Slope method

The analyses showed a main effect of workload on $\Delta[HbO_2]$ and on Δ[HHb]: $F(2,18)=15.8$; $p<0.01$; $\eta^2_p=0.63$ and $F(2,18)=20.12$; $p<0.01$; $\eta^2_p=0.69$, respectively. *Post hoc* analyses showed that there was a significant difference between each workload for both $\Delta[HbO_2]$ and Δ[HHb] slope coefficients (Fig. 2).

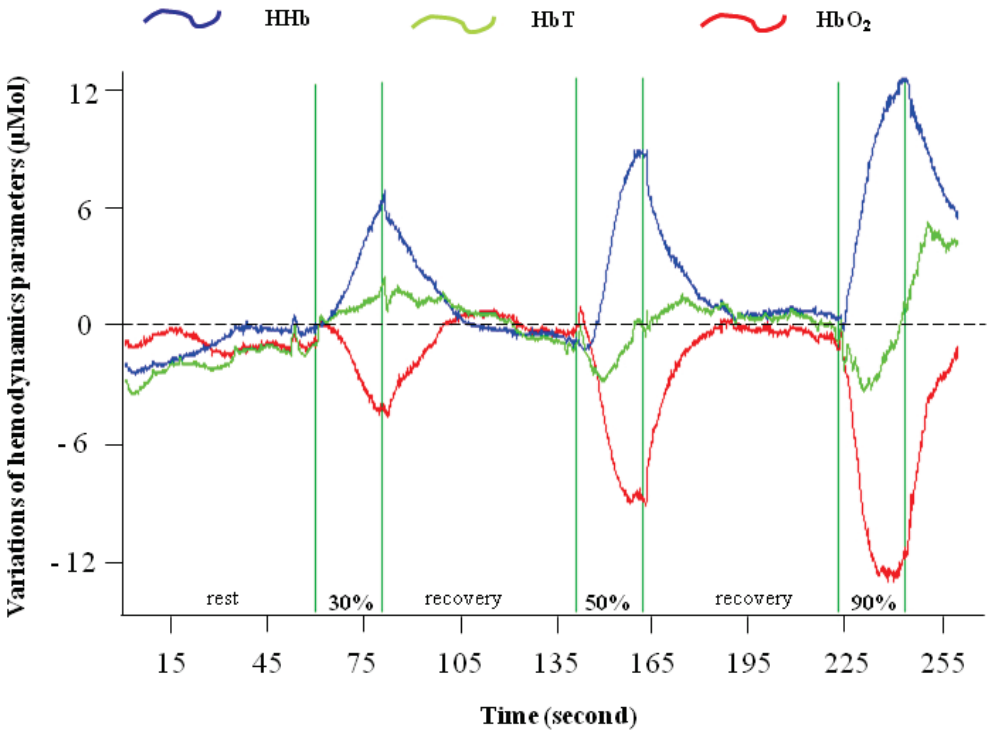

Fig. 1. Illustration of the typical variations of hemodynamic parameters as a function of workload for one participant.

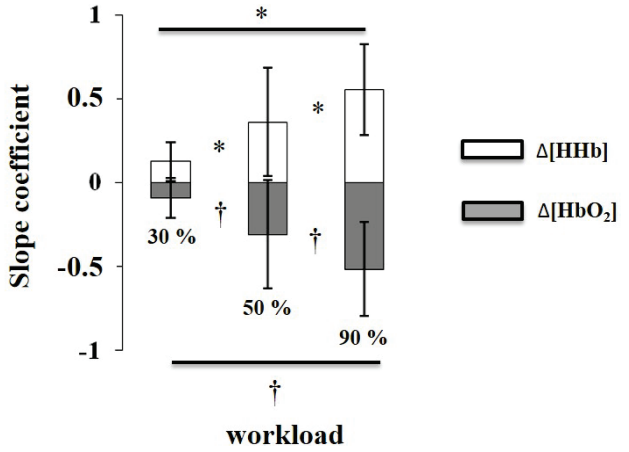

Fig. 2. Mean results using the slope method as a function of workload. Bars represent standard deviation. * $p<0.05$ for HHb and † $p<0.05$ for HbO_2.

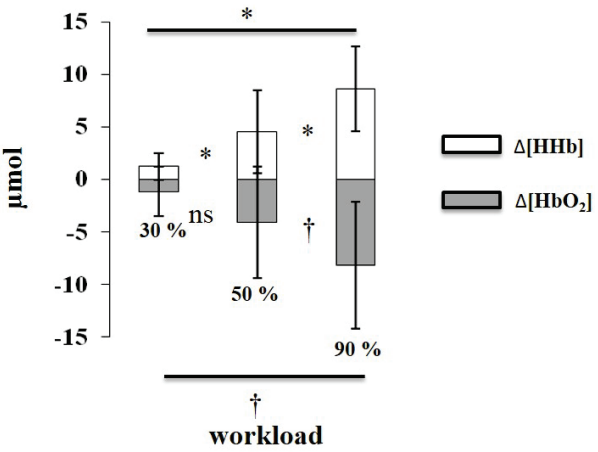

Fig. 3. Mean results using the amplitude method as a function of workload. Bars represent standard deviation. * $p<0.05$ for HHb and † $p<0.05$ for HbO_2.

Amplitude method

The analyses showed a main effect of workload on $\Delta[HbO_2]$ and on $\Delta[HHb]$: $F(2,18)=14.51$; $p<0.01$; $\eta^2_p=0.61$ and $F(2,18)=30.04$; $p<0.01$; $\eta^2_p=0.76$, respectively. For $\Delta[HbO_2]$ data, *post hoc* analyses showed that there was a significant difference between 30 % and 90 % and between 50 % and 90 %, but not between 30 % and 50 % ($p=0.1$; Fig. 3). For $\Delta[HHb]$ data, *post hoc* analyses showed that there was a significant difference between each workload.

Area under the curve method

The analyses showed a main effect of workload on $\Delta[HbO_2]$ and on $\Delta[HHb]$: $F(2,18)=10.73$; $p<0.01$; $\eta^2_p=0.54$ and $F(2,18)=32.48$; $p<0.01$; $\eta^2_p=0.78$, respectively. For $\Delta[HbO_2]$ data, *post hoc* analyses showed that there was only a significant difference between 30 % and 90 % (Fig. 4). For $\Delta[HHb]$ data, *post hoc* analyses showed that there was a significant difference between each workload.

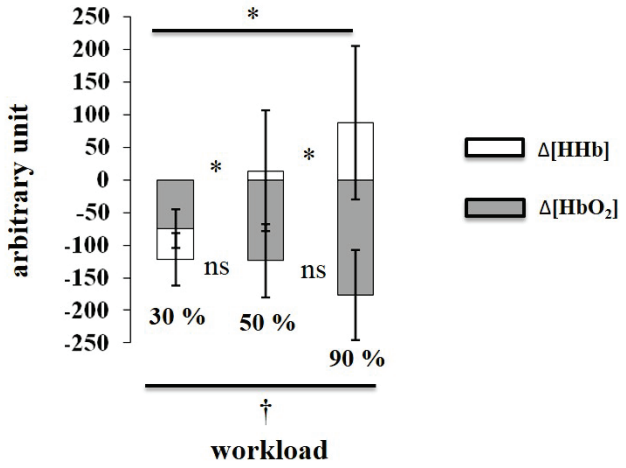

Fig. 4. Mean results using the area under the curve method as a function of workload. Bars represent standard deviation. * $p<0.05$ for HHb and † $p<0.05$ for HbO_2.

Comparison of the three methods

The MANOVA performed on $\Delta[HbO_2]$ *z*-scores showed a significant effect of workload and a main effect of the method: Wilk's lambda=0.22; F(2,9)=15.85; p=0.001 and Wilk's lambda=0.32; F(2,9)=9.53; p=0.005, respectively. A significant workload × method interaction was also revealed: Wilk's lambda=0.12; F(4,7)=11.73; p=0.003. *Post hoc* analyses showed that only the SM was sufficiently sensitive to indicate a significant difference between 30 % and 50 % workloads ($p<0.01$).

For Δ[HHb] *z*-scores, a significant main effect of workload was revealed: Wilk's lambda=0.17; F(2,9)=21.43; p=0.0003. The main effect of the method was not significant (p=0.22).

Discussion

In the present study, NIRS was used to examine relative changes in muscle oxygenation parameters as a function of three workloads during an isometric exercise. These muscle oxygenation changes were calculated using three different methods that are often reported in the literature to investigate their respective sensitivity for determining the magnitude of hemodynamic parameters as a function of workload.

In regard to the effect of workload, the results showed a consistent decrease in $\Delta[HbO_2]$ and an increase in Δ[HHb] induced by the isometric contractions as a function of workload (Fig. 1). This result is important because no study has specifically examined the sensitivity of the hemodynamic parameters as a function of workload.

This evolution of the hemodynamic parameters during muscular contraction can be explained by an important and local consumption of oxygen. For example, it was shown that at 10 % of maximum voluntary contraction, the oxygen consumption of the superficial flexor and brachioradial is five times higher than the quantity of oxygen needed at rest (Van Beekvelt *et al.* 2001). Recent studies also confirmed the increase in oxygen consumption during a voluntary isometric contraction (Ryan *et al.* 2012, Ryan *et al.* 2013). These changes in oxygenation concentration would represent oxygen needed by the muscle mitochondria during the contraction. Van Beekvelt *et al.* (2002) showed that local muscle oxygen consumption at rest, as well as during exercise, can be reliably measured by NIRS. Our results confirm this reliability of the continuous-wave NIRS technique.

The comparison of the sensitivity to workload between the three different methods used in the present study showed that only the SM provided a significant decrease of $\Delta[HbO_2]$ concentration between 30 % and 50 % of MVS. The AM and the AUC method only detected significant variations between 30 % and 90 % and between 50 % and 90 % of MVS. However, the three methods appeared to be equivalent in detecting variations in [HHb] as a function of the three manipulated workloads. During an isometric contraction with forces varying between 12 %, 18 % and 24 % of the maximum voluntary isometric contraction, Sako *et al.* (2001) calculated the changes in $[HbO_2]$ using the least-square method and showed important and significant decreases. The diminution in $[HbO_2]$ was strongly correlated (r=0.97) with the decrease in phosphocreatine (Pcr), measured with P-MRS (Sako *et al.* 2001). This result shows that a slight increase of workload contraction can induce an important variation of hemodynamic parameters. Using the SM, the variation of workload contraction between 30 % and 50 % induces important changes in hemodynamic parameters. Therefore, it is important to use a method that is sufficiently sensitive to detect slight changes, particularly in the case of a small sample of participants. In our study, only the SM method was sufficiently sensitive to detect the changes between 30 % and 50 % of MVS. Globally, using the SM, Δ[HHb] increased by 23 %, 20 % and 43 % between 30 %, 50 % and 90 % of MVS, respectively, and $\Delta[HbO_2]$ decreased by 22 %, 21 % and 42 % between 30 %, 50 % and 90 % of MVS, respectively.

In the present study, the SM was shown to be the

most sensitive method for detecting significant muscular [HbO_2] decreases at low levels of workload (between 30 % and 50 % of MVS) and for a small sample of participants. The sensitivity of this method could be explained by the characteristics of the [HbO_2] signal, which increases linearly during the entire activation period (Fig. 1) and thus appears particularly appropriate for this type of method. However, the basis of the AM, which computes the mean difference between rest and contraction periods during 10 s windows, is that only a portion of the entire signal, thought to be representative of the complete hemodynamics, is captured. This method seems particularly appropriate when the signal reaches and maintains a plateau, which was clearly not the case for the hemodynamic data in the present study. As such, this could indicate that this method is not well adapted for detecting significant [HbO_2] decreases as a function of slight changes in low levels of MVS. The AUC method, similar to the SM, uses the entire activation period for computing hemodynamic changes but was shown in our study to be the less sensitive method for detecting [HbO_2] changes. This result deserves future studies to more specifically examine this lack of sensitivity. Taken together, the results of this study favor the use of the SM, which is more sensitive and well adapted to this type of signal because NIRS signals have a linear evolution in most investigations of muscle oxygenation (Ferrari *et al.* 1997). Moreover, the SM is easy to use because it requires only a linear regression on the NIRS signals.

Some potential limitations of the present study should be addressed. First, quantifying intramuscular oxygenation changes with NIRS requires the occlusion technique to control the circulation (Van Beekvelt *et al.* 2001). This technique estimates the microcirculation status of muscle (Gerovasili *et al.* 2010). In our study, we did not use this method. However, this method is used to precisely calculate mVO_2 (oxygen consumption of muscle). In our study, this index was not the index of interest because the parameters that we have examined were Δ[HbO_2] and Δ[HHb]. Second, we did not counterbalance the three workloads during the experiment. Although this could be a clear limitation to ascertain that muscle oxygenation changes were actually due to workload increases and not due to time on task or fatigue, this non-counterbalancing had no effect on the estimation of the sensitivity of the three methods, which was the principal aim of the study. Finally, although sufficiently powered to detect significant differences, our study's sample size was quite small and a replication of these results using a larger population with a direct measure of fat thickness would strengthen the conclusion.

In conclusion, the important difference between the three methods (SM, AM, AUC) used in this investigation is their sensitivity in the quantification of variations in muscle [HbO_2] according to workload. The SM appears to be a well-adapted, user-friendly method to determine slight changes in hemodynamic parameters.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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