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Assessing muscular oxygenation during incremental exercise using near-infrared spectroscopy: Comparison of three different methods

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Short title: Measures of muscular oxygenation by NIRS

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 Δ[HbO2]. Theoretical and methodological implications of these results are discussed.

Keywords: NIRS, muscle hemodynamics, linear slope, amplitude, area under the curve.

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 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

64 metabolism is independent of the oxygen source, hemoglobin is then the term used to name
65 the two chromophores (Lacroix et al., 2012). In general, the main recorded parameters
66 using NIRS to study muscular oxidative metabolism are the following: (1) changes in
67 oxyhemoglobin concentrations ($\Delta[HbO_2]$) and deoxyhemoglobin concentrations (Δ[HHb]);
68 (2) changes in total hemoglobin concentrations (Δ[HbT]); and (3) muscle oxygen
69 saturation (SmO_2) (Ferrari et al., 2011).
70 To determine the level of change in hemodynamic parameters, previous studies have used
71 different methods such as the slope method (SM) (Quaresima et al., 2001), the amplitude
72 method (AM) (Celie et al., 2012) and the area under the curve (AUC) method (Manfredini
73 et al., 2009). The SM consists of calculating, in the entire recorded signals window, a
74 linear regression to obtain the slope coefficient, which indicates the magnitude and
75 direction of the hemodynamic parameters (e.g., $\Delta[HbO_2]$, Δ[HHb]). The AM consists of
76 subtracting the level obtained during a resting state (typically the last 10 seconds of the rest
77 period) from an activation period (typically the last 10 seconds of the contraction) after
78 reaching a plateau for each trial. To determine the amplitude of changes of the
79 hemodynamic parameters, the AUC method integrates the surface obtained under the curve
80 of the hemodynamic parameter changes during the entire recorded signal window.
81 As stated above, few studies have used NIRS to investigate muscle oxygen consumption
82 during active contraction, and those studies have used different quantification methods
83 (Quaresima et al., 2001; Celie et al., 2012; Manfredini et al., 2009). Among these studies,
84 only Celie et al. (2012) used different workloads in their protocol. Their results indicate
85 that the muscle's hemodynamic response (increase in Δ[HHb] and decrease in $\Delta[HbO_2]$)
86 appears somewhat proportional to workload. However, these authors did not specifically
87 addressed this issue in their study. To our knowledge, no study has yet examined the
88 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/m², respectively. Because of potential effects of subcutaneous fat on NIRS signals (McCully & Hamaoka, 2000), we recruited non-obese subjects whose BMI was no more than 25 kg/m² (range 17-24 kg/m²). 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 ±2kg. 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, four 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

114 right hand in supination so that their forearm formed an angle of approximately 130° with
115 their arm. The width of the handle was adjusted to the size of the hand to ensure that the
116 middle phalanx rested on the inner handle. The participants were allowed to perform one
117 test trial. Then, after a complete 30-second period of rest, the participants performed one
118 isometric contraction at each of three different workloads: 30%, 50% and 90% of maximal
119 voluntary strength (MVS). Isometric contraction was chosen because this form of
120 contraction is more prevalent in the studies (Quaresima et al., 2001; Muthalib et al., 2010;
121 Celie et al., 2012) and minimizes noise due to movements. The duration of each isometric
122 contraction was 20 seconds with a rest period equal to 60 seconds between each
123 contraction.
124 A continuous-wave NIRS (Oxymon Mk III, Artinis Medical Systems ^{BV}, Zetten, The
125 Netherlands) was used to measure the changes in concentration of oxygenated hemoglobin
126 ($\Delta[HbO_2]$), deoxygenated hemoglobin (Δ[HHb]) and total hemoglobin (Δ[HbT]), with a
127 sampling rate set at 10 Hz (Quaresima et al., 2001; Muthalib et al., 2010). This device
128 measures only hemodynamic relative changes and may be relatively sensitive to movement
129 artifacts (Wolf et al., 2007). This constraint was limited by using isometric contractions.
130 The measurement of changes in concentration involves the determination of optical
131 densities of two wavelengths (857 nm and 764 nm) in the near-infrared range once they
132 have passed through the muscle. The optical density of these two wavelengths, which are
133 absorbed by oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (HHb),
134 respectively, are converted to concentrations of oxygenated hemoglobin [HbO_2] and
135 deoxygenated hemoglobin [HHb] by the modified Beer-Lambert law (Villringer &
136 Chance, 1997 - see Eq. [1]):
137 $$A = \varepsilon \times c \times d \times DPF + G \qquad [1]$$

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 a commercially available software (Oxysoft, Artinis Medical Systems ^{BV}, 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-second 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 seconds of the rest period) from the one of the activation period (mean of the last 10 seconds of the isometric contraction). Finally, for the AUC, we determined the area under the curve of each hemodynamic parameter for the 20-second 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 $\Delta[HHb]$ 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; $\Delta[HHb]$ increased and $\Delta[HbO_2]$ decreased as a function of workload. Globally, the standardized Cronbach alphas were 0.87 for $\Delta[HHb]$ 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 .66 and .98) with the exception of the AUC method and the SM at 30% of workload for both $\Delta[HbO_2]$ and $\Delta[HHb]$ (all r = .5, ns) and the AUC method and the AM for $\Delta[HHb]$ at 30% of workload (r = .57, ns).

Insert figure1 about here

The SM

The analyses showed a main effect of workload on $\Delta[HbO_2]$ and on $\Delta[HHb]$: $F(2,18) = 15.8$; $p < 0.01$; $\eta^2_p = 0.63$ 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 $\Delta[HHb]$ slope coefficients (see Figure 2).

Insert Figure 2 about here

The AM

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

188 30% and 90% and between 50% and 90%, but not between 30% and 50% ($p = 0.1$; see
189 Figure 3). For Δ[HHb] data, post-hoc analyses showed that there was a significant
190 difference between each workload.

191 *Insert Figure 3 about here*

192 The AUC

193 The analyses showed a main effect of workload on $\Delta[HbO_2]$ and on Δ[HHb]: $F(2,18) =$
194 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
195 $\Delta[HbO_2]$ data, post-hoc analyses showed that there was only a significant difference
196 between 30% and 90% (see Figure 4). For Δ[HHb] data, post-hoc analyses showed that
197 there was a significant difference between each workload.

198 *Insert Figure 4 about here*

199 Comparison of the three methods

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

206 For Δ[HHb] *z*-scores, a significant main effect of workload was revealed: Wilk's lambda =
207 0.17; $F(2,9) = 21.43$; $p = 0.0003$. The main effect of the method was not significant ($p =$
208 0.22).

209 **Discussion**

210 In the present study, NIRS was used to examine relative changes in muscle oxygenation
211 parameters as a function of three workloads during an isometric exercise. These muscle
212 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 (see Figure 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 =

238 0.97) with the decrease in phosphocreatine (Pcr), measured with P-MRS (Sako et al.,
239 2001). This result shows that a slight increase of workload contraction can induce an
240 important variation of hemodynamic parameters. Using the SM, the variation of workload
241 contraction between 30% and 50% induces important changes in hemodynamic
242 parameters. Therefore, it is important to use a method that is sufficiently sensitive to detect
243 slight changes, particularly in the case of a small sample of participants. In our study, only
244 the SM method was sufficiently sensitive to detect the changes between 30 and 50% of
245 MVS. Globally, using the SM, Δ[HHb] increased by 23%, 20% and 43% between 30%,
246 50% and 90% of MVS, respectively, and Δ[HbO_2] decreased by 22%, 21% and 42%
247 between 30%, 50% and 90% of MVS, respectively.
248 In the present study, the SM was shown to be the most sensitive method for detecting
249 significant muscular [HbO_2] decreases at low levels of workload (between 30 and 50% of
250 MVS) and for a small sample of participants. The sensitivity of this method could be
251 explained by the characteristics of the [HbO_2] signal, which increases linearly during the
252 entire activation period (see Figure 1) and thus appears particularly appropriate for this
253 type of method. However, the basis of the AM, which computes the mean difference
254 between rest and contraction periods during 10 second windows, is that only a portion of
255 the entire signal, thought to be representative of the complete hemodynamics, is captured.
256 This method seems particularly appropriate when the signal reaches and maintains a
257 plateau, which was clearly not the case for the hemodynamic data in the present study. As
258 such, this could indicate that this method is not well adapted for detecting significant
259 [HbO_2] decreases as a function of slight changes in low levels of MVS. The AUC method,
260 similar to the SM, uses the entire activation period for computing hemodynamic changes
261 but was shown in our study to be the less sensitive method for detecting [HbO_2] changes.
262 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 $\Delta[HbO_2]$ and $\Delta[HHb]$. 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.

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Conflict of Interest

The authors have no conflict of interest.

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Figure captions

Figure1: Illustration of the typical variations of hemodynamic parameters as a function of workload for one participant.

Figure 2: Mean results using the SM as a function of workload. Bars represent standard deviation. * $p < .05$ for HHb and † $p < .05$ for HbO_2.

Figure 3: Mean results using the AM as a function of workload. Bars represent standard deviation. * $p < .05$ for HHb and † $p < .05$ for HbO_2.

Figure 4: Mean results using the AUC as a function of workload. Bars represent standard deviation. * $p < .05$ for HHb and † $p < .05$ for HbO_2.

Figure 1

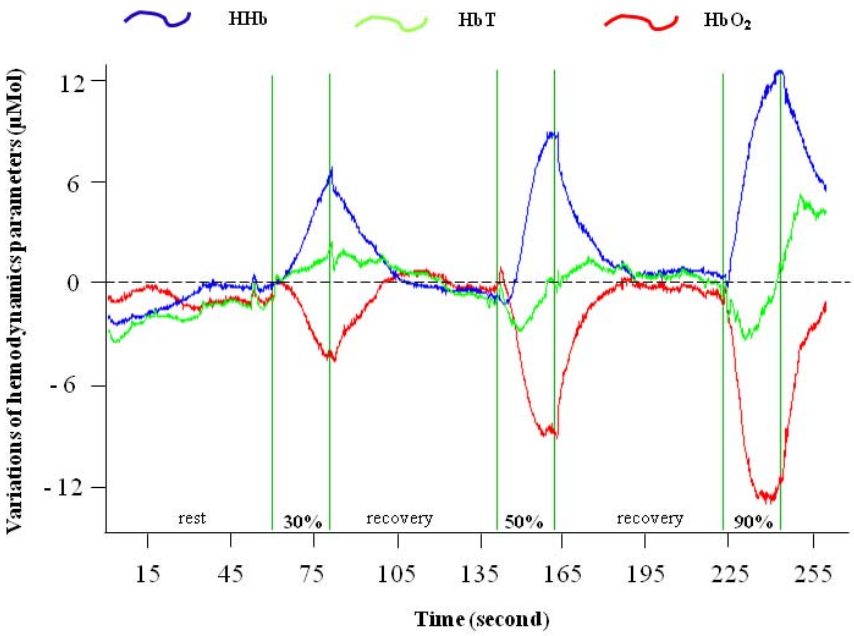
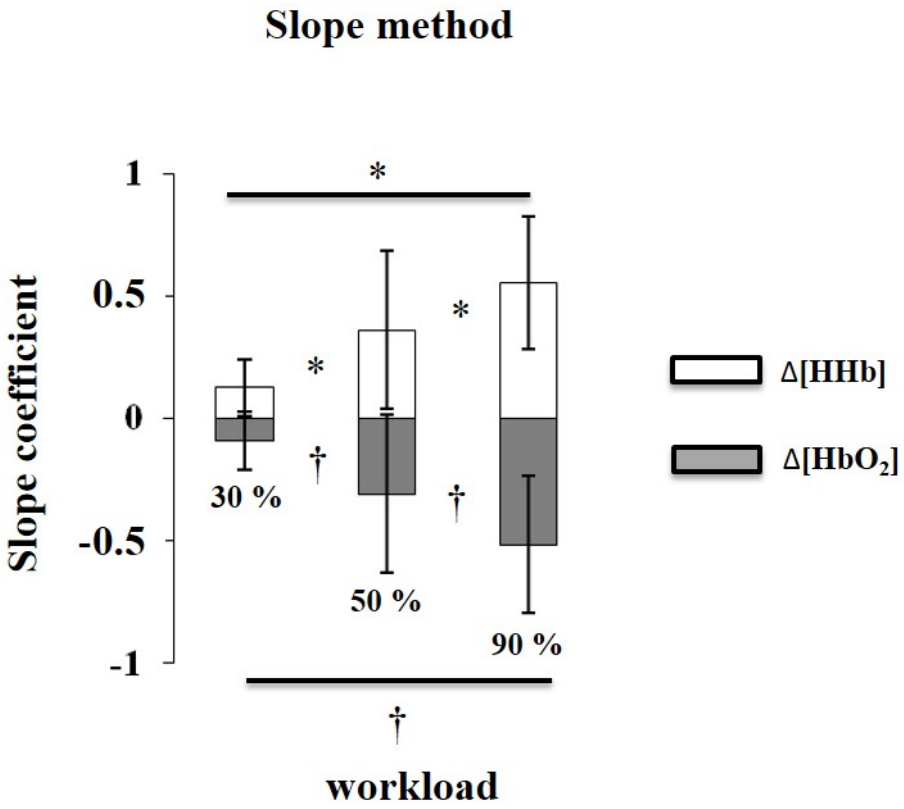

HHb
HbT
HbO2
Variations of hemodynamics parameters (uMol)
12
6
0
-6
-12
rest
30%
recovery
50%
recovery
90%
15
45
75
105
135
165
195
225
255
Time (second)

Figure 2


Slope method
Slope coefficient
1
0.5
0
-0.5
-1
*
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30 %
50 %
90 %
workload
Δ[HHb]
Δ[HbO2]

384 Figure 3

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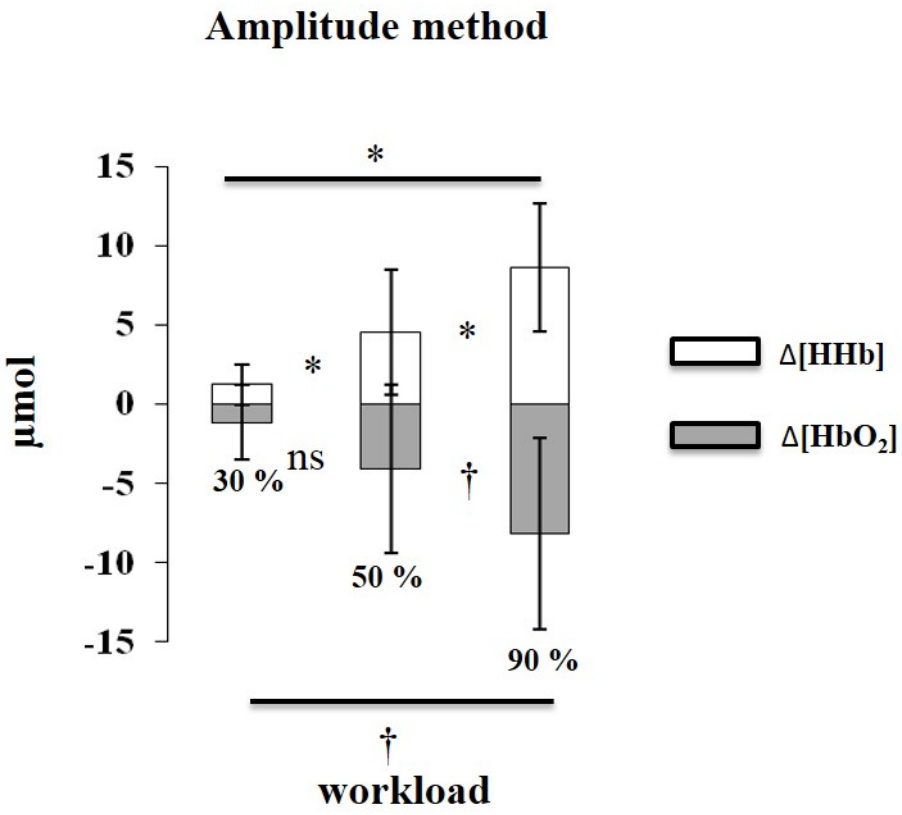

397 Figure 4

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