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SHORT COMMUNICATION

Reliability of near infrared spectroscopy (NIRS) for measuring forearm oxygenation during incremental handgrip exercise

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Abstract The purpose of this study was to test the reliability of a new handgrip exercise protocol measuring forearm oxygenation in 20 healthy subjects on two occasions. The retest took place 48 h later and at the same time of the day. The incremental exercise consisted of 2 min steps of cyclic handgrip contraction (1/2 Hz) separated by 1 min of recovery. The exercise started at 20% MVC, was increased with 10% MVC each step and was performed until exhaustion (69.5 and 73% MVC). Near infrared spectroscopy (NIRS) was used to measure deoxygenation (deoxy[Hb + Mb]) and oxygen saturation (SmO_2) in the forearm muscles. Prior to the exercise protocol an arterial occlusion of the forearm was performed until deoxy(Hb + Mb) did no longer increase. Maximal increase in deoxy[Hb + Mb] during 10 s of each exercise bout was expressed relative to the occlusion amplitude. ICC was used to examine the test–retest reliability. Significant ICC's were reported at 50% ($r = 0.466$, $p = 0.017$) and 60% MVC ($r = 0.553$, $p = 0.005$). The group mean of the maximum increase in oxygen extraction was 45.6 ± 16.7% and at the retest 44.9 ± 17.0% with an ICC of $r = 0.867$ ($p < 0.001$) which could be classified (Landis and Koch 1979) as almost perfect. The absolute SmO_2 values showed reliable ICC's for every submaximal intensity except at 60% MVC. An ICC of $r = 0.774$ ($p < 0.001$) was found at maximal intensity. The results of the present study show that deoxy[Hb + Mb] and SmO_2 responses during this protocol are highly reliable and indicate that this protocol could be used to get insight into deoxygenation and oxygen saturation in a population with low exercise tolerance.

Keywords Deoxy[Hb + Mb] · O_2-extraction · SmO_2 · Cyclic contraction · Forearm muscle

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Introduction

Near infrared spectroscopy (NIRS) is a well established technique to measure tissue oxygenation. In biological tissues like bone, muscle and skin, the NIRS-signal can be affected by absorbing and scattering of the NIR light at a particular wavelength. Three molecules account for most of the NIRS light absorption: oxygenated and deoxygenated hemoglobin (Hb), myoglobin (Mb) and cytochrome oxidase c (Boushel and Piantadosi 2001). The concentration of cytochrome oxidase can be neglected. No distinction can be made between Hb and Mb because there is an overlap of the NIR spectrum. Since the past decade this technique has frequently been used to measure blood perfusion and oxygenation index of several tissues. The validity of NIRS as an appropriate technique to measure oxygenation status at the level of microcirculation has been demonstrated in the past, both in the brain and in muscle tissue (Huppert et al. 2006; Mancini et al. 1994; Mehagnoul-Schipper et al. 2002). Besides, validation of NIRS other studies have shown that

NIRS provides a reliable technique for measurement of the oxygenation status of active muscles during exercise (Kell et al. 2004; Pereira et al. 2007). Significant test–retest intraclass correlations (ICCs) ($r = 0.69$–0.84) were found for total blood volume and oxygenation index of the erector spinae muscle during a static endurance protocol (Kell et al. 2004). Limb oxygenation measurements in the vastus lateralis during knee extensions at slow ($r = 0.73$–0.76) and fast ($r = 0.85$–0.97) velocities were shown to be reliable (Pereira et al. 2007). Considering the muscle oxygen saturation (SmO_2) measurements with NIRS, reliable results were found in a recent study (Tew et al. 2010). Blood volume and SmO_2 in the vastus lateralis at rest were affected by changes in skin blood flow. However, SmO_2 during exercise was not influenced. Significant test–retest ICCs ($r = 0.93$ and $r = 0.96$) for the rest and end-exercise periods, respectively, were reported for SmO_2 (Tew et al. 2010). This means that SmO_2 is relevant and reliable to observe in an exercise protocol.

All these previous studies focused on perfusion and oxygenation in large muscle groups. However, little is known about the reliability of NIRS measurements in small muscles. Muthalib et al. (2010) tested the reliability of NIRS measuring the M. biceps brachii oxygenation. They concluded that the level of reliability is acceptable. Their protocol consisted of sustained isometric contractions at 30 and 100% of the maximal voluntary contraction (MVC) for 10 s and of repeated isometric contractions (1 s contraction, 1 s relaxation). A second study of Van Beekvelt et al. (2002) established the reliability of measuring local muscle oxygen consumption (mVO_2) in the forearm muscles. In this study, however, our interest is not the mVO_2, but rather the oxygenation measurements in the forearm muscles. Also the arterial occlusion in Van Beekvelt et al. (2002) was used to calculate the mVO_2 and was performed on the subjects directly after the handgrip exercise. In the present study we wanted to test the reliability of deoxygenation measurements on the forearm muscles during an incremental handgrip protocol, without calculating the mVO_2. We focused on the deoxy(Hb + Mb) because it is often considered as an accurate indication for oxygen extraction into the muscle (Grassi et al. 2007). Our study was different to Van Beekvelt et al. (2002) both in that the arterial occlusion occurred prior to the exercise and also in our interest in deoxy[Hb + Mb] as an indication for arteriovenous O_2 difference in the local muscle, which is only one parameter of Fick's law. Further research to establish the NIRS reliability on small muscles is necessary as it could be used to study muscle oxygenation without inducing a high cardiac stress in several populations. The purpose of this study is to test the reliability of the NIRS on forearm muscles in a newly developed incremental exercise protocol.

Methods

Subjects

Twenty healthy subjects (8 males, 12 females) participated in this study, and their mean (±SD) age, height, and body mass were 24.4 ± 7.9 years, 172.6 ± 8.2 cm and 66.5 ± 9.5 kg, respectively. Skinfold thickness at the forearm was measured at the location under the NIRS probe using a skinfold caliper (Holtain Ltd., Crymmych, UK). The mean skinfold thickness was 6.0 ± 2.2 mm at the proximal section of the forearm. All subjects reported no health problems or upper extremity injuries. The subjects were asked not to perform strenuous exercise 48 h prior to and during the test–retest experimental period. The study conformed to the recommendations of the local Human Research Ethics Committee in accordance with the Declaration of Helsinki. A written informed consent was signed by each subject.

Near infrared spectroscopy (NIRS)

During the exercise test, muscle tissue oxygenation was measured with a near infrared spectroscopy (NIRS) system (Oxiplex TS, ISS, Champaign, IL, USA). This system is based on an infrared light absorption method, where the infrared light is emitted at different wavelengths. The NIRS probe consisted of eight light-emitting diodes operating at wavelengths 750 and 830 nm and one detector fiber bundle (source–detector distance = 2.0–3.5 cm). The deoxy[Hb + Mb] was stored at a frequency of 25 Hz and afterwards digitally averaged into 1 s values. The probe was positioned longitudinally on the M. flexor carpi radialis and ulnaris and on the M. flexor digitorum superficialis and secured with Velcro straps around the upper arm. Pen marks were made over the skin to detect movement of the probe during the exercise and in order to ensure that the probe could be positioned in exactly the same location during the retest. This retest took place 48 h after the first test at the same time of the day.

Study design and protocol

Preceding the test the MVC force was determined on the hydraulic handgrip dynamometer (Saehan corporation, Masan, Korea) for all subjects (best of the three attempts). Each attempt was terminated when the force showed a clear stagnation or a decrease. Between every attempt there was a 5 min rest period. The NIRS probe was placed on the M. flexor carpi radialis and ulnaris and a pneumatic cuff on the upper arm. The subjects performed the complete exercise protocol in supine position. The protocol started with an arterial occlusion of the forearm. There was no

specific timeframe for this occlusion period as it was executed until a steady state in deoxy[Hb + Mb] was reached. This occlusion was carried out with a cuff inflated at about 260 mmHg on the upper arm. After the occlusion there was a 5 min rest period before the exercise task started. This task consisted of 2 min periods of an incremental cyclic contractions protocol (ICCP) at 0.5 Hz (1 s contraction, 1 s relaxation) at different intensities of maximal voluntary contraction (% MVC). These contraction periods were separated by a 60 s rest period. The work intensity was increased by 10% MVC each step. This protocol was executed until exhaustion was reached and the subjects were not able to produce the required force. The cyclic contractions for all subjects used the same (dominant) hand in each period and in each of the two sessions, which were separated by 48 h.

Data analysis

When muscle tissue is exposed to an arterial occlusion, acute local hypoxia is induced. Following Fick's law muscular oxygen consumption (VO_{2m}) is the resultant of the product of muscle blood perfusion (Q_m) and oxygen extraction (Δ a-v O_2). To determine the mean values within this protocol a smoothing procedure was performed in which the 30 s and 10 s mean values were determined by means of a moving average (Boone et al. 2010). The amplitude of the deoxy[Hb + Mb] response (i.e., the difference between the highest 30 s average of deoxy[Hb + Mb] during the occlusion and the 30 s average of deoxy[Hb + Mb] preceding the occlusion) was used as an index for maximal O_2 extraction and was set to 100%. The changes in deoxy[Hb + Mb] during each work step (i.e., the mean of the maximum 10 s) were expressed relative to this amplitude.

The SmO_2 is a calculation of oxy[Hb + Mb]/(oxy[Hb + Mb] + deoxy[Hb + Mb]). As the NIRS instrument provides absolute measurements of these parameters and the oxy[Hb + Mb] is set relative to the tot[Hb + Mb], values can be reported without the need for a physiologic calibration using arterial occlusion. At every intensity (%MVC) interval, the 10 s mean minimum values of the SmO_2 were subtracted from the 30 s baseline value before the exercise protocol started.

Statistical analyses

To measure significant differences in the MVC and amplitude of the occlusion steady state a paired samples *T*-test was used. The same analysis was used to compare the increase (in %) in deoxy[Hb + Mb] as a function of the amplitude in deoxy[Hb + Mb] and the decrease in SmO_2 between the test and the retest for each work step. In addition, single measure intraclass correlation coefficients (ICCs) and the kappa test for agreement were used to examine the test–retest reliability of this new protocol. The ICCs were interpreted following Landis and Koch's (1977) benchmarks of 0.00–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, 0.81–1.0 almost perfect. Significance was set at $p < 0.05$. Statistical computations were performed using SPSS® software (version 18; SPSS Lead Technologies Inc., Chicago, IL, USA). All data are presented as mean ± SD.

Results

No significant differences ($p = 0.188$) were found between the MVC at the test ($x = 492 \pm 125$ N) and at the retest ($x = 475 \pm 127$ N). The mean final step when the exercise was finished was at 69% MVC for the first test and 73% MVC for the retest. Typical deoxy[Hb + Mb] outputs as a response on the arterial occlusion and the incremental cyclic contractions protocol (ICCP) are shown in Fig. 1. The mean amplitude of the maximal deoxy [Hb + Mb] during the arterial occlusion between the test ($x = 44.05 \pm 5.22$ μM) and the retest ($x = 47.16 \pm 4.65$ μM) showed no significant difference ($p = 0.156$). For the amplitude of the arterial occlusion an ICC of $r = 0.952$ ($p < 0.001$) was found.

The submaximal and maximal deoxy[Hb + Mb] values (in %) at the test and the retest are presented in Figs. 2, 3. No significant differences were found for the mean values of the submaximal increase (in %) in deoxy[Hb + Mb] for 20% ($p = 0.419$), 30% ($p = 0.331$), 40% ($p = 0.947$), 50% ($p = 0.12$) and 60% MVC ($p = 0.894$). The ICC and *p* values are presented in Table 1 for all submaximal and maximal increases relative to the occlusion value. In addition, no significant differences were found ($p = 0.907$) for the increase in deoxy[Hb + Mb] (in %) during the final bout (i.e., 69.5–73%) between the test ($n = 45.6 \pm 16.7$%) and the retest ($n = 44.9 \pm 17$%). For the maximal increase in oxygen extraction during the ICCP, an ICC of $r = 0.873$ ($p < 0.001$) was found between the test and the retest values (see Fig. 4). The pattern (in %) at these intensities at the test and the retest is given in Fig. 2. Following Landis and Koch's (1977) benchmarks the reliability for the submaximal intensities is slight for an intensity of 30%, fair for the intensities of 20% and 40% MVC and moderate for the intensities of 50 and 60%. For the maximal O_2 extraction (in %) the reliability of the measurements is almost perfect (Landis and Koch 1977).

The submaximal and maximal SmO_2 values (subtracted from baseline) at the test and the retest are presented in Fig. 5. No significant differences were found for the SmO_2 values between the test and the retest. The ICC and *p* values are presented in Table 2 for all submaximal and

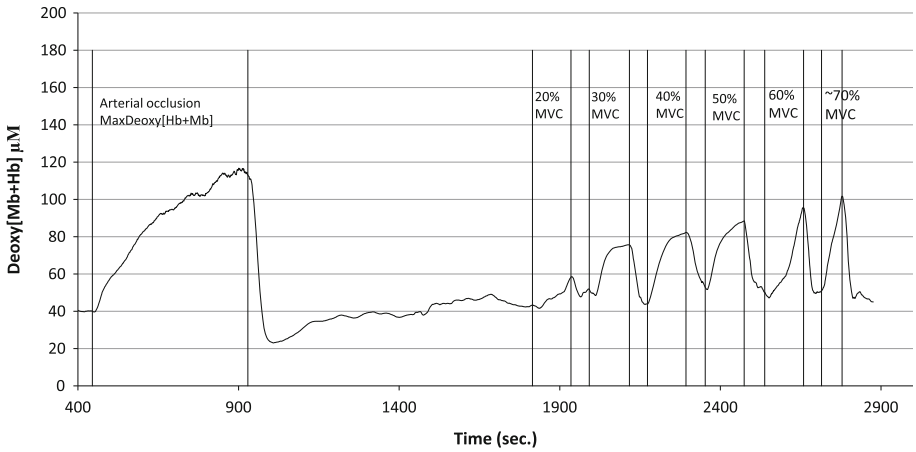

Fig. 1 Typical changes in the values of deoxygenated haemoglobin and myoglobin (deoxy[Hb + Mb])) of one subject during arterial occlusion and the incremental cyclic contractions protocol (ICCP) until exhaustion

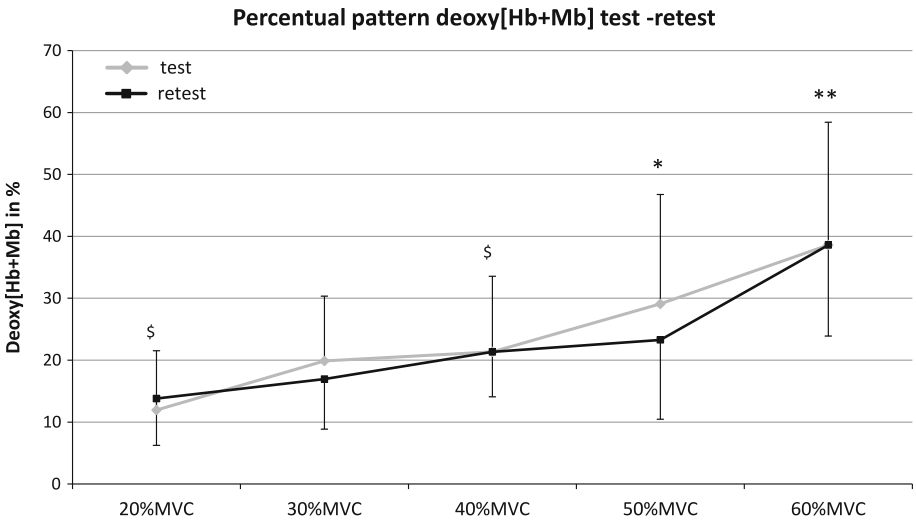

Fig. 2 Mean submaximal percentual deoxy[Hb + Mb] values at different intensities (% MVC) at the test and the retest for all 20 subjects. Trends of significant ICC's ($\$ \leq 0.1$) and ICC's at the significance levels of $*p \leq 0.05$, $**p \leq 0.01$ and $***p \leq 0.001$ are presented between the test and retest

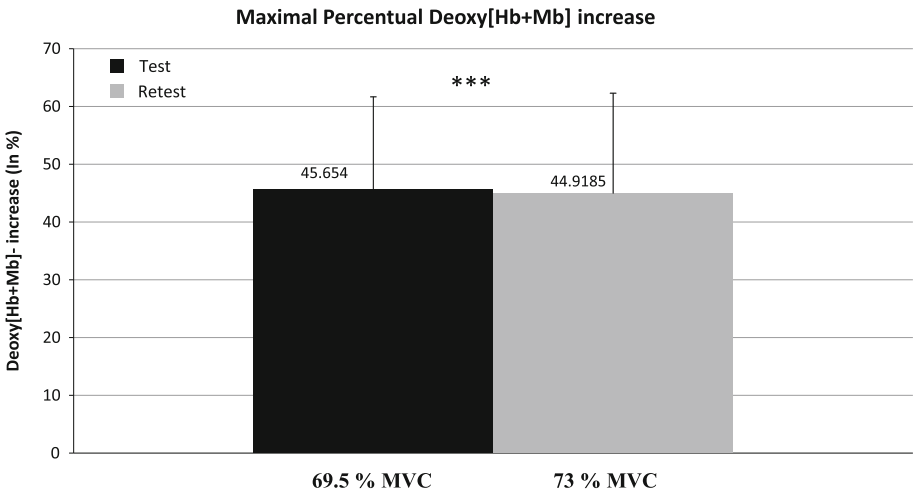

Fig. 3 Mean maximal percentual deoxy[Hb + Mb] values at the maximal intensity at the test and retest for all 20 subjects. ICC at the significance level $***p \leq 0.001$ is presented between the test and the retest

maximal decreases in SmO_2. Following Landis and Koch's (1977) benchmarks the reliability for the submaximal intensities is fair for the intensities of 30, 50 and 60% MVC, moderate for the intensity of 40% and substantial for 20% MVC. For the maximal SmO_2 the reliability of the measurements is substantial (Landis and Koch 1977).

Discussion

To our knowledge, this is the first study reporting a significant test–retest ICC for the deoxy[Hb + Mb] and SmO_2 response in forearm muscles with near infrared spectroscopy (NIRS). Other researchers have concluded that NIRS

Table 1 ICC and p values for the deoxy[Hb + Mb] values relative to the maximum value (during arterial occlusion) at the submaximal and maximal intensities

	20% MVC	30% MVC	40% MVC	50% MVC	60% MVC	69.5–73%
ICC	0.321	0.025	0.334	0.466	0.553	0.873
p value	0.078	0.457	0.069	0.017	0.005	<0.001

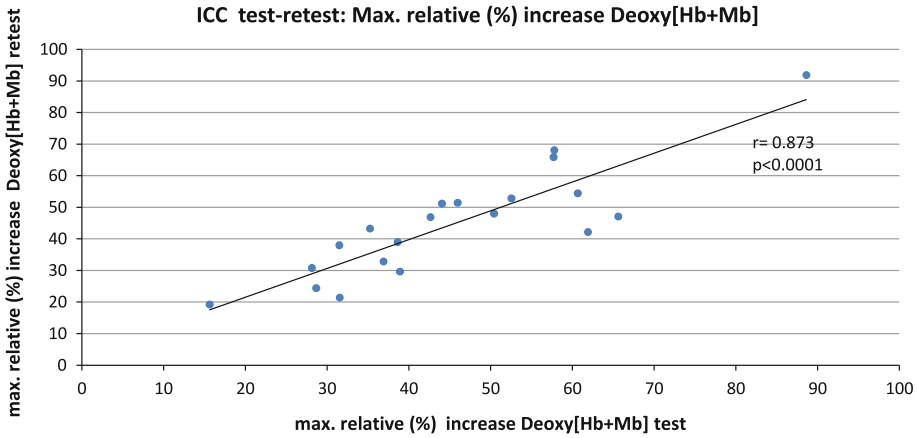

Fig. 4 The ICC between the maximal deoxy[Hb + Mb] increase (in %) between the test and the retest

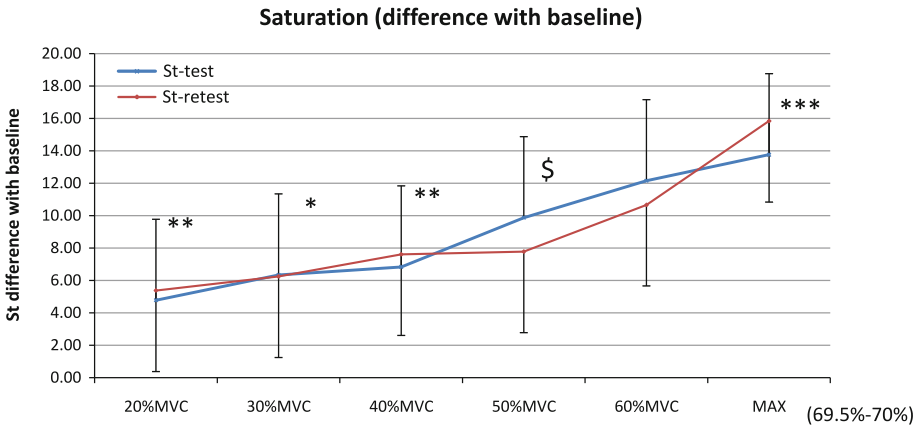

Fig. 5 Mean submaximal and maximal absolute SmO_2 values (subtracted from baseline values) at different intensities (% MVC) at the test and the retest for all 20 subjects. Trends of significant ICCs ($\$ \leq 0.1$) and ICC's at the significance levels of $*p \leq 0.05$, $**p \leq 0.01$ and $***p \leq 0.001$ are presented between the test and retest

Table 2 ICC and p values for the SmO2 (differences between the lowest 10-s mean values and 30-s baseline values) at the submaximal and maximal intensities

	20% MVC	30% MVC	40% MVC	50% MVC	60% MVC	69.5–73%
ICC	0.602	0.39	0.579	0.345	0.22	0.774
p value	0.002	0.04	0.003	0.063	0.171	<0.001

is a reliable tool for measuring hemodynamics, but specific to large muscles (limb) or brain (Kell et al. 2004; Pereira et al. 2007). The main finding of our study was that according to our protocol NIRS is a reliable tool for measuring deoxygenation in small muscles. The deoxy[Hb + Mb] was studied, to gain a better insight into peripheral extraction mechanisms. The only calculation in this study was to compare the increases of deoxy[Hb + Mb] relative to the maximal deoxygenation. It is important to mention that this arterial occlusion was very important in our protocol as a maximal deoxygenation value. The difference between our protocol and other studies is that the occlusion was maintained until a steady state of the deoxy[Hb + Mb] was reached. Our underlying rationale for the use of this protocol is that as it is expressed relatively, it can be used for comparison between individuals. This is because other factors (i.e., adipose tissue) can influence the absolute value. In our opinion, an arterial occlusion standardized in time is not effective because the time to reach a steady state differs greatly

between individuals. When an arterial occlusion is induced, muscle blood perfusion (Q_m) of the forearm is stopped. As a consequence local available oxygen is consumed to respond to the metabolic demands of the forearm muscles at that moment. The increased O_2 extraction from the blood into the muscle can be observed in our deoxy[Hb + Mb] output because more oxygen is released from the haemoglobin molecules. The maximal increase (in %) in the protocol was the most important output from this test: a high value represents in fact a high extraction rate of oxygen from the blood into the muscle.

In the research of Van Beekvelt et al. (2002) the occlusion was imposed for 45 s and the muscular VO_2 (mVO_2) was calculated. The protocol and the outcome examined were very different in this study compared to that of Van Beekvelt et al. (2002). In this research our interest was not to calculate the mVO_2, but to determine the oxygen extraction in the forearm muscles. Van Beekvelt et al. (2002) used the 45 s arterial occlusion to calculate the mVO_2. In our study, as mentioned previously, the occlusion had a different purpose: as a maximal deoxy[Hb + Mb] value to analyze the other data relative to, and not as a calculation parameter. Despite the fact that both studies investigated the reliability of NIRS on forearm muscles, both outcome and methods were very different.

Grassi et al. (2007) were the only researchers that used a similar protocol and outcome to compare the oxygen extraction between a mitochondrial myopathy and a control population. The fact that this protocol was executed on a cycle ergometer was a major difference compared to our study. The deoxy[Hb + Mb] increases in the incremental cycloergometric test were also examined relative to the maximal steady state deoxy[Hb + Mb]. Grassi et al. (2007) concluded that the NIRS showed an impaired oxygen extraction in a mitochondrial myopathy and a McArdle population. Our method is very easy to execute and could be useful to study oxygen extraction reliably in a population with high exercise intolerance as well as in a healthy population.

The fact that substantial reliability was found considering the oxygen saturation supports the fact that reliable results were found with the deoxy[Hb + Mb] values. The SmO_2 is a calculation of oxy[Hb + Mb]/Tot[Hb + Mb] or oxy[Hb + Mb]/(oxy[Hb + Mb] + deoxy[Hb + Mb]). Although the deoxy[Hb + Mb] output is the most important as a measure for microvascular oxygen extraction, it is useful to compare the SmO_2 as well. Physiologic calibration using arterial occlusion is not necessary. The relation between the SmO_2 and the deoxy[Hb + Mb] values are inverse. When the deoxy[Hb + Mb] values are increasing, the SmO_2 values decrease. The fact that the decrease of SmO_2 shows similar reliability with the deoxy[Hb + Mb] values supports the fact that the measurements of forearm oxygenation during this protocol are reliable.

To our knowledge Van Beekvelt et al. (2002) is the only study that addressed the reliability of NIRS on the forearm muscles, using a different protocol and outcome. Using this protocol, this is the first study that finds a highly reliable outcome for measuring the deoxy[Hb + Mb] relatively and the SmO_2. In conclusion we propose that this protocol can be used to compare muscle deoxygenation and oxygen saturation between different subjects.

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