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Reproducibility of Parameters of Postocclusive Reactive Hyperemia Measured by Near Infrared Spectroscopy and Transcutaneous Oximetry

RUDI KRAGELJ, TOMAŽ JARM, and DAMIJAN MIKLAVČIČ

Faculty of Electrical Engineering, University of Ljubljana, Tržaška, Slovenia

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Abstract—The purpose of this study was to investigate postocclusive hyperemic response using near infrared spectroscopy (NIRS) and transcutaneous oximetry ($TcpO_2$). Five minute arterial occlusion on the calf muscle was performed in six healthy volunteers (mean age 29, range 23–34 years, mean $TcpO_2$ at rest 53 mm Hg, range 47–58 mm Hg, and ankle brachial index between 1 and 1.2). Oxygen partial pressure at rest, oxygen consumption (VO_2) during ischemia, recovery times and resaturation rates after arterial occlusion were determined and new parameters for evaluation of the level of vascular disorders of lower limbs are suggested. The reproducibility of the signals was studied by repeating the same protocol on each subject four to six times. Repeated measurements showed no significant difference among trials, indicating that the measurements were reproducible. The mean values of the coefficient of variability for suggested parameters varied between 6% and 30% (mean value 17%). Interindividual variations of parameters are higher and can be explained by differences in fat/muscle ratio and in the measured tissue volume of the NIRS signal. Simultaneous measurements of NIRS and $TcpO_2$ showed different responses to ischemic conditions, due to the different physiological levels of oxygen assessment. The combined use of both methods yields deeper insight into conditions of blood flow and tissue oxygenation. *© 2000 Biomedical Engineering Society.* [S0090-6964(00)00302-7]

Keywords—Noninvasive method, Oxygen consumption, Tissue oxygenation, Skin perfusion, Ischemia, Peripheral circulation.

INTRODUCTION

In 1977 Jöbsis first described *in vivo* application of near infrared spectroscopy (NIRS) to monitor changes in the oxygenation of the brain in the intact cat head.[12] Initial studies using this optical technique showed that cerebral oxygenation and blood flow in preterm babies can be monitored.[1,10,19,20] Technical developments in the instrumentation made it possible to measure absorption changes across a total of 10 optical densities equivalent to 8–9 cm of brain tissue.[4] The method offered promise in measurements of changes in intravascular (hemoglobin) and mitochondrial (cytochrome $aa3$) oxygenation of the limb muscles. It was shown that it is possible to quantify muscle oxygen consumption VO_2,[3,5] muscle blood flow[6,8] and venous saturation.[21]

NIRS measurements in muscle have been used to investigate diseases associated with impaired tissue oxygenation, like heart failure and peripheral vascular disease.[9] Many studies were made to investigate peripheral vascular diseases (PVDs). It was found that, at rest, VO_2 in patients with PVD was half that found in healthy controls.[2] Another method for assessing PVD using NIRS following walking exercise is to determine oxygen resaturation as an indicator of oxygen debt and arterial inflow capacity.[13,15] The patients with severe impairment showed an earlier decrease of muscle oxygenation. The hyperemic response after arterial occlusion was significantly lower in patients with PVD.[14] In the same study hyperemic responses and recovery times following arterial occlusion were reported to be in good correlation with the ankle brachial index (ABI).

Postocclusive reactive hyperemia (PORH) is a reproducible transient increase in blood flow after the release of arterial occlusion. Although it is one of a few well-known tests in clinical practice for evaluating the functional aspects of arterial blood flow in the lower limb, not many studies have dealt with the determination of the parameters that could provide important information about tissue oxygenation and the severity of the PVD. For validation of the parameters obtained a study of reproducibility of the NIRS measurement is necessary.

The purpose of this study was therefore to explore the postocclusive hyperemic response using NIRS in combination with transcutaneous partial pressure oximetry ($TcpO_2$). We (1) examined the applicability of NIRS for quantification of PORH of healthy human limbs, (2) suggested parameters which can be used to evaluate the state of peripheral vasculature, (3) calculated the reproducibility of the measured parameters and (4) compared

Address correspondence to Rudi Kragelj, Faculty of Electrical Engineering, Tržaška 25, SI-1001, Slovenia. Electronic mail: rudi@svarun.fe.uni-lj.si

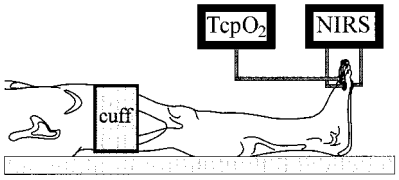

FIGURE 1. Experimental setup.

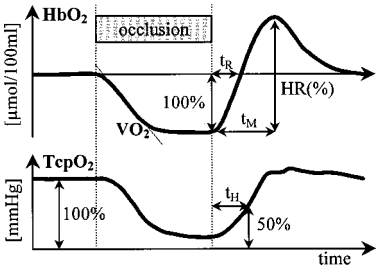

FIGURE 2. Parameters evaluated.

the NIRS signals with the results of $TcpO_2$ measurements.

MATERIALS AND METHODS

Subjects

Six healthy, nonsmoking volunteers (mean age 29, range 23–34 years, mean $TcpO_2$ at rest 53 mm Hg, range 47–58 mm Hg, and ABI between 1 and 1.2) took part in the study, after giving informed consent. All subjects were asked not to perform any extensive physical activity on the day of the test. All the experiments were carried out at the ambient temperature of 21 °C.

The study was approved by the Ethical Committee of the Institute of the Republic of Slovenia for Rehabilitation.

Methods

NIRS is a noninvasive and continuous optical method for measuring tissue oxygenation and hemodynamics.[12,18,20] In this study it was used for the assessment of tissue oxygenation in the distal parts of the lower limbs. The technique is based on two fundamental characteristics: (a) the relative transparency of human tissue to light in the near infrared region (700–1000 nm) and (b) the oxygenation-dependent absorption of oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb) and cytochrome-c-oxidase (CytOx). By measuring changes in light absorption at different wavelengths tissue oxygenation can be measured continuously. The relation between light absorption and concentration changes of the chromophore is described by the modified Beer-Lambert law.[7]

A $NIRO_2$-X2 instrument (Keele University, UK) was used for simultaneous monitoring of concentration changes of oxy- and deoxyhemoglobin (HbO_2 and Hb). Summation of the changes in the concentrations of HbO_2 and Hb provides a measure of changes in the total tissue hemoglobin Hbtot and reflects changes in the tissue blood volume. From the difference of HbO_2 and Hb signals the oxygenation index (OI) signal can be derived. It gives an indication of the net hemoglobin oxygenation status.[16]

Experimental Protocol

Figure 1 shows the experimental setup. All subjects were in a supine position during the measurement. The optical fibers (optodes) of the NIRS instrument were positioned on the dorsal and lateral surfaces of the foot between fourth and fifth digits. The measurement was performed in the transmission mode. The optodes were attached to the skin by a support that allowed both the distance and the angle between the optodes to be maintained constant. The distance between optodes was approximately 4 cm in all subjects. The sampling time was 1 s. Data collected by NIRS were transferred on line to the computer (IBM compatible XT/AT) for storage and subsequent analysis. Data collection program NIRDCU 4.81 (Keele University, UK) was used for instrument control and data acquisition. A transcutaneous oxygen partial pressure meter (TCM2, Radiometer, Denmark) was used for oxygen partial pressure ($TcpO_2$) monitoring. The electrode was positioned on the upper surface of

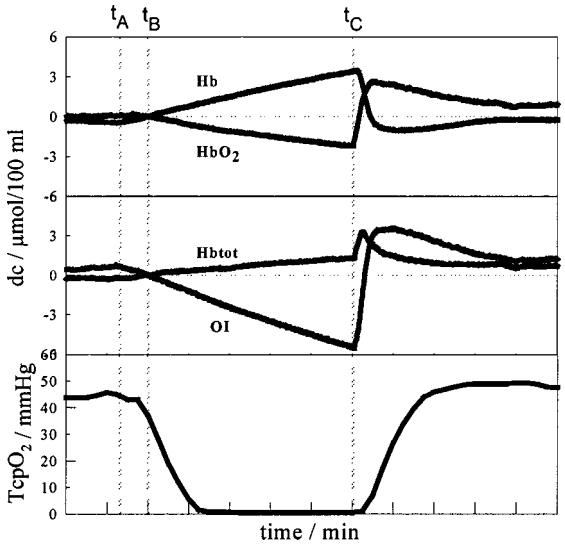

FIGURE 3. NIRS and $TcpO_2$ responses from the foot of a representative subject during 5 min arterial occlusion and subsequent recovery (t_A, time point of cuff inflation; t_B, time point of the start of arterial occlusion; t_C, time point of the end of arterial occlusion).

TABLE 1. Mean individual values of the parameters of oxygenation (AVG±S.E.). [S.E., standard error; range (min/max).]

	HbO_2				Hb			OI			$TcpO_2$	
Subject	VO_2 ml l^{-1} min^{-1}	t_R (s)	t_M (s)	HR (%)	t_R (s)	t_M (s)	HR (%)	t_R (s)	t_M (s)	HR (%)	$TcpO_2$ mm Hg	t_H (s)
1	0.80±0.12	16±2	39±5	194±17	29±2	66±1	146±8	21±1	54±3	163±9	48±1	58±2
2	0.79±0.09	17±4	50±6	236±20	31±2	64±8	159±5	24±3	56±7	193±13	58±2	76±3
3	0.70±0.10	23±3	53±5	224±25	33±3	75±3	161±6	27±3	57±3	182±11	51±1	100±7
4	0.81±0.06	19±2	51±6	178±12	41±5	68±6	126±1	28±2	60±8	142±4	53±3	54±1
5	0.79±0.06	23±4	55±6	213±20	37±4	80±1	159±5	29±4	66±6	177±7	58±4	56±1
6	0.78±0.05	15±1	53±6	172±24	40±4	78±9	132±5	25±3	63±5	138±4	47±4	70±1
Mean	0.78	19	50	203	35	72	147	26	59	166	53	69
S.E.	0.02	1	2	10	2	3	6	1	2	9	2	7
Range	0.70/0.81	15/23	39/55	172/236	29/41	64/80	126/161	21/29	54/66	138/193	47/58	54/100

the same foot between the second and the third digits. The measurements were performed at an electrode temperature of 43 °C.

Arterial occlusion was achieved by inflating a thigh cuff (CC17, Hokanson) to a pressure of 30 mm Hg above the value of the individual systolic pressure of each subject. The cuff was placed above knee on the thigh and inflated, using a standard cuff inflator (TD312, Hokanson), in less than 1 min to the pressure needed for the arterial occlusion. The cuff remained inflated for 5 min and then rapidly released.

A 10 min rest period was allowed after the placement of all necessary equipment. In order to check the reproducibility of the measurements all subjects underwent the same protocol from four to six times on different days during a period of 3 months.

Signal Analysis

Different parameters obtained by the two noninvasive methods were studied (Fig. 2):

(1) VO_2, oxygen consumption, calculated from the gradient of the HbO_2 signal during the first 60 s of decrease of the HbO_2 signal during arterial occlusion and converted to ml l^{-1} min^{-1} as was already described by Cheatle *et al.*,[2]
(2) t_R, time of recovery, time after release of the cuff until the initial values of HbO_2, Hb and OI signals before the test are reached,
(3) t_M, time to peak value, time after release of the cuff until the peak value of the signals are reached,
(4) HR, hyperemic response (μmol/100 ml), maximum change of the signal after the release of the cuff, expressed as the percentage of change of the signal during arterial occlusion,
(5) $TcpO_2$, oxygen partial pressure value (mm Hg) obtained before the arterial occlusion started,
(6) t_H, the time after release of the cuff until 50% of the initial $TcpO_2$ is reached.

Statistical Analysis

The coefficient of variability (CV) was used as the measure of the reproducibility of the chosen parameters. It was determined as the ratio between the standard deviation and average value of the parameter in each individual.

The reproducibility of the measurement in each subject and the homogeneity of the group of volunteers were statistically evaluated by Friedman repeated measures ANOVA on ranks test. The statistically significant level of difference was considered to be at $p<0.05$.

RESULTS

Figure 3 shows a typical example of $TcpO_2$ and NIRS measurements. At the time point t_A inflation of the cuff was started. In less than 60 s the pressure in the cuff reached the desired value needed for arterial occlusion (time point t_B). The HbO_2 signal decreased from the beginning of the arterial occlusion. This decrease was mirrored by the increase of the Hb signal. During the occlusion a small increase in the Hbtot signal was observed. At time point t_C the cuff was released and a hyperemic response was observed. The Hbtot signal, which is correlated with total blood volume, increased above the base line within 30 s after the release of arterial occlusion. The increase in the HbO_2 signal exceeded the decrease in the Hb signal in the first minute of hyperemia. All signals returned to or near the starting base line by the end of the 7 min recovery time. The value of $TcpO_2$ decreased faster than the HbO_2 signal and reached the minimum value within 2–3 min of arterial occlusion. After the relief of occlusion $TcpO_2$ recovered more slowly than the HbO_2 signals.

Oxygen consumption (VO_2), hyperemic response (HR) and recovery times (t_R,t_M,t_H) after arterial occlusion as well as the absolute $TcpO_2$ values at rest are listed in Table 1. The parameters of oxygenation of repeated measurements in a single individual were aver-

TABLE 2. Coefficients of variability (CV in %) for all evaluated parameters of NIRS and $TcpO_2$ measurements. [S.E., standard error; range (min/max).]

	HbO_2				Hb			OI			$TcpO_2$	
Subject	VO_2	t_R	t_M	HR	t_R	t_M	HR	t_R	t_M	HR	$TcpO_2$	t_H
1	33	23	27	19	14	4	12	14	13	12	3	6
2	29	55	29	21	18	32	7	27	31	17	7	12
3	28	27	19	23	20	7	7	20	12	12	3	9
4	15	22	22	13	22	16	2	15	28	5	9	2
5	15	36	23	19	21	3	6	28	18	8	15	5
6	14	18	28	35	10	28	8	25	18	18	22	4
Range	14/33	18/55	19/29	13/35	10/22	4/32	2/12	14/28	12/31	5/18	3/22	2/12

aged. For the group of subjects the mean values, standard errors and the range for each parameter are also given in Table 1.

The coefficients of variability were calculated from the set of repeated measurements in each subject. Individual results and range values are presented in Table 2. The coefficients of variability varied from 2% to 35% except for one parameter in one subject where the value was higher than 50%.

The Friedman repeated measures ANOVA on ranks test was used to calculate the differences among measurements repeated on different days and to calculate the difference among the subjects for each parameter. Results of statistical analysis of the reproducibility of NIRS and $TcpO_2$ measurements, expressed in p values, are presented in Table 3. The results showed no significant differences between repeated measurements on different days, indicating that the measurements were reproducible. The results of the test among different subjects showed no significant differences in 8 out of 12 determined parameters, suggesting that the group of subjects was not entirely homogeneous.

DISCUSSION

In the study we described the measurement and the derivation of different parameters of tissue oxygenation. The results proved to be reproducible. Statistical analysis showed that there is good reproducibility among the repeated measurements. The interindividual variation was larger, indicated by lower p values, compared to the difference among the repeated measurements. A statistically significant difference among the subjects was found in four parameters in spite of the fact that the group of healthy volunteers was carefully chosen and supposed to be homogeneous with regard to their age, mean $TcpO_2$ value at rest and ankle brachial index. One of the factors which could play a role in the interindividual variation is the optical pathlength although special care was taken to position the optodes on the same place in all subjects. Probably a more important factor could be the interindividual difference in the amount of tissue contributing to the NIRS signal.[5]

In almost all subjects an increase in total blood volume, increase in Hbtot signal, was observed, although it is almost impossible that there was still arterial inflow into the leg. An increase in the Hbtot signal was found also in other studies and explained as the consequence of blood redistribution in the muscle during arterial occlusion.[2,3,14]

One criticism of the study could be the relatively long time needed to inflate the cuff to the pressure of arterial occlusion. More than 30 s of inflation with the standard cuff inflator, widely used in the clinical environment, probably caused undesired venous occlusion prior to arterial occlusion. Using a rapid cuff inflator, which is capable of inflating the cuff within 1 s, would probably lead to more precise observations and improved reproducibility.

TABLE 3. Statistical analysis of differences among repeated sets of treatments and among different subjects.

Statistical analysis	HbO_2				Hb			OI			$TcpO_2$	
	VO_2	t_R	t_M	HR	t_R	t_M	HR	t_R	t_M	HR	$TcpO_2$	t_H
Among treatments	0.43	0.26	0.39	0.88	0.07	0.20	0.65	0.33	0.52	0.89	0.72	0.96
Among subjects	0.85	0.56	0.36	0.28	0.03*	0.33	<0.01*	0.52	0.69	<0.01*	0.11	<0.01*

*Statistically significant difference ($p<0.05$).

In this study we found lower VO_2 values (mean value 0.78, range 0.70–0.81 $ml\,l^{-1}\,min^{-1}$) than those reported in previous studies.[2,14,15] The difference can be explained by the different location of the optodes of NIRS. In the above mentioned studies the optodes were positioned either on gastrocnemius muscle or on the forearm. We performed the measurement on the distal part of the foot where there is a relative paucity of muscle tissue and blood flow is lower than in calf muscle.

NIRS was compared in this study with a technique currently used clinically for noninvasive monitoring of oxygenation. Simultaneous with the NIRS measurement the oxygen partial pressure was measured transcutaneously. Signals obtained from both methods showed different responses to ischemic conditions. Whereas NIRS assesses O_2 sufficiency at the deep tissue level, transcutaneous O_2 monitoring measures oxygen diffusion predominantly from skin.[11] During recovery from ischemia $TcpO_2$ returned to the base line slower than the HbO_2 signal. Although the results of $TcpO_2$ showed better reproducibility compared to the NIRS measurement they should be interpreted with caution. Namely, $TcpO_2$ does not reflect oxygen transport from the capillaries at physiological circumstances but in a state of thermally induced vasodilation.[17]

The mean time taken to reach maximum HbO_2 levels was 50 s (range 39–55 s). It is longer than recovery times reported in other studies,[2,14] where it was around 38 s. This is probably due to a larger distance between the thigh occlusion site and the site of the measurement of the foot. In this study we introduced a new parameter which has not yet been studied by others. Hyperemic response could be used as the measure of resaturation and can give some additional information on blood flow and oxygen delivery to tissue after the release of the occlusion. Although we presented the parameters calculated from different NIRS signals, the parameters obtained from HbO_2 signals are of the greatest importance.

From the results of this study it can be concluded that NIRS can be used to obtain valuable new information about the condition of peripheral vasculature, which cannot be obtained by $TcpO_2$ alone. An important advantage of NIRS over $TcpO_2$ is that the more dynamic nature of the NIRS signals in comparison to the $TcpO_2$ signal (Fig. 3) reflects more closely the actual response of the peripheral vasculature to the occlusive provocation. Furthermore it takes considerably less time to perform the described NIRS measurement due to the very long time needed for $TcpO_2$ signal stabilization prior to the start of measurement. The combined parameters of both methods obtained from the hyperemic response following arterial occlusion can yield deeper insight into conditions of blood flow and tissue oxygenation in lower limbs than any of the methods alone. Even though the NIRS measurements are nearly as reproducible as the $TcpO_2$ measurement, the origin of the NIRS signal should be known better.

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