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Abstract. Near-infrared spectroscopy (NIRS) is a noninvasive technique evaluating microvascular function. The aim of this study was to assess the reproducibility of NIRS parameters during reactive hyperemia induced by a 5 min brachial artery occlusion. Twenty-four healthy young males (mean 34 ± 8 years old) had two microvascular function evaluations by NIRS over a 7 to 30-day period (mean 16 ± 10 days). Intra-subject and inter-observer reproducibility were evaluated with intraclass correlation coefficient (ICC), coefficient of variation (CV), and standard error of measurement (SEM%) for every parameter. Mean NIRS parameters did not differ between both evaluations. Reproducibility was greatest for muscle oxygen consumption (ICC: 0.84; CV: 6.51%; SEM: 7.11%), time to basal O_2Hb (ICC: 0.63, CV: 20.04%, SEM 27.22%), time to maximal O_2Hb (ICC: 0.71; CV: 15.61%; SEM: 19.27%), peak of O_2Hb (ICC: 0.63, CV: 6.68%, SEM 8.53%), time to maximal tHb (ICC: 0.73, CV: 19,61%, SEM 24.56%) and area under the O_2Hb and tHb curves (ICC: 0.68, CV: 16.15%, SEM 22.93% and ICC: 0.62, CV: 18.59%, SEM 26.64%, respectively). Moreover, inter-observer reproducibility ranged from excellent to perfect (ICC from 0.85 to 1.00) for every parameter. NIRS parameters during reactive hyperemia are highly reproducible which enables their repeated measurement to study microvascular function in healthy subjects. [DOI: 10.1117/1.JBO.17.7.077010]

Keywords: reproducibility; near-infrared spectroscopy; reactive hyperemia; cardiometabolic risk; microvascular function.

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1 Introduction

Microvascular function is structurally dependent of microvascular density and capillary recruitment, and refers to small vessels (less than 150 μm) including arterioles, capillaries, and venules.[1] Microvascular dysfunction is thought to contribute to insulin resistance and hypertension associated with obesity, and potentially links central adiposity with cardiovascular disease risk.[1] An impairment of microvascular function (measured by hyperemic velocity) was found to be predictive of future cardiovascular events in apparently healthy men.[2] Microvascular function assessed by fingertip temperature variation upon brachial artery occlusion was shown to correlate with coronary artery calcification, myocardial perfusion, and insulin resistance in asymptomatic individuals.[3–5]

Recently, assessment of microvascular function using near-infrared spectroscopy (NIRS) during a 5 min brachial artery postocclusive reactive hyperemia (PORH) was performed in healthy adults and in patients with peripheral arterial disease and Chronic Heart Failure (CHF).[6–10] It was also shown by our group that microvascular function measured by NIRS during PORH was impaired in patients with metabolic syndrome and coronary heart disease and that the degree of impairment was related to the number of cardiovascular risk factors.[11]

However, only one previous small study performed with six healthy subjects has documented the reproducibility of lower limb NIRS PORH parameters.[8] Thus, studies reporting the reproducibility of NIRS parameters in evaluating microvascular function during forearm PORH are lacking. Such data would be of great interest since forearm PORH evaluation by NIRS is a simple, noninvasive, low-cost, and a less uncomfortable technique than lower limb microvascular function evaluation. This makes forearm PORH evaluation by NIRS the method of choice to evaluate microvascular function in situations outside lower limb peripheral vascular disease. Furthermore, the inter-observer reproducibility of NIRS parameters measured during PORH has also not been established. The aims of this study were therefore: 1. to evaluate the intra-subject reproducibility, and 2. to evaluate the inter-observer reproducibility of forearm NIRS parameters measured during PORH in young healthy males.

2 Methods

2.1 *Subjects*

Twenty-four young healthy male subjects were recruited in the study. Participants were included if they were healthy

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nonsmoking men, aged between 18 and 50 years, and exempt from any cardiovascular risk factors or diseases. Exclusion criteria were LDL-cholesterol > 3.50 mmol/L, diagnosed (treated/untreated) hypertension (blood pressure ≥ 140/90 mmHg), abdominal obesity (waist circumference ≥ 102 cm), diabetes mellitus, and/or previous cardiovascular disease (coronary heart disease and/or heart failure). Subjects taking any pharmacological drugs, vitamin C, or omega-3 polyunsaturated fatty acid supplements were also excluded from the study to control for potential impact on vascular function. Participants were told to refrain from any strenuous exercise and alcohol consumption the day prior to testing. This study was approved by the Montreal Heart Institute Ethics Committee and all subjects provided written informed consents.

2.2 *Study Protocol*

Each subject underwent a medical history, a physical examination with measurement of height, weight, waist circumference, and body composition with bio-electrical impedance (Tanita, model BC418, Japan). A blood draw was also done to measure fasting blood glucose, insulin, and lipid profile. Microvascular function assessment by NIRS during forearm PORH was repeated on two different occasions separated by a minimum of 7 and a maximum of 30 days (16 ± 10 days). Assessments were performed in the early morning (8:30 to 9:00 am) while subjects were overnight-fasted and resting in a supine position in a quiet, dimly lit and temperature controlled room (22°C to 25°C). An automated pneumatic cuff inflator (Hokanson model E20) was positioned above the right elbow and NIRS optodes were positioned below the elbow on the right brachio-radialis muscle with an inter-optode distance of 45 mm. The optodes were attached to the skin of participants with adhesive stickers so the angle and position of the optodes were kept constant. Optodes were connected to a continuous-wave NIRS (Oxymon, Artinis Medical Systems, Nijmegen, Netherlands) that generated light at 905, 850, and 770 nm, which differentiated between oxy- and deoxyhemoglobin/myoglobin (O_2Hb/O_2Mb and HHb/HMb, respectively).[12,13] For convenience, both hemoglobin and myoglobin will be referred to as hemoglobin since their respective spectrum cannot be distinguished. The changes in absorption at the discrete wavelengths were converted into concentration changes of O_2Hb and HHb (μM) using a modified Lambert—Beer law in which a path-length factor was incorporated to correct for scattering of photons in the tissue.[14] The sum of both signals represented total hemoglobin (tHb). To correct for scattering of photons in the tissue, a differential path-length factor of 4.0 was used for the calculation of absolute concentration changes.[13,15] NIRS acquisition was done continuously during a 2 min preocclusive rest period, 5 min occlusion (pressure cuff inflated at 100 mm Hg over the systolic blood pressure), and a 5 min postocclusive period.[7,11] NIRS signals were sampled at 10 Hz, displayed in real time and stored on a hard drive for off-line software analysis (Oxysoft, Artinis Medical Systems, Netherlands).

Figure 1 shows typical NIRS signals for O_2Hb, HHb, and tHb that are used for off-line NIRS parameters analysis. (1) Muscle oxygen consumption (mVO_2) (ml O_2/min/100 g) was measured by evaluating the rate of decrease of O_2Hb during arterial occlusion [$-d(O_2Hb)/dt$].[7,11–13] Concentration changes of O_2Hb were expressed in μM/s and converted to ml O_2/min/100 g.[12,13] A value of 1.04 kg/L was used for muscle density.[12,13] The following NIRS parameters were measured during

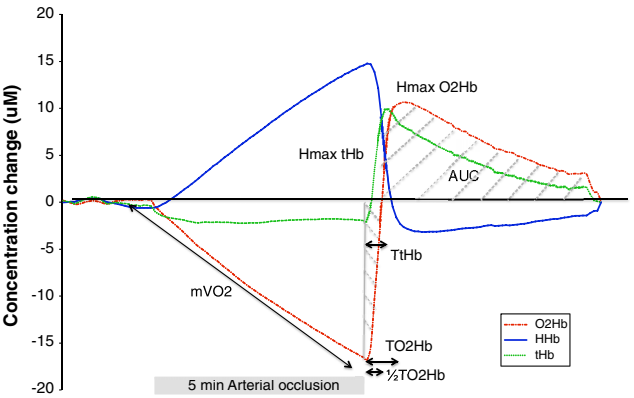

Fig. 1 Typical representation of NIRS signals during arterial occlusion and postocclusive reactive hyperemia.

PORH: (2) ½ time recovery of the O_2Hb ($1/2T\ O_2Hb$) (s): time after release of the cuff until the initial preocclusion O_2Hb values are reached, (3) time to maximal O_2Hb (TO_2Hb) (s): time between the release of the cuff and the maximum value of O_2Hb is reach, (4) maximal amplitude of O_2Hb (Hmax O_2Hb) (μm): maximal amplitude of postocclusion O_2Hb signal, (5) maximal amplitude of tHb (Hmax tHb) (μm): peak value of postocclusive tHb, (6) time to maximal tHb (TtHb) (s): time between the release of the cuff and the maximum value of tHb is reach, (7) increase rate to max O_2Hb (μM/s) was calculated by dividing maximal amplitude of O_2Hb by time to maximal O_2Hb, (8) increase rate to max tHb (μM/s) was calculated by dividing HRmax of tHb by time to Hmax of tHb, (9) post-occlusion area under the curve of O_2Hb (AUC O_2Hb) (arbitrary unit; a.u.) is the area under the 5 min postocclusion O_2Hb curve, (10) post-occlusion area under the curve of HHb (AUC HHb) (a.u.) is the area under the 5 min postocclusion HHb curve, (11) post-deflation area under the curve of tHb (AUC tHb) (a.u.) is the area under the 5 min postocclusion tHb curve.[7,11]

2.3 *Statistical Analysis*

Data were analyzed using Statview software (SAS, USA, version 5.0) and are presented as mean ± standard deviation except where otherwise indicated. Normal distribution of the data was verified by a Shapiro-Wilk test and data were transformed logarithmically when this criteria was not met. For continuous variables, statistical differences between tests were evaluated by a one-way ANOVA with repeated measure. A p-value ≤ 0.05 was considered significant. Intra-subject reproducibility was obtained by comparing NIRS parameters evaluated on two different occasions, in the same subjects. Inter-observer reproducibility was obtained by having two observers familiar with the methodology calculate each NIRS parameter for the same NIRS evaluation of every subject. The relative reproducibility, defined by the degree to which individuals maintain their rank order in a sample with repeated measurements,[16,17] was assessed by the intraclass correlation coefficient (ICC).[16] An ICC ≤ 0.20 was defined as poor, an ICC between 0.21 to 0.40 was defined as fair, an ICC between 0.41 and 0.60 was defined as satisfactory, an ICC between 0.61 and 0.80 was defined as good, and an ICC ≥ 0.81 was defined as an excellent agreement between both evaluations.[18] Absolute reproducibility was evaluated with

coefficient of variation (CV) [SD/average of every parameter × 100] and standard error of measurement (SEM) calculated as recommended by Hopkins [SEM(%) = SEM/average of both evaluations × 100].[16] SEM represents the degree to which repeated measurements vary for a given individual (i.e., trial-to-trial noise) and will be presented as a percentage of the average of both evaluations.[16,17]

Table 1 Baseline characteristics of study subjects ($n = 24$).

Age (yr)	34.0 ± 8.2
Weight (kg)	77.88 ± 10.12
Height (cm)	176.54 ± 5.79
BMI (kg/m^2)	25.04 ± 3.25
Waist circumference (cm)	91.43 ± 9.56
Body fat (%)	18.10 ± 6.87
Glucose (mmol/L)	4.72 ± 0.37
Insulin (pmol/L)	40 ± 23
HOMA-IR	8.08 ± 5.09
Total cholesterol (mmol/L)	4.62 ± 0.97
LDL cholesterol (mmol/L)	2.84 ± 0.89
HDL cholesterol (mmol/L)	1.33 ± 0.39
Triglycerides (mmol/L)	0.99 ± 0.59
Total cholesterol/HDL-C	3.78 ± 1.40
Blood pressure (mmHg)	119/73 ± 7/7
FMD (%)	10.66 ± 3.20
Active smoker	0

3 Results

3.1 *Baseline Characteristics*

Baseline characteristics of the study population are given in Table 1. Subjects were healthy young males, without any cardiovascular risk factors or diseases. Study subjects had normal body composition and blood glucose, insulin, and lipid levels.

3.2 *Microvascular Function Reproducibility*

Table 2 describes mean NIRS parameters measured during both PORH and shows that mean NIRS parameters did not differ significantly. Table 3 describes intra-subject reproducibility of NIRS parameters measured during both PORH. Most parameters had good (½TO_2Hb, TO_2Hb, Hmax O_2Hb, TtHb, AUC O_2Hb, and AUC tHb, ICC ranging from 0.62 to 0.73) to excellent (mVO_2, ICC of 0.84) intra-subject reproducibility. The less reproducible NIRS parameters classified as poor to satisfactory were: Hmax tHb, increase rates to max. tHb and O_2Hb and AUC HHb; ICC: 0.31 to 0.59. Table 4 describes inter-observer reproducibility of NIRS parameters measured during both PORH. Inter-observer reproducibility of all NIRS parameters ranged from excellent to perfect (ICC ranging from 0.85 to 1.00).

4 Discussion

The main findings of the present study were that 1. intra-subject relative reproducibility of microvascular function assessed by NIRS during PORH ranged from good to excellent for most parameters and that 2. excellent inter-observer relative reproducibility of microvascular function assessed by NIRS during PORH was obtained for all parameters. This study is the first to document intra-subject relative and absolute reproducibility of such a variety of NIRS parameters during brachial PORH and is the first to document their inter-observer relative and absolute reproducibility.

Table 2 NIRS parameters measured during postocclusive reactive hyperemia.

	1st test	2nd test	*p*-value
mVO_2 (ml O_2/ min /100 g)	0.0602 ± 0.010	0.0602 ± 0.011	0.99
½TO_2Hb (s)	18.2 ± 8.1	20.1 ± 8.9	0.44
TO_2Hb (s)	41.1 ± 16.0	42.7 ± 13.9	0.72
Hmax O_2Hb (μM)	28.05 ± 3.15	27.92 ± 4.55	0.91
Hmax tHb (μM)	10.56 ± 1.80	10.18 ± 1.92	0.49
TtHb (s)	22.8 ± 11.3	23.9 ± 10.9	0.75
Increase rate to max O_2Hb (μM/s)	0.75 ± 0.22	0.73 ± 0.27	0.73
Increase rate to max tHb (μM/s)	0.52 ± 0.16	0.49 ± 0.20	0.58
AUC O_2Hb (a.u.)	995 ± 372	889 ± 388	0.34
AUC HHb (a.u.)	161 ± 251	167 ± 239	0.93
AUC tHb (a.u.)	834 ± 359	722 ± 315	0.26

Table 3 Intra-subjects reproducibility of NIRS parameters during postocclusive reactive hyperemia.

	ICC	CV (%)	SEM (%)
mVO_2 (ml O_2/ min /100 g)	0.84	6.51	7.11
½TO_2Hb (s)	0.63	20.04	27.22
TO_2Hb (s)	0.71	15.61	19.27
Hmax O_2Hb (μM)	0.63	6.68	8.53
Hmax tHb (μM)	0.31	13.32	14.91
TtHb (s)	0.73	19.61	24.56
Increase rate to max O_2Hb (μM/s)	0.59	15.30	21.34
Increase rate to max tHb (μM/s)	0.42	21.71	27.18
AUC O_2Hb (a.u.)	0.68	16.15	22.93
AUC HHb (a.u.)	0.41	20.37	114.10
AUC tHb (a.u.)	0.62	18.59	26.64

Table 4 Inter-observer reproducibility of NIRS parameters during postocclusive reactive hyperemia.

	ICC	CV (%)	SEM (%)
mVO_2 (ml O_2/ min /100 g)	0.98	1.14	2.10
½TO_2Hb (s)	0.99	3.83	4.21
TO_2Hb (s)	0.95	3.21	8.83
Hmax O_2Hb (μM)	1.00	0.73	0.91
Hmax tHb (μM)	1.00	0.52	0.82
TtHb (s)	0.92	4.70	12.99
Increase rate to max O_2Hb (μM/s)	0.98	3.31	5.21
Increase rate to max tHb (μM/s)	0.85	5.11	14.78
AUC O_2Hb (a.u.)	0.97	8.32	9.50
AUC HHb (a.u.)	0.97	50.75	35.19
AUC tHb (a.u.)	0.96	7.74	8.99

4.1 *Relative Intra-Subject Reproducibility*

Relative intra-subject reproducibility (ICC) of mVO_2, ½TO_2Hb, TO_2Hb, Hmax O_2Hb, TtHb, AUC O_2Hb, and AUC tHb ranged from good to excellent when assessed by NIRS during PORH in young healthy males. Among these parameters, mVO_2 is the most often studied since it is the ultimate measure of resting muscle metabolic rate and reflects resting muscle oxygen consumption.[6] The average mVO_2 obtained from our study (0.0602 ± 0.01 ml O_2/ min /100) agreed with previously published data measured in the forearm of healthy subjects (0.05 ± 0.01 to 0.21 ± 0.01 ml O_2/ min /100 g).[13] The assessment of forearm mVO_2 is of particular interest since it was shown to be significantly reduced in patients with CHF,[6,9] metabolic syndrome,[11] and with chronic or acute smoking.[10] Lowered mVO_2 reflects lower muscular ability to extract oxygen and impaired mitochondrial function.[6]

Other NIRS parameters that showed good relative intra-subject reproducibility reflect the ability of muscle to recruit arterioles and capillaries during reperfusion. Of these parameters, Hmax O_2Hb, Hmax tHb, and AUC O_2Hb were recently shown by our group to be impaired in patients with metabolic syndrome and/or coronary heart disease.[11] Additionally, impairment of these parameters was related to the number of cardiovascular risk factor (ranging from 0 to 4–5 risk factors) independently of cardiovascular status (healthy, metabolic syndrome, or coronary artery disease patients).[11] Time to maximal O_2Hb, TtHb, and Hmax O_2Hb are also significantly affected by peripheral arterial disease (longer TO_2Hb and TtHb and lower Hmax O_2Hb values).[7,19] A parameter similar to increased rate O_2Hb (named tissue oxygen saturation rate) was impaired in CHF and is predictive of reduced VO_2 peak and inefficient ventilatory capacity.[9,20] Microvascular function can also be improved by lifestyle interventions[21] (such as exercise training) and NIRS can be useful in assessing the impact of such interventions.[22] In fact, a three-month exercise-training program in patients with CHF increased the AUC O2Hb and increased rate O2Hb during PORH.[21,22] This demonstrates that increased physical activity improved muscular arteriolar and capillary recruitment and increased the speed of reperfusion[9] and adds to the interest of having a simple and reliable method to evaluate such microvascular improvements in the clinical setting.

4.2 *Absolute Intra-Subject Reproducibility*

Absolute intra-subject reproducibility assessed with CV and SEM is important to determine the usefulness of NIRS parameters in evaluating the impact of therapeutic interventions (lifestyle or treatment) on microvascular function. We obtained CVs ranging from 6.51% (mVO_2) to 21.71%. To our knowledge, only Van Beekvelt et al. calculated CVs for forearm PORH parameters and obtained a CV of 17.6% for mVO_2.[13] Absolute reproducibility was also studied for other NIRS parameters by Kragelj et al. who obtained higher CV for mVO_2, ½TO_2Hb, TO_2Hb, Hmax O_2Hb, and TtHb following lower limb ischemia in healthy subjects.[8] Our study therefore shows improved CV, which could be attributed to our larger sample size and to methodological differences (i.e., ease of NIRS measurement at the forearm, longer occlusion period and use of an automated pressure cuff inflator). In fact, a rapid cuff pneumatic inflator eliminating the short venous occlusion inevitable when inflating by hand could have lowered variation of measurement.[7,8]

The SEM allows for identification of the minimal detectable change needed to identify statistically important variations between two evaluations of a single subject that cannot be attributed to measurement error. Standard error of measure has already been calculated for some NIRS parameters upon leg artery occlusion[7,8] but has never been assessed during forearm PORH.

4.3 *Inter-Observer Reproducibility*

Inter-observer relative reproducibility of all NIRS parameters was found to be excellent, meaning that evaluation of the same NIRS signal by two observers is expected to yield very

similar results. Absolute inter-observer reproducibility (CV and SEM) of most NIRS parameters was very small and was lower than that found for intra-subject reproducibility. These important findings indicate that NIRS testing can be performed by different trained-observers and add to the ease of use of this method by eliminating the need for analysis to be done centrally in multicenter studies.

4.4 *Limitations*

Firstly, this sub-study was part of a principal study only including young male subjects, free of cardiovascular risk factors. Females were excluded from the main study to eliminate potential variations in endothelial function related to the menstrual cycle. There is, however, no reason to believe that reproducibility of microvascular function using NIRS during PORH would be affected by gender. Secondly, there was a broad variation in the length of time between evaluations (from 7 to 30 days). Shorter and constant length between visits, reducing the potential impact of changes in dietary habits, sleep patterns, and stress level variations, might have yielded more reproducible results. On the other hand, the present study demonstrated NIRS parameters to be reproducible over a 7 to 30-day period, which enables their use in studies spanning such periods of time.

In conclusion, intra-subject and inter-observer reproducibility of NIRS parameters during PORH was assessed and showed that a majority of parameters had good to excellent intra-subject reproducibility and that every parameters had excellent inter-observer reproducibility.[23] Further studies are now needed in patients with cardiovascular risk factors or diseases and in elderly subjects. However, reproducibility of repeated measurement of fasting NIRS parameters in patients with stable conditions and medications should be similar to what was described previously. The impact of lifestyle interventions such as dietary habit modifications and physical activity and therapeutic interventions on NIRS parameters should also be evaluated. There is also a need for standardization of NIRS parameter nomenclature and occlusion protocols to enhance the comparability of future studies.

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