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Near Infrared Spectroscopy is not a Surrogate of Venous Occlusion Plethysmography to Assess Microvascular Resting Blood Flow and Function

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ABSTRACT

International Journal of Exercise Science 15(2): 1616-1626, 2022. Near-infrared spectroscopy (NIRS) is a non-invasive technique that measures tissue perfusion using red blood cells oxygen saturation and venous occlusion plethysmography (VOP) is the gold standard to assess microvascular blood flow and function. The purpose of this study was to determine if NIRS can surrogate the microvascular blood flow assessment after an ischemic challenge obtained via VOP. Twenty apparently healthy subjects (10 males and 10 females), aged 18 to 35 years, were recruited for this single session study. NIRS probes were placed 40mm apart along the epicondylar muscles on the right forearm and on the tibialis anterior on the right lower leg, while VOP strain gauges were placed on the largest circumference on both right forearm and calf. Blood flow via VOP and NIRS variables (hemoglobin saturation (SO2), oxygenated hemoglobin (HbO2), and deoxyhemoglobin (HHb) slopes) were assessed before and after 5-min ischemic challenge. Person's correlations and intra-class correlations (ICC2k) were conducted for each of the NIRS variables vs VOP. There were moderate associations between of SO2 and HbO2 slopes and VOP (r = 0.59, p < 0.01 and r = 0.53, p < 0.05, respectively) at the lower body during resting conditions. There was a poor agreement between NIRS SO2 and VOP at the resting condition in the lower body (ICC2k = 0.45). There were no other associations between any of the other NIRS variables and VOP of the lower and upper body at resting or post-ischemic conditions. In conclusion, NIRS cannot surrogate VOP for measurements of microvascular blood flow at resting or post-ischemic conditions.

KEY WORDS: NIRS, VOP, hemoglobin, deoxyhemoglobin, oxygen saturation, agreement

INTRODUCTION

Heart diseases, including coronary heart disease, hypertension, and stroke, remain the number one cause of death worldwide [42]. Vascular impairments at both the macro-and the micro-circulatory levels are known to be associated with cardiovascular diseases [45]. Monitoring of vascular reactivity and blood oxygenated hemoglobin (HbO2) in tissues has been utilized to determine health status in different clinical settings [27, 36]. For example, venous occlusion plethysmography (VOP) has been used to assess microvascular function in patients with pre-

hypertension [5] and to monitor vascular changes during the menstrual cycle in females [3, 30]. Moreover, assessment of tissue HbO2 via near-infrared spectroscopy (NIRS) has helped to understand mitochondrial function in patients with post-thrombotic venous claudication [25], chronic disease [2], Friedreich ataxia [9], and multiple sclerosis [19].

VOP is a non-invasive technique for the measurement of tissue blood flow [23, 24, 44]. VOP works under the principle of momentarily interrupting venous drainage while allowing arterial inflow to continue, which results in a linear increase in volume over time that is proportional to arterial blood inflow until occluding pressure is achieved [18, 43]. Moreover, VOP is considered the gold standard in measuring blood flow in the assessment of vascular function of the microvasculature *in vivo* in health and in disease [4, 23]. On the other hand, NIRS is a non-invasive technique that measures tissue perfusion and HbO2 [1, 2], which uses two different light wavelengths to measure differential light absorption of HbO2 and deoxyhemoglobin (HHb) [37, 38]. NIRS has been used to monitor perfusion changes in brain and muscle microcirculation during reconstructive surgery and in shock and trauma medicine [11, 13, 38, 39, 44].

Previous reports have linked blood flow [11, 41] and vascular function with tissue HbO2 based on the Fick principle and the concept that the rate of tissue re-HbO2 will be associated with microvascular blood flow after an ischemic challenge, respectively. For example, some studies have shown that there is a significant correlation between the saturation of microvascular hemoglobin (SO2) slope, measured via NIRS, and popliteal flow-mediated dilation (FMD) and that SO2 slope reliability makes it a good candidate to measure vascular reactivity [31-33], even though FMD is a biomarker for conduit, rather larger arteries [20]. Based on our best knowledge, previous studies have assessed different vessel types, with no studies assessing the relationship or the agreement between NIRS and VOP at the same vascular bed. Therefore, the purpose of the current study was to determine if NIRS can measure and surrogate the microvascular blood flow assessment after an ischemic challenge obtained via the gold standard.

METHODS

Participants

Twenty apparently healthy subjects (10 males and 10 females), aged 18 to 35 years, were recruited for the current *in vivo* study. Exclusion criteria included known history of cardiovascular, pulmonary, or metabolic disease, taking prescription medications, any extremity injury that could affect attaching a blood pressure cuff to the arm or leg, and pregnant females. Participants that were taking over-the-counter painkillers or nutritional supplements containing antioxidants were required to abstain from their use 12 hours prior to the lab visit. In addition, participants were asked to refrain from exercising and caffeine consumption for at least 8 hours before testing. A priori power analysis was conducted in Rstudio (version 1.4.1103) using the "pwr" package and data from who found a correlation of r = 0.63 between the slope of SO2 and flow mediated dilation, indicated that a total of a minimum of 14 participants were needed to obtain a power (1-β) of 0.80 at an alpha level of 0.05. The study was approved by the

Institutional Review Board of The University of Texas at El Paso, followed previous ethical guidelines [34] and all individual participants included in the study signed the informed consent before scheduling the visit to the Clinical Applied Physiology (CAPh) Lab.

Protocol

During the lab visit, assessment of weight (WB-110A Class III, Tanita, Japan), height (Seca Medical, Germany), and body mass index (BMI) was performed. Then, participants laid supine on an examination table in the anatomical position. NIRS and VOP were performed simultaneously in the right forearm and right calf using 2-channel NIRS unit (moorVMS-NIRS, Moor Instruments, London, England) and a validated plethysmograph (AI6, Hokanson, Bellevue, WA), respectively. NIRS used two photodetectors per channel operating at two wavelengths (750nm and 850nm), while VOP used 2 strain gauges placed around the largest perimeter of both forearm and calf. Pressure cuffs were placed on the arm (above the elbow), wrist, thigh (above the knee), and at the ankle. For NIRS, two probes were used, one for the forearm and one for the calf, according to company guidelines. The forearm probe was placed perpendicular to the longitudinal arm axis, between the strain gauge and the wrist, over the epicondylar muscles. The calf probe was placed perpendicular to the longitudinal lower leg axis, between the strain gauge and the ankle, over the tibialis anterior muscle. Both diodes were placed 40 mm a part, inside a probe holder (NPH1-40). To secure probe holder from moving and to obstruct light from reaching the emitter and detector head, black adhesive tape was taped around the probe holder.

NIRS and VOP data were collected during resting and post-ischemic stress conditions. After pressure cuffs, strain gauges, and diode probes were placed, participants were allowed to relax for 15 minutes to ensure that increased sympathetic activity due to nervousness did not alter blood pressure readings [24]. During the resting period, four peripheral blood pressure values were recorded using an automated brachial blood pressure cuff (BP760, Omnron Healthcare, Inc., Lake Forest, IL). After this resting period, resting conditions data was collected every 10 seconds for 1 minute (Resting measurements). Then, arm and thigh cuffs were inflated to suprasystolic pressure (i.e., 220 mm Hg) for 5 minutes. Immediately before cuffs deflation, wrist and ankle cuffs were inflated to suprasystolic pressure to isolate the hand and foot vascular response. After arm and thigh cuffs deflation, post-ischemic stress data were collected every 10 seconds for 1 minute.

The NIRS output signal was transmitted to the device's software program (moorVMS-PC v4.2.0, Moor Instruments, London, England). The slopes for SO2, HbO2, and HHb were determined automatically during resting conditions and within 5 seconds post cuff deflation for the post-ischemic condition.

The plethysmograph output signal was transmitted to a calibrated software program (NIVP3, D.E. Hokanson, Inc) on a personal computer and the slope was expressed as milliliters per minute per 100 mL of tissue (mL/min per 100 mL tissue). Absolute blood flow was determined by the rate of change of limb circumference (e.g., slope) during the seven-second venous

occlusion, within 15-sec measurement cycles, which correlates highly to arterial blood inflow into the limb [18, 22]. One channel was connected to the forearm gauge to determine forearm blood flow (FBF) and one channel was connected to the calf gauge to determine calf blood flow (CBF). The six measurements obtained during resting conditions were averaged to determine resting FBF and CBF. Peak post-ischemic FBF and CBF were determine by the peak VOP slope within the first 5 seconds post deflation.

Statistical Analysis

Data was imported into Rstudio (version 1.4.1103) integrated development environment and analyzed with R statistical programming language using a custom-built script. A series of independent t-tests were conducted to determine differences on the demographic variables (Age, Height, Weight, BMI) between male and female participants. Pearson's product-moment correlations were conducted between NIRS variables (SO2, HbO2, and HHb) and VOP. The magnitude the associations were interpreted as follows: 0.0 as "trivial", 0.10-0.29 as "small", 0.30-0.49" as "moderate", 0.50-0.69 as "large", 0.70-0.89 as "very large", 0.90-0.99 as "nearly perfect [21]. Additionally, a two-way mixed model intra-class correlation (ICC_{2k}, "Agreement") was also conducted. Data were presented as means and standard deviations (sd), medians and standard errors (se), and lower and upper bounds of 95% confidence intervals (CI). The magnitude of ICC was interpreted as follows: < 0.50 as "poor", 0.50 – 0.74 as "moderate", 0.75-0.89 as "good", 0.90-1.0 as "excellent" [16].

RESULTS

General characteristics of the sample are presented in Table 1. The independent t-tests revealed no differences between sex on age, weight, height, or BMI. Additionally, descriptive data for each variable are displayed in Table 2.

Table 1. General characteristics of the sample.

	All *n* = 20		Male *n* = 10		Female *n* = 10	
	mean	sd	mean	sd	mean	sd
Age	24.0	4.5	25.6	4.8	22.4	3.8
Height (m)	1.6	0.0	1.7	0.0	1.6	0.0
Weight (kg)	69.7	14.6	75.8	16.7	63.7	9.4
BMI (kg/m^2)	24.4	5.4	25.8	6.8	23.1	3.2

Table 2. Mean, standard deviation (sd), median, and standard error (se) of each variable for all, male, and female participants. (Near Infra-Red Spectroscopy (NIRS), Venous Occlusion Plethysmography (VOP), Hemoglobin saturation (SO2), oxygenated hemoglobin (HbO2), and deoxyhemoglobin (HHb).

	mean	sd	median	se
All				
NIRS SO2 resting	1.07	3.67	0.09	0.82
NIRS OXY resting	5.89	21.50	0.03	4.81
NIRS HHb resting	-6.84	18.05	-3.26	4.04
VOP_resting	2.18	1.01	2.20	0.23
NIRS SO2 post ischemia	2.66	1.52	2.70	0.34
NIRS OXY post ischemia	8.94	4.23	8.88	0.95
VOP post ischemia	17.75	6.86	16.81	1.53
Males				
NIRS SO2 resting	1.98	5.14	0.12	1.62
NIRS OXY resting	10.93	30.22	-0.29	9.56
NIRS HHb resting	-10.76	25.52	-3.26	8.07
VOP resting	2.61	0.99	2.55	0.31
NIRS peak OXY post ischemia	9.42	4.44	9.04	1.40
NIRS peak SO2 post ischemia	3.02	1.84	2.87	0.58
NIRS peak HHb post ischemia	-9.65	10.48	-7.27	3.31
VOP post ischemia	20.74	7.41	21.46	2.34
Females				
NIRS SO2 resting	0.16	0.41	0.08	0.13
NIRS OXY resting	0.86	2.53	0.20	0.80
NIRS HHb resting	-2.91	1.58	-2.86	0.50
VOP resting	1.74	0.89	1.68	0.28
NIRS peak OXY post ischemia	8.47	4.20	8.68	1.33
NIRS peak SO2 post ischemia	2.31	1.11	2.37	0.35
NIRS peak HHb post ischemia	-12.10	9.36	-10.47	2.96
VOP post ischemia	14.75	4.95	13.91	1.57

Moderate associations between SO2 slope and VOP ($r = 0.59$, $p < 0.01$) and HbO2 slope and VOP ($r = 0.53$, $p < 0.05$) were observed only on the lower body at resting conditions (Table 2). Additionally, there was a poor ICC_{2k} agreement between SO2 and VOP at the resting conditions in the lower body ($ICC_{2k} = 0.45$; $p < 0.05$). There were no more associations or agreements between any of the other NIRS variables and VOP of the lower and upper body at resting or post-ischemic conditions (Table 3).

Table 3. Pearson's r correlation coefficients and Intra-Class Correlation coefficients comparing Venous Occlusion Plethysmography and Near Infra-Red Spectroscopy variables. (Venous Occlusion Plethysmography (VOP), Hemoglobin saturation (SO2), oxygenated hemoglobin (HbO2), and deoxyhemoglobin (HHb).

Variables	Pearson's *r* correlation coefficient			Intra-Class Correlation coefficient		
	r	Lower 95 CI	Upper 95 CI	ICC_{2k}	Lower 95 CI	Upper 95 CI
Resting Upper Body						
SO2 slope and VOP	0.07	-0.39	0.50	0.07	-0.50	0.48
HbO2 slope and VOP	0.07	-0.39	0.50	0.06	-1.03	0.57
HHb slope and VOP	0.10	-0.36	0.52	0.06	-1.03	0.57
Post-Ischemia Upper Body						
SO2 slope and VOP	0.08	-0.37	0.51	0.05	-0.15	0.30
HbO2 slope and VOP	-0.17	-0.57	0.30	0.00	-1.17	0.54
HHb slope and VOP	0.09	-0.37	0.51	0.02	-0.17	0.28
Resting Lower Body						
SO2 slope and VOP	0.59**	0.20	0.82	0.45*	-0.13	0.74
HbO2 slope and VOP	0.53*	0.12	0.79	0.11	-0.92	0.59
HHb slope and VOP	0.02	-0.42	0.46	0.00	-0.81	-0.81
Post-Ischemia Lower Body						
SO2 slope and VOP	0.04	-0.41	0.48	0.26	-0.33	0.62
HbO2 slope and VOP	0.31	-0.16	0.66	0.23	-0.17	0.55
HHb slope and VOP	0.17	-0.29	0.57	0.05	-0.07	0.22

*Denotes significance below $p < 0.05$; **Denotes significance below $p < 0.05$

DISCUSSION

The purpose of this study was to determine if NIRS variables can measure and surrogate the microvascular blood flow assessment at resting and after an ischemic challenge obtained via the gold standard VOP. The main results of the current study show poor correlation and poor agreement at resting in the lower body between two NIRS variables (SO2 and HbO2 slopes) and VOP, but no correlation and no agreement between any of the NIRS variables and VOP post-ischemia. These findings confirm the lack of association between blood flow measurements or vascular reactivity with tissue oxygenation/de-oxygenation/re-oxygenation. NIRS technology does not directly measure microcirculatory blood flow, so that the increase in SO2 after temporary arterial occlusion does not necessarily reflect the local increase in oxygen delivery characterizing the reactive hyperemic response [11].

The lack of association between NIRS variables and VOP might be explained by the measurement principles of each instrumentation. First, the principle of VOP is the linear increase in the body segment volume (i.e. forearm or calf) overtime when venous drainage is interrupted and the arterial flow is unaltered [43]. In contrast, NIRS is mostly based on monitoring two different light wavelengths, which are used to measure differential absorption properties of oxygenated and deoxygenated hemoglobin [38]. Changes in HbO2 and HHb can be measured continuously, while SO2 can be calculated (defined as [HbO2]/[HbO2 + HHb]) [34], even

though current NIRS software calculated it automatically. It appears that the linear increase in the body segment volume observed in the VOP is not associated with the light absorption slope in the red blood cells, for either HbO2, HHb, or SO2. Moreover, recent work by Abay and Kyriacou [1] found that venous occlusion affects total tissue oxygenation (ratio of oxygenated and total hemoglobin) from NIRS measurements. Similar findings were corroborated by Cross and Sabapathy [12], who also found that NIRS derived variables, underestimate muscle flow (Qmus) at the flexor digitorum superficialis muscle after a venous occlusion procedure. This indicating that the venous occlusion method affects NIRS measurements. Interestingly, De Blasi et al. [8] reported a good correlation between NIRS and VOP (r = 0.93) for forearm blood flow at rest and after 6-min ischemia in a similar sample that the current study. The most probable explanation for the differences recorded in their study, is that the authors were able to compare blood flows and not just the slopes as the current study performed. However, blood flow obtained via NIRS, needs blood samples, which makes it an invasive procedure.

In their seminal study, Edwards et al. [14] found an almost perfect forearm blood flow correlation between NIRS and VOP. In their study, they estimated blood flow with NIRS based on the Fick principle, estimated tissue density, and whole blood hemoglobin concentration, which was invasively measured. Even though their results were promising, the invasive nature of their study shadows NIRS's non-invasive nature. On a similar study, using the Fick principle and invasive determination of hemoglobin concentration, Van Beekvelt et al. [41] determined regional blood flows via NIRS and VOP in young, healthy volunteers. Their findings show a significant difference between both methods with more than twice the blood flow when using VOP compared to NIRS. Interestingly, both methods were able to measure a ~1.4-fold increase in blood flow after exercise, partially confirming some of the results from Edwards et al. [22]. The present study showed only a poor correlation between NIRS and VOP during baseline conditions in the lower body (Table 2). This contrast with the studies described above might be associated with the non-invasive model used here. The present study only used the slope after venous occlusion of different NIRS variables, without using the Fick principle and/or invasive determination of hemoglobin concentration.

Even though assessing direct blood flow via NIRS would need to be invasive, using non-invasive NIRS to assess microvascular function might be a viable alternative. For example, Kragelj et al. [26] measured NIRS before and after a 5-min ischemic challenge in patients with peripheral vascular disease and normal controls. Their findings were aligned with studies using VOP in clinical populations [5], where apparently normal individuals have a much larger vascular reactivity (i.e., steeper VOP and/or SO2 slopes and faster back to normal values) than in the clinical populations. Unfortunately, Kragelj et al. did not perform a validation study comparing NIRS versus another validated test for vascular reactivity [26]. Similar findings were observed by McLay et al. [32] when comparing healthy young trained and healthy young untrained men. Trained participants showed a steeper SO2 slope than untrained participants in all 5 different durations of vascular occlusion, confirming that apparently healthier individuals have faster re-oxygenation after any ischemic challenge [32].

To the best of our knowledge, there are only two studies seeking for an association between NIRS and a validated test for vascular reactivity [31, 33]. McLay, Fontana, et al. [31] correlated SO2 slope of the tibialis anterior muscle after 5-min ischemic challenge and popliteal flow mediated dilation (FMD) in 20 healthy young men. Even though their results showed a significant association between both variables (r = 0.63, p = 0.003), it is important to note that correlation does not necessarily mean agreement [6, 7, 28]. The present study showed a similar, moderate correlation between SO2 slope and VOP in the lower body during resting conditions (Table 2); however, the agreement was poor (ICC2k = 0.45). There might be two sources of error in McLay, Fontana, et al. study [31]. First, the fact that the popliteal artery is a conduit artery and measuring the tibialis anterior is more of a microvascular bed could bring some differences in vascular reactivity [3, 5, 23]. Secondly, the authors did not use an automatic edge-detection system and did not specify if the ultrasound images analysis was blind, what could have biased their results [20, 35, 40]. Finally, McLay et al. [32] studied the reproducibility of NIRS and FMD between multiple measurements within one visit and between single measurements within 5 days. Following a similar protocol than their previous study [31] the authors showed a better reproducibility of NIRS (SO2 slope) when compared with FMD. Both agreement and coefficient of variations (CV) were stronger in NIRS versus FMD (NIRS agreement and CV: 0.92-0.94, 9-14%; FMD agreement and CV: 0.36-0.25, 40-44%). Even though this study did not directly compare both methods for vascular reactivity, both methods showed similar behaviors over time. Interestingly, their FMD reproducibility was considerably lower than previous reports on FMD reproducibility [20, 35, 40], which have used automatic edge-detection systems to increase reproducibility.

Our study is not without limitations. First, previous studies have highlighted that the accuracy of the NIRS signal is dependent on the individual's regional adipose tissue thickness [15, 41]. We did not measure regional adipose tissue or body composition; however, both lateral forearm and anterior calf are 2 areas with very low subcutaneous tissue [10, 29]. Anyways, it is the possibility that the values obtained by our NIRS measures could be corrected depending on previously proposed algorithms that account for regional adipose tissue [17]. Secondly, we tested only young apparently healthy participants. Testing different clinical populations (e.g., patients with coronary artery disease, stroke, or mitochondrial diseases) might show different results. Finally, we were unable to obtain reliability measurements, as participants were involved in a one-time visit to our lab. Future studies might want to obtain several measures of the same individual at similar conditions to obtain intra and inter-test reliability.

In conclusion, NIRS has clinical and field applications to study mitochondrial function and tissue oxygen consumption. However, and according to the present results, NIRS should not be considered as a surrogate for microvascular blood flow or function.

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