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Near-Infrared Spectroscopy Does Not Track Forearm Blood Flow during Venous Occlusion Plethysmography

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Featured Application: Near-infrared spectroscopy (NIRS) has gained popularity in biomedical and sport sciences to non-invasively assess the microvascular responses and health in men and women. Venous occlusion plethysmography (VOP) is also capable of assessing microvascular health. However, due to the ease of assessment and portability of a NIRS sensor, this method may be more favorable to some individuals. Currently, there are limited direct comparisons between each approach, and thus, the ability to interchange values is not clear. Here, it was reported that NIRS responses do not correlate well with strain-gauge VOP values.

Abstract: Background: Venous occlusion plethysmography (VOP) non-invasively measures forearm blood flow (FBF), whereas near-infrared spectroscopy (NIRS) assesses skeletal muscle oxygenation. Using these techniques has revealed sex differences in microvascular responses. However, it is not clear if NIRS and VOP results are interchangeable under various conditions like reactive hyperemia (RH). Our purpose was to evaluate sex-specific associations between FBF and NIRS-derived parameters: oxygenated hemoglobin, deoxygenated hemoglobin, total hemoglobin, and hemoglobin difference (O_2Hb, HHb, tHb, and HbDiff). Methods: In total, 29 adults (15 men) participated, and a strain-gauge was placed on the forearm for VOP and a NIRS device was distally attached. Slopes for FBF and NIRS parameters were quantified during venous occlusion intervals at rest and during RH. Pearson's correlations were assessed between VOP and NIRS slopes. Intraclass correlation coefficients ($ICC_{2,1}$) examined the sex-specific consistency of the slopes at rest. $p \leq 0.05$ was considered significant. Results: During RH, FBF was not correlated with O_2Hb (r = −0.126), HHb (r = 0.228), tHb (r = 0.061), or HbDiff (r = 0.046). Seemingly, there were no sex differences. Resting FBF and NIRS-derived variables, except for HbDiff, displayed suitable consistency as suggested by the reliability results ($ICC_{2,1}$ = 0.115–0.577). Conclusions: The NIRS values collected did not match the strain-gauge slopes. Individuals should practice caution when generating blood flow inferences from NIRS-based data during VOP.

Keywords: microvascular; reactive hyperemia; vascular occlusion; muscle oxygenation; health technology; health promotion; sex differences

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1. Introduction

Cardiovascular disease (CVD) remains the global leading cause of death for men and women [1], which highlights the growing need to focus on a variety of applied methods and devices capable of providing early predictions of the risks associated with the onset and severity of CVD [2–8]. Devices and approaches are currently available to examine the health and function of the microvasculature, which have been identified as early indicators

of CVD [2]. For example, a marker of microvascular function was shown to independently predict future (5-year follow-up) CVD in a large cohort of adult men who were, at the time, free of vascular disease [2]. Most commonly, reactive hyperemia (RH) has been used to define microvascular function and is represented by an exaggerated reperfusion immediately following transient ischemia, which is caused by various local myogenic, metabolic, and/or endothelial factors within arterioles [7,9–11]. It is acknowledged that many approaches are available to capture associated RH responses [7,12], but, here, strain-gauge venous occlusion plethysmography (VOP) [10] and near-infrared spectroscopy (NIRS) [3,11,13–15] are of particular interest. These two approaches seemingly garner substantial interest as demonstrated by their high usage among research scientists [16–19]. Venous occlusion plethysmography uses a strain-gauge to record changes in circumference (i.e., limb volume) provoked by venous occlusion to provide measures of the arterial inflow rate [20,21]. Indeed, VOP has demonstrated clinical importance as lower values (e.g., impaired peripheral endothelial function) were shown to be an independent predictor of mortality in patients with heart failure [22]. Although VOP is highly valid and reproducible, there have been some notable technological advancements since the advent of VOP [7]. One such technology includes NIRS, which is able to provide data describing oxygenated hemoglobin (O_2Hb), deoxygenated hemoglobin (HHb), total hemoglobin (tHb), hemoglobin difference (HbDiff) [7,23]. Previously, it has been reported that changes in tHb were correlated with peak blood flow following fast and slow muscle contractions, which the investigators interpreted as evidence that this NIRS variable may be interpreted as changes in muscle blood flow provoked by active hyperemia [24]. However, there remain inconclusive data concerning whether or not NIRS-derived parameters, including tHb, can be used as surrogate measures to values obtained by a plethysmograph, like forearm blood flow (FBF). De Blasi et al. [13] and Olamaei et al. [5] previously suggested that NIRS and VOP correlated well during rest and RH. In contrast and more recently, Gomez et al. [3] reported that NIRS was not a suitable surrogate of strain-gauge VOP measures. Thus, there remains a gap in the literature regarding the match between NIRS and strain-gauge measures, which requires additional investigation, especially for investigators aiming to specifically capture blood-flow-related results that inform preventative measures and health promotion.

Determining the match between NIRS and strain-gauge measures collected during VOP remains important, especially during studies that assess sex-specific relations and responses. When considering the routinely reported limitations of NIRS- and plethysmograph-derived measures, there is rationale to hypothesize that men and women may demonstrate different strengths of associations between these two techniques, which requires investigation. For example, Shoemaker et al. [15] hypothesized that greater adipose tissue thickness (ATT) of women may contribute to lower reliability in NIRS-based measures compared to men. However, it is interesting to note that ATT likely remained unchanged between their two reported visits [15], suggesting that some other factor primarily influenced the lower reliability for the women (e.g., metabolic rate, oxygen affinity, etc.). This currently unknown factor may influence NIRS and strain-gauge measures differently and in a sex-specific manner. There has also been numerous conflicting reports regarding the sex-related differences in the magnitude of NIRS- and plethysmograph-based measures of RH [11,14,25–27]. Thus, there are inconsistencies pertaining to possible superior microvascular function for men versus women. This is of particular importance due to known sex differences in diminished vascular function in response to unhealthy behavior (e.g., prolonged sitting) [28]. That is, men or women may be more or less protected against the development of early CVD risk factors and this possible intrinsic difference may influence the match between the assessment of changes in oxygenation and rates of blood flow. Considerable evidence already exists demonstrating different mitochondrial function and vasodilatory capacity between men and women [29–31]. Taken together, the associations/match between different vascular measures must be considered on a biological male versus female basis to identify preventative measures and to promote health.

Therefore, the purpose of the current study was to simultaneously evaluate sex-specific microvascular FBF and NIRS parameters during resting and RH conditions throughout a typical VOP protocol. Based on previous studies [5,13,32], it was hypothesized that FBF and NIRS-derived parameters would exhibit moderate to high correlations (i.e., match) during rest and RH. Additionally, it was hypothesized that the men would demonstrate greater associations between strain-gauge and NIRS measures as well as greater reliability (i.e., consistency) in consecutive measures. These hypotheses were important to test due to the growing use of NIRS technology in both biomedical and sport sciences, and to clarify current uncertainty regarding the appropriateness of inferring blood flow from NIRS-based results, especially during VOP.

2. Materials and Methods

Ethics Approval. The current study was conducted in accordance with the ethics and standards set forth by the Declaration of Helsinki 2013 and was approved by the Institutional Review Board of the University of South Alabama (IRB# 22-333/2087315-2) on 31 August 2023. However, this study was not registered in a public database. Prior to the initiation of any experimental protocol, all participants gave written signed consent and completed a health history document.

Experimental Design. Participants reported to the Integrative Laboratory of Exercise and Applied Physiology (iLEAP) to complete paperwork and then were led to the body composition laboratory for recording of anthropometrics and body composition. The same single researcher then led participants to the body composition laboratory for recording of anthropometrics and body composition. Once these characteristics were measured, the participants were instructed to quietly lay still in the supine position on a padded patient table within the iLEAP. Their dominant arm was abducted 90° and supported at heart level by a foam base on a height-adjustable table. Ultrasonography was used to evaluate the brachial artery and forearm ATT of the abducted limb. A blood pressure cuff was attached to the non-dominant arm to measure mean arterial pressure (MAP) and heart rate (HR) with a patient monitor (Datascope Passport 2 Patient Monitor, Mindray DS USA, Inc., Mahwah, NJ, USA). After measures were collected with the ultrasound device, a mercury in-silastic strain-gauge (based on forearm circumference size), NIRS device, and pneumatic cuffs were attached to the dominant arm for the simultaneous recording of FBF and muscle oxygenation parameters (i.e., O_2Hb) at rest and in response to transient ischemia (Figure 1). That is, the NIRS device and a strain-gauge were both attached to the same forearm and simultaneously collected data during VOP resting and RH assessments. These simultaneously collected values were then analyzed to determine match/correlation. Protocols associated with this experimental design are described below, and this concluded the visit.

Human Participants. Thirty-three young (19–31 years old), healthy adults were recruited for this single-visit investigation. Data from two participants were not properly recorded due to NIRS device signal interruptions. Subsequently, an evaluation of potential influential outliers was performed, which utilized Cook's D versus studentized residuals (SREDIS) plots. These plots were visually inspected under the specific evaluation criteria of a Cook's D > 1.5 and SREDIS > ±2. This identified two influential outliers. Thus, a total of four participants were removed from all further statistical analyses. The characteristics of this final sample (n = 29) are presented in Table 1 (male = 15, female = 14). These characteristics included a surrogate measure of physical activity, step count. This value was self-reported by each participant as a seven-day average calculated by participant-owned smart devices (e.g., Apple Watch, Garmin, or Apple iPhone). Further, all participants were classified as being recreationally active as defined by the World Health Organization activity guidelines, which included completing a minimum of 150–300 min of moderate-intensity activity or 75–150 min of vigorous-intensity activity per week, and/or muscle-strengthening activities two or more days a week [33]. The participants were instructed to continue their typical dietary, hydration, and medication habits while abstaining from exercise for at least

48 h before arriving to their experimental visit. Although no attempts were made to control for the menstrual cycle, four female participants reported the use of pharmaceutical contraceptives. Based on the currently stated purpose and prior research assessing microvascular reperfusion in women during various stages of the menstrual cycle as well as with/without the use of an oral contraceptive [34], it was our interpretation that the likely variation in the menstrual cycle did not substantially affect the present results.

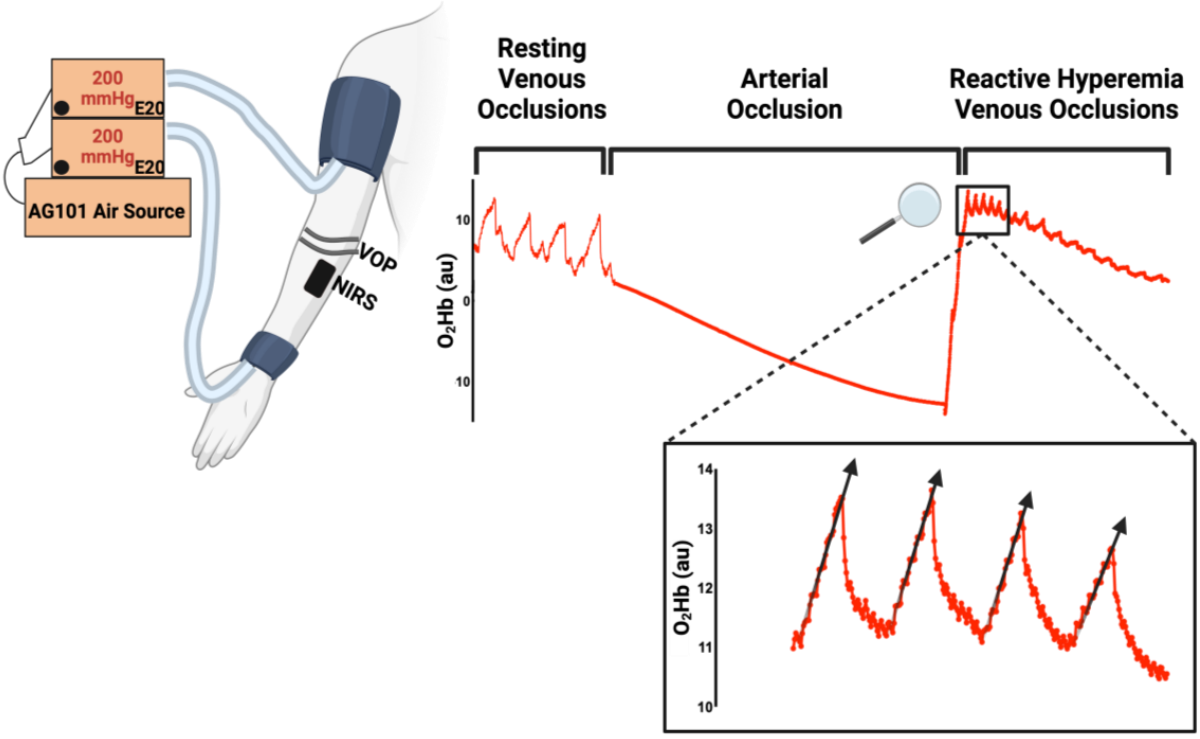

Figure 1. Experimental design. Figure 1 depicts the raw data (n = 1) for the NIRS-derived variable, O_2Hb during resting venous occlusion intervals, the 5 min arterial occlusion, and the reactive hyperemic venous occlusion intervals. An example of a participant's forearm is depicted with the strain-gauge located on the forearm, and the NIRS device just below the strain-gauge. The boxed tracings depict reactive hyperemia captured by venous occlusions intervals with progressively declining rates of increase. VOP = venous occlusion plethysmography, NIRS = near-infrared spectroscopy, O_2Hb = oxygenated hemoglobin.

Table 1. Participant characteristics.

Participant Characteristics	Men (n = 15)	Women (n = 14)	Lower-Upper $CI_{95\%}$; *p*
Age (yrs)	21 ± 3	21 ± 2	−2.3 to 1.8; 0.08
Height (cm)	174.7 ± 10.2	164.2 ± 11.2	−18.7 to −2.4; 0.01
Weight (kg)	83.7 ± 12.0	65.0 ± 9.4	−27.0 to −10.5; <0.001
Step Count	9031.7 ± 3433.1	7582.0 ± 2748.9	−2880.0 to 1227.1; 0.42
Body Composition			
Body Mass Index (kg/m^2)	27.4 ± 2.6	24.5 ± 23.7	−5.3 to −0.5; 0.02
Body Fat (%)	17.8 ± 3.5	24.6 ± 6.2	3.0 to 10.6; 0.001
Fat Mass (kg)	14.8 ± 3.8	15.9 ± 6.1	−2.8 to 4.9; 0.584
Lean Mass (kg)	68.7 ± 10.5	48.8 ± 5.2	−26.4 to −13.3; <0.001
Ultrasound Measures			
Brachial Artery Diameter (cm)	0.36 ± 0.05	0.29 ± 0.05	0.03 to 0.11; 0.002
Brachial Artery Velocity (cm/s)	30.9 ± 14.3	24.2 ± 11.1	−6.7 to 4.8; 0.17
Forearm Adipose Tissue (cm)	0.30 ± 0.06	0.22 ± 0.1	−0.09 to −0.04; 0.02

Independent *t*-tests, bolded *p*-values correspond to significant at the ≤0.05 level. $CI_{95\%}$ = 95% confidence interval of mean differences (men–women).

Power Analysis. Based on a previous study [3], an a priori power analysis was conducted using a moderate effect size. G*Power (v. 3.1.9.7) was used to evaluate a bivariate normal correlation model with the following inputs: (1-β = 0.80, η^2 = 0.59, α = 0.05). This recommended a total sample size of 17, but to ensure adequate power as well as to meaningfully assess potential sex differences, a total of 33 participants were recruited for the current investigation. Therefore, the current sample size, even with the removal of four participants, was likely sufficient for the testing of the stated hypotheses.

Body Composition. Prior to experimental testing, the participants underwent body composition analysis to determine percent body fat, fat mass, and lean mass via a FIT3D scan based on the guidelines of the manufacturer (FIT3D156 ProScanner, Redwood City, CA, USA). The 3D optical scanning has been shown to produce similar results compared to the accepted gold-standard four-compartment model [35]. A single researcher was responsible for collecting and inputting height, age, and sex into the Fit3D software (version 2.1.0), and the participants were instructed to all remove clothing, except undergarments. Further instructions were to stand tall, to center their feet on the markers on the turntable, and to grasp the hand grips with arms abducted at 45°.

Ultrasonography. Once in a rested state, brachial artery diameter and blood flow velocity were assessed with a digital ultrasound machine (Logiq E R7-ext Gen, GE Healthcare, Chicago, IL, USA). The diameter of the vessel was first measured with brightness (B)-mode via a screen shot image and the linear distance function. Next, blood flow velocity was determined via Doppler mode and a multi-frequency linear array probe (12L-Rs; 5–13 MHz; 38.4 mm field-of-view). Velocity was assessed at a constant insonation angle of 60° and the Doppler gate was set to the width of the vessel to promote complete insonation. Furthermore, velocity was recorded as the maximum frequency (TAMAX), or peak velocity, averaged across three complete cardiac cycles. Subsequently, this value was divided by two to produce a mean velocity [36,37]. The mean velocity value was then multiplied by the cross-sectional area of the vessel to yield a measure of BABF [38]. Specifically, the equation used to determine BABF was as follows:

$$BABF = \left(\frac{blood\ velocity\ (\mathrm{cm/s})}{2}\right) \times \pi \left(\frac{diameter\ (\mathrm{cm})}{2}\right)^2 \times 60 \quad (1)$$

A single researcher was responsible for collecting data with the ultrasound, and great care was given to avoid excess pressure on the vessel. A generous amount of water-soluble transmission gel was also used to enhance acoustic coupling and reduce artifact.

Near-Infrared Spectroscopy. Measures derived from NIRS are based on tissue transparency for light in the near-infrared region (~700–900 nm), which allows devices to record the relative concentrations and oxygenation status of light-absorbing chromophores (e.g., hemoglobin, myoglobin) in skeletal muscle tissue [23]. The modified Beer–Lambert Law, as follows:

$$A(OD) = Log(I_0/I) = \varepsilon\ [c]\ L \cdot DPF + G \quad (2)$$

was used to account for light scattering [23], and, here, we used a differential pathlength factor (DPF) of 4 (i.e., the extended path length due to scattering) and a *G* constant (i.e., the loss of light due to scattering) [23,32]. In the present study, the NIRS parameters (e.g., O_2Hb, HHb, etc.) were collected from the T × 2 channel at a sampling rate of 10 Hz, and the measures were tracked as changes from baseline [23,32]. It is relevant to note the used channel given that this dictates signal depth. All values were relative to the initial baseline, and not absolute values. These values were used to calculate tHb (O_2Hb + HHb) and HbDiff (O_2Hb–HHb). To support the appropriateness of the NIRS forearm placement site, the ultrasound machine was used to quantify ATT. It was determined that each subject exhibited less than 2 cm of subcutaneous fat (mean ± SD: 0.26 ± 0.10 cm), rending T × 2 an appropriate channel. The NIRS device was attached to the dominant forearm flexor digitorum superficialis muscle with black self-adhesive Coban™ in order to minimize exogenous light from influencing the signal. The specific device used was a portable,

dual-wavelength (760 nm and 850 nm) continuous-wave, spatially resolved NIRS (Portalite, Artinis Medical Systems, Elst, The Netherlands). Throughout data collection, signal quality was assessed with continuous inspection of the signal fit factor and the percentage of light reaching the photodiode.

Venous Occlusion Plethysmography. This procedure involved the application of a mercury in-silastic strain-gauge wrapped around the widest portion of the participant's dominant forearm, adjacent to the NIRS device. Great care was taken to minimize potential sources of error, such that the same researcher was responsible for the placement of the NIRS device and mercury in-silastic strain-gauge. The mercury in-silastic strain-gauge was selected based on measured forearm circumference. Prior to attaching the strain-gauge, the equipment was calibrated. A 2-point calibration was used to zero the baseline and set the high voltage point to 60. Afterwards, the units reflected mL of blood per 100 mL of tissue per minute (mL·dL FAV^{-1}·min^{-1}) [9,39]. Thus, the strain-gauge provided a measure of blood flow via changes in circumference (i.e., strain-gauge length). The change in circumference was recorded as voltage via a plethysmograph (EC6 Strain-Gauge Plethysmograph, Hokanson Inc., Bellevue, WA, USA). Following the calibration and attachment of the strain-gauge, two independently controlled rapid (<0.3 s) inflator and deflator cuffs (E20 Rapid Cuff Inflator, cuff: SC5DTM 11 × 85 cm, Hokanson, Belleview, WA, USA) were attached to the upper arm and wrist and inflated using the Hokanson air source (AG101 Hokanson, Belleview, WA, USA). To assess resting FBF, the upper arm cuff was inflated to 50 mmHg, ensuring sufficient pressure to occlude venous return in all participants, while the wrist cuff was inflated to a suprasystolic (~200 mmHg) value to occlude arterial and venous flow. The upper cuff was set to alternate between inflation and deflation at a duty cycle of 7 s inflated/8 s deflated. That is, one measure of FBF was recorded every 15 s [10]. This continued for four minutes. The last minute (i.e., last four resting FBF measures) were averaged and used to define a single resting FBF value. After the resting FBF phase, one minute of rest was provided without either cuff inflated. Subsequently, the upper cuff and wrist cuff were inflated to a suprasystolic (~200 mmHg) value for five minutes to induce transient ischemia. After precisely five minutes of ischemia, the upper arm cuff was deflated and immediately set to alternate between 4 s of inflation and 3 s of deflation, again at a pressure to only occlude venous return (50 mmHg). That is, eight FBF measures were collected during the first minute following ischemia. This period was defined as RH. Peak hyperemia was defined as the value that was recorded during the first inflation period (i.e., initial 7 s). After this first minute, the duty cycle was adjusted to the resting settings (7 s inflation, 8 s deflation) for an additional two minutes. Throughout the entire post-ischemia phase (three min), the wrist cuff remained inflated at ~200 mmHg.

As previously mentioned, the collected strain-gauge signals corresponded to changes in limb circumference (i.e., volume, due to venous pooling). Signals were transmitted to a personal computer (ThinkBook 15-IML, Lenovo, Beijing, China) with data acquisition hardware (PowerLab 8/35SP, ADInstruments, Inc., Colorado Springs, CO, USA) and then processed as the rate of increase (i.e., slope) (LabChart Pro v8.1.16). The resulting slopes have been described to represent FBF (mL^{-1}·dl FAV^{-1}·min^{-1}) [9]. We have previously demonstrated suitable day-to-day reliability with this procedure [16], and this methodology has previously been supported [7]. To be clear, the NIRS device, as described above, was attached during the entirety of this VOP protocol adjacent and distal to the strain-gauge. Thus, simultaneous slopes were collected for FBF and all skeletal muscle oxygenation parameters. Additionally, throughout this procedure (four min rest, one min without occlusion, five min ischemia, and three min reperfusion phase), MAP and HR were recorded at set timepoints, the midpoint of rest and the initial point of RH.

Statistical Analyses. Independent *t*-tests were used to examine potential mean sex differences among the participant characteristics (Table 1). The data used for analysis were normally distributed, as suggested by the Kolmogorov–Smirnov test. Two separate 2 (Sex: men and women) × 2 (Time: rest and reactive hyperemia) mixed factorial ANOVAs were used to assess mean differences in MAP and HR. Partial eta squared $\left(\eta_p^2\right)$ was reported

as a measure of effect for all ANOVA models (Table 2). Individual peak values of FBF, O_2Hb, HHB, tHb, and HbDiff were normalized to their respective resting value. Pearson's correlation coefficients were used to evaluate the relationships between the normalized FBF and the NIRS-derived variables (Table 3). Additional Pearson's correlation coefficients were calculated for normalized FBF and NIRS-derived variables separated by sex. Pearson's correlation coefficients were also used to evaluate the relationships between BABF and FBF, as well as NIRS-derived variables (Table 4). The magnitudes for all correlations were interpreted as follows: 0.0–0.2 as "poor", 0.3–0.5 as "fair", 0.6–0.7 as "moderate", 0.8–09 as "very strong", and 1.00 as "perfect" [40]. To determine consistency, intraclass correlation coefficients with the 2,1 model ($ICC_{2,1}$) were reported for the four resting values used to calculate the average resting values for each variable: FBF, O_2Hb, HHB, tHb, and HbDiff [41]. Again, this was carried out for the total sample, as well as independently for each sex (Table 5). Standard error of the mean (SEM) was reported for all $ICCs_{2,1}$. In addition, 95% confidence intervals ($CI_{95\%}$) were reported for all mean differences and correlation coefficients. The collected data were exported to a custom Microsoft Excel template that allowed for organization and normalization. All calculations and statistical analyses were conducted using JASP version 0.14.1 (University of Amsterdam, Amsterdam, The Netherlands). A value of $p \leq 0.05$ was considered statistically significant. All data were reported as mean ± SD.

Table 2. Sex-specific hemodynamics.

Variables	ANOVA	*p* Value	η_p^2	Effect
Mean Arterial Pressure (mmHg)	Sex × Time	0.352	0.032	
	Sex	0.137	0.080	
	Time	**0.047**	0.138	Resting > Peak Reactive Hyperemia
Heart Rate (bpm)	Sex × Time	0.287	0.042	
	Sex	0.204	0.059	
	Time	**0.007**	0.240	Resting < Peak Reactive Hyperemia

ANOVA models for resting and reactive hyperemic physiological variables between sexes. Bolded *p*-values correspond to significant at the ≤0.05 level. Resting was the time prior to complete occlusion. Peak reactive hyperemia was the 1st seven seconds following complete occlusion.

Table 3. Relationships between reactive hyperemic forearm blood flow and oxygenation parameters.

Reactive Hyperemia Variables	*r*	Lower-Upper $CI_{95\%}$; *p*
Total (n = 29)		
FBF and O_2Hb	−0.126	−0.47 to 0.25; 0.516
FBF and HHb	0.228	−0.15 to 0.55; 0.235
FBF and tHb	0.061	−0.31 to 0.42; 0.753
FBF and HbDiff	0.046	−0.33 to 0.41; 0.811
Men (n = 15)		
FBF and O_2Hb	−0.219	−0.66 to 0.33; 0.433
FBF and HHb	0.217	−0.33 to 0.66; 0.438
FBF and tHb	0.010	−0.51 to 0.52; 0.972
FBF and HbDiff	0.237	−0.31 to 0.67; 0.395
Women (n = 14)		
FBF and O_2Hb	−0.150	−0.63 to 0.41; 0.608
FBF and HHb	0.207	−0.36 to 0.67; 0.477
FBF and tHb	−0.128	−0.62 to 0.43; 0.663
FBF and HbDiff	−0.048	−0.56 to 0.50; 0.871

FBF, forearm blood flow; O_2Hb, oxygenated hemoglobin; HHb, deoxygenated hemoglobin, tHb, total hemoglobin; HbDiff, hemoglobin difference. $CI_{95\%}$ = 95% confidence interval. $p \leq 0.05$ is significant.

Table 4. Relationships between resting brachial artery blood flow and oxygenation parameters.

Resting Variables	*r*	Lower-Upper $CI_{95\%}$; *p*
Total (n = 29)		
FBF and BABF	−0.043	−0.40 to 0.33; 0.823
O_2Hb and BABF	0.217	−0.16 to 0.54; 0.259
HHb and BABF	0.187	−0.19 to 0.52; 0.332
tHb and BABF	0.221	−0.16 to 0.54; 0.249
HbDiff and BABF	0.219	−0.16 to 0.54; 0.254
Men (n = 15)		
FBF and BABF	0.393	−0.15 to 0.75; 0.148
O_2Hb and BABF	0.191	−0.36 to 0.64; 0.495
HHb and BABF	0.303	−0.25 to 0.71; 0.273
tHb and BABF	0.269	−0.28 to 0.69; 0.332
HbDiff and BABF	0.004	−0.51 to 0.52; 0.988
Women (n = 14)		
FBF and BABF	−0.196	−0.66 to 0.37; 0.501
O_2Hb and BABF	0.228	−0.35 to 0.68; 0.432
HHb and BABF	0.069	−0.48 to 0.58; 0.814
tHb and BABF	0.174	−0.39 to 0.65; 0.552
HbDiff and BABF	0.303	−0.27 to 0.72; 0.292

FBF, forearm blood flow; BABF, brachial artery blood flow; O_2Hb, oxygenated hemoglobin; HHb, deoxygenated hemoglobin, tHb, total hemoglobin; HbDiff, hemoglobin difference. $CI_{95\%}$ = 95% confidence interval. $p \leq 0.05$ is significant.

Table 5. Intra-class correlation coefficients ($ICC_{2,1}$) for forearm blood flow and oxygenation parameters variables during baseline.

Resting Variables	Baseline 1	Baseline 2	Baseline 3	Baseline 4	$ICC_{2,1}$	*p*	SEM
Total (n = 29)							
FBF	1.74 ± 0.65	1.73 ± 0.66	1.68 ± 0.62	1.75 ± 0.59	0.919	0.491	0.367
O_2Hb	0.10 ± 0.08	0.10 ± 0.07	0.11 ± 0.10	0.09 ± 0.09	0.758	0.453	0.085
HHb	0.06 ± 0.06	0.06 ± 0.05	0.06 ± 0.06	0.05 ± 0.06	0.877	0.825	0.041
tHb	0.16 ± 0.14	0.15 ± 0.12	0.17 ± 0.15	0.14 ± 0.14	0.844	0.478	0.109
HbDiff	0.04 ± 0.05	0.040 ± 0.04	0.05 ± 0.06	0.04 ± 0.06	0.377	0.536	0.083
Men (n = 15)							
FBF	1.85 ± 0.51	1.81 ± 0.42	1.74 ± 0.40	1.81 ± 0.51	0.836	0.483	0.377
O_2Hb	0.10 ± 0.08	0.10 ± 0.07	0.10 ± 0.10	0.09 ± 0.09	0.732	0.743	0.117
HHb	0.06 ± 0.07	0.06 ± 0.06	0.05 ± 0.07	0.05 ± 0.07	0.873	0.597	0.048
tHb	0.16 ± 0.14	0.15 ± 0.13	0.15 ± 0.17	0.13 ± 0.14	0.853	0.474	0.113
HbDiff	0.04 ± 0.05	0.04 ± 0.03	0.05 ± 0.05	0.04 ± 0.06	0.115	0.971	0.092
Women (n = 14)							
FBF	1.62 ± 0.77	1.64 ± 0.86	1.61 ± 0.81	1.68 ± 0.68	0.947	0.724	0.360
O_2Hb	0.11 ± 0.09	0.09 ± 0.08	0.12 ± 0.10	0.10 ± 0.10	0.777	0.483	0.084
HHb	0.06 ± 0.05	0.06 ± 0.05	0.06 ± 0.06	0.06 ± 0.05	0.887	0.970	0.033
tHb	0.17 ± 0.13	0.15 ± 0.12	0.18 ± 0.15	0.16 ± 0.14	0.830	0.680	0.111
HbDiff	0.05 ± 0.05	0.03 ± 0.04	0.06 ± 0.08	0.04 ± 0.06	0.577	0.230	0.077

FBF, forearm blood flow; O_2Hb, oxygenated hemoglobin; HHb, deoxygenated hemoglobin, tHb, total hemoglobin; HbDiff, hemoglobin difference. $ICC_{2,1}$ = intraclass correlation coefficient. SEM = standard error of the mean. $p \leq 0.05$ is significant.

3. Results

3.1. Participant Characteristics

The data from 29 participants were reported and analyzed (male = 15, female = 14). Independent *t*-tests revealed that the men exhibited significantly ($p < 0.05$) greater height, weight, body mass index, lean mass, brachial artery diameter, and ATT than the women. The women, however, exhibited significantly ($p < 0.05$) greater values of body fat percentage compared to the men (Table 1).

3.2. Sex-Specific Hemodynamics

There were no significant ($p > 0.05$) two-way interactions for either MAP or HR (Table 2). However, for MAP, there was a significant ($p = 0.047$; $\eta_p^2 = 0.138$) main effect for time, such that the rest timepoint (87.4 ± 7.3 mmHg) was greater than the RH phase (85.4 ± 7.5 mmHg). Similarly, for HR, there was a significant ($p = 0.007$; $\eta_p^2 = 0.240$) main effect for time, such that HR was lower during rest (67.0 ± 13.5 BPM) compared to the RH phase (70.0 ± 14.0 BPM).

3.3. Relationships among Strain-Gauge and NIRS Parameters

There were no significant ($p > 0.05$) relationships between FBF and the NIRS-derived parameters (O_2Hb, HHb, tHb, HbDiff) during RH (Figure 2, Table 3). Additionally, there were no significant ($p > 0.05$) relationships when the total sample was separated by sex (Table 3).

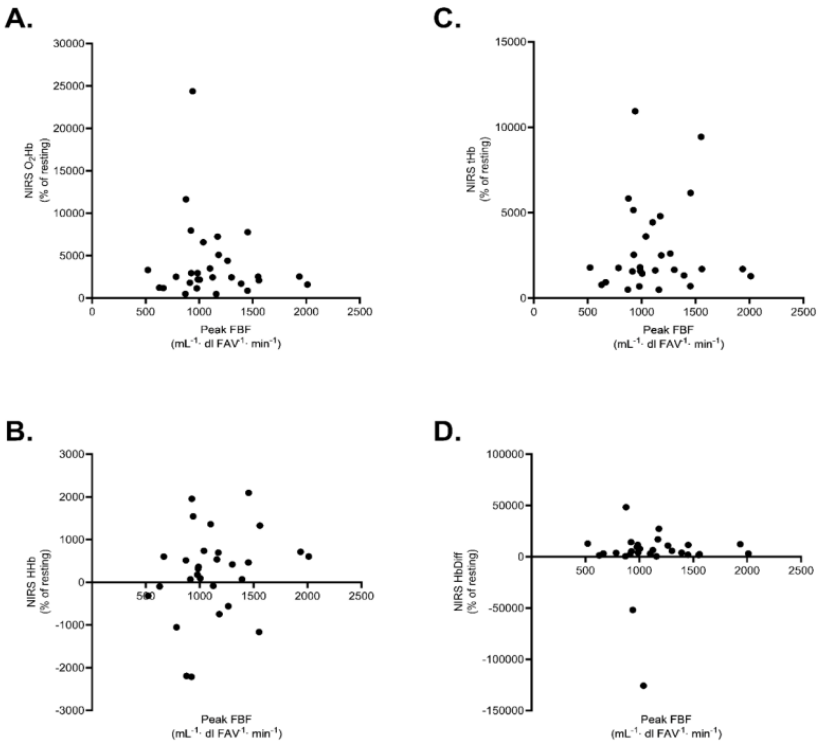

Figure 2. Correlations of strain-gauge and NIRS-derived variables. Panel (**A**) depicts the correlation of forearm blood flow (FBF) mL^{-1}·dl FAV^{-1}·min^{-1} and oxygenated hemoglobin (O_2Hb) % of resting. Panel (**B**) depicts FBF mL^{-1}·dl FAV^{-1}·min^{-1} and deoxygenated hemoglobin (HHb) % of resting. Panel (**C**) depicts FBF mL^{-1}·dl FAV^{-1}·min^{-1} and total hemoglobin (tHb) % of resting. Panel (**D**) depicts FBF mL^{-1}·dl FAV^{-1}·min^{-1} and hemoglobin difference (HbDiff) % of resting. All four correlations were considered poor and not significant.

3.4. Relationships among Resting VOP, NIRS Parameters and Ultrasound Measures

There were no significant ($p > 0.05$) relationships between BABF and FBF or the NIRS-derived parameters (O_2Hb, HHb, tHb, HbDiff) (Table 4). Additionally, there were no significant ($p > 0.05$) relationships when the total sample was separated by sex (Table 4).

3.5. Consistency of Resting Parameters

There were strong $ICC_{2,1}$ for resting FBF ($ICC_{2,1} = 0.919$), O_2Hb ($ICC_{2,1} = 0.758$), HHb ($ICC_{2,1} = 0.877$), and tHb ($ICC_{2,1} = 0.844$), whereas HbDiff ($ICC_{2,1} = 0.377$) was much weaker (Table 5). These were again assessed but separated by sex. For the men, there were strong $ICC_{2,1}$ for resting FBF ($ICC_{2,1} = 0.836$), O_2Hb ($ICC_{2,1} = 0.732$), HHb ($ICC_{2,1} = 0.873$), and tHb ($ICC_{2,1} = 0.853$), but not for HbDiff ($ICC_{2,1} = 0.115$). For the women, the consistency analysis

indicated similar results as the men, such that strong values were observed for resting FBF ($ICC_{2,1}$ = 0.947), O_2Hb ($ICC_{2,1}$ = 0.777), HHb ($ICC_{2,1}$ = 0.887), and tHb ($ICC_{2,1}$ = 0.830), but again not for HbDiff ($ICC_{2,1}$ = 0.577) (Table 5).

4. Discussion

The main objective of the current investigation was to assess the ability of NIRS-derived parameters to serve as a surrogate measure to a well-accepted approach of microvascular blood flow, strain-gauge plethysmography [42]. This was tested by performing a widely used, standardized protocol of VOP while simultaneously recording NIRS from a device fixed just distal to the strain-gauge (Figure 1). The major findings from our study included that (1) peak FBF (i.e., measure of RH) was poorly associated with all of the simultaneously collected NIRS variables (O_2Hb, HHb, tHb, and HbDiff) and (2) there was suitable agreement for the men and women among the four consecutive resting values for each outcome variable (FBF, O_2Hb, HHb, tHb, and HbDiff). These findings may help inform future microvascular-focused research studies as reported responses and measures of RH likely vary depending on the technique utilized (i.e., NIRS vs. strain-gauge plethysmography). Thus, the predictive power of RH for future onset and severity of CVD [2,43] will likely need to be evaluated separately for each technique. Below, these findings are discussed in the context of previous, relevant findings.

Strain-gauge and NIRS association. We hypothesized that the NIRS-derived variables (O_2Hb, HHb, tHb, and HbDiff) would match the slopes of FBF pre- and post-transient ischemia, yet the results of the current study did not support this. Our findings were, however, similar to those of Gomez et al. [3], who also reported poor associations between FBF and NIRS-derived measures of resting tissue saturation index, O_2Hb, and HHb values. These investigators mainly attributed the mismatch between NIRS and strain-gauge measures to the non-invasive approach of quantifying the rate of change (i.e., slope) in each parameter as opposed to more invasive procedures directly applying the Fick principle. In fact, earlier work [13,44,45] all reported a nearly perfect match between strain-gauge plethysmography and NIRS when utilizing the more invasive procedures. As previously noted, here, we only assessed the rate of change (i.e., slope values) in FBF and NIRS parameters in response to venous occlusion pre- and post-ischemia, and no invasive measures were included. In addition, our findings did not agree with those of Olamaei et al. [5], who reported a strong correlation for NIRS and strain-gauge measures, however, only after the use of a unique correction factor. Our findings also did not agree with the report of Harel et al. [46], who showed a strong correlation between values captured from NIRS and the strain-gauge, yet there was disagreement during faster flow rates (e.g., RH), with NIRS consistently yielding underestimates of values. Furthermore, the slope values of the NIRS parameters in the current study were atypical analytical outcomes, such that generally a vascular occlusion test (absent of intermittent venous occlusion phases) is used to generate NIRS-based outcome measures (e.g., maximum tissue saturation). Despite the differences in methodological approaches, here, we were still able to suggest additional explanations to those previously presented by Gomez et al. [3]. Based on the present results, it was proposed that various reperfusion kinetics along the oxygen cascade contributed to the lack of association between FBF and NIRS parameters. As seen in Table 5, there were generally greater (i.e., faster) slopes for FBF compared to the NIRS measures, which indicated that the forearm increased in volume (due to venous pooling) faster than the forearm muscle group re-saturated. It is well-established that diffusive oxygen transport (capillary to myocyte) is a limiting factor in the genesis of aerobically derived cellular energy [47]. This diffusion capacity is affected by numerous factors, including capillary characteristics (e.g., density), red blood cell abundance and transit time, surface area, and solubility of oxygen [48–50]. Alternatively, the magnitude of perfusion (i.e., peak FBF), termed RH, is largely related to the ability of an individual to vasodilate the microvasculature via various myogenic and local metabolic/endothelial factors within arterioles [7,9,10]. Taken together, it remains quite possible that the separate, but linked, factors specifically contributing to reperfusion and

re-saturation may not be aligned intra-individually. That is, perhaps the major promoter of the mismatch between NIRS- and strain-gauge-derived measures included inconsistent relationships between discrete factors influencing diffusion capacity and RH. Thus, caution should be used when extending the predictive value of actual microvascular blood flow [2,9,10,43] to measures of microvascular oxygenation. However, it is still important to note that NIRS-parameters have demonstrated value and usefulness, as evidenced by their sensitivity to various chronic conditions such as obesity and CVD [51,52], as well as sex- and age-specific differences [11,38,53–57]. Ultimately, future work remains needed to fully establish the potential prognostic value of NIRS-based microvascular oxygenation values.

Associations among micro- and macrovascular measures. The current results indicated that resting hemodynamics assessed by BABF did not correlate with either FBF or the NIRS measures (O_2Hb, HHb, tHb, HbDiff) (Table 4). The lack of a clear association of either FBF or the NIRS-derived variables with BABF suggested that the resting macrovascular measures did not track the microvascular blood flow measures. This was particularly interesting given the role of the brachial artery in supplying the downstream micro-vessels (e.g., arterioles). Previously, Ives et al. [58] provided evidence that NIRS (microvascular) appropriately tracked pharmacologically induced changes in blood flow as compared to Doppler ultrasound (macrovascular) measures. Thus, there is some evidence that simultaneous measures from different locations along the vascular tree should be associated. The current results did, however, partially demonstrate this notion. When examining associations separately for the men and women, HHb correlated "fairly" with BABF in men (r = 0.303), but not women (r = 0.069), whereas HbDiff exhibited a stronger correlation in women (r = 0.303) than men (r = 0.004) (Table 4). Specifically, HHb is a proxy variable for the degree of deoxygenation in arterial and venous blood (e.g., the perfusive component of the Fick equation) [23]. Related, but distinctly different, HbDiff represents the collective changes in oxygenation in response to supply and demand, and thus, may serve as an index of oxygen delivery [59]. It has previously been advised that these measures should be used in tandem to provide a more comprehensive indicator of metabolic changes [60]. Notably, the NIRS–Doppler ultrasound relation was determined between O_2Hb and femoral blood flow and from a sample of 10 healthy men [58]. Potential underlying sex differences such as intrinsic respiration, efficiency, oxidative capacity and/or oxygen affinity should likely not be ignored when assessing NIRS-derived values [29,30,54]. In support of these intrinsic factors likely promoting the observed sex differences, the men and women did not exhibit any difference in changes of HR or MAP during the experiment. Overall, in conjunction with a previous report [61], the current results suggest using caution when attempting to generalize vascular findings of one specific region to another, and underlying differences between men and women should be considered.

Consistency of FBF and NIRS variables at rest. Limited data exist concerning the consistency of consecutive slopes of FBF and NIRS-derived variables induced by venous occlusion during resting conditions. Previous investigations, like the current study, have reported methodology including a calculated resting mean value derived from four recordings [62]. However, the present analysis indicated that there was suitable consistency among the four consecutive slope values for the FBF and NIRS-derived variables (O_2Hb, HHb, tHb, and HbDiff). This agreed well with the previous work of Harel et al. [46], but these investigators only examined tissue oxygenation in a mixed-sex sample of 13 CVD patients. Here, the current study extended these findings to a healthy cohort and examined sex differences. Specifically, our results demonstrated acceptable consistency for FBF (ICC = 0.919) and for NIRS-derived variables (ICCs = 0.758–0.877) except for HbDiff (ICC = 0.377) (Table 5). Additionally, when separated by sex, there were generally higher ICC values for the women (ICCs = 0.777–0.947) compared to men (ICCs = 0.732–0.873). Notably, HbDiff remained much lower than the other ICCs, women: ICC = 0.577 and men: ICC = 0.115. Gomez et al. [3] also reported ICC values; however, their analysis aimed to determine match between tissue saturation index, O_2Hb, and HHb to FBF, not consistency across four consecutive measures. These authors reported poor ICCs (i.e., match) for all

comparisons (ICCs 0.00–0.07) at rest. Taken together, the current results, in conjunction with those of Gomez et al. [3], strongly suggest that FBF and NIRS variables should not be interchanged, as well as that a single measure of the slope value may be sufficient given the high consistency among four consecutive measures.

Experimental considerations. Readers are encouraged to consider the following aspects when evaluating the presented interpretations of the current data. This study was conducted using an apparently healthy, young adult population, and therefore, this limits the results' generalizability to additional populations. Although the participants were advised to maintain a regular diet and other habits, as well as complete a standardized rest phase, it is possible that other external factors beyond our current ability to control may have influenced resting blood flow. In addition, it is possible that the currently used sample size may be perceived as a limitation and lessen the enthusiasm related to the conclusions. Additionally, it has previously been suggested that NIRS variables should only be assessed across the first cardiac cycle during venous occlusion [63], but the current study assessed slopes across multiple cardiac cycles. The present study did not include a time-aligned electrocardiogram (ECG), so the ability to only assess the first cycle was not possible. It is our recommendation that future studies include time-aligned ECG tracings to determine if FBF and NIRS-derived outcomes match better than the currently reported results. We also did not apply a unique correction factor for NIRS signals as used by Olamaei et al. [5]. Finally, we acknowledge that our decision not to control for the menstrual cycle in the female participants may be viewed as a limitation. While acknowledging this, based on available evidence [34], it remains unlikely that natural interindividual variability in menstrual cycle phase substantially affected the current results.

5. Conclusions

In summary, the current study reported that FBF and NIRS-derived outcomes linearly changed in response to venous occlusion, yet these changes were not meaningfully associated. This was in contrast to our stated hypotheses. It was interpreted that this mismatch most likely suggested that the measures from strain-gauge plethysmography and NIRS reflected different underpinnings of blood flow and oxygen delivery. The strain-gauge plethysmography provided measures of forearm circumference change (i.e., rate of FBF) and NIRS gave insights into skeletal muscle oxygenation changes. Thus, the rate of blood arriving to the forearm did not associate with the skeletal muscle re-saturating following transient ischemia. Ultimately, based on previous findings, strain-gauge plethysmography and NIRS remain important and useful measures, but likely should not be used interchangeably or as surrogates of each other. However, it is acknowledged that future research remains necessary to confirm our findings with larger, more diverse samples to improve the generalizability of the current conclusions. Future work is clearly needed to determine the predictive ability of NIRS-derived measures of RH for future CVD. Enthusiastically, our future research will aim to help elucidate this potential predictive value in a sex-specific manner.

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