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Measuring reactive hyperemia in the lower limb using near-infrared spectroscopy

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Abstract. Near-infrared spectroscopy (NIRS) has been used to measure reactive hyperemia following a vascular occlusion. However, the procedures and methods of analysis used have varied. The purpose of the present study is to identify reproducible methods for measuring reactive hyperemia using HbO_2 NIRS signals in the calf and foot. Healthy participants (10 male, 10 female) aged 19 to 28 years performed one of two tests: reproducibility trials or elevation protocol (30 and 60 cm limb elevation above the heart). The time to 50% reperfusion ($T_{1/2}$) and the second (R_{2q}) quartile rates of reperfusion were found to be the most reproducible parameters (coefficient of variation= 7.12 to 14.1%). The time to 95% reperfusion (T_{95}) was 12.7% more reproducible on average than the previously reported parameter of time to peak hyperemia. Measures of reperfusion time and rate slowed with increasing limb elevation. Correlations were identified between the calf and foot in the measurements of R_{2q} ($R^2 = 0.713$, $p = 0.021$), $T_{1/2}$ ($R^2 = 0.673$, $p = 0.033$), and T_{95} ($R^2 = 0.792$, $p = 0.006$). Half and 95% recovery times and second and third quartile rates expressed good reproducibility and sensitivity to change with reduced perfusion pressure. NIRS measures of reactive hyperemia have the potential to evaluate microvascular perfusion in clinical populations. [DOI: 10.1117/1.JBO.21.9.091302]

Keywords: near-infrared spectroscopy; reactive hyperemia; lower limb perfusion; vascular occlusion test; reproducibility.

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1 Introduction

Near-infrared spectroscopy (NIRS) has been well established as a method of measuring blood flow, oxygen saturation, and oxidative capacity in skeletal muscle and cerebral tissue.[1–11] NIRS measures of perfusion have also been used to identify impaired microvascular perfusion in clinical populations with conditions such as peripheral vascular disease, diabetes, and hypovolemia.[12–18] Specifically, continuous-wave NIRS (CW-NIRS) and frequency-domain NIRS devices have been used to noninvasively measure microvasculature function by observing the kinetics of NIRS signals during reactive hyperemia following a vascular occlusion.[5,12,16,19–24] NIRS measures of reactive hyperemia correlate well with other measures of tissue perfusion, including conduit artery blood flow kinetics, transcutaneous oximetry, and plethysmography.[12,13,20,25–27] Additionally, studies have found baseline oxygen saturation and reperfusion rates during reactive hyperemia to be predictors of mortality in critically ill patients.[28–31]

Patients with peripheral vascular disease and other pathologies experience impaired circulation in the lower extremities, particularly in the calf and foot.[18,32–38] CW-NIRS devices have been used to study recovery kinetics during reactive hyperemia in the lower limbs of these patient populations.[12,18,23] The CW-NIRS probe is typically placed over the calf muscle or foot pad, and reperfusion is observed following 5 min of ischemia. Recovery kinetics have been characterized by reperfusion rates and times of the hemoglobin (HbO_2), deoxygenated hemoglobin (HHb), and oxygen saturation signals; however, HbO_2 is consistently reported in studies measuring reactive hyperemia in patient populations.[12,18,39]

A limitation to the previous studies is the use of different methods of analysis, and very few studies have reported the reproducibility of their measurements. Only one previous study has reported the reproducibility of CW-NIRS HbO_2 kinetics in the lower limb, and that study measured only reactive hyperemia in the foot of six subjects.[24] Another recent study reported the reproducibility of CW-NIRS HbO_2 kinetics in the forearm, but the sensitivity to perfusion pressure of the parameters was not investigated.[5] The purpose of the present study was to identify the most reproducible methods for measuring reactive hyperemia using CW-NIRS and to characterize reactive hyperemia in two different tissue types (in calf muscle and in the foot pad). We quantified the reproducibility of reperfusion times and reperfusion rates of HbO_2 recovery kinetics as measured by CW-NIRS. Furthermore, we hypothesized that reperfusion rates and times of HbO_2 would change with perfusion pressure.

2 Materials and Methods

2.1 *Participants*

Twenty participants (10 male, 10 female) aged 19 to 28 years performed one of two tests: reproducibility trials or elevation protocol. The study was conducted with the approval of the institutional review board at the University of Georgia (Athens, Georgia), and all subjects gave written, informed consent before testing. Participant characteristics are presented in Table 1.

2.2 *Near-Infrared Spectroscopy*

Reactive hyperemia was measured in the left medial gastrocnemius (calf) and the plantar midline of the left foot using CW-NIRS

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Table 1 Characteristics of study participants.

	Reproducibility	Elevation protocol
N	10	10
Gender (M/F)	5/5	5/5
Age (year)	22.3 (2.4)	21.5 (1.4)
Height (cm)	172.1 (7.3)	174.9 (7.8)
Weight (kg)	71.1 (7.6)	72.9 (14.8)
Body mass index ($kg\,m^{-2}$)	24.1 (3.1)	23.8 (4.1)

(Oxymon Mk III, Artinis Medical Systems). One transmitter and two receivers were placed at each measurement location. At the calf muscle, the interoptode distance was set to measure NIRS signals from a tissue depth of at least twice that of the adipose tissue thickness (ATT). The distance between the NIRS transmitter and receivers (interoptode distance) was adjusted (25 to 55 mm) according to each individual's respective ATT. ATT was measured using ultrasound (LOGIQ, GE HealthCare) as previously described.[40] The two receivers were always separated by a distance greater than 10 mm. For the plantar foot measurements, a custom-made rubber bracket was used to secure the transmitter and receivers to the foot, and the interoptode distances were constant at 35 and 45 mm for each participant. The optode distance of 35 to 45 mm was selected to provide measures of oxygen kinetics at a depth similar to previous studies measuring reactive hyperemia in the foot.[12,18,24] NIRS measurements were digitally recorded in real time throughout the duration of the protocol at an acquisition frequency of 10 Hz.

2.3 *Vascular Occlusions*

Vascular occlusions were performed on the left lower extremity with the participant in the supine position and the foot transmitter positioned 2 cm above the level of the heart. A blood pressure cuff (Hokanson, 20c, Bellevue, Washington) was placed proximal to the knee and rapidly inflated to 250 to 300 mm Hg using a rapid cuff inflation system (Hokanson, E20, Bellevue). The cuff was inflated for 5 min, and HbO_2 signals were monitored during this period to ensure that the arterial occlusion was maintained throughout the test. Following the 5-min arterial occlusion, the cuff was rapidly deflated and reactive hyperemia was observed until oxygen levels returned to baseline. A representative test result is shown in Fig. 1.

2.4 *Experimental Protocols*

The first aim of the study was to assess the test-retest reliability of reactive hyperemia kinetics in the calf muscle and the foot by performing two vascular occlusions at a baseline elevation of 2 cm above the level of the heart. The tests were performed ~5 min apart on the same day by the same tester. The second aim of this study was to assess the influence of reduced perfusion pressure on reactive hyperemia kinetics in the foot. A model of reduced perfusion pressure was achieved by conducting the elevation protocol consisting of three separate vascular occlusions performed at three different limb elevations (baseline, 30 cm, and 60 cm). All elevations were calculated as the elevation of the NIRS transmitter above the level of the heart. Ankle segmental pressures were measured at baseline and at each level of elevation. Limb elevation was achieved by supporting the heel of the foot with padded rubber blocks to provide comfort and prevent the restriction of blood flow throughout the elevation protocol. HbO_2 signals were monitored after each test to ensure that oxygen levels returned to baseline before proceeding to the next test.

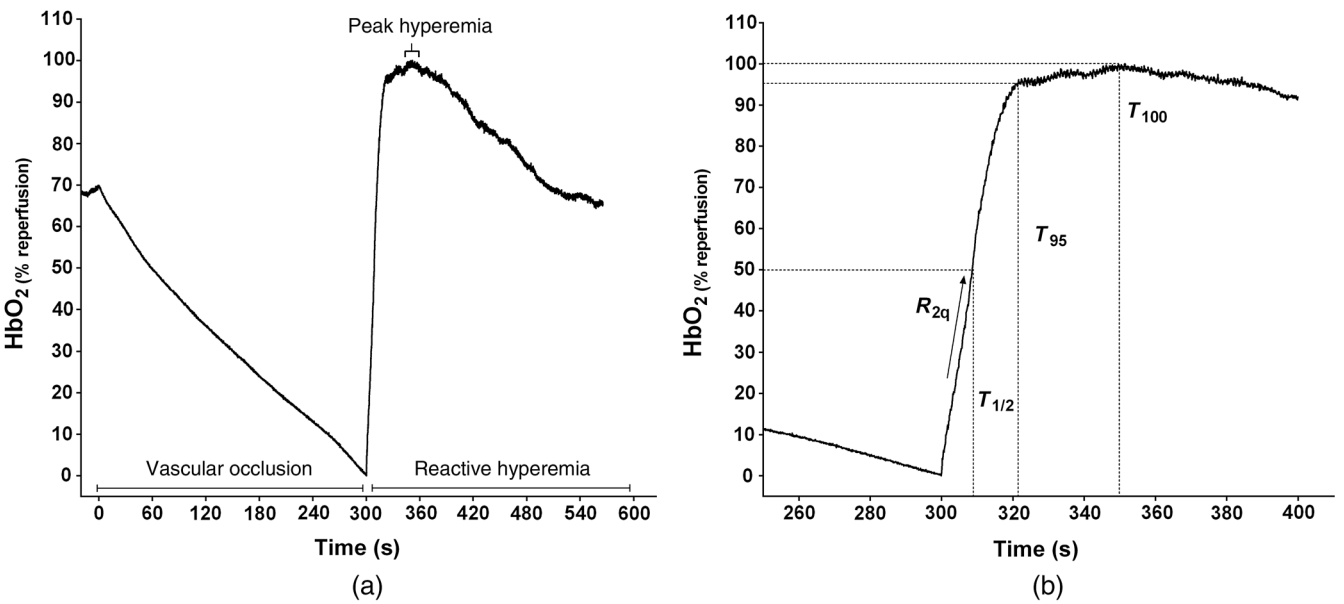

Fig. 1 Representative data (a) showing the oxygenated hemoglobin/myoglobin (HbO_2) NIRS signal during arterial occlusion and reperfusion. (b) Measures of reactive hyperemia. Broken lines indicate temporal reperfusion parameters: time to 50% magnitude ($T_{1/2}$), time to 95% magnitude (T_{95}), and time to the peak hyperemic signal (T_{100}). Solid arrow indicates the second quartile rate (R_{2q}).

2.5 *Data Analysis*

The oxygenated hemoglobin signal (HbO_2) was selected as a measure of wash-in kinetics of oxygen reperfusion during reactive hyperemia.[5,18,39,41] The raw data collected from the NIRS device were exported and analyzed using custom-written routines in MATLAB® R2014b (MathWorks Inc.). Four reperfusion times of the HbO_2 signal were calculated: $T_{1/2}$ as the time from release of the cuff to return to 50% magnitude, T_{50-100} as the time from 50% magnitude to the maximal hyperemic signal, T_{95} as the time from release of the cuff to 95% of the magnitude, and T_{100} as the time from release of the cuff to the peak hyperemic signal (Fig. 1). A series of reperfusion slopes of the HbO_2 signal were calculated, including the slope of the complete recovery response and slopes of segments of the recovery response (increments of 25, 10, and 5%). Only the most reproducible incremental rates were reported. To control for differences in the range of reperfusion and in NIRS signal calibration between tests, all slope measurements were normalized to the range of reperfusion (end occlusion to peak hyperemia) and expressed as a percent of the range.

Statistical analysis was performed using IBM SPSS Statistics 22 (IBM®, Armonk, New York).

Differences between measurements were identified using one-way analysis of variance (ANOVA) comparing means of measures at different anatomical locations and between channels at the same anatomical locations. One-way repeated measures ANOVA was performed to identify differences in reactive hyperemia and ankle blood pressure during the elevation protocol. Bonferroni corrections for multiple comparisons were performed to identify differences from baseline at each elevation. Data reported at means (± standard deviation) unless otherwise specified. Significance was accepted at $p < 0.05$ for all comparisons.

3 Results

3.1 *Reproducibility*

Test-retest reproducibility was performed on 10 participants. One-way repeated measures ANOVA indicated no significant differences in the means of any measures between the two trials. Interoptode distance had no significant effect on measures of reperfusion time or rate of reperfusion at the calf, and the signal from the receiver with the smaller interoptode distance consistently produced stronger signal-to-noise values. Therefore, the signal from the receiver with the smaller interoptode distance was selected for further analysis.

Overall, measurements from the first half of the reactive hyperemia response were most reproducible (Table 2). $T_{1/2}$ was the most reliable measure of reperfusion time in the calf and foot ($CV_{calf} = 9.68 \pm 4.19$, $CV_{foot} = 14.1 \pm 8.61$), and the second quartile rate (R_{2q}) was consistently the most reliable measure of rate at both measurement sites [coefficient of variation$(CV)_{calf} = 7.12 \pm 3.95$, $CV_{foot} = 8.86 \pm 6.80$]. Measures of second half reperfusion time (T_{50-100}) were 25% more variable and significantly slower in the calf ($p < 0.01$) and foot ($p < 0.01$) compared to the measure of $T_{1/2}$ (Table 2). The highest variability was found in the last 5% of the reperfusion curve. Furthermore, the measure of T_{95} ($CV_{calf} = 15.8 \pm 9.12$, $CV_{foot} = 17.0 \pm 6.18$) was found to be more reproducible than the measure of T_{100} ($CV_{calf} = 33.3 \pm 9.46$, $CV_{foot} = 25.8 \pm 9.65$) at both measurement sites (Table 2).

Selected analysis of HHb recovery kinetics was also performed. HHb measures of $T_{1/2}$ ($CV_{calf} = 9.43 \pm 7.31$, $CV_{foot} = 13.4 \pm 8.92$) and T_{95} ($CV_{calf} = 11.8 \pm 10.9$, $CV_{foot} = 23.5 \pm 18.4$) expressed similar reproducibility compared to HbO_2. While HHb reperfusion times of $T_{1/2}$ (calf $= 10.3 \pm 3.35$ s, foot $= 23.9 \pm 6.75$ s) and T_{95} (calf $= 28.3 \pm 7.7$ s, foot $= 54.2 \pm 16.4$ s) were significantly slower compared to HbO_2, the measurements consistently correlated with HbO_2 measures at the calf ($T_{1/2}$: $R^2 = 0.88$, $p < 0.01$; T_{95}: $R^2 = 0.91$, $p < 0.01$) and foot ($T_{1/2}$: $R^2 = 0.93$, $p < 0.01$; T_{95}: $R^2 = 0.75$, $p < 0.01$).

3.2 *Anatomical Comparisons of Reactive Hyperemia*

At baseline, measures of HbO_2 reperfusion time and rate were significantly slower in the foot compared to the calf (Fig. 2). However, correlational analysis did identify significant relationships between the calf and foot in the measures of $T_{1/2}$ ($R^2 = 0.673$, $p = 0.033$), T_{95} ($R^2 = 0.792$, $p = 0.006$), and R_{2q} ($R^2 = 0.713$, $p = 0.021$). No correlational relationships were identified in the measures of total rate or T_{100} between the two anatomical locations.

3.3 *Reperfusion Parameters During Elevation*

Elevation significantly reduced ankle systolic blood pressure from baseline (108 ± 10.9 mm Hg) by 16.0 mm Hg at 30 cm ($p < 0.01$) and 28.9 mm Hg at 60 cm ($p < 0.01$) on average. The temporal measures of $T_{1/2}$ and T_{95} were significantly increased and the measure of second quartile rate was significantly decreased at 30 and 60 cm elevation (Fig. 3). Significant correlations between the most reproducible time and rate measurements were consistently identified at all levels of elevation (Fig. 4). HHb measures of $T_{1/2}$ and T_{95} were also significantly slower at each level of limb elevation.

4 Discussion

This study systematically examined reperfusion kinetics of HbO_2 as measured by CW-NIRS to determine the most reproducible measures of reactive hyperemia. Our results identified the previously reported parameter of $T_{1/2}$ as the most reproducible measure of reperfusion time and the novel measure of R_{2q} as the most reliable measure of rate.[14,24,42] T_{95} was also found to be a more reproducible measure of reperfusion time compared to the commonly reported parameter of time to peak

Table 2 Coefficients of variation (%) of measures of reperfusion.

	T_{100}	T_{95}	$T_{1/2}$	T_{50-100}	R_{1q}	R_{2q}	R_{3q}	R_{4q}
Calf	33.3 (9.46)	15.8 (9.12)	9.68 (4.19)	42.4 (13.3)	10.6 (6.13)	7.12 (3.95)	8.35 (6.38)	15.23 (17.6)
Foot	25.8 (9.65)	17.0 (6.18)	14.1 (8.61)	31.10 (13.8)	15.82 (8.54)	8.86 (6.80)	11.75 (11.9)	30.35 (18.8)

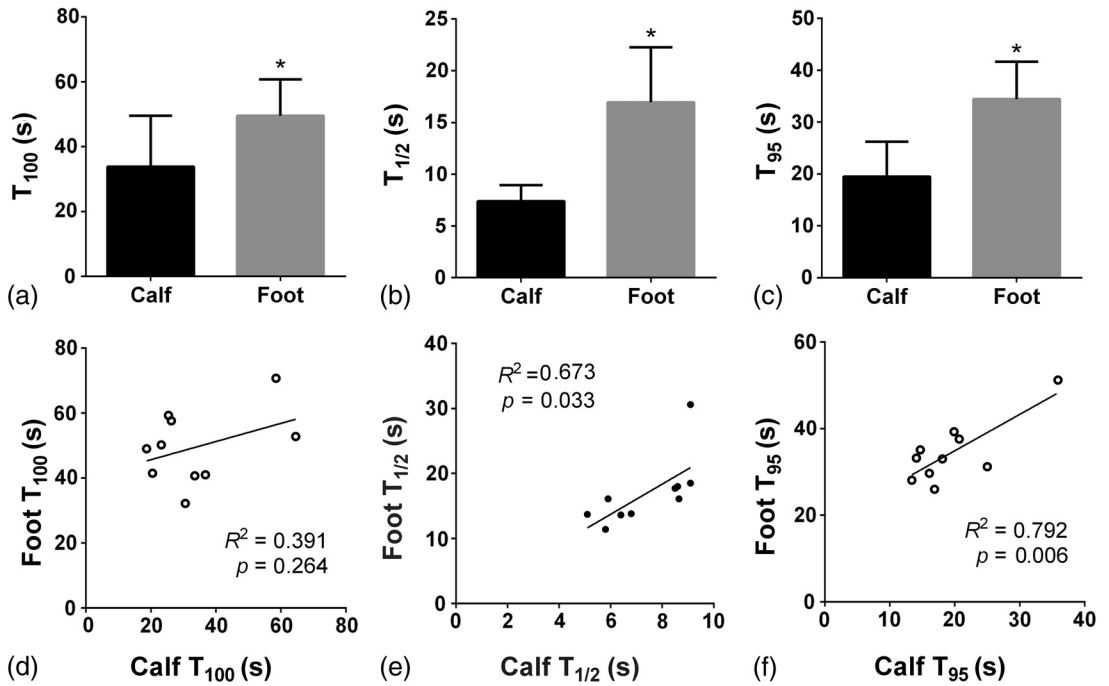

Fig. 2 Perfusion time and rate in foot compared to calf. (a) to (c) Values are means ± SD; temporal reperfusion parameters: time to 50% magnitude ($T_{1/2}$), time to 95% magnitude (T_{95}), and time to the peak hyperemic signal (T_{100}) at the foot and the calf. * indicates significantly different from calf. (d) to (f) Correlational analysis of temporal measures at the foot and the calf. Significance accepted at $p < 0.05$.

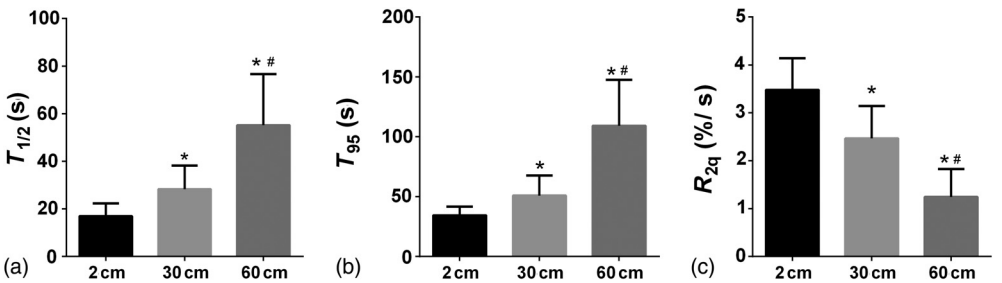

Fig. 3 Values are means ± SD; measures of (a) time to 50% magnitude ($T_{1/2}$), (b) time to 95% magnitude (T_{95}), and (c) time to the peak hyperemic signal (T_{100}) in the foot at three difference levels of elevation. * indicates significant difference from 2 cm; # indicates significant difference from 2 and 30 cm. Significance accepted at $p < 0.05$.

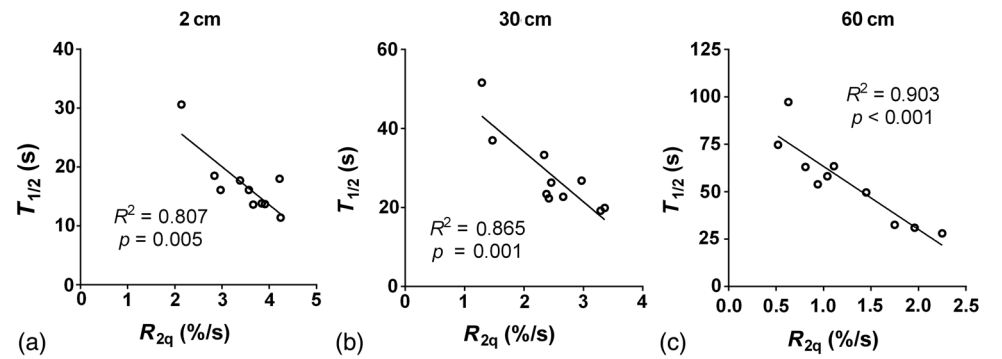

Fig. 4 Correlational analysis between measures of time to 50% magnitude ($T_{1/2}$) and second quartile rate (R_{2q}) in the foot at (a) 2 cm, (b) 30 cm, and (c) 60 cm of limb elevation. Significance accepted at $p < 0.05$.

hyperemia.[5,12,18,23,24] The present study further demonstrated the sensitivity to perfusion pressure of each reproducible parameter, suggesting that either time or rate variables can be used to characterize reactive hyperemia in healthy and diseased populations.

4.1 *Reproducibility*

Various recovery times have been used to characterize reactive hyperemia using NIRS, but few studies have assessed the reproducibility of these measures.[5,12,18,23,24,43] The present study found values and CVs of total HbO_2 reperfusion times similar to those previously reported.[5,24] In comparison, we found the temporal measures of T_{95} and $T_{1/2}$ to be more reproducible parameters than the previously reported parameter of T_{100}.[18,23,24] By excluding the highly variable top 5% of the reperfusion response in the calculation of T_{95}, the reliability of measuring reperfusion time increased by 17% in the calf and 8% in the foot when comparing T_{95} to T_{100}. T_{95} has been reported once before, but the reproducibility was not defined.[12] Our results suggest that T_{95} may serve as a more reliable, and therefore clinically relevant, measure of the total reperfusion time than measures that include 100% of the recovery period. A significant correlation between $T_{1/2}$ and T_{95} was also identified at the calf ($R^2 = 0.75$, $p = 0.013$) and foot ($R^2 = 0.879$, $p = 0.001$), indicating that similar information can be obtained from either measurement.

Recovery rates of NIRS oxygen signals have also been used to characterize reactive hyperemia, and we found the variability of the total rate of reperfusion to be similar to the CV values previously reported ($CV_{calf} = 12.5 \pm 10.7$, $CV_{foot} = 17.1 \pm 10.9$).[5,12,21,23,24,31,43–45] Alternatively, our results identified R_{2q} as consistently the most reproducible measure of rate at both measurement sites. Furthermore, the R_{3q} measure was identified as the most reproducible second half rate with a CV of 8.35 and 11.75% in the calf and foot, respectively. Reperfusion rates derived from the first and second half of the reperfusion curve may have different physiological mechanisms associated with them, and the present results identify R_{2q} and R_{3q} as reproducible markers of primary and secondary reperfusion rates, respectively. We also found significant correlations of measures of R_{2q} with measures of $T_{1/2}$ at both measurement sites, indicating that the measures of rate and time may be used interchangeably. However, ATT has not been found to influence temporal parameters of reactive hyperemia, so $T_{1/2}$ and T_{95} could potentially be more reliable measures of reperfusion in populations with high ATT compared to measures of rate.[19,20]

4.2 *Perfusion Pressure Sensitivity*

We found that the reproducible reperfusion times and rates were also robustly related to perfusion pressures associated with limb elevation. Although a recent study demonstrated the utility of changing limb elevation using NIRS, the study only evaluated resting muscle oxygen saturation.[46] In our study, increases in reperfusion time and decreases in rates are consistent with perfusion parameters reported for patients with cardiovascular and metabolic disease.[14,15,17,30,47] Specifically, we observed a 2.2-fold increase in reperfusion time with 60 cm elevation, which was comparable to the magnitude of changes seen in patients with mild-to-moderate peripheral arterial disease.[12] The results of the elevation protocol support the use of the proposed measures in populations that experience decreased blood flow as an index of perfusion impairment.

4.3 *Anatomical Differences*

We found that our measures of reperfusion time and rate were consistently shorter and faster in the calf muscles compared to the foot tissue, suggesting that the microvasculature of the calf muscle has a faster responsiveness to hypoxic stimuli. The differences in reperfusion between the calf and the foot are likely a result of differences in tissue composition. The signal from the CW-NIRS device at the calf measurement site is assumed to reflect the microvasculature of predominantly skeletal muscle tissue, which may have higher vascular reactivity compared to the heterogeneous composition of fascia, tendon, and muscle under the probe placed on the foot pad. While the values of reperfusion times in the calf and foot measured in the present study are similar to the times previously reported, not all studies have found differences in recovery rates between the calf and foot.[23,24,39] Despite the observed differences, we found correlational relationships in both time and rate parameters between the two measurement sites, indicating preserved relative reperfusion kinetics in the proximal and distal lower limb.

4.4 *Methodology*

Reactive hyperemia measured by various methodologies has been used to characterize the magnitude of diseases as well as to predict future health outcomes.[48–52] The reactive hyperemia measured by NIRS is largely mediated by the same mechanisms that drive reactive hyperemia in larger resistance arteries; however, NIRS measures are specific to oxygen delivery in the microvasculature of the skeletal muscle.[1,7,8,20,26,53–55] Other methodologies such as laser Doppler have been used to study microvascular perfusion, but these measurements represent superficial skin blood flow and not skeletal muscle microvascular beds.[56,57] Magnetic resonance imaging (MRI) studies using muscle BOLD technology have reported microvascular perfusion parameters similar to NIRS measures in this study, but MRI methodology is far more expensive and methodologically involved.[58–60] NIRS technology provides a much simpler and less expensive alternative in assessing the endothelial function of the microvasculature. Specifically, NIRS measures of reactive hyperemia may be particularly beneficial in studying vascular pathology and intervention outcomes in populations with impairments in nutritive flow.[1,17,49,61]

The present study reported HbO_2 as a measure of wash-in kinetics during reactive hyperemia. Several previous studies using CW-NIRS to measure reactive hyperemia have also reported parameters of HHb and oxygen saturation.[5,23] The slower HHb reperfusion times measured in the present study suggest that these signals may be influenced by accumulations of blood in the tissue. Interestingly, parameters of HHb had similar reproducibility and expressed sensitivity to changes in perfusion pressure at 30 and 60 cm of elevation.

4.5 *Limitations*

A key to NIRS measurements of the microvascular is accounting for tissue heterogeneity over the sampling site as the intensity of the HbO_2 signal may be influenced by optical measurement calibration and tissue composition.[7,26] The present study

minimized the potential influence of varying ATT on the rate measurements by normalizing all HbO_2 signals to their respective ranges of reperfusion.[40] Some of the variations in the finding of studies using NIRS to measure reactive hyperemia kinetics could be a result of using un-normalized NIRS signals and different lengths of occlusion time.[43,44,62] While some studies have used occlusion lengths shorter and longer than 5 min, reactive hyperemia is typically examined following a 5 min occlusion as longer or shorter durations of ischemia may alter recovery times and rates.[12,15,19,63,64] Vascular occlusions up to 5 min have been shown not to result in significant depletion of phosphocreatine stores, which can potentially influence oxygen kinetics during recovery.[65] A plateau in the oxygen signal may occur in longer occlusion lengths and could indicate the use of phosphocreatine stores. Furthermore, the 5 min occlusion used in the present study did not appear to result in any long-term changes to the vascular reactivity as indicated by the reproducibility of our results in sequential trials. Future studies should investigate the reproducibility of reperfusion kinetics following exercise. Although the two groups were not significantly different in age, BMI, or disease status, it should be noted that the reproducibility and elevation protocol were measured in two separate cohorts. The use of young, healthy participants may also be a potential limitation to our study, and future studies will need to examine the reproducibility and utility of these measurements in diseased/injured populations.

5 Conclusion

This study employed a systematic analysis of reperfusion kinetics during reactive hyperemia to develop reproducible, standardized, and physiologically relevant measures of reactive hyperemia as assessed by NIRS. Furthermore, this study demonstrated the ability of the NIRS measures of reactive hyperemia to detect changes in perfusion pressure in the lower limb, revealing potential clinical applications. The capacity of NIRS to consistently measure reactive hyperemia in the presence of impaired blood flow is imperative to the use of this methodology in diseased populations and its implementation in clinical practice. Future studies should seek to further validate the application of the measures presented in this study and explore the applicability to diseased populations.

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