

CHANGES IN MICROVASCULAR HEMATOCRIT DURING POST-OCCLUSIVE
REACTIVE HYPEREMIA: DESCRIPTIONS AND MECHANISMS

by

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B.S., University of Massachusetts-Lowell, 1997

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Anatomy and Physiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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ABSTRACT

The primary aim of this dissertation was to describe the changes in microvascular hematocrit, as total[hemoglobin+myoglobin] (T[Hb+Mb]) measured with near-infrared spectroscopy (NIRS), during post-occlusive reactive hyperemia (PORH). Mechanisms of reactive hyperemia within skeletal muscle were also explored. The investigation detailed in Chapter 2 of this dissertation found that the differing time courses of the kinetic responses of both oxy- and deoxy[Hb+Mb], are related to changes in T[Hb+Mb]. We also determined that adipose tissue thickness had no effect on a purely temporal analysis of NIRS data. In Chapter 3 we observed that brachial artery reactive hyperemia preceded changes in T[Hb+Mb] during reactive hyperemia. Assuming that myoglobin remained constant, we posited that changes in T[Hb+Mb] must reflect alterations in red blood cell concentration in the microvasculature, i.e., microvascular hematocrit. In Chapter 4 comparisons were made between brachial artery blood flow, cutaneous and skeletal muscle flux and T[Hb+Mb]. The conduit artery response was faster than the microvascular responses in all tissues. Within skeletal muscle, time to peak and the time constant for the on-kinetics were faster in T[Hb+Mb] compared with intramuscular flux as measured with intramuscular laser-Doppler. We observed no differences in temporal responses between cutaneous and intramuscular measures and suggested that in a purely temporal analysis the cutaneous microvasculature could serve as an analog for the skeletal muscle microvasculature. Finally, in Chapter 5 we found that prostaglandin inhibition with ibuprofen altered the initial T[Hb+Mb] response during PORH without impacting cutaneous flux or brachial artery blood flow. Chapter 5 also discussed that the addition of a wrist cuff to our standard instrumentation prevented the accumulation of T[Hb+Mb] during the occlusion period.

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Preface

Chapters 2-5 of this dissertation represent original research papers that have been published or are currently under review in peer-reviewed journals (citations are listed below).

Bopp, C.M., D.K. Townsend, and T.J. Barstow, *Characterizing near-infrared spectroscopy responses to forearm post-occlusive reactive hyperemia in healthy subjects*. Eur J Appl Physiol, 2011. **111**(11): p. 2753-61.

Bopp, C.M., et al., *Relationship between brachial artery blood flow and total [hemoglobin+myoglobin] during post-occlusive reactive hyperemia*. Microvasc Res, 2014. **91**: p. 37-43.

Bopp, CM et al., The association between cutaneous and intramuscular microvascular measures during post-occlusive reactive hyperemia. *Clinical Hemorheology and Microcirculation*. In Press.

Bopp, CM et al., Ibuprofen alters the initial post-occlusive reactive hyperemic response in skeletal muscle, but not cutaneous tissue, in the human forearm. Ready for submission.

Chapter 1 - Introduction

Following a period of arterial occlusion there is a transient increase in blood flow called post-occlusive reactive hyperemia (PORH), or ischemia/reperfusion. During the period of occlusion, vasodilation of downstream arterioles occurs due to various vasodilators; when the occlusion is removed the decreased vascular resistance results in a temporary hyperemic state; eventually these vasodilators are flushed, vasodilation wanes and blood flow returns to pre-occlusion levels. Reactive hyperemia has traditionally been assessed in the brachial artery via Doppler ultrasound[1, 2] and in the forearm as a whole with strain gauge plethysmography[3]. However, these early studies could not assess reactive hyperemia within skeletal muscle. In light of recent evidence suggesting that microvascular reactive hyperemia is more predictive of cardiovascular disease than measures derived from the conduit artery[1, 4, 5], the ability to assess microvascular reactive hyperemia within skeletal muscle could offer clinical utility beyond traditional measures.

For over 40 years, near-infrared spectroscopy (NIRS) has been used to non-invasively monitor oxygen status in various tissues, including skeletal muscle [6-8]. NIRS has been used to assess reactive hyperemia in the lower limb in healthy subjects and in patients with peripheral artery disease.[9, 10] These authors suggested that the dynamic changes in oxyhemoglobin+myoglobin (oxy[Hb+Mb]) as measured with NIRS during PORH were a better measure for determining microvascular dysfunction than was the amplitude of the PORH response. These authors were also the first to observe a transient increase in total hemoglobin+myoglobin (T[Hb+Mb]) during PORH. However, they provided no framework for the evaluation of the dynamic changes in the NIRS related signals T[Hb+Mb], oxy[Hb+Mb] and

deoxyhemoglobin +myoglobin (deoxy[Hb+Mb]). The investigation described in Chapter 2 sought to rectify this issue and to provide a technique for the evaluation and quantification of both the dynamic changes in oxy[Hb+Mb] and deoxy[Hb+Mb] signals and their signal strength and to determine which had the greatest impact on T[Hb+Mb]. Briefly, this investigation showed that distinct sigmoidal functions are best used to describe each NIRS response (oxy[Hb+Mb] and deoxy[Hb+Mb]) and that the changes in T[Hb+Mb] are related to both the dynamic components of each response and their amplitude. We also demonstrated that correction for adipose tissue thickness is not required during a purely temporal analysis of NIRS data.

During PORH, reactive hyperemia results in a transient increase in conduit artery blood flow above baseline levels; microvascular blood flow is also increased in the skin.[11, 12] Within the skeletal muscle microvasculature, T[Hb+Mb] also demonstrates a transient increase above baseline.[9, 13] A limitation of these previous studies was that no comparisons were made between conduit artery reactive hyperemia and reactive hyperemia within skeletal muscle itself. Therefore, the purpose of the investigation described in Chapter 3 was to compare the kinetic relationship between T[Hb+Mb] within the skeletal muscle microvasculature and the conduit artery macrovascular response during PORH. Recovery kinetics for both conduit artery and T[Hb+Mb] responses were moderately correlated and not different from one another. Our results suggest that the T[Hb+Mb] response was preceded by brachial artery reactive hyperemia. T[Hb+Mb] reflects both skeletal muscle myoglobin and erythrocyte hemoglobin in the microvasculature in the area of interrogation. Assuming that myoglobin remains constant,

changes in T[Hb+Mb] must reflect alterations in red blood cell concentration in the microvasculature, i.e., microvascular hematocrit.[14, 15]

Comparisons between microvascular and macrovascular reactive hyperemia had previously demonstrated poor degrees of association,[11, 12] but our analysis demonstrated a moderate degree of correlation between the two and suggested causality.[16] Previous comparisons had only compared the cutaneous microvasculature to conduit artery during reactive hyperemia. The study described in Chapter 4 added cutaneous and intramuscular assessments of erythrocyte flux measured as with laser-Doppler flowmetry to our instrumentation. We found that both time to peak and the time constant of the on-kinetics during PORH were faster in the conduit artery compared with all microvascular measures (both cutaneous and skeletal muscle). Within skeletal muscle, these same parameters were faster for T[Hb+Mb] compared to intramuscular laser-Doppler. No differences in temporal profiles were observed between cutaneous and intramuscular measures, either by NIRS or IMLD, during PORH in the forearm.

Many vasodilators have been implicated in reactive hyperemia following arterial occlusion. End products of arachidonic acid metabolism, such as prostaglandins, are an often cited causal agent of reactive hyperemia as measured in the entire forearm with strain gauge plethysmography and in the conduit artery.[3, 17-19] Prostanoid production can be curtailed with the administration of non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen.[20] The investigation described in Chapter 5 examined the effects of prostaglandin inhibition with 1200 mg of oral ibuprofen on the temporal parameters of the overall reactive hyperemic response in skeletal muscle, cutaneous tissue and the brachial artery. Our results confirm that ibuprofen

alters the initial T[Hb+Mb] response during PORH, resulting in a longer time to peak and a longer on-kinetic time constant. These results also showed that ibuprofen had no impact on the cutaneous or conduit artery blood flow during PORH. Chapter 5 also addresses the accumulation of T[Hb+Mb] during the occlusion period, a common problem in NIRS research seen in the first known assessment of reactive hyperemia with NIRS,[9] and in previous work in our lab[13, 16]. The addition of a wrist cuff, as is typically used in strain gauge plethysmography,[21] eliminated this problem in our study population.

Chapters 2 and 3 have been previously published and thus are presented in the formatting style of the chosen journal. Chapters 4 and 5 are currently in review. Chapter 6 provides a summary of the dissertation findings and offers potential future directions. References for this dissertation have been appended to the end of the document and are combined for simplicity.

**Chapter 2 - Characterizing near-infrared
spectroscopy responses to forearm post-occlusive
reactive hyperemia in healthy subjects**

ABSTRACT

During post-occlusive reactive hyperemia (PORH) there is a temporary increase in the total hemoglobin + myoglobin (T[Hb+Mb]) signal as measured by near-infrared spectroscopy (NIRS). This transient increase predicts differences in the kinetic responses of deoxy[Hb+Mb] and oxy[Hb+Mb] during PORH. The purpose of this study was to determine whether sigmoidal (Gompertz or logistic) or exponential functions better describe these response curves during PORH. The fit of the three functions (exponential, Gompertz and logistic) to the NIRS responses, as determined from residual sum of squares, was compared using repeated measures ANOVA on Ranks. The Gompertz function provided a better fit to the oxy[Hb+Mb] response curve than did either the exponential or logistic function ($\chi^2 = 21.7$, $df = 2$, $p < 0.001$). The logistic function provided a better fit for the deoxy[Hb+Mb] response ($\chi^2 = 22.9$, $df = 2$, $p < 0.001$) than did either the Gompertz or exponential functions. For both NIRS signals, the better fitting sigmoidal functions fit the data well, with an average r value of 0.99 or greater. Adipose tissue thickness was correlated with parameters related to signal strength (amplitude, $r = 0.86$ – 0.89 ; baseline, $r = 0.67$ – 0.75 ; all $p < 0.001$) but was not related to kinetic parameters (time constant and inflection point; $p > 0.05$ for all comparisons). These results suggest that during PORH distinct sigmoidal mathematical functions best describe the responses of the oxy[Hb+Mb] (Gompertz) and deoxy[Hb+Mb] (logistic) as measured by NIRS. Further, differences in both the kinetic and amplitude aspects for the responses of oxy[Hb+Mb] and deoxy [Hb+Mb] predict the observed

transient change in T[Hb+Mb]. Our methods provide a technique to evaluate and quantify NIRS responses during PORH, which may have clinical utility.

INTRODUCTION

The magnitude of the hyperemic response to brachial artery occlusion, post-occlusive reactive hyperemia (PORH), has been used to evaluate the relative health of the vasculature in both diseased and apparently healthy populations [22-24]. The limitation in these earlier studies is that the downstream hyperemia within skeletal muscle was not assessed; only the relationships between conduit artery and other easily accessible peripheral vascular beds (i.e., skin) were explored. An advantage of using near-infrared spectroscopy (NIRS) to assess PORH responses is the ability to detect changes in microvascular function within skeletal muscle itself, which could have significant clinical implications. For example, much of the insulin resistance found in obesity and type II diabetes mellitus is thought to occur in response to endothelial dysfunction within the capillaries of skeletal muscle[25]. The ability to compare information regarding microvascular function obtained from NIRS across patients or within the same patient over time could help inform treatment decisions. What is needed is a systematic process that facilitates the comparison of these microvascular responses as measured by NIRS; this project seeks to lay the groundwork for such a paradigm.

At near-infrared wavelengths the primary chromophores present in the human forearm are hemoglobin, myoglobin and cytochrome aa3[6]. There is an overlap in

the absorbance spectra of hemoglobin and myoglobin in the near-infrared spectra, rendering them almost indistinguishable with traditional commercial NIRS systems (but see [26]). Near-infrared spectroscopy (NIRS) allows for the non-invasive assessment of the concentration of oxygenated (oxy[Hb+Mb]) and deoxygenated hemoglobin + myoglobin (deoxy[Hb+Mb]) in skeletal muscle [7]. Total hemoglobin plus myoglobin (T[Hb + Mb]) is the sum of oxy[Hb+Mb] and deoxy[Hb+Mb]. Changes in the concentrations of these chromophores within skeletal muscle may provide information about microvascular function.

During PORH if oxy[Hb+Mb] and deoxy[Hb+Mb] change with the same amplitude and time course, we would expect T[Hb+Mb] to remain constant. In fact, a transient increase in T[Hb+Mb] is observed during PORH [27]. Visual inspection of these responses following cuff release suggest that both have a sigmoidal shape, but that the oxy[Hb+Mb] signal changes more rapidly than does the deoxy[Hb+Mb] signal. This implies that oxy[Hb+Mb] and deoxy[Hb+Mb] change with different time courses and predicts that the oxy[Hb+Mb] and deoxy[Hb+Mb] response curves would be best fit by two distinct sigmoidal functions during PORH. A logistic function has a constant rate of change which is relatively sluggish early in the response, such as immediately post cuff release. This may make it a good descriptor of the changes in deoxy[Hb+Mb] response, but inappropriate to describe the rapid early changes observed in the oxy[Hb+Mb] response. On looking for an alternative function to characterize the rapid acceleration observed in the oxy[Hb+Mb] signal post cuff release, we chose the Gompertz function due to its earlier inflection point and resultant increased rate of change early in the response. Therefore, the purposes of this study were to: (a) Determine the best descriptor of the

oxy[Hb+Mb] and deoxy[Hb+Mb] responses during PORH, and (b) determine which of the characteristics (kinetic vs. signal strength) of the oxy[Hb+Mb] and deoxy[Hb+Mb] responses contributed to the transient change in T[Hb+Mb] during PORH. Specifically, we tested these three hypotheses: (1) a logistic function would best describe the response of deoxy[Hb+Mb] during PORH, (2) a Gompertz function would best describe the response of oxy[Hb+Mb], and (3) the peak amplitude of change in oxy[Hb+Mb] and deoxy[Hb+Mb] would be similar, implying that the observed changes in T[Hb+Mb] would be related only to the distinct kinetics of each response.

METHODS

Subjects

Twenty subjects between the ages of 18 and 26 (10 males, average age 21.6 years) were recruited from the general student body at Kansas State University. Informed consent was obtained after both written and verbal explanation about the possible risks and discomforts of the experimental protocol were given. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University. These subjects were apparently healthy and sedentary. Average adipose tissue thickness (ATT), measured with pulsed Doppler ultra-sound, under the NIRS probe was 4.62 ± 1.45 mm. Average Body Mass Index was 23.6 ± 3.7 kg/m², mean resting heart rate was 66 ± 8 beats/min, mean systolic blood pressure was 119 ± 9 mmHg and mean diastolic blood pressure was 72 ± 7 mmHg.

Protocol

All tests were conducted between 0700 and 0830 hours, and were performed in a quiet, temperature-controlled room. Participants were instructed to refrain from alcohol and exercise for 24 h and caffeine for 12 h and were fasted. Subjects were supine and the sampled arm was abducted to a 45° angle. The forearm was supinated to expose the sampling site. Skeletal muscle oxygenation was monitored continuously using an OxiplexTS, Model 96208 (ISS, Champaign, IL, USA) system with the probe placed over the brachialis, flexor digitorum superficialis and flexor carpi radialis by a trained anatomist. This placed the distal edge of the NIRS probe housing approximately halfway between the wrist and the antecubital fold, with the lateral edge of the probe approximately along the midline of the forearm. Characterization of the OxiplexTS probe has been previously described in detail [28]. Briefly, the system operates at wavelengths of 692 and 834 nm with a modulation frequency of the light-source intensity of 110 MHz. The probe utilized source-detector separations of 2.0, 2.5, 3.0 and 3.5 cm. The probe was fixed in place with an elastic strap tightened to prevent movement. The entire forearm was covered with an opaque cloth to prevent ambient light from influencing the NIRS results. For each trial, NIRS measurements were collected continuously during 1 min of rest, 5 min of brachial artery occlusion and 150 s of reactive hyperemia. Total collection time per trial was 8.5 min. The protocol was repeated a total of three times with a 10 min rest period between trials. Occlusion of the brachial artery was accomplished by rapid (within 2 s) inflation, by hand, of a blood pressure cuff to [250 mmHg. The cuff was placed just proximal to the elbow. Complete occlusion of the

brachial artery was assumed if the T[Hb+Mb] signal did not increase following cuff inflation. All NIRS data were time aligned to cuff release ($t = 0$) and averaged across trials.

Statistical analysis

All data were imported into SigmaPlot 10 with SigmaStat 3.5 (Systat Software, Inc., SanJose, CA, USA). Logistic (Eq. 1), Gompertz (Eq. 2) and exponential (eq. 3) functions were fit to both the oxy[Hb+Mb] and deoxy[Hb+Mb] responses producing six response curves per subject.

$$Y = Y_0 + a / (1 + e^{-(X-X_0)/b}) \quad (1)$$

$$Y = Y_0 + a e^{-e^{-(X-X_0)/b}} \quad (2)$$

$$Y = Y_0 + a(1 - e^{-(X-X_0)/b}) \quad (3)$$

For each equation, “ Y_0 ” represents the baseline (end-cuff occlusion) value, “ a ” represents the signal amplitude, “ X ” is time, “ b ” represents a time constant, and “ X_0 ” represents the time at the inflection point of each curve (Eqs. 1, 2) or time delay (Eq. 3) (Fig. 1). Y_0 was determined from the last 20 s of the 5-minute cuff occlusion. Goodness of fit for each function was determined by examining residual sum of square (RSS) values. We had originally planned to use the Δ Akaike method to compare our response curves (since the three functions are not nested models), but since all three models had the same number (four) of parameters, the equation reduced to RSS times a similar constant across models and subjects. Repeated measures ANOVA on ranks was thus used to compare the RSS values from each function within each response.

Parameter estimates from the best fitting function for each response were plotted against ATT for each subject. Pearson product moment correlation was then performed between ATT

and the uncorrected parameter estimates. The slope of this line was used to correct each parameter estimate affected by ATT (amplitude a , and baseline Y_0). Due to unequal variance, for corrected and uncorrected amplitude (a) values a Kruskal–Wallis One Way Analysis of Variance on Ranks analysis was performed. One way ANOVA was performed on the corrected and uncorrected baseline (Y_0) values. Tukey’s post-hoc analysis was used to determine group differences for both variables. Kinetic parameters (time constant b and inflection point X_0) were not influenced by ATT and, therefore, correction for ATT was not necessary. These parameters were compared with paired t tests.

RESULTS

Figure 2 shows the T[Hb+Mb], oxy[Hb+Mb] and deoxy[Hb+Mb] responses across the entire experiment for one representative subject through baseline data collection, cuff occlusion and PORH following cuff release. Upon cuff release, while the time course of changes in oxy[Hb+Mb] and deoxy[Hb+Mb] appeared similar, they were not identical as demonstrated in Fig. 3. Oxy[Hb+Mb] increased as soon as the cuff was released. However, the deoxy[Hb+Mb] response had a slower rate of change for approximately the first 20 s before the dynamic hyperemic response occurred. The result of these disparate kinetics was that T[Hb+Mb] shows a transient increase following cuff release (Fig. 1).

Figure 4 shows combined deoxy[Hb+Mb] and oxy[Hb+Mb] response curves and residuals for the same subject as in Figs. 2 and 3 fit with Gompertz, logistic and exponential functions. Tables 1 and 2 show the RSS data for all subjects for the three functions fit to the deoxy[Hb+Mb] and oxy[Hb+Mb] responses, respectively. For both responses a sigmoidal curve

provided the better fit on average compared to the exponential function (deoxy[Hb+Mb]: $\chi^2 = 22.9$, $df = 2$, $p < 0.001$; oxy[Hb+Mb]: $\chi^2 = 21.7$, $df = 2$, $p < 0.001$). Post-hoc analysis revealed that the logistic function provided the best fit of the three functions tested for the deoxy[Hb+Mb] response; there was no significant difference between the fit of the exponential and the Gompertz functions. Conversely, the Gompertz function provided the best fit for the oxy[Hb+Mb] response when compared with both the exponential and logistic functions; there was no significant difference between the RSS values for the logistic and exponential functions. Since the exponential function was not the best fit on average for either response, it was excluded from further analyses.

Amplitude (a) and baseline (Y_0), but not time constant (b) and time of inflection point (X_0) (data not shown) were significantly correlated with ATT for all response curves (Fig. 5). Table 3 gives the average parameter estimates, uncorrected- and corrected-for-ATT where appropriate, for the best fit model (logistic for the deoxy[Hb+Mb] response and Gompertz for the oxy[Hb+Mb] response) across all subjects. Correcting for ATT resulted in a significant increase in the baseline (Y_0) and amplitude (a) values for both the oxy[Hb+Mb] and deoxy[Hb+Mb] responses ($F = 367.82$, $df = 3$, $p < 0.001$). For the Gompertz function fit to the oxy[Hb+Mb] response, the time constant (b) was significantly greater, and the inflection point (X_0) was significantly smaller (both $p < 0.001$) compared to the corresponding value for the logistic function fit to the deoxy[Hb+Mb] (Table 3).

The relationship between the amplitude for deoxy[Hb+Mb] and oxy[Hb+Mb], both for the uncorrected and corrected-for-ATT values, for all subjects is further illustrated in Fig. 6.

Note that while there was a strong relationship between the amplitudes of the two responses across subjects, most of the amplitudes for oxy[Hb+Mb] fell below amplitude of deoxy[Hb+Mb]), for both uncorrected and corrected data. Figure 7 shows the relationships for the best fit model (oxy[Hb+Mb] fit with the Gompertz function and deoxy[Hb+Mb] fit with the logistic function) for both kinetic parameters (b and X_0) across all subjects. While both b and X_0 were consistently above the line of identity (i.e., deoxy[Hb+Mb] values smaller than the corresponding oxy[Hb+Mb] for b , but greater for X_0), nonetheless there were strong correlations across the range of values (i.e., subjects with faster responses (smaller b and X_0) in one response also showed faster responses in the other).

DISCUSSION

The main findings of this investigation are that, consistent with hypotheses 1 and 2, a Gompertz function provided a better fit, on average, for the oxy[Hb+Mb], while a logistic function fit the deoxy[Hb+Mb] responses best, during PORH. Further, in contrast to hypothesis 3, while the amplitudes of change during PORH for both oxy- and deoxy[Hb+Mb] were similar, they were not identical. This difference in amplitudes, combined with the difference in kinetics of adjustment between the Gompertz and logistic functions, would predictably lead to a transient change in the T[Hb+Mb] signal, as was seen in the present study.

Exponential functions have typically been used to describe NIRS responses during dynamic exercise[29-31], following maximal 3 s contractions in the human forearm and during 120 s of 1 Hz twitch contractions at various intensities in the rat hindlimb preparation[32-34]. In contrast, we found that the sigmoidal functions provided a better fit to NIRS data during PORH

than the exponential function in 85% (34 of 40) of the responses modeled here (17 of 20 responses each for oxy[Hb+Mb] and deoxy[Hb+Mb]). Interestingly, two of the three exceptions occurred in the same subjects (subjects 5 and 13 in Tables 1, 2) (i.e., both oxy[Hb+Mb] and deoxy[Hb+Mb] responses were initially best described as exponential in these two subjects). However, when these exponential fits were examined more closely, it was noted that the amplitudes of the best fit function greatly exceeded the actual amplitude of the response (e.g., see Fig. 1). When the amplitude of the exponential function was constrained to the real amplitude of the responses, the resulting RSS was greater than that of the corresponding best fit sigmoidal function. We, thus, conclude that our overall observations that the logistic function best fit the deoxy[Hb+Mb] data, while the oxy[Hb+Mb] data were best described by the Gompertz function, was also true for these subjects (#5, 13, 14 and 16). These results demonstrate that modeling techniques that are appropriate for data obtained during or in recovery from exercise may not provide an appropriate description of the NIRS data obtained following 5 min of ischemia during PORH. Taken together, these findings suggest that care must be taken when selecting a function for mathematical modeling of NIRS response data.

To our knowledge, only Kragelj et al.[27] have previously examined tissue oxygenation during PORH using NIRS. Their model was tissue oxygenation of the foot during and after cuff occlusion of the popliteal artery. While their data analysis was model-independent, their overall observations were similar to those of the present study, i.e., that the kinetics of the oxy[Hb+Mb] response were faster than those of deoxy[Hb+Mb] during PORH.

Amplitude and baseline values which are related to signal strength, were significantly correlated with ATT, while the kinetic parameters, slope and inflection point, were not. These results suggest that ATT does not affect the kinetic characteristics of the response, only the signal strength, and that correction for ATT is not required when performing a purely temporal analysis.

The NIRS signal primarily emanates from skeletal muscle [Mb] and the microvasculature [Hb] (Liu et al. 1995)[35]. Assuming all of the transient increase in T[Hb+Mb] is due to increased [Hb] (with skeletal muscle [Mb] remaining constant) the increased T[Hb+Mb] signal likely represents increased microvascular hematocrit[36-38]. However, the underlying reasons for the temporal differences observed in the oxy- and deoxy[Hb+Mb] responses during PORH are not known. One potential mechanism for the delayed response of deoxy[Hb+Mb] compared to oxy[Hb+Mb] during PORH may involve additional unloading of oxygen during the early recovery phase of PORH to refill tissue Mb stores utilized during the ischemic period. Tran et al.[39] demonstrated that during cuff ischemia of the lower leg, Mb desaturation by NMR reached a maximum value by *4.5 min. Given that Mb content of human skeletal muscle is greater than previously thought[26, 40], the dynamics of Mb de/resaturation may contribute significantly to the overall kinetics of the NIRS responses during PORH. Consistent with this, the observed time to the inflection point (X_0) for deoxy[Hb+Mb] in the present study (16.9 s) is similar to the time constants for Mb resaturation reported by Duteil et al.[41].

It should be noted that one limitation of the present analysis is that only 3 models (exponential, logistic and Gompertz) were compared. There could be another, unidentified model

which may provide an even better fit to the response curve data. In addition, our subjects were a relatively young, healthy cohort. It is currently unclear to what extent our findings and approach to data analysis may be applicable to clinical populations. We expect any pathological condition affecting vascular function, such as diabetes, heart failure and peripheral vascular disease, would alter the kinetics and possibly amplitude of the observed deoxy[Hb+Mb] and oxy[Hb+Mb] responses, which in turn might alter the T[Hb+Mb] response. In fact, Kragelj et al.[27] observed slowing of the NIRS responses in patients with peripheral vascular disease. Many of the signs of microvascular dysfunction predate a clinical diagnosis and may, therefore, be useful as novel markers of disease risk, disease progression and treatment efficacy[25]. Evaluation of the impact of diseases which affect microvascular circulation must await future studies.

CONCLUSION

Our findings support our initial hypotheses that during PORH, sigmoidal functions do fit the NIRS data better than an exponential function and specifically that the oxy[Hb+Mb] response is best described by a Gompertz function, while the logistic function provides a better fit for the deoxy[Hb+Mb] response. The distinct time courses of these responses in combination with their different response amplitudes, leads to a transient increase in T[Hb+Mb]. While the exact mechanism for the delayed response of deoxy[Hb+Mb] relative to that of oxy[Hb+Mb] is currently unknown, we speculate that it likely relates to sustained unloading of O₂ from Hb during the early phase of PORH to restore tissue MbO₂ stores.

ACKNOWLEDGMENTS

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Table 2-1: RSS values for all functions fir to the deoxy[Hb+Mb] response

Subject	RSS _G	RSS _L	RSS _E	N
1	11.1	3.3	10.2	64
2	34.7	16.2	77.9	70
3	402	294	310	60
4	1335	1189	1267	63
5	194	94.8	59.8	124
6	339	224	240	77
7	11.5	6.3	19.3	67
8	6.9	2.5	13.6	69
9	304	261	453	68
10	237	127	132	63
11	329	255	328	93
12	4.8	1.9	16.5	83
13	14.6	7.2	2.3	101
14	5.8	2.2	20.6	87
15	226	121	124	115
16	325	196	175	97
17	35.3	13.4	27.6	88
18	63.9	23.0	75.7	123
19	211	153	202	63
20	48.7	21.5	67.8	59
Ave	207	150 *	181	82
SD	300	265	285	21

N is the number of data points in regression, equal to 20s baseline data plus time from cuff release until peak response. RSS is residual sum of squared errors for Gompertz (RSS_G), logistic (RSS_L) and exponential (RSS_E) functions

*Significantly different from both RSS_G and RSS_E

Table 2-2: RSS values for all functions fit to the oxy[Hb+Mb] response

Subject	RSS _G	RSS _L	RSS _E	N
1	17.0	22.3	23.5	51
2	83.2	99.9	102	57
3	668	678	841	55
4	1424	1611	1594	61
5	247	315	226	109
6	339	347	368	47
7	32.6	38.1	32.9	52
8	10.9	17.0	12.8	60
9	844	854	958	56
10	383	383	556	63
11	587	666	806	46
12	14.8	16.8	17.6	58
13	13.8	18.2	11.2	95
14	29.9	45.6	19.4	78
15	144	164	233	72
16	440	455	489	62
17	27.0	37.6	34.3	74
18	95.0	97.2	159	74
19	273	285	559	55
20	89.4	131	95.3	52
Ave	288*	314	357	64
SD	363	396	424	16

N is the number of data points in regression, equal to 20s baseline data plus time from cuff release until peak response. RSS is residual sum of squared errors for Gompertz (RSS_G), logistic (RSS_L) and exponential (RSS_E) functions*Significantly different from both RSS_L and RSS_E

Table 2-3: Parameter estimates for the best fit model for deoxy[Hb+Mb] and oxy[Hb+Mb], uncorrected- and corrected-for-ATT

		Y ₀ (μM)	a (μM)	b (s)	X ₀ (s)
Deoxy[Hb+Mb] (Logistic)					
	Uncorrected	12.7±4.5	30.2±15.7	-5.3±2.0	16.9±6.8
	Corrected	21.5±3.3 ^c	71.8±7.2 ^c		
Oxy[Hb+Mb] (Gompertz)					
	Uncorrected	37.5±7.2 ^b	33.8±17.3 ^a	7.4±2.4 ^b	9.4±4.0 ^b
	Corrected	53.8±4.7 ^{bc}	75.1±8.9 ^{ac}		

Values are mean± standard deviation. See eqs. 1 and 2 for meaning of symbols

Significant difference between Oxy[Hb+Mb] and Deoxy[Hb+Mb] vales:^a p<0.02 and

^b p<0.001. ^cSignificantly different from uncorrected-for-ATT values (p<0.001).

Figure 2-1: Schematic showing Gompertz (A) and exponential (B) functions fit to the oxy[Hb+Mb] data in Fig. 2. Y_0 denotes baseline, a is the amplitude of the response, b is the time constant, and X_0 is the time of the inflection point (A) or the time delay (B). Parameters have same meaning for logistic function as for Gompertz. See text and Eqs. 1-3 for more information.

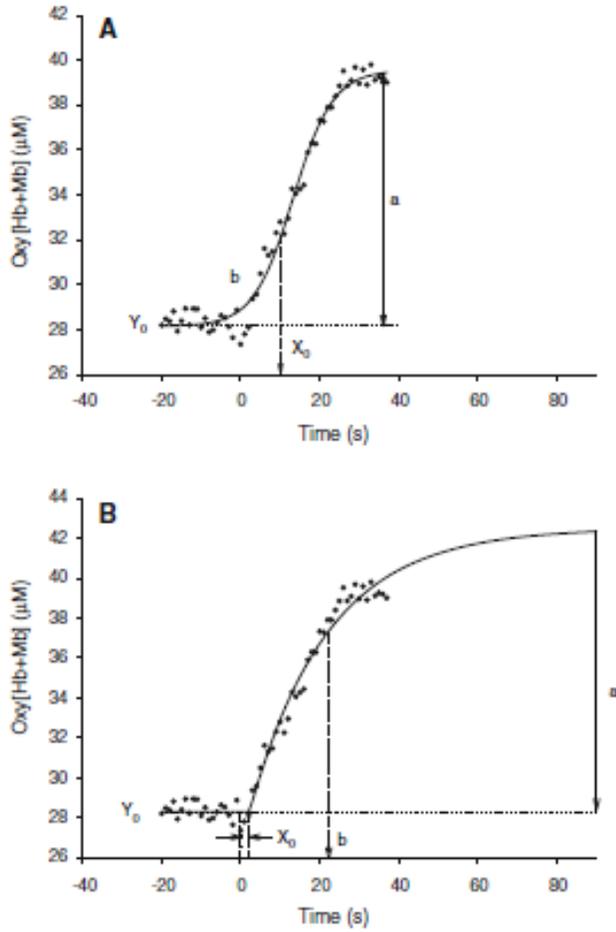


Figure 2-2: Complete experiment for one subject. Cuff inflation occurred after 60s of baseline data collection, and was maintained for 5 min. Post-occlusive reactive hyperemia (PORH) was monitored for 120s in this example.

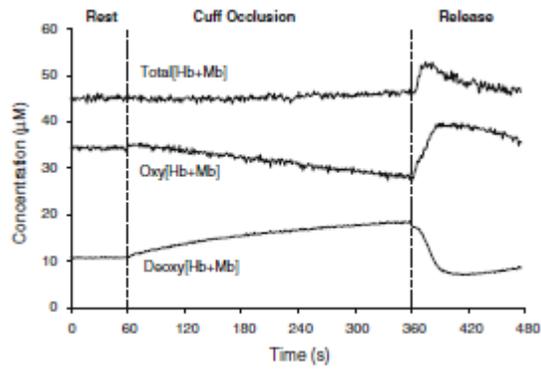


Figure 2-3: : Oxy[Hb+Mb] and deoxy[Hb+Mb] response curves during PORH from the same subject as Fig. 1. Deoxy[Hb+Mb] response has been inverted for comparison. Time point 0 indicates cuff release.

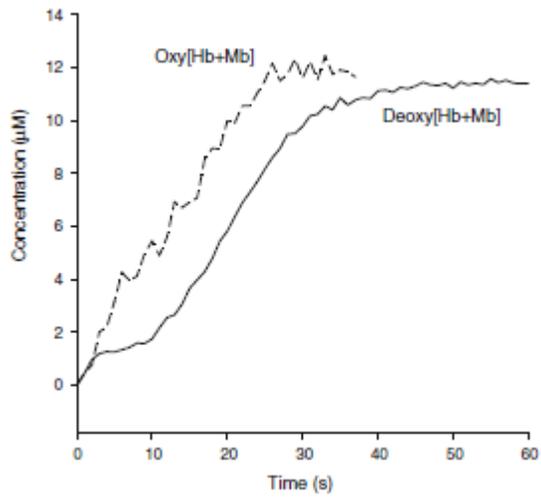


Figure 2-4: Example of Gompertz, logistic and exponential fits to data. Note especially the poor fit of the exponential function to the deoxy[Hb+Mb] signal, and the projection of exponential fit past plateau of both responses (shown by arrows)

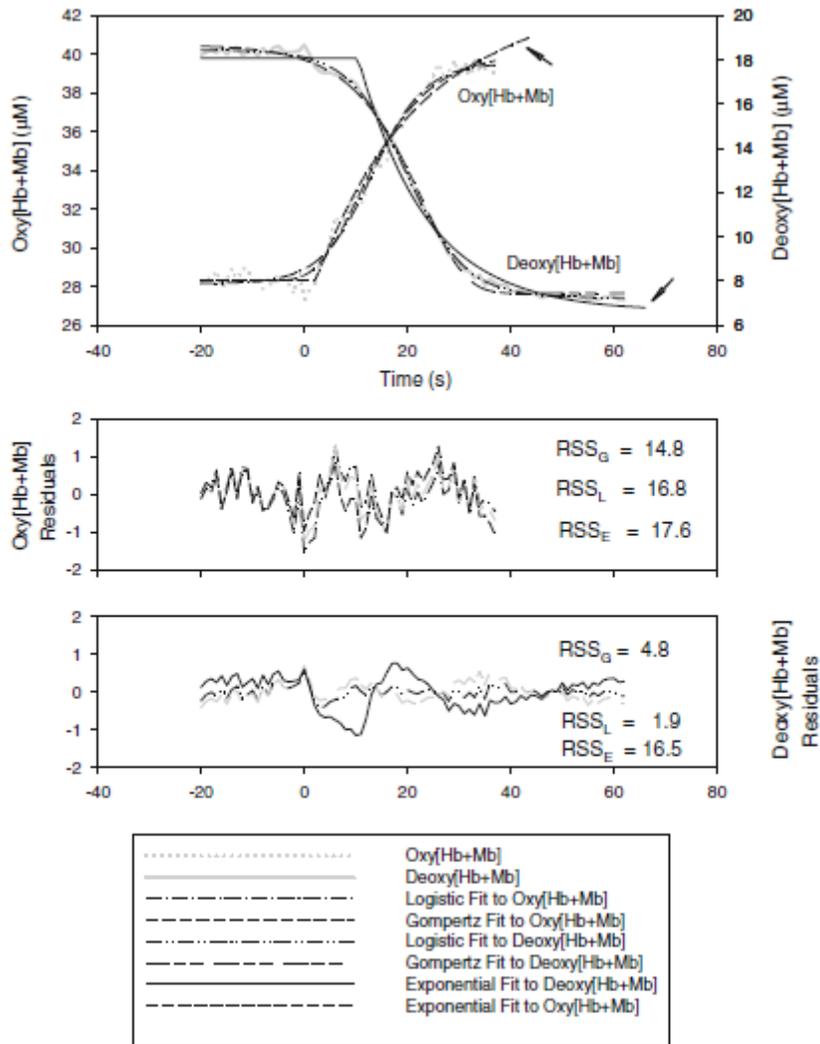


Figure 2-5 Original (circles) and corrected (triangles) baseline (Y_0), amplitude (a) and time constant (b) values plotted against ATT:

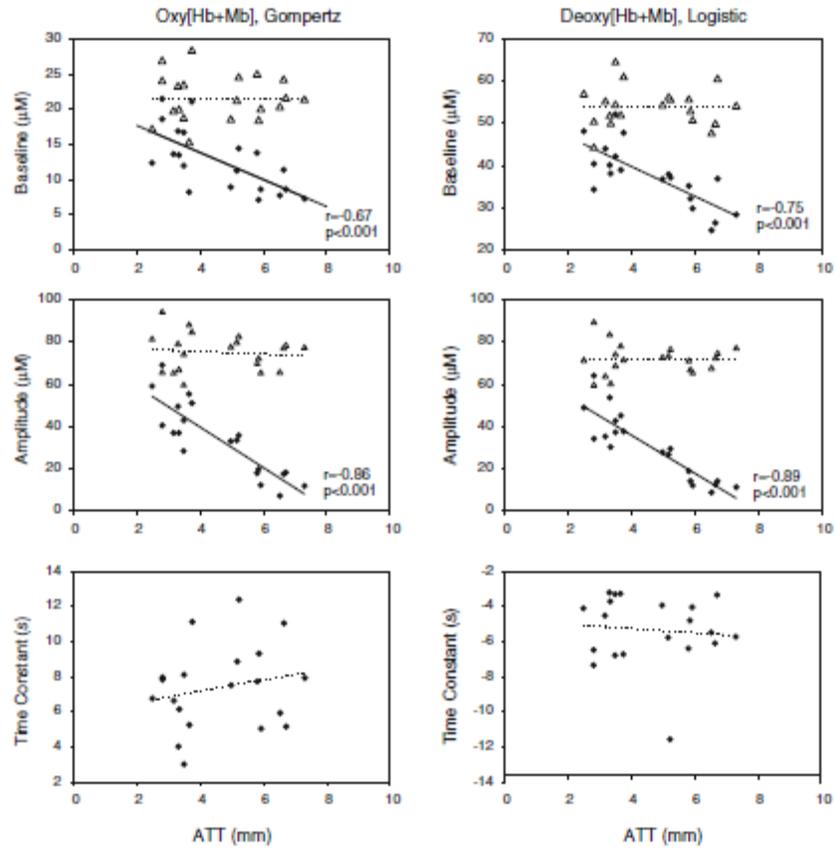


Figure 2-6: Amplitudes (*a*) for deoxy[Hb+Mb] compared to oxy[Hb+Mb] for uncorrected- (top panel) and corrected-for-ATT (bottom panel). Dotted line represents line of identity

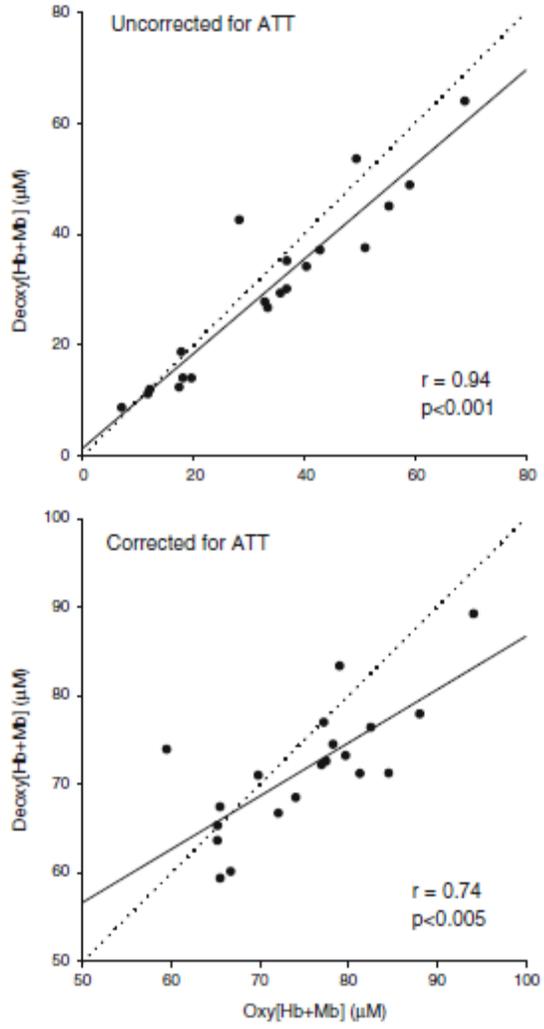


Figure 2-7: Time constant (b) and time inflection point (X_0) during PORH for deoxy[Hb+Mb] compared to oxy[Hb+Mb]. Dotted line represents line of identity.

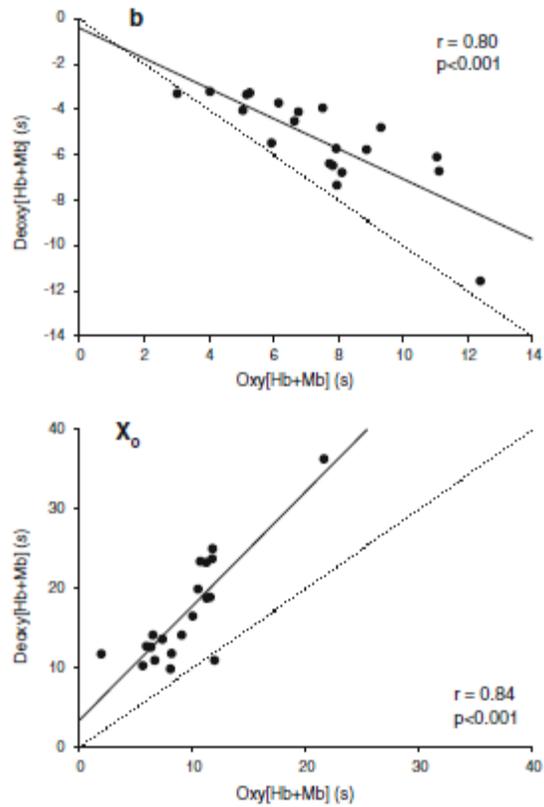


Fig. 7 Time constant (b) and time inflection point (X_0) during PORH for deoxy[Hb+Mb] compared to oxy[Hb+Mb]. Dotted line represents line of identity

**Chapter 3 - Relationship between brachial
artery blood flow and total
[hemoglobin+myoglobin] during post-
occlusive reactive hyperemia**

ABSTRACT

The associations between macrovascular and microvascular responses reported previously during post-occlusive reactive hyperemia have been inconsistent. The purpose of this study was therefore to determine the temporal relationship between the reactive hyperemic responses within a conduit artery and the downstream microvessels. Conduit artery blood flow was measured in the brachial artery with pulsed Doppler ultrasound. A potential analog of microvascular flow, changes in skeletal muscle total[hemoglobin + myoglobin] (T[Hb + Mb]), was assessed with near-infrared spectroscopy (NIRS). We found a high degree of correlation between these two measures ($r = 0.91$). Cross-correlation analysis revealed two distinct response patterns. In 10 of our 15 subjects there was time displacement between peak brachial artery blood flow (BABF) and T[Hb + Mb] responses; in the remaining 5 the peaks were coincident. Granger causality testing suggested that reactive hyperemia in the macrovessel determined hyperemia in the downstream microvessels in all 15 study subjects. Time constants for the on (τ_1) and off (τ_2) kinetics of each response were calculated; our initial hypothesis was that τ_1 and τ_2 for T[Hb + Mb] would correlate with τ_1 and τ_2 for BABF, respectively. However, only for τ_2 was this observed ($r = 0.52$; $p < 0.05$). No similar relationship was observed for τ_1 . Adipose tissue thickness did not influence either time constant for T[Hb + Mb]. Taken together, our results show that the temporal characteristics of the hyperemic response in the conduit artery are qualitatively reflected in the downstream microvasculature, but mechanisms for quantitative differences remain to be identified.

INTRODUCTION

Post-occlusive reactive hyperemia (PORH) has been used to assess both microvascular and conduit artery vascular reactivity[2, 42]. Reactive hyperemia and flow-mediated dilation (FMD) are related phenomena. During occlusion of a conduit artery there is dilation of downstream arterioles, caused by metabolic and prostaglandin dilators[17, 43] which decreases vascular resistance. Upon cuff release, the decreased resistance to flow results in hyperemia which, in turn, increases shear rate in the conduit artery, resulting in FMD[44]. Abnormal FMD has been linked to increased cardiovascular disease risk in apparently healthy individuals[24] and in clinical populations including post-menopausal women[22], cardiac patients[45-47] and those with metabolic syndrome[23]. In addition, type II diabetes results in slowed time to peak response for cutaneous blood flow as measured with laser Doppler flowmetry[48], suggesting abnormal microvascular reactive hyperemia.

Although a transient increase in both conduit artery and microvascular blood flow occurs during PORH, the relationship between the two remains unclear. Previous comparisons between the reactive hyperemic responses in the microvasculature and the macrovasculature have shown little association[12, 49]. Dhindsa and colleagues compared 7 different measures of microvascular and macrovascular reactivity[11]. For microvascular measures, the response was characterized either by a steady-state change or by the peak response observed during the reactive hyperemia. While the majority of their comparisons were not significant, both FMD and macrovascular reactive hyperemia were significantly correlated with measures of cutaneous microvascular reactive hyperemia, as measured at the fingertip. Yet, no technique was employed

that was capable of measuring reactive hyperemia within the skeletal muscle microvasculature. This is an important distinction as it has been speculated that cutaneous vascular reactivity may not be a good surrogate for skeletal muscle microvascular reactivity[50]. Since endothelial dysfunction in the microvasculature of skeletal muscle is thought to be a major contributor to much of the elevated plasma glucose and insulin resistance seen in type II diabetes and obesity[25], a significant advance would be a non-invasive technique that would allow for the assessment of microvascular reactivity in skeletal muscle.

Near-infrared spectroscopy (NIRS) is an emerging clinical tool that is capable of non-invasive determination of the oxygenation of skeletal muscle. One advantage of NIRS is the ability to measure the oxygenation changes of hemoglobin + myoglobin ([Hb + Mb]) within skeletal muscle microvasculature. During PORH there is a transient increase in both brachial artery blood flow and T[Hb + Mb] as determined by NIRS[27]. Currently, NIRS has found clinical utility in the qualitative assessment of oxygenation at the thenar eminence during PORH[51]. This application has shown utility in distinguishing severely ill patients, but is not without its limitations. While the thenar eminence is readily accessible, some authors suggest that the forearm measurement sites are more sensitive than the thenar site to alterations in blood flow[52].

The inconsistent correlations between microvascular and macrovascular responses during PORH suggest that comparing peak responses among disparate variables may not be the most sensitive analytical approach. We propose that analyzing the time course of changes in microvascular T[Hb + Mb] as a potential surrogate for microvascular flow and conduit artery

blood flow may provide a better technique for investigating these relationships between micro- and macrovascular reactivity. To our knowledge, the kinetic relationship between the skeletal muscle microvascular and limb macrovascular reactive hyperemia during PORH has not been assessed. Therefore the purpose of this study was to determine the temporal relationship between forearm skeletal muscle T[Hb + Mb] and brachial artery blood flow (BABF) during PORH. Specifically, we hypothesized that the time constants for both the on and off kinetics for T[Hb + Mb] would correlate with the respective on and off kinetics for BABF.

METHODS

Subjects

Fifteen subjects (8 males) between the ages of 18 and 26 (average 21.4 years) were recruited from the general student body at Kansas State University. [Table 1](#) shows subject characteristics. These subjects were sedentary and apparently healthy. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University. No significant differences were observed between males and females in terms of reactive hyperemic responses, so all data were pooled.

Protocol

All tests were conducted between 7:00 and 8:30 am, and were performed in a quiet, temperature-controlled room. Participants were fasted overnight, had abstained from alcohol and exercise for 24 h and from caffeine for 12 h.

Microvascular reactive hyperemia

Skeletal muscle oxygenation was monitored continuously using an OxiplexTS, model 96208 (ISS, Inc., Champaign, IL, USA) system with the probe placed over the brachioradialis, flexor carpi radialis, flexor digitorum superficialis and pronator teres muscles. The OxiplexTS system has been previously described[28]. NIRS measurements were collected continuously during a 1 min baseline period, 5 min of brachial artery occlusion and 150 s of reactive hyperemia. The protocol was repeated a total of three times with a 10 min rest period between trials. Occlusion of the brachial artery was accomplished by rapid inflation by hand pump and within 1–2 s of a blood pressure cuff to N 250 mm Hg. Complete occlusion of the brachial artery was assumed if the T[Hb + Mb] signal did not increase more than 10% following cuff inflation. All NIRS data were time aligned to cuff release ($t = 0$) and averaged across the three trials within each subject.

Macrovascular reactive hyperemia

Brachial artery blood velocity was measured with pulsed Doppler ultrasound (GE Vivid3, GE Medical Systems). Discontinuous longitudinal BA images and beat-by-beat velocity tracings (three at rest, one during the last minute of occlusion, one every 5 s for the first 30 s of post- occlusion, and then every 10 s for the remainder of post-occlusion) were obtained, coded, and stored on the ultrasound hard drive. BA diameter was measured with electronic calipers as the anterior to posterior endothelial–lumen interface and determined from an average of 3 successive cardiac cycles, 3 measurements / cardiac cycle, both for the widest diameter and

narrowest diameter. BABF for each time point was calculated as average velocity $\times \pi(\text{average diameter}/2)^2$.

Data analysis

All data were transferred to SigmaPlot 10 (Systat Software, Inc., San Jose, CA, USA) and response curves were plotted for both BABF and T[Hb + Mb]. A double exponential function (Eq. (1)) with no time delay was fit to each response curve and time constants (τ) for BABF and T[Hb + Mb] were recorded for both the rise from baseline to peak value (τ_1) and for the return from peak to baseline values (τ_2). Also recorded were baseline values (Y_0) and two amplitude values (A_1 and A_2) corresponding to the amplitude of the rise to peak and the subsequent return towards baseline from peak, respectively (see [Fig. 1](#)).

$$Y = Y_0 + A_1 * (1 - e^{-(t/\tau_1)}) - A_2 * (1 - \exp^{-(t/\tau_2)}) \quad (1)$$

We have previously found that correction for adipose tissue thickness (ATT) is not required when performing a temporal analysis of the parameters (i.e., time constants) on oxy- and deoxy[Hb + Mb] response curves during PORH[13]. However, parameters (τ_1 , τ_2 , A_1 , A_2 , and Y_0) from the T[Hb + Mb] response were plotted against ATT to determine if ATT influenced the results and if correction for adipose tissue thickness was required for these parameters.

Statistical analysis

Both τ_1 and τ_2 were compared between BABF and T[Hb + Mb] using a 2-tailed, paired *t*-test. Ratios of τ_2/τ_1 for both response curves were calculated. Correlation among time constants was assessed with Pearson product moment correlation; these relationships were demonstrated with scatter plots. Significance was declared for $p < 0.05$. Data were also imported into SPSS

version 18 (SPSS, Chicago, IL, USA) for cross-correlation analysis to determine the lead or lag time between T[Hb + Mb] and BABF. In this analysis the BABF data was anchored and the NIRS data was displaced. Cross-correlation analysis requires an equal time interval between data points. While NIRS data was captured continuously, BABF data was not; therefore the BABF data was interpolated to generate second by second data. Cross-correlation analysis to ± 15 lags was then performed to determine the degree of association between BABF and T[Hb + Mb] and the time displacement between the peak values of BABF and T[Hb + Mb]. Finally, data was imported into Stata 12 (College Station, Texas, USA) and Granger causality testing was performed to determine if past events in one response variable predicted future events in the other response variable.

RESULTS

Two distinct response profiles were seen in our subjects. [Fig. 1](#) shows a temporal displacement between brachial artery blood flow and T[Hb + Mb] responses in one subject; this profile was seen in 10 of 15 subjects. The average time displacement between peak values for these 10 subjects was 3.7 s (range 1–7 s). [Fig. 2](#) shows no temporal displacement between the responses in another subject (true for 5 subjects). [Table 2](#) shows the correlation and cross-correlation coefficients and time displacement data for all subjects. The correlation between brachial artery blood flow and T[Hb + Mb] responses for individual subjects ranged between 0.79 and 0.98 (for all, $p < 0.0001$), while the average correlation for pooled data was 0.91 ($p < 0.0001$). In no subject did T[Hb + Mb] peak before BABF.

Relationships among the temporal parameters are illustrated in [Fig. 3](#). There was no relationship between the rate of rise in T[Hb + Mb] following cuff release (τ_1 T[Hb + Mb]) and that of BABF (τ_1 BABF) ([Fig. 3A](#)). In contrast, the subsequent recoveries of both variables towards resting baseline (τ_2 for both, [Fig 3B](#)) were significantly related ($r = 0.52$, $p < 0.05$). τ_1 and τ_2 for BABF showed a moderate inverse association ($r = -0.63$, $p = 0.01$) ([Fig. 3](#), panel C); while τ_1 and τ_2 for T[Hb + Mb] did not ($r = 0.4$) ([Fig. 3](#), panel D). [Table 3](#) shows the individual time constants (τ_1 and τ_2) for both response curves. τ_1 was significantly faster than τ_2 for both BABF and T[Hb + Mb] ($p \leq 0.001$, for both). τ_1 for BABF was faster than τ_1 for T[Hb + Mb] ($p \leq 0.001$). However, there was no significant difference between the recovery kinetics (τ_2). Lag time in seconds was positively correlated with τ_1 T[Hb+Mb] ($r = 0.55$, $p = 0.03$). Finally, the ratios of τ_2 BABF/ τ_1 and τ_2 T[Hb + Mb]/ τ_1 showed large intersubject variability and were not significantly different from each other; they were significantly correlated ($r = 0.52$, $p = 0.049$). ATT did not influence either time constant for T[Hb + Mb] as demonstrated by the poor degree of association between ATT and both τ_1 ($r = 0.30$) and τ_2 ($r = 0.43$).

[Table 4](#) lists non-temporal model parameters. A_1 was highly correlated with A_2 for BABF and for T[Hb + Mb] (for both $r = 0.99$, $p < 0.0001$). However, unlike the time constants, there were no significant correlations for the corresponding amplitude values between BABF and T[Hb + Mb]. As expected, baseline T[Hb + Mb] (Y_0) was influenced by ATT ($r = -0.91$, $p < 0.0001$) as was both A_1 ($r = 0.58$, $p = 0.024$) and A_2 ($r = 0.58$, $p = 0.03$). [Fig. 4](#) shows the uncorrected- and corrected-for-ATT baseline (Y_0), and A_1 and A_2 values for T[Hb + Mb] plotted against ATT.

DISCUSSION

This is the first investigation that we are aware of to compare BABF and T[Hb + Mb] during reactive hyperemia. As predicted, these two response variables were highly correlated ($r = 0.91$, $p < 0.0001$). Although no correlation was noted between the time constants for the on kinetics, the main finding of this investigation was the correlation between the time constants for the off kinetics. In addition, we found a spectrum of relationships between the peaks of BABF and T[Hb + Mb] ranging from temporal coincidence (lag = 0 s) to a lag in T [Hb + Mb] of 7 s. Our investigation also confirmed that temporal parameters of the NIRS signal were not influenced by ATT.

Previous investigators have reported little association between measures of microvascular and macrovascular reactivity[11, 53]. However, microvascular reactivity in these studies was determined in primarily cutaneous vascular beds, which may have different mechanisms of control and sensitivity to stimuli (e.g., ischemia) than the microvasculature of skeletal muscle[50]. If a lack of association between measures of vascular reactivity in these previous investigations indicates that each technique is providing information on distinct physiological mechanisms, the high level of association between the BABF and T[Hb + Mb] responses in the present investigation leads us to conclude that similar mechanisms are responsible for both the BABF and T[Hb + Mb] responses during PORH. Further, our results suggested that the brachial artery reactive hyperemia preceded the reactive hyperemic response in the downstream skeletal muscle. Nakamura et al. reported that blood flow is coordinated between upstream conduit vessels and downstream peripheral tissue in the lower limb [54]. We utilized Granger causality

testing to determine if this relationship existed in the forearm, and found that for all 15 of our subjects the conduit artery response predicted the microvascular response ($p < 0.001$). However, in 3 of our subjects, the reverse was also true, i.e., the microvascular response also predicted the conduit artery response. All 3 of these subjects were among the group where there was no lag between peak responses. Therefore, based upon Granger criteria, the conduit artery reactive hyperemia caused the microvascular hyperemia in 80% of our sample. Granger causality testing is used to evaluate if past events in one data set predict future events in another set of data. It is possible that this technique failed in these 3 subjects because the peak response in both vascular compartments was achieved at the same time [55].

We hypothesized that the time constants for the on and off kinetics for T[Hb + Mb] would correlate with the on and off kinetics for BABF. While comparisons between microvascular and macrovascular blood flow in the human forearm have been made previously, we believe that this investigation is the first to compare the temporal parameters of T[Hb + Mb] during reactive hyperemia, as measured via near-infrared spectroscopy, and brachial artery blood flow. We found no correlation between the on kinetics of the two responses, possibly because the time to attain peak values was quite short and contained a limited number of data points; we therefore had poor confidence in τ_1 estimates. However, the off kinetics (as τ_2) for our two measures were not significantly different from each other and were moderately, positively correlated (see [Fig. 3](#)), i.e., individuals with longer conduit artery recovery times also had longer recovery times for T[Hb + Mb]. We believe that this association is related to vascular compliance in the forearm.

While conduit artery and microvascular reactive hyperemic responses were highly correlated; correlation coefficients between these two responses did vary among subjects (range = 0.79–0.98). The lowest correlation coefficients were seen in those subjects with the largest time displacement between the attainment of peak responses. In only 5 of our 15 subjects was the peak response in these measures achieved at the same time (3 if we used the Granger causality results). In the remaining 10 subjects there was displacement between peak BABF and peak T[Hb + Mb] responses. We believe that this reflects differences in compliance somewhere in the arterial tree between the brachial artery and the forearm microvasculature. In support of this, there was a positive correlation ($r = 0.553$, $p = 0.03$) between lag duration and τ_1 T[Hb + Mb], e.g., the longer lag was associated with a longer time to peak response. Those subjects who demonstrated no lag between peak responses could have reduced compliance in the forearm arterial tree. None of the subjects reported any symptoms nor displayed signs of metabolic or cardiovascular disease during the screening process which could have affected arterial compliance, but we cannot rule out that subclinical disease was present. The lag between peak responses in the remaining 10 subjects likely reflects a normal range of arterial compliance.

T[Hb + Mb] reflects both skeletal muscle myoglobin and erythrocyte hemoglobin in the microvasculature beneath the probe. Assuming that myoglobin remains constant, any changes in T[Hb + Mb] must reflect changes in microvascular erythrocyte concentration (microvascular hematocrit)[14]. At rest, microvasculature hematocrit can be far lower than systemic values[38], a result of the Fahraeus effect and impedance to plasma entry in the capillary networks due to the

glycocalyx. Capillary hematocrits of 10.4%, arteriolar hematocrit of 13.9% and conduit artery hematocrit of 53.2% have been reported at rest in the rat[38].

The increase in T[Hb + Mb] during reactive hyperemia represents a temporary abatement of the Fahraeus effect as the hyperemic blood flow and increased blood velocity in the brachial artery are sufficient to homogenize blood flow through the downstream microvasculature of the forearm. Hyperemia, both reactive and active, will increase micro-vascular hematocrit towards systemic levels[37, 56]. Hyperemia is also accompanied by an increase in the T [Hb + Mb] signal obtained from the NIRS device[13, 28]. As the hyperemic flow in the brachial artery wanes, blood volume and velocity will decrease resulting in restoration of heterogeneous blood flow through downstream vascular beds, a restoration of the Fahraeus effect, and possible influence of the restoration of any influence of the glycocalyx. As a result capillary hematocrit values will return towards baseline values, and T[Hb + Mb] will return to pre-occlusion levels.

There are other putative mechanisms that could explain the lag time between peak responses of BABF and T[Hb + Mb] which are related to the broad area of interrogation of the NIRS probe. The hemoglobin portion of the signal reflects the average Hb within the arterioles, capillaries and venules with diameters less than 1 mm within the area of interrogation[14, 57]. Microvascular hematocrit is proportional to the ratio of red blood cell flux to red blood cell velocity[37, 58]. During and in recovery from muscle contractions[58] or ischemia[59] the rate of change in these two measures is not the same; the change in red blood cell velocity is faster than the change in red cell flux and varied across muscles resulting in a discrepancy between the changes in flux (flow) and hematocrit. In the present study, the volume of interrogation of the

NIRS probe included multiple muscles, specifically the brachioradialis, flexor carpi radialis, flexor digitorum superficialis and pronator teres. It is possible that the different response rates at distinct levels of the microvascular tree, and the differential response rates of red blood cell flux and velocity found in different muscles could all affect the global microvascular response within the tissue volume of NIRS interrogation, and therefore mechanistically underlie the differences in the temporal characteristics of the microvascular responses of T[Hb + Mb] and its relationship to BABF across individuals.

The characteristics of the arterial tree can be described in terms of compliance (C) and resistance (R) where $R * C = \tau$. The 4 muscles that are within the area of interrogation of the NIRS probe are fed by different arterial systems. The brachioradialis is fed by the radial artery, the pronator teres by both the ulnar and radial arteries, while the flexor digitorum superficialis and the flexor carpi radialis are both fed by the ulnar artery. Each of these arteries receives inflow from the brachial artery. These arteries (brachial, radial and ulnar) have different diameters and would likely have different compliance and resistance values, resulting in 3 different time constants[60, 61]. Thus, the lumped-parameter microvascular time constants determined here represent weighted integration of the blood flow through all three arteries. Unfortunately, current technology does not permit the distinction of each of the arteries to the final lumped-parameters.

The microvascular rheologic responses observed here during reactive hyperemia differ from those reported in other studies during active hyperemia. In the rat model, there was no change in capillary hematocrit for approximately 20 s after the initiation of contractions; once hematocrit changed it did so with a biphasic temporal profile[37]. In contrast, our results during

occlusive reactive hyperemia demonstrated an immediate, exponential increase in T[Hb + Mb], reflecting an increase in microvascular hematocrit, following cuff release. This different response profile could be related to the absence of the muscle pump in reactive hyperemia and the vasodilation that exponential (both τ_1 and τ_2) were not influenced by ATT and support our earlier findings that correction for ATT is not required for purely kinetic analysis of NIRS data. In contrast, we found that baseline (Y_0) values and both amplitude measures of T[Hb + Mb] were influenced by ATT (Fig. 4). We had previously shown that only those parameters associated with signal strength, like baseline T[Hb + Mb] and amplitude values, but not temporal parameters such as time constants, were influenced by ATT[13]. In our previous study we used exponential and sigmoidal functions to examine the temporal parameters of the oxy[Hb + Mb] and deoxy[Hb + Mb] responses during PORH. In the current investigation we used a double exponential function to examine the temporal parameters of the T[Hb + Mb] and BABF responses during PORH. The current investigation showed that time constants do not require correction for ATT when using a double exponential function as well.

CONCLUSION

In conclusion, macrovascular and microvascular responses in the forearm during reactive hyperemia have similar temporal profiles. Our results suggest that during reactive hyperemia the macrovascular response determines the microvascular response in the human forearm. The strong association between our two responses, including cross correlations, the Granger causality results and the correlation between τ_2 values for each response suggest that the temporal characteristics of the conduit artery blood flow are reflected in the downstream microvasculature.

Finally, our results confirm that correction for adipose tissue thickness is not required when performing a purely temporal analysis of NIRS data.

Table 3-1: Subject characteristics. Data is presented as average \pm SD.

Subject characteristic	N = 15 (8 males)
Age (years)	21.4 \pm 1.7
Height (cm)	174.7 \pm 9.3
Weight (Kg)	77.5 \pm 21.1
Body mass index (Kg/m ²)	23.9 \pm 4.3
Adipose tissue thickness (mm)	4.6 \pm 1.6

Table 3-2: Correlation, cross-correlation and time displacement data between BABF and T[Hb+Mb] for each subject.

Subject	Correlation coefficient	Cross-correlation	Lag time between peaks (s)
1	0.92	0.93	1
2	0.98	0.98	0
3	0.96	0.96	0
4	0.93	0.94	2
5	0.84	0.91	6
6	0.85	0.92	6
7	0.86	0.87	1
8	0.96	0.96	0
9	0.93	0.95	2
10	0.97	0.97	0
11	0.84	0.92	6
12	0.92	0.95	3
13	0.98	0.98	0
14	0.79	0.88	7
15	0.93	0.98	3
Average	0.91	0.94	3.7
SD	0.06	0.03	2.6

Correlation coefficient represents the correlation between BABF and T[Hb+Mb] responses.

Cross-correlation represents the correlation coefficient after accounting for lag times. Lag time between peaks represents the post-cuff release time displacement between T[Hb+Mb] and BABF.

Table 3-3: Temporal parameters and comparison ratios for all subjects.

Subject	τ_{1BAAF}	τ_{2BABF}	$\tau_{1(T[Hb+Mb])}$	$\tau_{2(T[Hb+Mb])}$	$\tau_{2BABF}/\tau_{1BAAF}$	$\tau_{2(T[Hb+Mb])}/\tau_{1(T[Hb+Mb])}$
1	3.2	27.4	3	40.3	8.5	13.6
2	5.4	22.3	6.6	19.8	4.1	3
3	3.2	27.6	9.4	14.7	8.6	1.6
4	5.9	25.7	14.7	29.7	4.4	2
5	4.3	40.8	9.8	78.5	9.4	8
6	4.3	16.7	13.3	20.2	3.9	1.5
7	3	24.7	2.7	81.2	8.2	30.3
8	5.3	13.1	4.8	14.6	2.5	3
9	2.6	34.7	5.8	23.8	13.1	4.1
10	3.7	34.7	4.8	60	9.3	12.4
11	2.1	45.6	9.4	51.9	21.3	5.5
12	6.5	18.6	13	18.6	2.9	1.4
13	3.1	42.2	12.3	18.5	13.6	1.5
14	3	19.3	15.1	19.2	6.4	1.3
15	6	9	8.8	11.4	1.5	1.3
Average	4.1 ^{ab}	26.8	8.9 ^a	33.5	7.8	6
SD	1.4	10.9	4.2	23.5	5.2	7.8

^a Significantly faster than τ_2 for each response variable, $p < 0.05$

^b Significantly faster than $\tau_{1(T[Hb+Mb])}$ $p < 0.05$

Table 3-4: ATT and non-temporal parameters.

Subject	ATT	BABF		Y ₀		T[Hb + Mb]			
		A ₁	A ₂	Raw	Corrected	A ₁	A ₁ corrected	A ₂	A ₂ corrected
1	6.7	919	895	59.3	143	73	131	62	130
2	5.0	758	744	72.8	135	244	117	249	117
3	2.5	1074	1055	109	140	1127	159	1136	160
4	2.8	1492	1471	112.7	148	808	132	893	141
5	5.2	591	605	81.8	147	176	113	18	114
6	5.9	2652	2538	52	126	378	147	371	146
7	5.9	1099	1033	46.8	120	51	114	42	113
8	3.3	3647	3581	99.3	141	507	112	494	110
9	3.7	1202	1285	87.9	134	364	105	292	98
10	7.3	1076	955	44.4	136	6	141	67	141
11	2.8	754	728	89.7	125	268	78	258	77
12	3.5	1704	1648	90.7	134	136	201	1328	197
13	5.8	942	1021	65.5	138	305	137	316	139
14	5.2	897	858	74.4	139	1329	229	131	227
15	3.3	5355	5302	78.1	119	1325	193	1283	189
AVG	4.6	1611	1581	78	135	56	141	55	140
SD	1.6	1315	1296	21	9	49	40	49	40

Figure 3-1: Representative graph showing lag between peak values in one representative subject. This pattern was observed in 10 of 15 subjects.

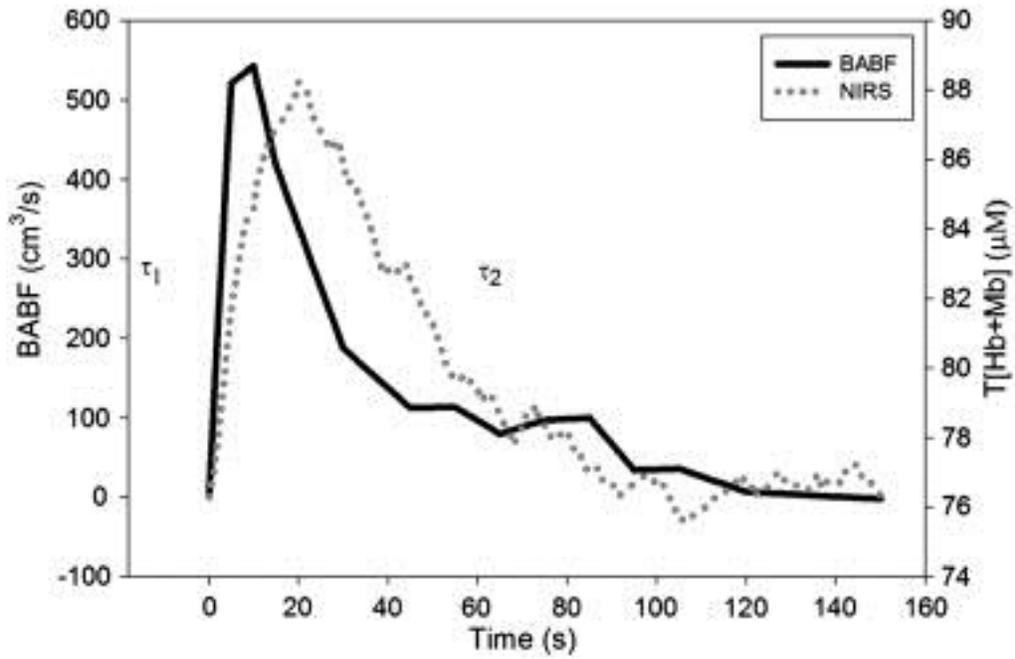


Figure 3-2: Representative graph showing coincident peak values in one representative subject. This pattern was observed in 5 of 15 subjects.

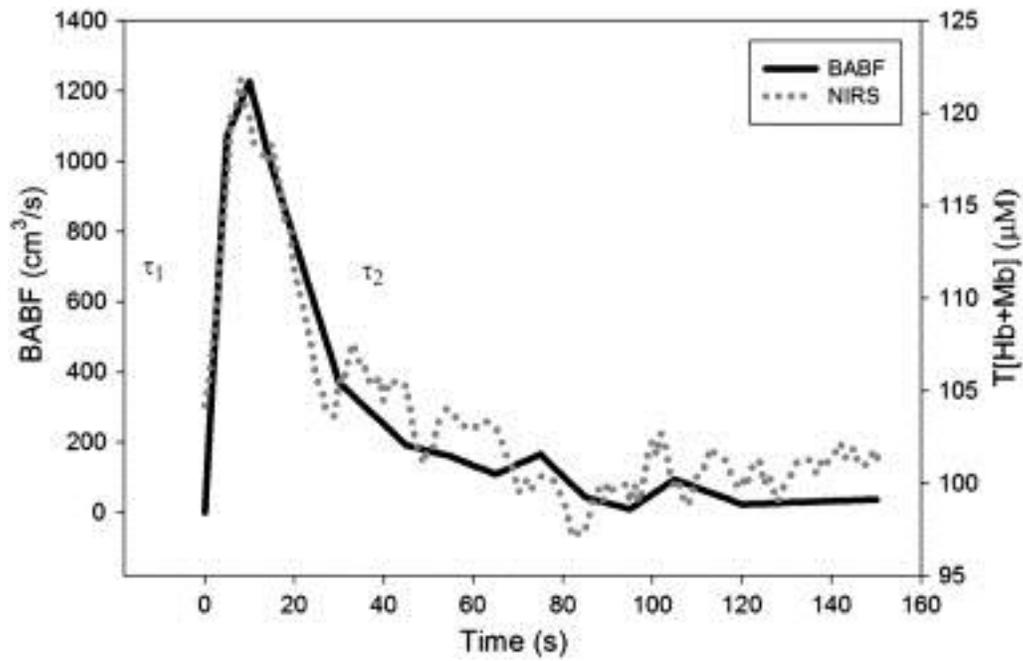


Figure 3-3: Relationships among time constants for BABF and T[Hb + Mb] during PORH. Trendlines are shown when significant correlation existed.

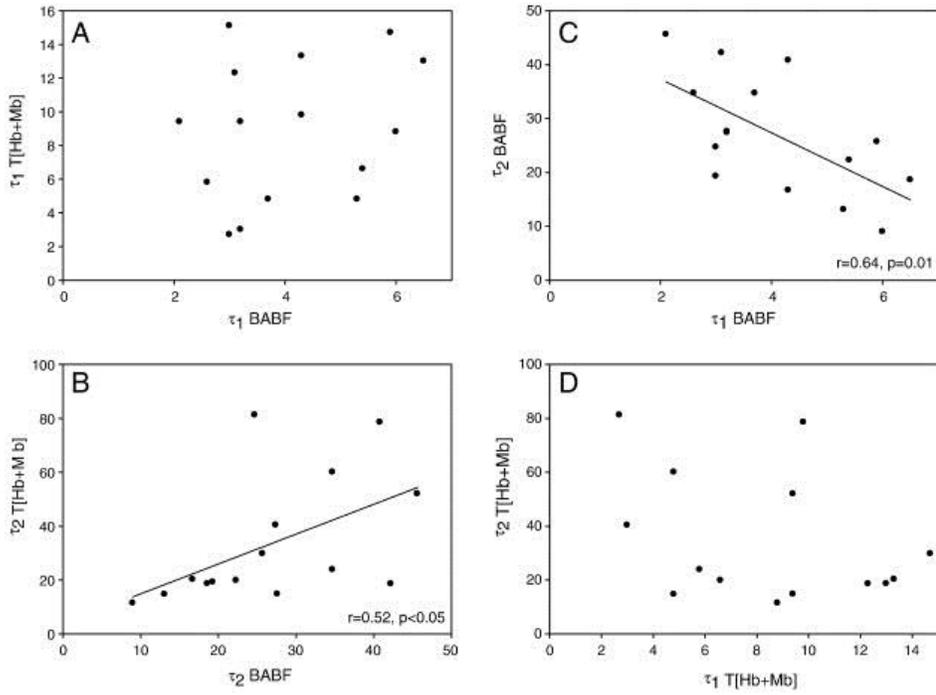
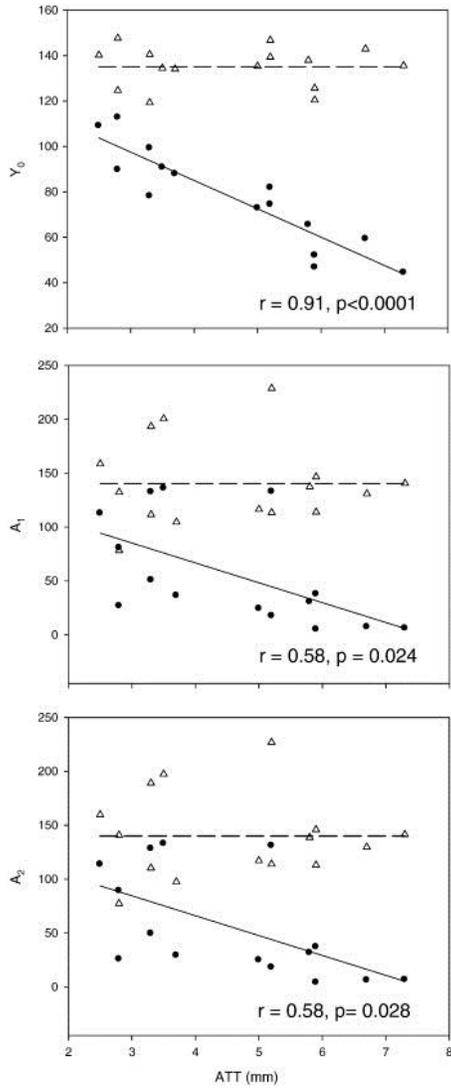


Figure 3-4: Relationship between original (●) and corrected (Δ) baseline T[Hb + Mb] (Y0), A₁ and A₂ plotted against ATT. Solid lines indicate linear regression model for uncorrected-for-ATT data. Dashed lines indicate linear regression model for corrected-for-ATT



Chapter 4 - The association between cutaneous and intramuscular microvascular measures during post-occlusive reactive hyperemia.

ABSTRACT

The purpose of this study was to compare microvascular kinetics during reactive hyperemia between skeletal muscle and cutaneous tissue. Cutaneous reactivity was assessed with laser-Doppler flowmetry. Intramuscular reactive hyperemia was assessed via two different techniques; single-fiber intramuscular laser-Doppler flowmetry (IMLD) was used to measure microvascular red blood cell flux, while near-infrared spectroscopy (NIRS) was used to measure changes in Total Hemoglobin+Myoglobin (T[Hb+Mb]). A double exponential function with no time delay was fit to each response curve and time constants (τ) for brachial artery blood flow (BABF), T[Hb+Mb], IMLD, and the proximal (SK_1) and distal cutaneous probes (SK_2) were recorded for both the rise from baseline to peak value (τ_1) and for the return from peak to baseline values (τ_2). Time to peak was also determined for each response. For all responses, τ_1 was significantly faster than τ_2 ($p < 0.001 - p = 0.002$). For both time to peak and τ_1 , BABF was significantly faster than T[Hb+Mb], IMLD, SK1 and SK2 ($p < 0.02$). In turn, T[Hb+Mb] was significantly faster than IMLD for both time to peak ($p = 0.024$) and τ_1 ($p < 0.01$). No differences were observed between all responses for τ_2 ($p = 0.3$). Finally, the temporal profile of the cutaneous reactive hyperemic response did not differ from that observed in the skeletal muscle via either NIRS or IMLD.

INTRODUCTION

The cutaneous microvasculature remains an appealing target for microvascular assessment due to its ready accessibility. It has been proposed that the cutaneous microvasculature could serve as model of generalized microvascular function[62]. For this to be true, changes in the cutaneous tissue would need to be quantitatively similar to the changes observed in other microvascular beds, such as skeletal muscle, in response to an intervention. There are several studies showing that this is not always the case. Addor et al. showed that the duration of reactive hyperemia was different between skin and skeletal muscle in the forearm[18]. Additionally, they demonstrated that aspirin administration increased the peak reactive hyperemic response in skeletal muscle, while in cutaneous tissue the duration of reactive hyperemia was reduced. Regardless of the mechanisms behind reactive hyperemia in cutaneous tissue and skeletal muscle, for one to serve as a surrogate of the other, the dynamic responses during post-occlusive reactive hyperemia (PORH) would have to show a high degree of symmetry.

Near-infrared spectroscopy (NIRS) has been used for over 40 years to non-invasively monitor oxygen status in various tissues, including skeletal muscle [6]. Despite this long tenure, there is still uncertainty regarding what this technique actually measures within skeletal muscle. Some of this uncertainty is due to the differing abilities of the commercially available devices to assess oxygenation status with NIRS. The OxiplexTS (ISS, Inc., Champlain, IL) device used in this project is able to quantify both oxy[hemoglobin+myoglobin] (oxy[Hb+mb]) and deoxy[hemoglobin+myoglobin] (deoxy[Hb+Mb]). The sum of these two measurements is equal

to total hemoglobin + myoglobin (T[Hb+Mb]). We have previously characterized the responses of oxy[Hb+Mb] and deoxy[Hb+Mb] and the resultant changes in T[Hb+Mb] during post-occlusive reactive hyperemia in the human forearm [16]. Assuming that tissue myoglobin remained constant, we suggested that changes in T[Hb+Mb] during PORH represented changes in skeletal muscle microvascular hemoglobin concentration (hematocrit), possibly due to an abatement of the Fahraeus effect [14, 16]. However, to date, the physiological meaning of changes in T[Hb+Mb] remain untested.

Intramuscular single-fiber laser Doppler (IMLD) is an invasive technique used to determine blood perfusion and erythrocyte flux (flow). Recently, this technique has been used to assess alterations in microvascular blood flow due to pain and musculoskeletal injuries [63-66]. Earlier studies using this technique compared bulk blood flow and red cell flux (an index of microvascular blood flow) in both skeletal muscle and cutaneous tissue during reactive hyperemia [67, 68]. What remains unclear is the temporal relationship between changes in microvascular hematocrit (T[Hb+Mb]) and erythrocyte flux (IMLD) during reactive hyperemia in the skeletal muscle of the forearm and the overlying cutaneous tissue. In the present study, we combined NIRS and IMLD to investigate these relationships.

Thus, one purpose of this investigation was to compare temporal responses between cutaneous and intramuscular vascular beds during PORH. We hypothesized that the time constants would be different in the skeletal muscle microvasculature compared to those observed in the cutaneous microvasculature. The second purpose of this investigation was to compare temporal characteristics of the reactive hyperemic responses within skeletal muscle as measured

with NIRS and IMLD. We hypothesized that there would be no differences between the temporal parameters of NIRS and IMLD during PORH.

METHODS

Subjects

Eight males (average age 20.6 ± 0.9 years) were recruited from the general student body at Kansas State University. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University. All subjects abstained from alcohol and strenuous exercise for 24 hours prior to testing. Subjects were fasted and had abstained from caffeine for 12 hours prior to testing. Subjects were also instructed to abstain from non-steroidal anti-inflammatory drugs for 24 hours.

Protocol

All tests were performed between 0900-1100. Each subject underwent 3 PORH trials consisting of 1 minute of baseline data collection, 5 minutes of brachial artery occlusion and 3 minutes of PORH monitoring. At least 15 minutes were allowed between trials for responses to return to baseline.

Intramuscular monitoring

Both NIRS and IMLD flowmetry were used to evaluate reactive hyperemia within the skeletal muscle of the forearm. Quantitative measures of the changes in total hemoglobin+myoglobin (T[Hb+Mb]) were assessed with NIRS(OxiplexTS, ISS, Inc., Champlain, IL). With the subject supine, the NIRS probe was placed on the supinated left forearm over the brachioradialis and the flexor carpi radialis muscles. IMLD flowmetry was used

to measures changes in red cell flux within the same sampled muscle. The single fiber intramuscular laser Doppler probe (Periflux 5010 laser Doppler perfusion monitor, with Master probe 418-1 with MT A500-0 (120mm), Perimed; Jarfalla, Sweden) was placed through a 22-gauge catheter inserted into the muscle adjacent to the NIRS probe. Thirty minutes were allowed post probe insertion for restoration of baseline. We had intended to insert the intramuscular probe directly beneath the NIRS probe to sample the same muscle volume, but in pilot studies this placement scheme generated artifacts in both signals. The probe was therefore inserted just adjacent to the NIRS probe, but still within the same muscle tissue being monitored by NIRS, which eliminated the interference. Laser-Doppler results were recorded in mV.

Cutaneous monitoring

Changes in cutaneous red cell flux were also assessed with laser Doppler flowmetry. Two integrated laser Doppler probes (Probe 413, Perimed) were placed on the forearm and secured with a probe holder. One was placed proximal to the NIRS probe (SK1) and one distal (SK2). As with the intramuscular laser-Doppler, cutaneous results were recorded in mV.

Brachial artery monitoring

Total blood flow into the forearm was measured beat by beat in the brachial artery (BABF) via pulsed-Doppler ultrasound (GE VIVID3, GE Medical Systems). Data was stored in 5 second intervals; vessel diameter was obtained discontinuously at the beginning of each 5 second collection interval. Blood flow was calculated as the velocity time integral (the distance in cm the blood travels per second) multiplied by cross sectional area ($0.785 * D^2$) to generate blood flow per second. All Doppler images were analyzed by the same investigator.

Brachial artery occlusion

The left arm was instrumented with a pressure cuff (SC12D) attached to a rapid inflator (E20 Rapid Cuff Inflator) with a dynamic air source (AG101) (for both, Hokanson, Bellevue, WA), which is capable of cuff inflation in less than 0.5 s. Following 1 minute of baseline data collection the 5-minute occlusion period was initiated by inflation of the cuff to suprasystolic pressure (250mmHg).

Continuous blood pressure monitoring

Continuous blood pressure monitoring on the right arm was carried out via photoplethysmography (NexfinHD; BMEYE,(Amsterdam, The Netherlands). These readings were confirmed via periodic automated blood pressure monitoring (S5 Light Monitor; Datex-Ohmeda, GE Healthcare; Madison,WI, USA).

Data management

All data were collected at 1Hz and stored for offline analysis. The NIRS system uses its own proprietary software, OxiTS (ISS, Inc.), for data management. The laser Doppler data were recorded using Windaq signal processing software (Dataq Instruments, Akron, OH). Brachial artery blood flow (BABF) data was calculated beat by beat, imported into Sigmaplot 10 and interpolated to 1Hz. We have previously reported that correction of NIRS data for adipose tissue thickness is not required when performing a temporal analysis,[16] therefore no correction for ATT was performed on our NIRS data. All red blood cell flux values obtained through laser-Doppler flowmetry were divided by mean arterial pressure to generate measures of intramuscular and cutaneous vascular conductance with units of mV/mmHg. T[Hb+Mb] data from 2 subjects

were excluded from the analysis because of significant accumulation of T[Hb+Mb] during the 5-min cuff occlusion, which eliminated the overshoot during PORH.

Statistical analysis

All subjects underwent 3 trials of the PORH protocol. Data from the 3 trials were time aligned and averaged. For one subject, only two trials were used due to equipment failure. A double exponential function (Equation 1) with no time delay was fit to each response curve and time constants (τ) for BABF, T[Hb+Mb], IMLD, SK1 and SK2 were determined for both the rise from baseline to peak value (τ_1) and for the return from peak to baseline values (τ_2). Time to peak was also determined for each response. Also recorded were baseline values (Y_0) and two amplitude values (A_1 and A_2) corresponding to the amplitude of the rise to peak and the subsequent return towards baseline from peak, respectively.

$$\text{Equation 1: } Y = Y_0 + A_1 * (1 - e^{(-t/\tau_1)}) - A_2 * (1 - \exp^{(-t/\tau_2)})$$

Comparisons among temporal responses were made with one way repeated measures analysis of variance. Student-Newman-Kuels post-hoc analysis was used where appropriate to look for individual differences in means. Individual paired t-tests with Bonferonni correction were performed where group differences were masked by missing data and/or high variance.

RESULTS

Figures 1 and 2 illustrate a complete protocol with intramuscular and cutaneous responses in the same subject. The 5-minute occlusion period was followed by cuff release and PORH monitoring until values returned to baseline (approximately 3 minutes). Figure 3 shows BABF, T[Hb+Mb] and IMLD responses during PORH in the same subject. Table 1 shows the time constants for the

on (τ_1) and off (τ_2) kinetic responses for all variables of interest. For all responses, τ_1 was significantly faster than τ_2 ($p < 0.001 - p = 0.002$).

τ_1 and Time to Peak

The overall responses for τ_1 , (Table 1) and Time to Peak (Table 2) were similar. BABF was significantly faster than the other 4 responses ($p < 0.02$). In turn, T[Hb+Mb] was significantly faster than IMLD for both τ_1 ($p < 0.01$) and time to peak ($p = 0.024$), which was not different from the two cutaneous measures.

τ_2

No significant differences were observed between any variables ($p = 0.30$).

DISCUSSION

The first main finding of this investigation was that, contrary to our first hypothesis, there were no significant differences in the temporal parameters of PORH between skeletal muscle (both NIRS and IMLD) compared with the cutaneous microvasculature. In contrast to our second hypothesis, significant differences were observed in the temporal parameters measured within skeletal muscle via two different techniques (NIRS and IMLD).

Holowatz et al. suggested that the cutaneous microvasculature can be used as an analog for total body microvascular function[62]. If true, this would predict that temporal responses to PORH within the cutaneous microvasculature would be similar to those observed in the skeletal muscle microvasculature. In the present study, neither T[Hb+Mb] nor IMLD responses were significantly different from those observed in the cutaneous microvasculature, consistent with the suggestions of Holowatz et al.

The validity of using the cutaneous microvasculature as a surrogate for coronary[69] and skeletal muscle[70, 71] microvascular beds has been questioned [72]. Ijzerman et al. stated that the assessment of intramuscular, cardiac and renal microvascular reactivity would be most relevant from a pathological standpoint, but that this would require invasive and complicated techniques [73]. Further, both Wilkins[74] and Edvinsson and Andersson[75] noted the need for direct comparisons between the cutaneous and skeletal muscle microvasculatures before these tissues could be used interchangeably. Our laboratory has previously suggested that there could be differences between the dynamic responses of the cutaneous and skeletal muscle circulations during PORH[71]. The current study resolves the problem identified by Ijzerman and colleagues by establishing that a simple, non-invasive technique (NIRS) does provide reliable intramuscular assessments. Further, by directly comparing the cutaneous and skeletal muscle microvasculature responses, we were able to establish that the cutaneous and skeletal muscle microvascular temporal responses during PORH were not significantly different, using LDF. Although the mechanisms underlying cutaneous versus skeletal muscle PORH may differ, our data suggest the resulting temporal responses are similar.

Neither changes in $T[\text{Hb}+\text{Mb}]$ nor intramuscular vascular conductance (IMLD) are true measures of microvascular blood flow. In turn, differences between the two techniques may account for the observed differences in temporal profiles between these measurements. It is currently unclear what the underlying mechanism is for the $T[\text{Hb}+\text{Mb}]$ changes during PORH. We have previously suggested that $T[\text{Hb}+\text{Mb}]$ is related to microvascular hematocrit [13]. Assuming that tissue myoglobin remains constant during our protocol, the transient increase in

T[Hb+Mb] following cuff release must come from increases in hemoglobin concentration (hematocrit) within the skeletal muscle microvasculature [14]. In contrast, laser-Doppler flowmetry examines the degree of Doppler shift of moving red blood cells.

The relationships among capillary hematocrit, RBC flux and RBC velocity have been examined in skeletal muscle during the transitions from rest to contraction and from contraction to recovery.[37, 58] These authors established that capillary hematocrit is proportional to the ratio of RBC Flux (flow) to RBC velocity. Kindig et al [37]. found that RBC velocity immediately increased to steady state following exercise onset, while flux increased in 2 phases; a rise to a plateau at 12-20 seconds followed by a secondary rise to steady state values. Capillary hematocrit did not significantly increase until 18 seconds after the initiation of contractions; this increase in hematocrit coincided with the onset of arteriolar dilation due to metabolic feedback. During recovery, Ferreria et al. found little or no change in RBC flux or velocity immediately following cessation of contractions [58]. Both flux and hematocrit dropped significantly at 5 seconds into recovery, while velocity did not change for 15-20 seconds. Both Kindig et al. [37] and Ferreria et al. [58] report little association between changes in hematocrit and changes in RBC velocity. Under both exercise and recovery conditions, flux changed to a greater extent than did velocity and was the determining factor in changes in hematocrit.

The results of the present study during PORH do not resemble the temporal profiles described for exercise. Both hematocrit (T[Hb+Mb]) and flux (IMLD) responses showed an immediate, exponential rise to peak upon cuff release. There was no time delay in either response, likely because at cuff release arterioles were already vasodilated due to the 5 minutes

of ischemia. Although this investigation did not measure microvascular RBC velocity, for an increase in hematocrit to be observed, the increase in flux would need to be greater than the increase in velocity and/or velocity must decrease as flux increases. Either scenario would result in increased hematocrit. House and Liposky [59] found that red cell velocity decreased as capillary hematocrit increased during PORH, likely due to increased blood viscosity as hematocrit increased [76]. Humeau et al., modeled laser Doppler flux responses during reactive hyperemia in a theoretical, simple microvessel [77]. Their models showed that red cell velocity achieved a steady state rapidly (within .001 s) upon cuff release, and that the increase in flux was due to an increasing number of RBCs in the area of interrogation due to vasodilation [78].

Active and reactive hyperemia have different causative mechanisms. In active hyperemia, as with exercise, the muscle pump produces a rapid increase in muscle blood flow [79]. Within 7-15 seconds of the start of exercise, vasodilators, such as nitric oxide and prostacyclin jointly induce vasodilation and regulate blood flow [37, 79-81]. These disparate mechanisms produce the two phase response observed by Kindig et al. [37] in the transition from rest to muscle contraction. In contrast, with reactive hyperemia of the forearm, brachial artery occlusion causes ischemia in the downstream vasculature. The resulting hypoxemia and metabolic dilators such as adenosine and H⁺, induce arteriolar dilation during the period of occlusion [82]. Previous authors have also suggested a role for both prostanoids (PGE₂, PGI₂ and Thromboxane) [3, 43, 83], nitric oxide [84] and endothelial hyperpolarization [85] in the reactive hyperemic response in skeletal muscle. These systems have a high degree of interaction and redundancy between them which causes difficulty in determining which has the greatest effect on PORH [81, 86-88].

Upon cuff release, first conduit artery and then microvascular blood flow overshoots resting, pre-cuff levels. With the increase in brachial artery blood flow, downstream microvascular flow will be homogenized, leading to reversal of the Fahraeus effect and a subsequent increase in red cell flux and velocity leading to increased shear stress on the vascular endothelium, causing flow-mediated dilation [89]. The reactive hyperemia is temporary; as metabolic dilators are flushed away, the arterioles will return to their resting diameters. Brachial artery blood flow returns to baseline and the Fahraeus effect is reinstated, microvascular blood flow heterogeneity returns and red cell flux and eventually velocity will return to baseline [90]. As flux returns to normal, microvascular hematocrit (and T[Hb+Mb]) will also return to baseline.

Changes in total limb blood flow, as during post-occlusive reactive hyperemia, of forearm skeletal muscle has historically been measured with strain gauge plethysmography [17, 91, 92]. One limitation of this technique is that the initial 3-5 seconds of data cannot be captured [18]. Both NIRS and IMLD overcome this limitation by collecting data continuously. Neither of these techniques measures flow per se; T[Hb+Mb] reflects changes in microvascular hematocrit while IMLD measures changes in RBC flux [16, 77]. The invasive nature of the IMLD probe compared with the non-invasive nature of the NIRS probe suggests that NIRS is the better choice for future evaluation of skeletal muscle microvasculature reactivity. Both the NIRS and IMLD systems sample from arterioles, capillaries and venules [15, 93]. A significant difference between the two techniques is the area of interrogation. The IMLD microtip probe samples within a small area of interrogation (roughly 1 mm³) directly in front of the probe tip [68]. In contrast, the OxiplexTS (NIRS) is able to penetrate to a depth of 17.5mm, and across

several muscles (brachioradialis, flexor carpi radialis, flexor digitorum superficialis and pronator teres); as such, NIRS provides a more generalized measure of PORH in the forearm [16]. The IMLD probe was inserted directly into the sampled muscle, parallel to the NIRS probe so that both devices were monitoring the same tissue. Microvascular blood flow demonstrates marked heterogeneity, thus, it is possible that the differences in the temporal profiles of NIRS versus IMLD result, in part, from sampling different regions within the skeletal muscle microvasculature [31]. The invasive nature of the IMLD probe combines with the limited area of interrogation suggests that NIRS is the better choice for future evaluation of skeletal muscle microvasculature reactivity.

Two of our eight subjects demonstrated accumulation of T[Hb+Mb] during the cuff occlusion period. We are confident that our brachial artery cuff completely occluded the artery and that flow into the forearm was zero as confirmed by loss of radial pulse and continuous measurement of brachial artery blood flow. Therefore, the accumulation of the T[Hb+Mb] signal over the course of the 5 minute occlusion period was likely caused by redistribution of red blood cells already within the forearm vasculature. These subjects may have experienced increased red blood cell accumulation in the venules during occlusion as has been previously reported [59]. Again, NIRS can only detect changes in vessels smaller than 1mm in diameter [14, 15] so this accumulation may represent movement of red blood cells from larger diameter vessels where they would be undetectable by NIRS into smaller vessels where they could be detected [94]. This movement may be facilitated by both the pressure gradient and difference in vessel compliance from the arterial to the venous vascular compartment.

T[Hb+Mb] accumulation is a major obstacle in the expansion of NIRS use in PORH research. In this investigation, 25% of our subjects experienced such accumulation. In a previous study from our lab, 3 of 26 (12%) participants demonstrated T[Hb+Mb] accumulation. To increase the utility of NIRS in the assessment of vascular reactivity within skeletal muscle, techniques must be developed to decrease the incidence of accumulation. One possible approach may be to add a wrist cuff to the protocol, as was traditionally done with strain gauge plethysmography, to exclude the hand circulation, thus forming a completely sealed forearm compartment which may minimize red cell redistribution across AV anastomoses between the distal ends of the radial and ulnar arteries and veins in the distal wrist and proximal hand[95]. However, use of such a cuff could alter the reactive hyperemic response in the forearm and may alter relationships between skeletal muscle, cutaneous and conduit artery responses during PORH in the forearm[96].

CONCLUSION

In conclusion, no differences in temporal profiles were observed between cutaneous and intramuscular measures, either by NIRS or IMLD, during PORH in the forearm. These results suggest that, at least in the human forearm and during a purely temporal analysis of PORH, the cutaneous microvasculature can serve as a surrogate for the skeletal muscle microvasculature. Significant differences were found between the temporal parameters measured within skeletal muscle using NIRS and IMLD. These results suggest that technical and physiological differences between NIRS and IMLD can result in discrepancies in measurements within the same vascular bed.

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Table 0-1: Time constants for all variables.

	Conduit Artery		Intramuscular microvasculature				Cutaneous Microvasculature			
	BABF	BABF	T[Hb+Mb]	T[Hb+Mb]	IMLD	IMLD	SK1 τ	SK1 τ	SK2 τ	SK2 τ
	τ_1	τ_2	τ_1	τ_2	τ_1	τ_2	1	2	1	2
Average										
e	1.4	30.7	4.5*	57.6	12.5*‡	40.8	10.5*	41.6	10.8*	48.6
SD	0.5	18.4	1.1	24.6	4.4	41.0	11.8	8.8	10.2	13.0

BABF- Brachial artery Blood Flow

T[Hb+Mb]- Total Hemoglobin+myoglobin

IMLD – Intramuscular Laser Doppler

SK1- Proximal skin probe

SK2 – Distal Skin probe

*Significantly longer than BABF (p<0.001)

‡Significantly longer than T[Hb+Mb] (p<0.01)

Table 0-2: Time to peak (s) for all variables.

	BABF	T[Hb+Mb]	IMLD	SK1	SK2
Average	6.6	13.2*	23.4‡*	22.0*	19.9*
SD	3.1	4.0	9.1	17.2	11.6

*Significantly longer than BABF ($p \leq 0.01$)

‡Significantly longer than T[Hb+Mb] ($p = 0.02$)

Figure 0-1: Responses for intramuscular laser Doppler (IMLD) and Total [Hb+Mb] from NIRS in a representative subject. Included are baseline, 5-minute cuff occlusion and PORH response to cuff release. Cuff release occurs at 320 seconds.

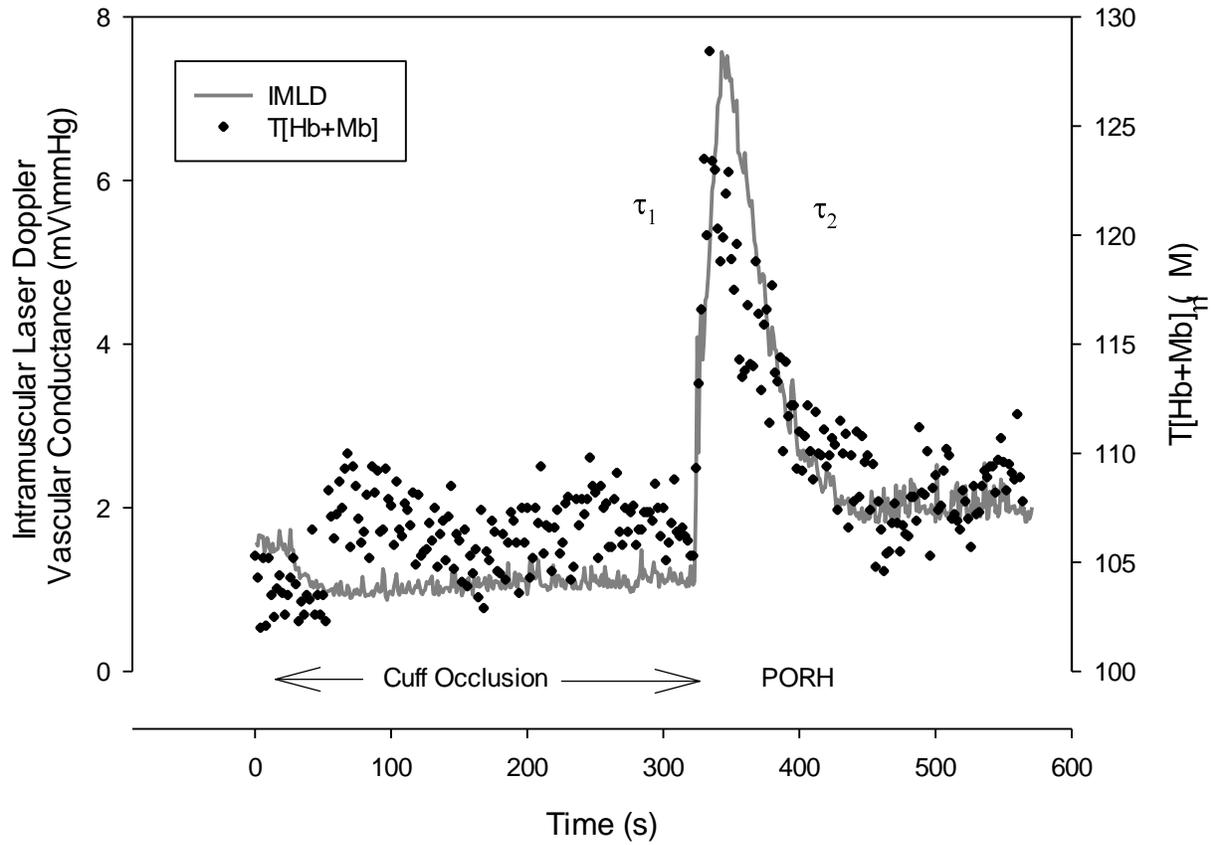


Figure 0-2: Laser-Doppler responses for cutaneous PORH in the same subject as Figure 1. Included are baseline, 5-minute cuff occlusion and PORH response to cuff release. Cuff release occurs at 320 seconds.

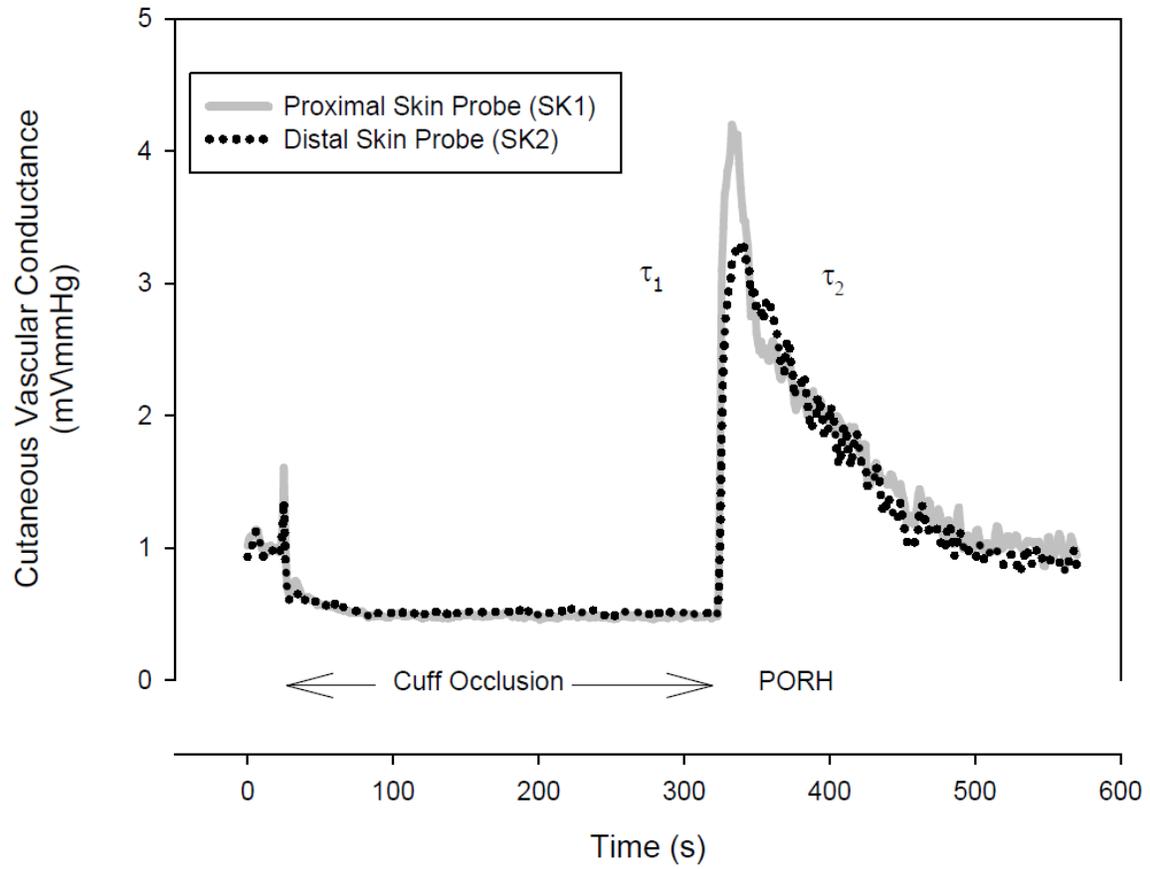
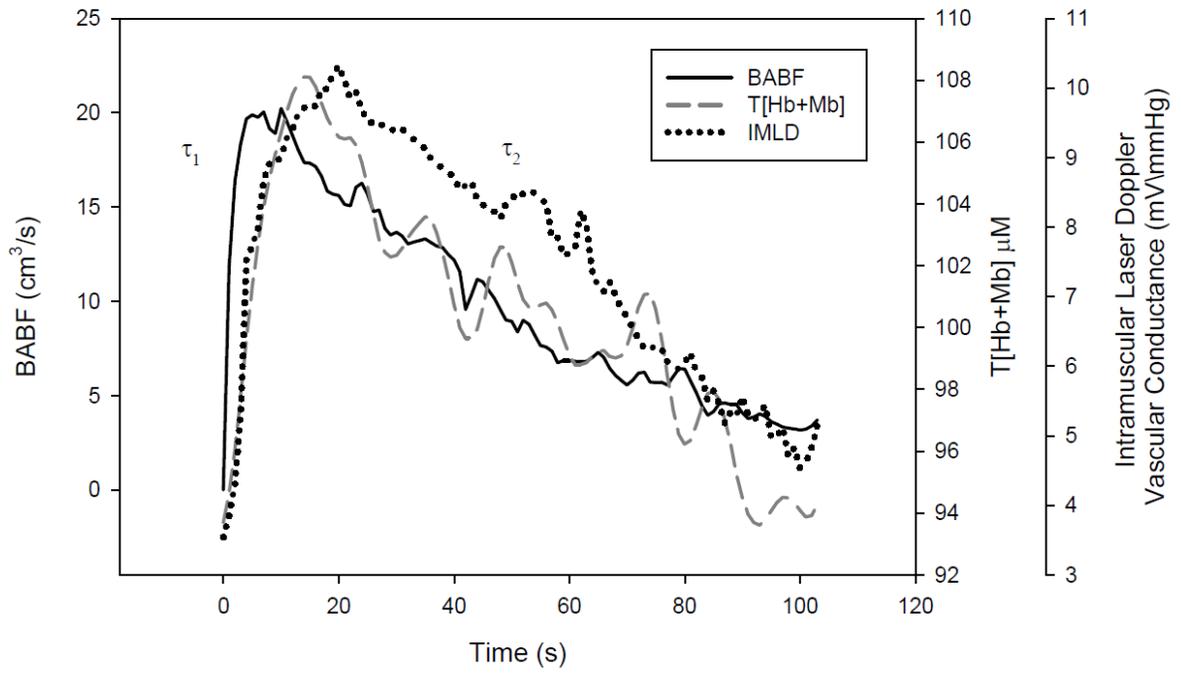


Figure 0-3: BABF, T[Hb+Mb] and IMLD PORH responses in a representative subject. Time 0 represents cuff release.



**Chapter 5 -Ibuprofen alters the initial post-occlusive
reactive hyperemic response in skeletal muscle, but not
cutaneous tissue, in the human forearm**

ABSTRACT

The purpose of this study was to compare the effects of 1200 mg of oral ibuprofen, a non-selective inhibitor of cyclooxygenase, on the post-occlusive reactive hyperemic response in skeletal muscle and cutaneous tissue. Skeletal muscle responses were measured as changes in T[Hb+Mb] with near-infrared spectroscopy. Cutaneous vascular conductance was measured with laser Doppler flowmetry. A double exponential function with no time delay was fit to each response curve and time constants (τ) for brachial artery blood flow (BABF), T[Hb+Mb], and the proximal (SK₁) and distal cutaneous probes (SK₂) were recorded for both the rise from baseline to peak value (τ_1) and for the return from peak to baseline values (τ_2). For all responses, τ_1 was faster than τ_2 ($p < 0.001$). Time to peak and total hyperemic responses were also determined for each signal. Inhibition of prostaglandin production with 1200 mg of ibuprofen slowed the initial changes for T[Hb+Mb] within skeletal muscle (τ_1 ($p = 0.014$)) and time to peak ($p = 0.002$), but had no effect on τ_2 . No effects were observed in cutaneous or conduit artery responses. Ibuprofen had no effect on total hyperemic response, measured as area under the response curve, in any tissue. These results suggest that vasodilator prostaglandins play a role in the microvascular hematocrit response within the skeletal muscle, but not in cutaneous tissue immediately cuff release following ischemic cuff occlusion.

INTRODUCTION

Post occlusive reactive hyperemia (PORH) is a temporary increase in blood flow and velocity above resting levels occurring after a period of ischemia. As a result of brachial artery occlusion, dilation of downstream arterioles occurs due to the presence of local metabolites, such as adenosine[97] and vasodilator prostaglandins.[17] When the occlusion is released, the decreased vascular resistance results in a transient increase in blood flow. This increase in blood flow and velocity serves as the stimulus for flow-mediated dilation of the conduit artery.[1, 5] The reactive hyperemia test procedure in the forearm is simple to perform compared with measurements of flow-mediated dilation (FMD), and recent studies suggest that reactive hyperemia is a more predictive measure of cardiovascular disease than is FMD itself.[5, 98]

Metabolism of arachidonic acid by either isoform of the cyclooxygenase enzyme (COX) leads to the formation of several vasoactive prostanoids including prostaglandin H₂ (PgH₂).[99] In endothelial cells, a potent vasodilator, prostacyclin (PgI₂), is formed from PgH₂ via the action of prostacyclin synthase. In platelets, thromboxane A₂ (a vasoconstrictor) is formed from PgH₂ via the actions of thromboxane synthase.[100] Non-steroidal anti-inflammatory drugs (NSAIDs), like ibuprofen, are potent inhibitors of prostaglandin synthesis due to its non-selective inhibition of COX.[20] Many studies have examined the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on reactive hyperemia in both skeletal muscle and cutaneous tissue. In skeletal muscle NSAIDs produce a blunted reactive hyperemic response.[3, 17-19] Specifically, these authors observed a decrease in peak and total hyperemic response to a 5-minute ischemic cuff occlusion.[17] [3] Later studies found that ibuprofen altered peak, but not total, hyperemic

flow.[18, 19] In cutaneous tissue there is no strong evidence to suggest that prostanoids, either vasodilatory [101, 102] or vasoconstrictive, [83] contribute to PORH.[103]

Near-infrared spectroscopy (NIRS) has been used to non-invasively monitor oxygen status in various tissues, including skeletal muscle, for over 40 years.[6] We have previously characterized the responses of oxy[Hb+Mb] and deoxy[Hb+Mb] and the resultant changes in total hemoglobin + myoglobin (T[Hb+Mb]) during post-occlusive reactive hyperemia in the human forearm.[13, 16] We suggested that changes in T[Hb+Mb] represent changes in skeletal muscle microvascular hematocrit, linking the increased brachial artery blood flow during PORH with an abatement of the Fahraeus effect and increased microvascular hematocrit [16]. Assuming that tissue myoglobin remains constant during PORH, the transient increase in T[Hb+Mb] following cuff release must come from changes in hemoglobin concentration (hematocrit) within the skeletal muscle microvasculature.[14]

Therefore, the purpose of this investigation was to determine the effects of prostaglandin inhibition on the temporal parameters of the overall reactive hyperemic response as brachial artery blood flow, microvascular hematocrit as T[Hb+Mb], as measured with NIRS, and cutaneous vascular conductance, as measured with laser Doppler flowmetry (LDF), in the forearm. We hypothesized that prostaglandin inhibition would alter the reactive hyperemic response to a 5-minute ischemic cuff occlusion in skeletal muscle while having no effect on cutaneous PORH.

METHODS

Subjects

Eight males (average age 20.6 ± 0.9 years) were recruited from the general student body at Kansas State University. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University. Subjects were fasted and instructed to abstain from alcohol, strenuous exercise and NSAID use for 24 hours and caffeine for 12 hours prior to testing.

Protocol

All tests were performed between 0900-1100. Subjects were administered 1200 mg of ibuprofen or a placebo 60-90 minutes prior to testing; they received the other treatment on their subsequent visit. Ibuprofen was chosen as the NSAID with the greatest effect on PORH.[3] When taken orally, peak values of ibuprofen appear in blood plasma within 60-120 minutes.[104, 105] Each subject underwent 3 PORH trials of the non-dominant arm consisting of 1 minute of baseline data collection, 5 minutes of brachial artery occlusion and 3 minutes of PORH monitoring. At least 15 minutes were allowed between trials for responses to return to baseline.

Intramuscular monitoring

NIRS was used to evaluate T[Hb+Mb] changes within the skeletal muscle of the forearm. Changes in T[Hb+Mb], are thought to be related to microvascular hematocrit.[14] With the subject supine, the NIRS probe (OxiplexTS, ISS, Inc., Champlain, IL) was placed on the supinated non-dominant forearm over the brachioradialis and the flexor carpi radialis muscles. The OxiplexTS provides quantitative measures of T[Hb+Mb] in μM .

Cutaneous monitoring

Changes in cutaneous red cell flux were assessed with laser Doppler flowmetry. Two integrated laser Doppler probes (Probe 413, Perimed) were placed on the forearm and secured with a probe holder. One was placed proximal to the NIRS probe (SK₁) and one distal (SK₂). Results were recorded in mV.

Brachial artery monitoring

Total blood flow into the forearm was measured, beat by beat, in the brachial artery (BABF) via pulsed-Doppler ultrasound (GE VIVID3, GE Medical Systems). Data was stored in 5 second intervals; vessel diameter was obtained discontinuously at the beginning of each 5-second collection interval. Blood flow was calculated as the velocity time integral (the distance in cm the blood travels per second) multiplied by cross sectional area ($0.785 * D^2$) to generate blood flow per second. All Doppler images were analyzed by the same investigator.

Brachial artery occlusion

The non-dominant upper arm was instrumented with a pressure cuff (SC12D) attached to a rapid inflator (E20 Rapid Cuff Inflator) with a dynamic air source (AG101) (for both, Hokanson, Bellevue, WA). Following 1 minute of baseline data collection the 5-minute occlusion period was initiated by inflation of the cuff to suprasystolic pressure (250mmHg) in less than 0.5 s.

Wrist occlusion

All subjects were fitted with a standard blood pressure cuff at the wrist. This cuff was inflated to a suprasystolic pressure 60 seconds prior to the start of each trial to seal the forearm and minimize the influence of the palmar arches anastomoses on microvascular blood flow.[21]

Continuous blood pressure monitoring

Continuous blood pressure monitoring on the dominant arm was carried out via photoplethysmography (NexfinHD; BMEYE,(Amsterdam, The Netherlands). These readings were confirmed via periodic automated blood pressure monitoring (S5 Light Monitor; Datex-Ohmeda, GE Healthcare; Madison,WI, USA).

Data management

NIRS and IMLD data were collected at 50Hz and 1Hz, respectively, and stored for offline analysis. The NIRS system uses its own proprietary software, OxiTS (ISS, Inc.), for data management. Cutaneous data were recorded using Windaq signal processing software (Dataq Instruments, Akron, OH). Brachial artery blood flow data was calculated beat by beat. BABF data was imported into Sigmaplot 10 and interpolated to 1HZ. We have previously reported that correction of NIRS data for adipose tissue thickness is not required for analysis of time constants.[16] The remaining NIRS parameters (amplitude and baseline) were corrected for adipose tissue thickness.[16] All red blood cell flux values obtained through laser-Doppler flowmetry were divided by mean arterial pressure to generate measures of cutaneous vascular conductance (CVC) with units of mV/mmHg.

Data Processing

All subjects underwent 3 trials of the PORH protocol. Data from the 3 trials were time aligned to cuff release and averaged. A double exponential function (Equation 1) with no time delay was fit to each response curve (BABF, T[Hb+Mb], SK₁ and SK₂) and time constants (τ) for each were determined for both the rise from

$$\text{Equation 1: } Y = Y_0 + A_1 * (1 - e^{(-t/\tau_1)}) - A_2 * (1 - \exp^{(-t/\tau_2)})$$

baseline to peak value (τ_1) and for the return from peak to baseline values (τ_2). Time to peak was also determined for each response. Also recorded were baseline values (Y_0) and two amplitude values (A_1 and A_2) corresponding to the amplitude of the rise to peak and the subsequent return towards baseline from peak, respectively. Area under the curve (AUC) for each hyperemic response was calculated using equation 2. Baseline (pre-occlusion) values for each response were multiplied by the duration of the hyperemic response following cuff release; this value was subtracted from calculated AUC to calculate total hyperemic response.

$$\text{Equation 2: Total Hyperemic Response} = \text{AUC} - (\text{baseline values} * \text{hyperemic duration})$$

Statistical analysis

Comparisons among time constants and AUC were made with one way or two way repeated measures analysis of variance. Student-Newman-Kuels post-hoc analysis was used, where appropriate, to look for individual differences in means. Individual paired t-tests with

Bonferonni correction were performed where group differences were masked by missing data and/or high variance. Associations among time constants, time to peak and AUC were evaluated using a Pearson product moment correlation with Bonferroni correction.

RESULTS

Figure 1 shows data from a complete protocol in cutaneous tissue as measured with laser Doppler flowmetry and includes a baseline period followed by a 5-minute cuff occlusion of the brachial artery, cuff release and the hyperemic response. Figure 2 shows microvascular hematocrit changes during PORH under both treatment conditions with the amplitude and baseline parameters labeled. For each tissue assessed, and regardless of treatment, τ_1 was faster than τ_2 ($p < 0.001$).

Table 1 shows time constants for the initial rise to peak (τ_1) and for the return to rest from peak (τ_2) for each response. For τ_1 , ibuprofen administration resulted in a longer time constant ($p = 0.014$) for T[Hb+Mb]. No differences were observed in τ_2 ($p = 0.2$). Ibuprofen had no effect on temporal parameters of reactive hyperemia measured in the cutaneous microvasculature or the brachial artery. Recovery kinetics (τ_2) were significantly faster for BABF than for T[Hb+Mb] and cutaneous measures ($p < 0.001$). Table 2 shows time to peak and total hyperemic response, while Table 3 shows baseline and amplitude values for each response; all NIRS amplitude variables were corrected for adipose tissue thickness as described previously.[16] Time to peak was increased significantly ($p = 0.002$) by ibuprofen administration for T[Hb+Mb], but not for cutaneous microvasculature or the conduit artery responses. Prostanoid inhibition had no effect on AUC, baseline or either amplitude in any response.

Significant associations were found among time constants, time to peak and total hyperemic response for T[Hb+Mb], cutaneous and conduit artery responses. For T[Hb+Mb], τ_1 ($r=0.78$, $p=0.02$), time to peak ($r=0.86$, $p=0.005$) and total hyperemic response ($r=0.77$, $p<0.025$) were significantly correlated between treatment conditions. Also in T[Hb+Mb], τ_1 was correlated with time to peak under the effects of ibuprofen ($r=0.85$, $p=0.007$), but not while untreated ($r=.075$, $p=0.03$). In cutaneous tissue, time to peak was correlated across treatment conditions in the distal skin probe ($r=0.81$, $p=0.02$), but not the proximal ($p>0.025$). Also for the distal skin probe, τ_1 was correlated with time to peak under the effects of ibuprofen ($r=0.86$, $p<0.001$) and under placebo ($r=0.83$, $p=.012$). In the brachial artery total hyperemic response ($r=0.93$, $p<0.001$) there was a significant correlation between treatment conditions. Neither τ_1 nor τ_2 was correlated with AUC within any signal.

DISCUSSION

To our knowledge, this study is the first to examine the effects of prostaglandin inhibition with NSAIDs on microvascular hematocrit (T[Hb+Mb]) during PORH with NIRS. In support of our hypothesis, prostaglandin inhibition with 1200 mg of ibuprofen altered temporal parameters of the microvascular hematocrit response in skeletal muscle, resulting in longer τ_1 time constant and time to peak in T[Hb+Mb] compared to placebo; τ_2 was unchanged. As expected, prostaglandin inhibition produced no changes in time constants, total hyperemic response, time to peak or peak response during PORH in cutaneous tissue. These results suggest that prostaglandin induced vasodilation during the 5-minute period of ischemic occlusion plays a role in the initial changes in microvascular hematocrit within skeletal muscle, but not within

cutaneous tissue, upon cuff release. Our findings of longer time constants during PORH for T[Hb+Mb] following NSAID administration agree with the results of previous authors who found blunted PORH following the administration of NSAIDs.[3, 17-19] In addition, our novel technique, using NIRS, highlighted differences between our findings and previously reported results. Earlier studies observed a decrease in both peak and total hyperemic response to a 5-minute ischemic cuff occlusion following NSAID administration.[17] [3] Later studies found that ibuprofen altered peak response, but not total hyperemic flow.[18, 19] In the current study, prostaglandin inhibition did not alter baseline, peak response (A_1), or total hyperemic response (AUC) in any variable. Only τ_1 and time to peak for T[Hb+Mb] was increased by ibuprofen.

The differences between our findings and those reported by previous authors could be due to methodological differences. PORH assessment in the forearm has commonly been performed via strain gauge plethysmography (SGP) [21] with results reported as total hyperemic response. One limitation of SGP is the loss of the first several seconds of the reactive hyperemia response,[21] precisely when we observed differences in T[Hb+Mb] within the skeletal muscle microvasculature of the forearm. The loss of the initial seconds of data could alter the calculated AUC values and decrease the accuracy of this technique compared to the continuous assessment of T[Hb+Mb] with NIRS. SGP also calculates blood flow as a function of a change in volume in a limb and is not able to distinguish between skeletal muscle and cutaneous tissue. Our results show that the effects of ibuprofen administration differ between skeletal muscle and cutaneous microvascular beds during PORH and suggests that earlier studies where ibuprofen blunted reactive hyperemia, as measured with SGP, could have been reflective of changes within the

skeletal muscle microvasculature alone. These results would suggest that an analysis of the kinetics of the T[Hb+Mb] response, as was performed in this investigation, is a more sensitive measure than a traditional assessment of total hyperemic response during PORH with SGP.

Previous authors have reported mixed findings on the effects of prostanoid inhibition on reactive hyperemia in cutaneous tissue using various routes of different NSAIDs (oral[18, 103, 106, 107], rectal[17, 108], intravenous[103], intradermal injection,[109] microdialysis [101]). Effects of NSAID administration on cutaneous reactive hyperemia ranged from a blunted response, [17, 18, 107-109] no effect,[103, 106] to increased response.[101] It is likely that some of these differences can be explained by the differing potency of the NSAIDS employed[3] and the different routes of administration. It is also possible that our dosage of ibuprofen was not sufficient to elicit prostanoid inhibition in the cutaneous tissue of our subjects. Previous research has shown that ibuprofen is the NSAID with the greatest impact on PORH and our chosen dosage of 1200 mg matched or exceeded previously used dosages[3, 19] which altered reactive hyperemia. However, these studies did not examine reactive hyperemia in muscle and cutaneous tissue separately; they used SGP. The one study which did examine the effects of ibuprofen (800mg) on cutaneous reactive hyperemia using LDF found no effect.[106] Concentrations of ibuprofen in skin and muscle tissue after oral dosing have been compared with mixed results.[110, 111] Tedeger et al.[110] found similar levels of ibuprofen in cutaneous and muscle tissue after oral dosing with 800 mg, however Dominkus et al.[111] showed that concentrations were lower in cutaneous tissue following oral dosing with 1200 mg. It is possible that we would have seen a blunted response in cutaneous flux with the use of a different NSAID.

Ibuprofen had no impact on the recovery kinetics (τ_2) of any response. Time constants for BABF were significantly faster than for T[Hb+Mb] and both cutaneous measures. This is possibly due to microvascular compliance where the hyperemic flow would be stored in the compliant vascular bed; additional time is required for blood to leave the system resulting in a longer time constant.[112] It is also possible that recovery kinetics were not altered due to redundant vasodilator systems that are present in the forearm such as nitric oxide and inwardly rectifying potassium (K_{IR}) channels and Na^+/K^+ -ATPase.[85] It is possible that once flow resumed, shear induced release of nitric oxide compensated for the lack of prostaglandin production during the ischemic period.[113] . These statements would only be true if T[Hb+Mb] is an analog of microvascular flow. However, we have previously posited that T[Hb+Mb] is related to microvascular hematocrit, not flow.[13]

Previous authors established that capillary hematocrit is proportional to the ratio of RBC flux (flow) to RBC velocity.[37, 58] Kindig et al.[37] found that RBC velocity immediately increased to steady state following exercise onset, while flux increased in 2 phases; a rise to a plateau at 12-20 seconds followed by a secondary rise to steady state values. Capillary hematocrit did not significantly increase until 18 seconds after the initiation of contractions; this increase in hematocrit coincided with the onset of arteriolar dilation due to metabolic feedback. In a previous investigation using both an intramuscular laser Doppler probe to measure flux and a NIRS probe to measure T[Hb+Mb], we observed skeletal muscle microvascular hematocrit and RBC flux increase exponentially upon cuff release and speculated that this rapid increase was due to the arteriolar vasodilation induced by a 5-minute ischemic cuff occlusion.[17] In the

current study, it is likely that inhibition of prostanoid formation by ibuprofen during ischemic conditions decreased the degree of arteriolar vasodilation achieved during ischemia, resulting in a decreased volume of erythrocytes in the area of interrogation as reflected by the blunted T[Hb+Mb] signal under treatment conditions but not placebo.

In healthy blood vessels, prostacyclin (PGI₂), a vasodilator prostanoid which inhibits platelet activation, is the primary prostanoid produced.[114] Thromboxane A₂ has opposite actions to prostacyclin, leading to platelet aggregation and vasoconstriction. There is a shift from prostacyclin production towards thromboxane A₂ during periods of ischemia[83] and in patients with atherosclerotic cardiovascular disease, diabetes mellitus, pulmonary fibrosis and hypertension, peripheral artery disease, smokers and the elderly.[86, 115-118] Pasce et al.[83] proposed that antagonizing the thromboxane prostanoid receptor would enhance post-ischemic vasodilation; the antagonist agent (S18886) had no effect in healthy, young subjects. However, when administered to heart disease patients, this same agent improved endothelial function and reactive hyperemia.[87] It is likely that our results would have been markedly different in a clinical or aged population as ibuprofen induced inhibition of thromboxane A₂ production decreased vasoconstriction resulting in enhanced vasodilation and increased T[Hb+Mb] responses following cuff release.

Accumulation of T[Hb+Mb] During Occlusion

In two previous investigations in our lab between 12% and 25% of our subjects experienced significant accumulation of T[Hb+Mb] such that upon cuff release there was no overshoot during PORH. NIRS can only detect changes in vessels smaller than 1mm in

diameter[14, 15] so this accumulation may represent movement of red blood cells from larger diameter vessels where they would be undetectable by NIRS into smaller vessels where they could be detected, driven by the pressure gradient from artery into capillaries and then into venules [94]. It could also represent vasodilation of the microvasculature under ischemic conditions leading to increased hematocrit.

In our current investigation this phenomenon was not observed in any of our 48 PORH trials. The major difference between the current investigation and these earlier studies was the inclusion of the wrist cuff, as is traditionally done with strain gauge plethysmography,[21] to exclude the hand circulation, thus forming a completely sealed forearm compartment. The wrist cuff may minimize red cell redistribution across AV anastomoses between the distal ends of the radial and ulnar arteries and veins in the distal wrist and proximal hand[95]. Figure 3 shows a single trial where the wrist cuff is inflated following a 60 second baseline reading. As the cuff is pressurized there was a temporary reduction of T[Hb+Mb], but it returned to baseline within 30-45 seconds. These results suggest that the addition of a wrist cuff inflated to suprasystolic pressure may eliminate the accumulation of T[Hb+Mb] without impacting baseline T[Hb+Mb] values in the long term.

CONCLUSION

In conclusion, our results show that the inhibition of prostaglandin formation with the non-selective COX inhibitor ibuprofen slows the initial T[Hb+Mb] response (τ_1 and time to peak) to a 5-minute ischemic cuff occlusion of the brachial artery. Our results also suggest that time series analysis of the microvascular hematocrit response may be a more sensitive technique

for detecting changes in microvascular reactivity that is the traditional measures of forearm blood flow collected with SGP. These results support claims that vasodilator prostaglandins play a role in the initial changes in T[Hb+Mb] during PORH within skeletal muscle, but not within cutaneous tissue. Our findings also suggest that the addition of a wrist cuff may minimize T[Hb+Mb] accumulation during ischemic cuff occlusion. Finally, our results suggest that caution is warranted when interpreting changes in NIRS responses including, microvascular hematocrit among clinical populations and among individuals using NSAIDs.

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Table 0-1: Time constants in seconds listed as mean(SD).

Time Constant	τ_1		τ_2	
	IBU	PLA	IBU	PLA
T[Hb+Mb]	4.3(2.2)*†	2.8(1.1)*	67.7(32.5)‡	55.2(20.2)‡
CVC, Proximal	3.3(1)*	2.7(1.3)*	46.2(19.2)‡	51.9(47.9)‡
CVC, Distal	8.0(11.9)*	11.5(13.4)*	71.2(54.2)‡	44(20.7)‡
BABF	8.1(7.3)*	7.4(3.9)*	19.1(8.8)	19.9(12.1)

T[Hb+Mb], Total hemoglobin+myoglobin

CVC, Cutaneous vascular conductance

BABF, Brachial artery blood flow

* =significantly faster than τ_2

† = significantly longer than placebo (p=0.014)

‡ = Significantly longer than BABF (p<0.001)

Table 0-2: Time to peak (s) and total hyperemic response (AUC) for each response listed as mean(SD).

	Treatment	IBU	PLA
Time to Peak	T[Hb+Mb]	14.5 (5.4) [†]	8.8 (2.9)
	CVC, Proximal	11.4 (3.4)	10.1 (3.9)
	CVC, Distal	16.6 (6)	15.1 (10.6)
	BABF	14.3 (5.9)	10.4 (6.3)
AUC	T[Hb+Mb]	931 (342)	1015 (262)
	CVC, Proximal	48.8 (16.9)	39.3 (15.2)
	CVC, Distal	38.0 (11.6)	30.8 (11.6)
	BABF	559 (246)	513 (275)

[†] = significantly longer than placebo (p=0.002)

Table 0-3: Baseline and amplitude parameters for all responses listed as mean (SD).

Treatment	Y ₀		A ₁		A ₂	
	IBU	PLA	IBU	PLA	IBU	PLA
T[Hb+Mb]*	123.5(7.4)	116.4(17.6)	40.1(1.5)	38.3(3.3)	43.9(7.8)	41.2(4.9)
CVC, Proximal	0.2(0.2)	0.1(0.1)	1.3(0.5)	1.2(0.3)	1.2(0.5)	1.0(0.3)
CVC, Distal	0.1(0.1)	0.2(0.2)	1.2(0.7)	1.1(0.6)	1.0(0.8)	1.0(0.6)
BABF	1.7(0.6)	1.7(0.8)	28.7(27.5)	35.0(34.1)	31.7(28.4)	35.1(33.4)

*-Corrected for ATT

Figure 0-1: Protocol schematic showing 60 second baseline period, 5-minutes of cuff occlusion, cuff release and PORH in cutaneous tissue, proximal skin probe, for both placebo and ibuprofen treatments.

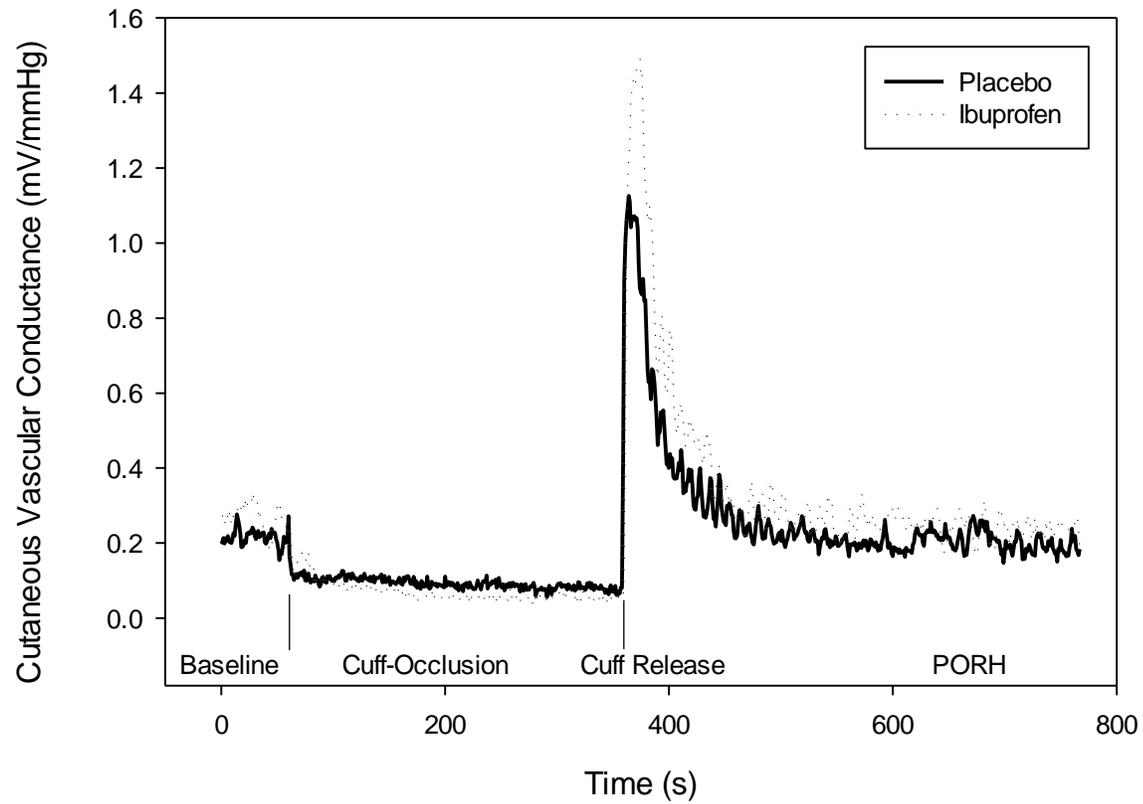


Figure 0-2: T[Hb+Mb] during PORH with baseline(Y_0) and amplitude indicators for both placebo and ibuprofen treatments. Same subject from Figure 1.

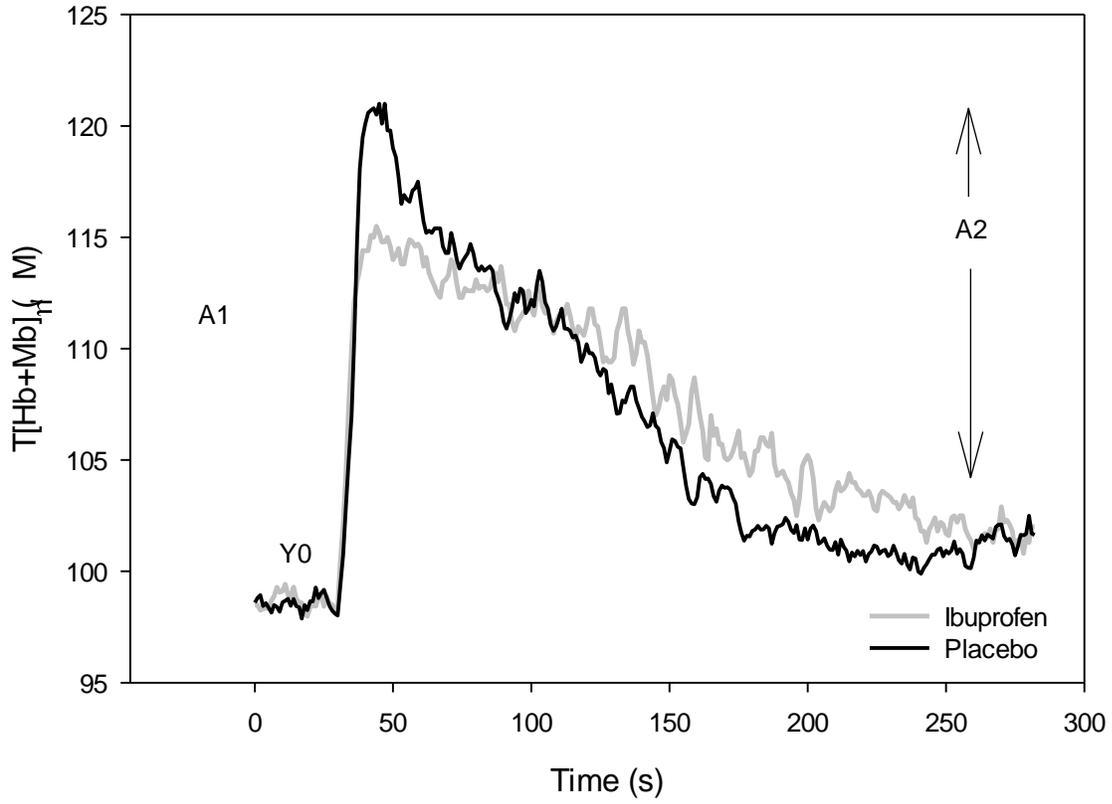
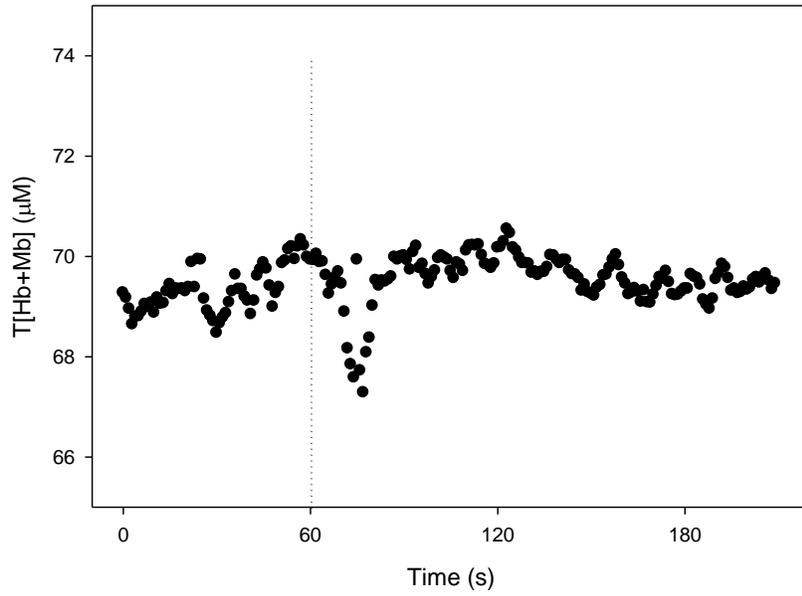


Figure 0-3: Wrist cuff inflation at 60 seconds resulted in a temporary alteration in T[Hb+Mb]. Values returned to baseline within 60-120 seconds.



Chapter 6 - Conclusion

In conclusion, during a period of ischemic cuff occlusion of the brachial artery, deoxy[Hb+Mb] increases and oxy[Hb+Mb] decreases as oxygen is removed from hemoglobin and myoglobin in the forearm vascular compartment.[9] Upon cuff release reactive hyperemia occurs in the brachial artery resulting in a temporary abatement of the Fahraeus effect and an increase in microvascular hematocrit, seen with NIRS as a transient increase in T[Hb+Mb]. As ischemia ends and oxygen rich blood once again flows through the brachial artery, oxy[Hb+Mb] increases and deoxy[Hb+Mb] decreases; the increased T[Hb+Mb] response is only seen because the oxy[Hb+Mb] response is faster than the deoxy[Hb+Mb] response. Recovery kinetics (τ_2) were correlated between T[Hb+Mb] and brachial artery blood flow, however, the brachial artery response is significantly faster than either cutaneous or skeletal muscle microvascular responses in τ_1 and time to peak. In comparisons between cutaneous and skeletal muscle microvascular responses no differences in temporal profiles were observed indicating that the cutaneous microvasculature can serve as a surrogate for the skeletal muscle microvasculature during a temporal analysis of PORH responses in the human forearm. Finally, inhibition of prostaglandin formation with oral ibuprofen slows the initial T[Hb+Mb] response, following a 5-minute ischemic episode indicating that prostaglandins are responsible, at least partially, for the initial T[Hb+Mb] response in skeletal muscle, but not within the cutaneous microvasculature. The addition of a wrist cuff to a PORH protocol can prevent the accumulation of T[Hb+Mb] during the ischemic cuff occlusion that has plagued this technique since it was first used.

While this dissertation has described and attempted to explain mechanisms behind the T[Hb+Mb] response during PORH, there are some questions that remain to be answered.

Chiefly, what exactly is the T[Hb+Mb] response measuring? We have made the case that changes in T[Hb+Mb] reflect changes in microvascular hematocrit, but to my knowledge, no direct comparisons between the two have been made. We also observed differences in temporal profiles within the same skeletal muscle microvasculature as measured with NIRS and intramuscular laser-Doppler devices. The technical and physiological differences behind these distinct profiles are unknown at this time. Lastly, in light of recent evidence suggesting that microvascular reactive hyperemia is more predictive of cardiovascular disease than measures derived from the conduit artery[1, 4, 5], does the ability to assess microvascular reactive hyperemia within skeletal muscle offer clinical utility beyond traditional measures. Of microvascular reactive hyperemia in the cutaneous tissue? The answers to these questions must await future work.

Chapter 7 - References

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113. Paniagua, O.A., M.B. Bryant, and J.A. Panza, *Role of endothelial nitric oxide in shear stress-induced vasodilation of human microvasculature: diminished activity in hypertensive and hypercholesterolemic patients*. *Circulation*, 2001. **103**(13): p. 1752-8.
114. Moncada, S., et al., *An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation*. *Nature*, 1976. **263**(5579): p. 663-5.
115. Udvardy, M., I. Torok, and K. Rak, *Plasma thromboxane and prostacyclin metabolite ratio in atherosclerosis and diabetes mellitus*. *Thromb Res*, 1987. **47**(4): p. 479-84.
116. Cruz-Gervis, R., et al., *Altered prostanoid production by fibroblasts cultured from the lungs of human subjects with idiopathic pulmonary fibrosis*. *Respir Res*, 2002. **3**: p. 17.
117. Carlsson, I., et al., *Differential inhibition of thromboxane A2 and prostacyclin synthesis by low dose acetylsalicylic acid in atherosclerotic patients*. *Thromb Res*, 1990. **57**(3): p. 437-44.
118. Adatia, I., et al., *Thromboxane A2 and prostacyclin biosynthesis in children and adolescents with pulmonary vascular disease*. *Circulation*, 1993. **88**(5 Pt 1): p. 2117-22.

Appendix A – Curriculum Vitae

Christopher Michael Bopp

146c Recreation Hall
Department of Kinesiology
Pennsylvania State University
University Park, PA 16802

814-863-9732

Cmb56@psu.edu

Education:

Ph.D. Candidate (ABD) Anatomy & Physiology **2015**

Kansas State University, Manhattan, KS

Advisor: Thomas J. Barstow

M.S. in Physical Education **2000**

East Stroudsburg University, East Stroudsburg, PA

Advisor: Shala E. Davis

M.S. in Cardiac Rehabilitation and Exercise Science **1999**

East Stroudsburg University, East Stroudsburg, PA

Advisors: Donald Cummings & Gregory Dwyer

B.S. in Exercise Physiology **1997**

University of Massachusetts-Lowell, Lowell, MA

Teaching Experience Pennsylvania State University

KINES 497K	EKG Interpretation	2015
KINES 350	Exercise Physiology	2012
KINES 202	Functional Human Anatomy	2011-14
KINES 298D	Fitness Precertification	2011-15
KINES 457	Exercise Prescription and Case Studies	2011-15
KINES 456	Fitness Appraisal	2010-15
KINES 492W	Programming for Business and Agencies	2010

Teaching Experience Kansas State University

KIN625	Exercise Testing and Prescription	2008-10
KIN520	Practicum in Fitness Settings	2007-10
KIN330	Human Biomechanics	2006-10
KIN605	Topics in Exercise Immunology	2006-07
KIN605	Topics in Clinical Exercise Physiology	2007
KIN603	Cardiovascular Physiology	2007
KIN607	Skeletal Muscle Physiology	2006

Teaching Workshops/Retreats:**Schreyer Institute Classroom Assessment Series**

Item Analysis: Improving multiple choice tests	Nov, 2010
Best Practices for Designing Effective Multiple Choice Tests	March, 2011
Best Practices for PowerPoint – Tips for Teachers	March, 2011

Wakonse Conference for College Teachers*Shelby, Michigan*

May 21-26, 2009

On the Road to Teaching Excellence: Our Journey as Teachers

Manhattan, KS

Jan, 2009

Books

Bopp, C.M. Describing patient adherence in a contemporary cohort of outpatient monitored cardiac rehabilitation patients. Masters Thesis. East Stroudsburg University, 2000.

Peer Reviewed Journal Articles

Bopp, M., **C.M. Bopp**, and M. Schuchert, Active Transportation to and on Campus is Associated With Objectively Measured Fitness Outcomes Among College Students. *J Phys Act Health*, 2015. 12(3): p. 418-23.

Bopp, M., **C. M. Bopp**, M. L. Duffey, R. Ganim and D. N. Proctor (2015). "Implementation and evaluation of an Exercise is Medicine on campus week." *Eval Program Plann* 52: 176-181.

Bopp, C. M. (Author), Townsend, D. K., Warren, S., & Barstow, T. J. (2014). Relationship between brachial artery blood flow and total [hemoglobin+myoglobin] during post-occlusive reactive hyperemia. *Microvasc Res*, 91, 37-43.

Gottschall, J., & **Bopp, C. M.** (2014). The Addition of High Intensity Interval Training Reduces Cardiovascular Disease Risk Factors and Enhances Strength in Active, Healthy Adults. *Open Journal of Preventive Medicine*.

Bopp, MJ, **Bopp, CM**, Schuchert, M. Active transportation to and on campus is associated with objectively measured fitness outcomes among college students. *Journal of Physical Activity & Health*. In Press.

Bopp CM, Townsend DK, Warren S, Barstow TJ. Relationship between brachial artery blood flow and total [hemoglobin+myoglobin] during post-occlusive reactive hyperemia *Microvasc Res.* 2014 Jan;91:37-43

Bopp, CM, Townsend, DK and Barstow, TJ. Characterizing Oxy(Hb+Mb) and Deoxy(Hb+Mb) changes during post-occlusive reactive hyperemia. *Eur J Appl Physiol.* 2011 Nov;111(11):2753-61.

Mohan, A., Hunt, M.C., Barstow, T.J., Houser, T.A., **Bopp, C.**, Hueber, D.M.(2010)Effects of fibre orientation, myoglobin redox form, and postmortem storage on NIR tissue oximeter measurements of beef longissimus muscle. *Meat Science.* 84(1); 79-85.

Wong BJ, Simmons G, **Bopp C**, Yeboah J, Casey DP, Austin C, Tanaka H, DeVan AE, Barnes JN, Atkinson G, Batterham AM, Green DJ, Thijssen DHJ, Hoeks APG, Reneman RS, Joannides R, Bellien J, Groot Pd, Hopman MTE, Hayman MA, Wray DW, Richardson RS, McGowan CL, MacDonald MJ, Pyke KE, Pierce GL, Donato AJ, Silber HA (2009) Commentaries on Viewpoint: Pick your Poiseuille: Normalizing the shear stimulus in studies of flow-mediated dilation. *J Appl Physiol* 107: 1360-1365

Bopp, C.M., Phillips, K.D., Mock, K.S. Mock & G.A. Hand. (2006). "Spiritual Well-being, Sleep Dysfunction and Health Related Quality of Life In HIV Infected Individuals." *Issues in Mental Health Nursing.* Feb-Mar;27(2):125-139.

Dudgeon, W.D., Phillips, K.D., **Bopp, C.M.**, & Hand, G.A. (2004). "Physiological and Psychological Effects of Exercise Interventions in HIV Disease." *AIDS Patient Care and STDs.* Feb; 18 (2):1-16.

Phillips, K.D., W.D. Dudgeon, J. Becker & **C.M. Bopp**. (2004). "Sexually transmitted diseases in men" *Nurs Clin North Am*. Jun;39(2):357-77.

VanVrancken, C., **Bopp, C.M.**, Reis, J.P., DuBose, K.D., Kirtland, K., & Ainsworth, B.E. (2004). "The prevalence of leisure-time physical activity among diabetics in South Carolina." *Southern Medical Journal*, Feb; 97(2): 141-144.

Bopp, C.M., Phillips, K.D., Dudgeon, W.D., Fulk, L.J., Sowell, R., & Hand, G.A. (2004). Physical activity and viral load in HIV-infected individuals. *AIDS Care*, April; 16(3): 387-93.

Bopp, C.M., Phillips, K.D., Fulk, L.J., & Hand, G.A. (2003). Clinical Implications of Therapeutic Exercise in HIV/AIDS. J Assoc Nurses AIDS Care. 14(1); 73-78.

Davis, S.E., Dwyer, G.B., Reed, K., **Bopp, C.M.**, Stosic, J., & Shepanski, M. (2002). Preliminary investigation: The impact of the NCAA Wrestling Weight Certification Program on weight cutting. *Journal of Strength and Conditioning Research*, 16(2); 305-307.

Durstine, J.L., **Bopp, C.**, & Thompson, R. (2001). Exercise Considerations for Diabetes. *Healthy Weight Journal*, 15(5).

Grants

Bopp, C. M. (Co-Principal Investigator), Duffey, M. L. (Co-Principal Investigator), Grant," Expansion of Project MORE²FIT² to State College Area High School," Mary Anna Mangino Community Service Endowment, Pennsylvania State University. Total requested: \$2270, total awarded \$2300.

Bopp, C. M. (Co-Principal Investigator), Duffey, M. L. (Co-Principal Investigator), Grant, "Mobile Outreach & Regional Expansion of EiM and Fitness Testing (MORE2FIT2)," Nardozzo

Community Service Endowment, Penn State. Total requested: \$2,000.00. Total awarded: \$2,000.00. (submitted: June 2014, date funding awarded: July 2014, funded: August 2014 - Present).

Bopp, C. M. (Co-Principal Investigator), Duffey, M. L. (Co-Principal Investigator), Grant, "Mobile Outreach & Regional Expansion of EiM and Fitness Testing (MORE2FIT2)," Schreyer Institute for Teaching Excellence, Penn State. Total requested: \$7,270.00. Total awarded: \$7,270.00. (submitted: March 2014, date funding awarded: April 2014, funded: April 2014 - Present).

Bopp, C. M. (Principal Investigator), Grant, "The Mary Anna Mangino Community Service Endowment," HHD / MAM, Penn State. Total requested: \$1,800.00. Total awarded: \$1,800.00. (submitted: December 2013, date funding awarded: January 2014).

Bopp, C. M. (Principal Investigator), Grant, "Development of an Online 12-Lead EKG Simulator," Schreyer Institute, Penn State. Total requested: \$6,940.00. Total awarded: \$6,940.00. (submitted: April 1, 2012, date funding awarded: June 11, 2012, funded: July 1, 2012 - Present).

Phillips, K. (Principal Investigator), **Bopp, CM** (Student Investigator), Grant, "The Effect of a Prescribed Exercise Protocol in Reducing Perceived and Physiological Stress, and Improving Mood and Sleep Quality in HIV-infected Individuals," Center for Health Promotion & Risk Reduction in Special Populations (National Institute of Nursing Research Funded Center). Amount: \$10,000. Funded 2002-2004.

Bopp, C.M., Grant, "Heart Failure and Creatine Usage," Gatorade Sport Science Institute Graduate Student Research Grant. Amount: \$1500.00

Other Funding

University of South Carolina Family Fund 2002-04
Faculty/Staff Stress Testing and Cholesterol Screening Program
Awarded to Preventive Exercise Program, Department of Exercise Science
Total Amount: \$17,000

Awards

First Place Student Award Winner

2003

“Differential time course for improvements in mental and physical health following exercise training in HIV-infected individuals.” The Cooper Institute Conference Series, Physical Activity and Mental Health: A Multidisciplinary Approach, Dallas, Texas.

Abstracts and Presentations

Bopp, CM, Duffey, ML, Z, Papalia. Prevalence of metabolic syndrome and dyslipidemia among college aged males and females. American College of Sports Medicine Annual Meeting, San Diego, CA, 2015.

Bopp, CM, Gottschall, JS, Hastings, B. Comparative effects of moderate-intensity, continuous training versus high-intensity interval training (HIIT) in the reduction of cardiovascular disease risk factors American College of Sports Medicine Annual Meeting, Indianapolis, IN, 2013

Bopp, CM, Wong, BJ, Ade, CA, Broxterman, RM, Wilcox, S., and Barstow, TJ. Ibuprofen alters initial time course of hyperemic response within skeletal muscle, but not cutaneous, microvasculature during post-occlusive reactive hyperemia, American College of Sports Medicine, Denver, CO. May 2011.

Bopp, CM, Townsend, DK, Wong, BJ, Ade, CJ & Barstow, TJ (FACSM), Variation in near-infrared spectroscopy and cutaneous and intramuscular laser-Doppler results during ischemia and

post-occlusive reactive hyperemia, American College of Sports Medicine Annual Meeting, Baltimore, MD, June 3rd, 2010.

Bopp, C.M., Townsend, D.K. and Barstow, T.J. “Relationship between brachial artery blood flow and total[hemoglobin+myoglobin] during post-occlusive reactive hyperemia.” American College of Sports Medicine, Seattle, WA, 2009.

Bopp, C.M., Townsend, D.K. and Barstow, T.J. “Characterizing Oxy(Hb+Mb) and Deoxy(Hb+Mb) changes during post-occlusive reactive hyperemia.” American College of Sports Medicine, Annual Meeting, Indianapolis, IN, 2008.

Bopp, C.M., Phillips, K.D., Burgess, S., Dudgeon, W.D., Durstine, J.L., & G.A. Hand. “Measuring physical activity in an HIV-infected individual.” Southeast American College of Sports Medicine Annual Meeting, Atlanta, Georgia. January 2004

Dudgeon, W.D., Phillips, K.D., **Bopp, C.M.**, Ewing, G., Durstine, L.J., & Hand, G.A. “Effects of 5 weeks of exercise on health of HIV-infected individuals.” Southeast American College of Sports Medicine Annual Meeting, Atlanta, Georgia. January 2004.

Bopp, C.M., Phillips, K.D., Dudgeon, W.D., Burgess, S., Ewing, G.B., Durstine, J.L., & G.A. Hand. “Differential time course for improvements in mental and physical health following exercise training in HIV-infected individuals.” The Cooper Institute Conference Series, Physical Activity and Mental Health: A Multidisciplinary Approach, Dallas, Texas. October 2003

Dudgeon, W.D., Phillips, K.D., **Bopp, C.M.**, Fulk, L.J., Burgess, S., Durstine, L.J., & Hand, G.A. (2003) “The association of body fat distribution with mental and physical health in HIV-infected adults.” The Cooper Institute Conference Series, Physical Activity and Mental Health: A Multidisciplinary Approach, Dallas, Texas. October 2003

Teas, J., Raghavan, S., Nitcheva, D., Lamb, L., Mech, P., **Bopp, C.M.**, et al. "Can eating seaweed make a high fat fast food breakfast healthier?" University of South Carolina, South Carolina Nutrition Research Summit, Columbia, SC. October 2003.

Phillips K.D., Skelton W.D., Fulk L.J., **Bopp C.M.**, and G.A. Hand. Acupuncture and HIV symptom management: The association of stress-reduction and the reduction of oral conditions. (Presentated at Association of Nurses in AIDS Care National Meeting, New York, NY 2003)

Phillips, K.D., **Bopp, C.M.**, Dudgeon, W.D., Fulk, L.J. & Hand, G.A. Therapeutic Exercise in HIV-infected Individuals: Effects on Mental and Physical Health (Presentated at Association of Nurses in AIDS Care National Meeting, New York, NY 2003)

Hand G.A., Phillips K.D., Skelton W.D., Fulk L.J., and **Bopp, C.M.** Reduction of stress-related oral disease by acupuncture performed in a group setting. (Presented at the International AIDS Society Annual Meeting, Paris, France, 2003).

Bopp, C.M., Phillips, K.D., Dudgeon, W.D., Fulk, L.J., Ewing, G., Durstine, J.L., and G.A. Hand: The effects of 6-weeks of exercise training on mental and physical health in HIV-infected men and women. *PNIRS Meeting Abstracts* 17: 162, 2003.

Dudgeon, W.D., Phillips, K.D., **Bopp, C.M.**, Fulk, L.J., Ewing, G., Durstine, J.L., and G.A. Hand: Relationships among mental health, physical health and regional body fat distribution in HIV-infected individuals. *PNIRS Meeting Abstracts* 17: 171, 2003.

Fulk L.J., Phillips K.D., **Bopp C.M.**, and G.A. Hand: Effects of acupuncture on emotional distress, salivary cortisol, and stress-related oral disease in HIV-infected subjects. *PNIRS Meeting Abstracts* 17: 176, 2003.

Brewer R.B., **Bopp C.M.**, Phillips K.D., Durstine J.L., and G.A. Hand: The Effects of Aerobic and Resistance Training on Muscle Strength in HIV-Infected Individuals. Alpha Xi Chapter, Sigma Theta Tau, *ViewPoints*, 2003.

Kane, B.E., Fulk, L.J., **Bopp, C.M.**, and G.A. Hand: Corticotropin-releasing hormone (CRH) release in the amygdala varies with different types of stressors. Alpha Xi Chapter, Sigma Theta Tau, *ViewPoints*, 2003.

Dudgeon, W.D., Phillips, K.D., **Bopp, C.M.**, and G.A. Hand: Effects of aerobic exercise on body composition of HIV-infected individuals. Alpha Xi Chapter, Sigma Theta Tau, *ViewPoints*, 2003.

Bopp, C.M., Phillips, K.D., Fulk, R.L., Sowell, R.L., Durstine, J.L., and G.A. Hand: Immunity and physical activity in HIV-infected individuals. Alpha Xi Chapter, Sigma Theta Tau, *ViewPoints*, 2003.

Bopp C.M., Phillips K.D., Fulk L.J., Sowell R.L., Durstine J.L., and G.A. Hand: Physical activity and immunity in HIV-infected individuals. *SEACSM Abstracts* 31: O42, 2003.

Fulk L.J., **Bopp C.M.**, Phillips K.D., and G.A. Hand: Effect of muscle contraction on medullary neuronal activity. *SEACSM Abstracts* 31: O37, 2003.

Brewer R.B., Fulk L.J., **Bopp C.M.**, Phillips K.D., Durstine J.L., and G.A. Hand: Spinal cholinergic activity and the exercise pressor reflex. *SEACSM Abstracts* 31: O36, 2003.

Kane B.K., Fulk L.J., **Bopp C.M.**, and G.A. Hand: Is rodent treadmill running a model for human exercise: CRH and the amygdala. *SEACSM Abstracts* 31: P20, 2003.

Phillips K.D., Sowell R.L., Fulk L.J., **Bopp C.M.**, and G.A. Hand: The relationship between sleep quality and determinants for health related quality of life in HIV+ women. *ANAC Abstracts* 14, P22, 2002.

Phillips K.D., Sowell R.L., **Bopp C.M.**, Rojas M., Fulk L.J., and G.A. Hand: Physical activity levels are inversely related to HIV-RNA viral load in HIV-infected individuals. *ANAC Abstracts* 14, P28, 2002.

Bopp, C., Davis, S.E., Dwyer, G., Smith, J. Describing Patient Adherence in A Contemporary Cohort of Outpatient Monitored Cardiac Rehabilitation Patients. (2000) Presented at Sigma Xi Student Research Symposium, East Stroudsburg University, East Stroudsburg, PA.

Bopp, C., Davis, S. E., FACSM, Dwyer, G. B., Stosic, J., Reed, K., Holtzapple, N., & Farin, J. Nutrition Knowledge of Selected Collegiate Athletic Teams. *Medicine and Science in Sport and Exercise*, 32(5), S285.,2000.

Davis, S.E., FACSM, Dwyer, G. B., Reed, K., Stosic, J., Farin, J., **Bopp, C.**, Shepanski, M., & Holtzapple, N. Nutrition Knowledge Evaluation of Collegiate Athletes. *Medicine and Science in Sport and Exercise*, 32(5), S286., 2000.

Dwyer, G. B., Davis, S.E., FACSM, Reed, K., Stosic, J., Holtzapple, N., **Bopp, C.** NCAA Mandated Wrestling Weight Certification Program Concerns. *Medicine and Science in Sport and Exercise*, 32(5), S209. 2000.

Reed, K., Dwyer, G.B., Davis, S.E., FACSM, **Bopp, C.**, Stosic, J., Holtzapple, N., Shepanski, M. 2000. Short Term Weight Loss History of Collegiate Wrestlers During NCAA Weight Certification. *Medicine and Science in Sport and Exercise*, 32(5), S180. 2000.

Abstracts and Presentations By Students

Erickson, CE, Bopp, CM. The Effects of a 15-Week Physical Activity Class on health-related physical fitness. Podium Presentation, Mid-Atlantic Regional Chapter of ACSM, November 1, 2014.

Orkin, SE, Bopp, CM. ACSM Risk Classification and Risk Factor Prevalence Rates Among College Students. Poster Presentation, Mid-Atlantic Regional Chapter of ACSM, November 1, 2014.

Dubiel, WD, Bopp, CM. Comparison of cholesterol and glucose levels between college-aged males and females. Poster Presentation, Mid-Atlantic Regional Chapter of ACSM, November 1, 2014.

Callestine, J., Bopp, MJ, Bopp, CM. College student work habits are related to physical activity and fitness. Society of Behavioral Medicine, Philadelphia, PA 2014.

Academic Service

2013-	Director, Center for Fitness and Wellness
2012-13	Co-Director, Student Fitness Assessment Center
2012-13	Talent Search/TRIO Program Coordinator, Kinesiology Department
2007-10	LIFE Program Director
2007-08	Kinesiology Curriculum Committee Member
2007-08	ACSM HFI Planning
2007-10	Human Resources Benefits Fair, Kinesiology Representative
2007-08	Project GROW: Activity planner and supervisor
2006-10	Department of Kinesiology University Wide Open House Representative
2004	Columbia Urban League Youth Empowerment Expo 2004 Arnold School of Public Health Representative

- 2001-04 ACSM Health/Fitness Instructor Workshop Planning Committee
University of South Carolina, Columbia, SC
- 2001-04 Undergraduate Student Admission Fair Department Representative
- 2000 ACSM Exercise Specialist Practicum Examiner
East Stroudsburg University, East Stroudsburg, PA

Clinical Experience

- 2005-06 Field Clinical Research Coordinator, ClinForce, St. Louis, MO
- 2004-05 Clinical Research Coordinator, Neem Research Group, *Columbia, SC*
- 2001-04 Manager, Preventive Exercise Program Supervised Exercise Facility, University of South Carolina, *Columbia, SC*
- 2000-04 Stress Testing Director, University of South Carolina, *Columbia, SC*
- 2001-02 Exercise Physiologist, The Imaging Company, *Columbia, SC*

Professional Affiliations

- American College of Sports Medicine **1998-15**
- ACSM Regional Chapter – Central States **2006-09**
- ACSM Regional Chapter – Southeast Region **2000-04**

Certifications

- | | | |
|--------------------------------------|--------------------|------------------|
| Clinical Exercise Specialist | ACSM | 1999-2015 |
| Exercise is Medicine, Level III | ACSM | 2012-2015 |
| Trainer for CPR/AED/FA/BBP | American Red Cross | 2014-16 |
| Clinical Research Coordinator | ACRP | 2005-07 |
| Phlebotomy | USC | 2000-04 |
| Certificate in Epidemiology (SS3030) | CDC | 1998 |