

# Characterizing near-infrared spectroscopy responses to forearm post-occlusive reactive hyperemia in healthy subjects

Christopher M. Bopp · Dana K. Townsend ·  
Thomas J. Barstow

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**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.

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T. J. Barstow  
Department of Kinesiology, Kansas State University,  
Manhattan, KS 66506, USA

C. M. Bopp · T. J. Barstow  
Department of Anatomy and Physiology,  
Kansas State University, Manhattan, KS 66506, USA

D. K. Townsend  
Division of Biology, Kansas State University,  
Manhattan, KS 66506, USA

C. M. Bopp  
Department of Kinesiology, Pennsylvania State University,  
State College, PA 16803, USA

T. J. Barstow (✉)  
Kansas State University, 1a Natatorium,  
Manhattan, KS 66506, USA  
e-mail: tbarsto@ksu.edu

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## 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 (Rossi et al. 2008; Shechter et al. 2009; Suzuki et al. 2008; Takase et al. 2008). 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 (Bakker et al. 2009). 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 (Jobsis 1977). 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 Marcinek et al. 2007). 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 (Hamaoka et al. 2007). 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 (Kragelj et al. 2001). 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 ultrasound, under the NIRS probe was  $4.62 \pm 1.45$  mm. Average Body Mass Index was  $23.6 \pm 3.7$  kg/m<sup>2</sup>, mean resting heart rate was  $66 \pm 8$  beats/min, mean systolic blood pressure was  $119 \pm 9$  mmHg and mean diastolic blood pressure was  $72 \pm 7$  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 (Ferreira et al. 2005). 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.

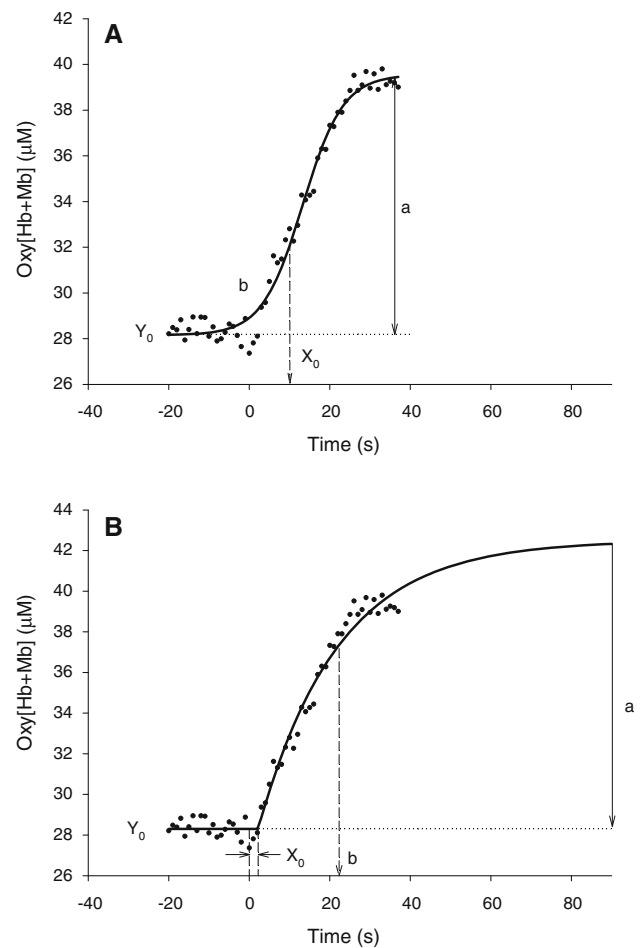
$$Y = Y_0 + a/(1 + e^{-(X-X_0)/b}) \tag{1}$$

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

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

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  $\Delta$ 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



**Fig. 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 time delay (b). Parameters have same meaning for logistic function as for Gompertz. See text and Eqs. 1–3 for more information

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.

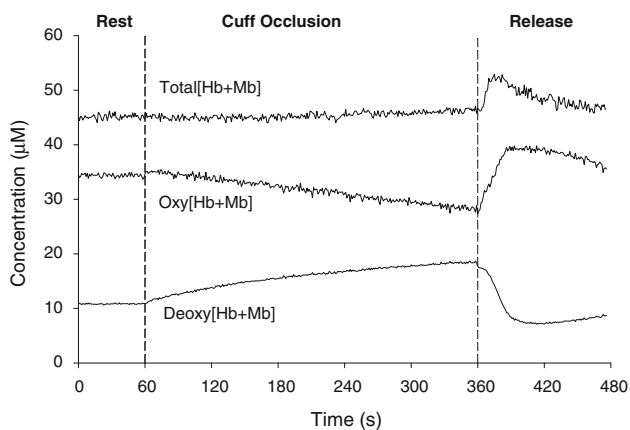
Results

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

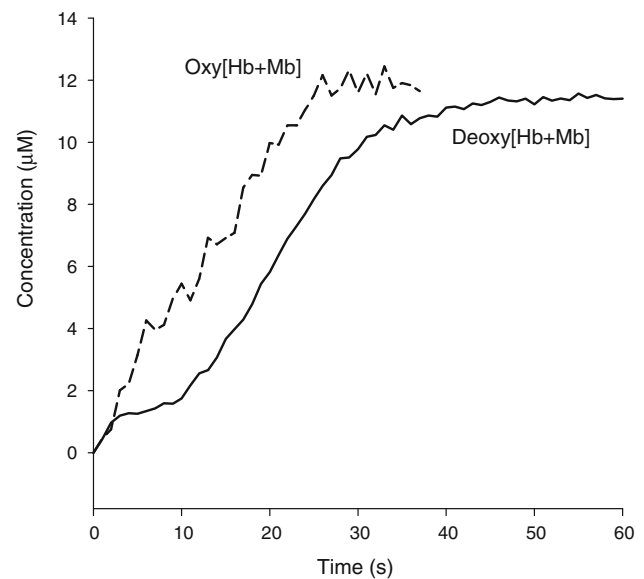
occlusion and PORH following cuff release. Upon cuff release, while the time course of changes in oxy[Hb+Mb] and deoxy[Hb+Mb] appeared similar, they were not identical as demonstrated in Fig. 3. Oxy[Hb+Mb] increased as soon as the cuff was released. However, the deoxy[Hb+Mb] response had a slower rate 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. 2** Complete experiment for one subject. Cuff inflation occurred after 60 s of baseline data collection, and was maintained for 5 min. Post-occlusive reactive hyperemia (PORH) was monitored for 120 s in this example

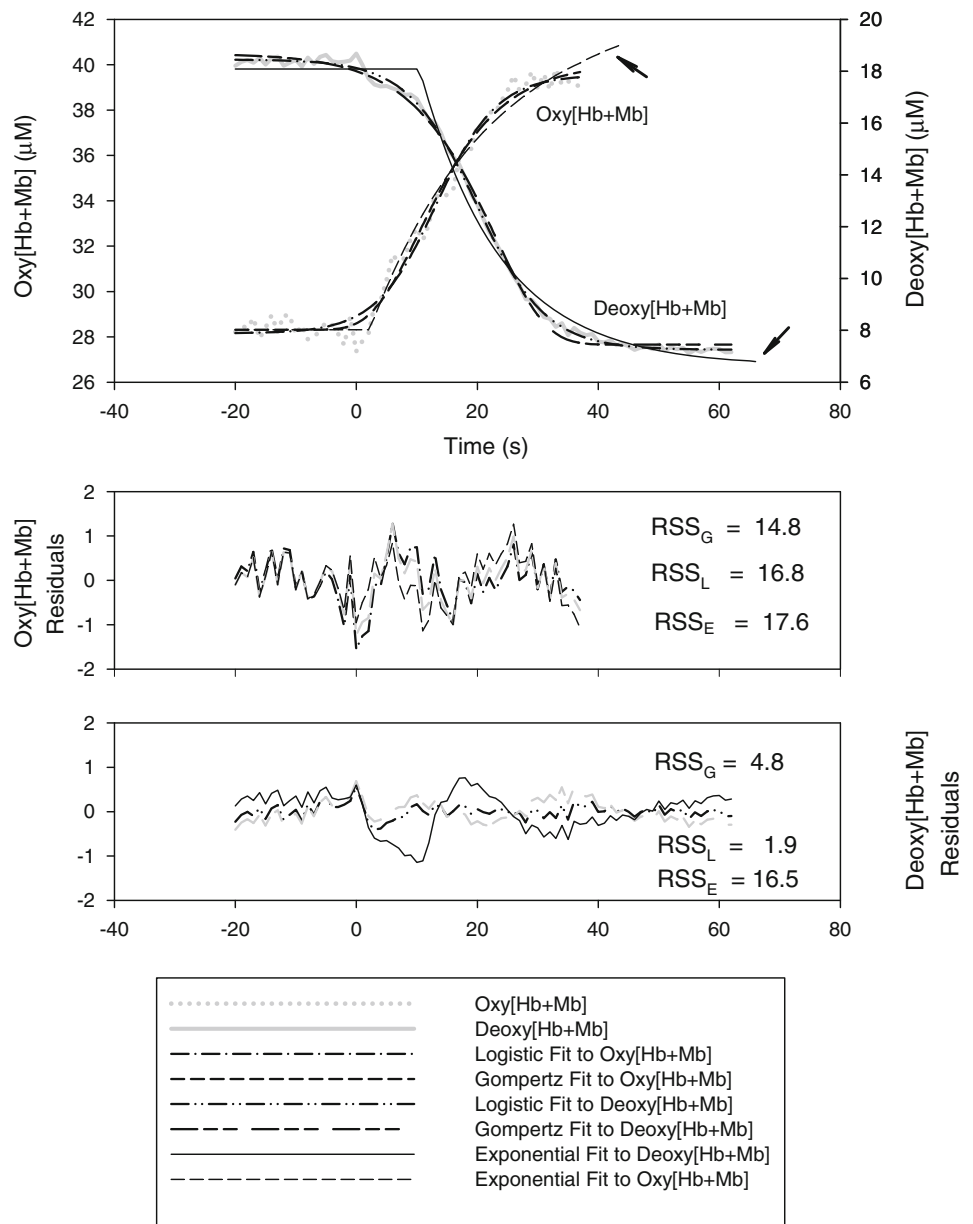


**Fig. 3** Oxy[Hb+Mb] and deoxy[Hb+Mb] response curves during PORH from the same subject as in Fig. 1. Deoxy[Hb+Mb] response has been inverted for comparison. Time point 0 indicates cuff release

(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 the line of identity (i.e., were greater than the corresponding 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).

**Fig. 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 projection of exponential fit past plateau of both responses (shown by *arrows*)



**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 (DeLorey et al. 2004; Grassi et al. 2003; Koga et al. 2007), 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 (Maguire et al. 2007; Takakura et al. 2010; Masuda et al. 2010). 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

**Table 1** RSS values for all functions fit to the deoxy[Hb+Mb] response

Subject	RSS <sub>G</sub>	RSS <sub>L</sub>	RSS <sub>E</sub>	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 number of data points in regression, equal to 20 s baseline data plus time from cuff release until peak response. RSS is residual sum of squared errors for Gompertz (RSS<sub>G</sub>), logistic (RSS<sub>L</sub>), and exponential (RSS<sub>E</sub>) functions

\* Significantly different from both RSS<sub>G</sub> and RSS<sub>E</sub>

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.

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

Subject	RSS <sub>G</sub>	RSS <sub>L</sub>	RSS <sub>E</sub>	N
1	17.0	22.3	23.5	51
2	83.2	99.9	102	57
3	668	678	841	55
4	1,424	1,611	1,594	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 number of data points in regression, equal to 20 s baseline data plus time from cuff release until peak response. RSS is residual sum of squared errors for Gompertz (RSS<sub>G</sub>), logistic (RSS<sub>L</sub>), and exponential (RSS<sub>E</sub>) functions

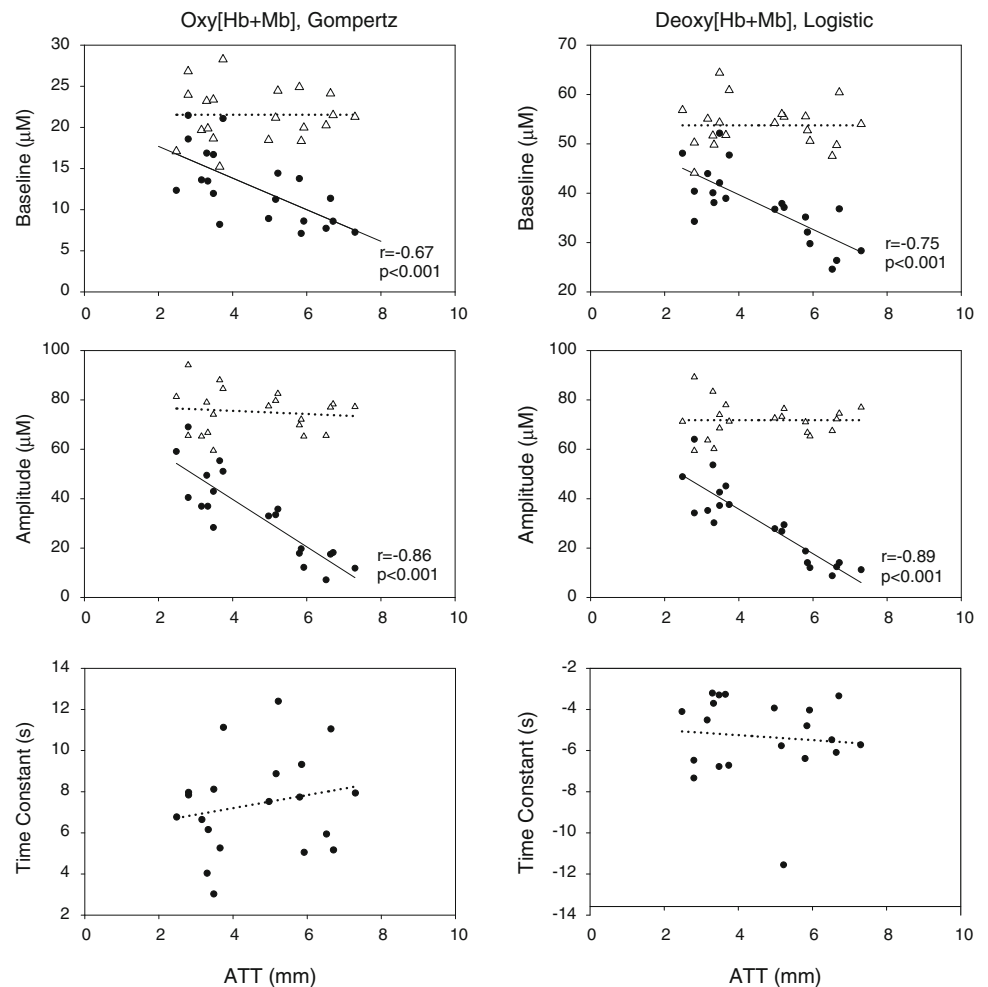
\* Significantly different from both RSS<sub>L</sub> and RSS<sub>E</sub>

To our knowledge, only Kragelj et al. (Kragelj et al. 2001) 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). 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

**Fig. 5** Original (circles) and corrected (triangles) baseline ( $Y_0$ ), amplitude ( $a$ ) and time constant ( $b$ ) values plotted against ATT



**Table 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	$12.7 \pm 4.5$	$30.2 \pm 15.7$	$-5.3 \pm 2.0$	$16.9 \pm 6.8$
Corrected	$21.5 \pm 3.3^c$	$71.8 \pm 7.2^c$		
Oxy[Hb + Mb] (Gompertz)				
Uncorrected	$37.5 \pm 7.2^b$	$33.8 \pm 17.3^a$	$7.4 \pm 2.4^b$	$9.4 \pm 4.0^b$
Corrected	$53.8 \pm 4.7^{bc}$	$75.1 \pm 8.9^{ac}$		

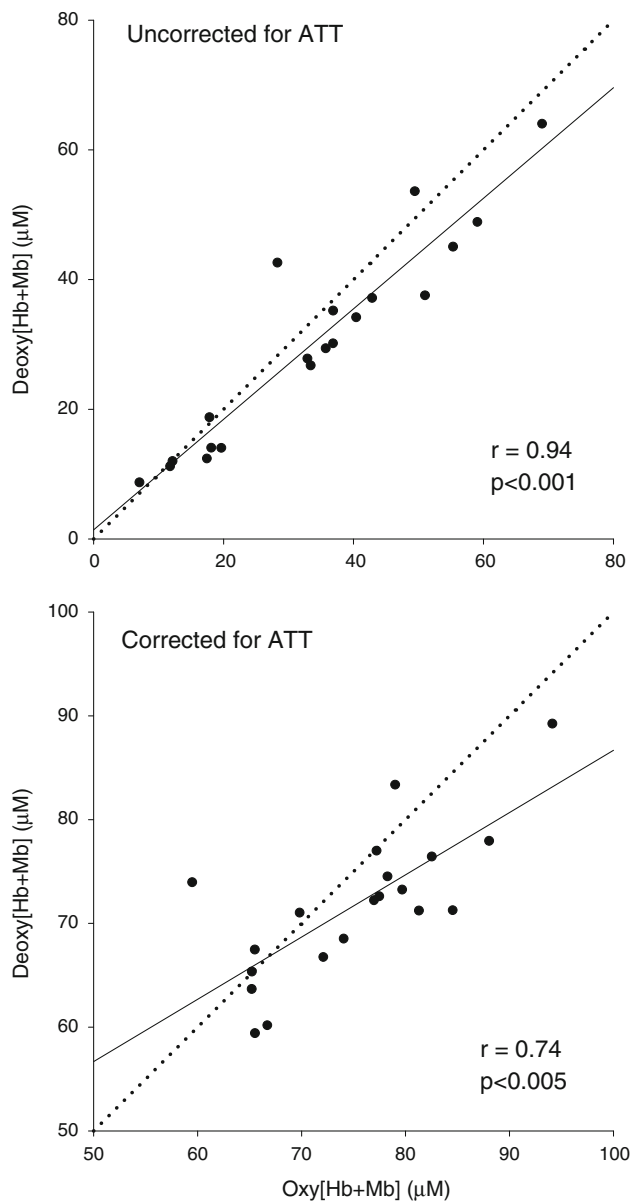
Values are mean  $\pm$  standard deviation. See Eqs. 1 and 2 for meaning of symbols

Significant difference between Oxy[Hb+Mb] and Deoxy[Hb+Mb] values: <sup>a</sup> $p < 0.02$  and <sup>b</sup> $p < 0.001$ . <sup>c</sup>Significantly different from uncorrected-for-ATT values ( $p < 0.001$ )

represents increased microvascular hematocrit (Boushel et al. 2001; Kindig et al. 2002; Klitzman and Duling 1979). 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

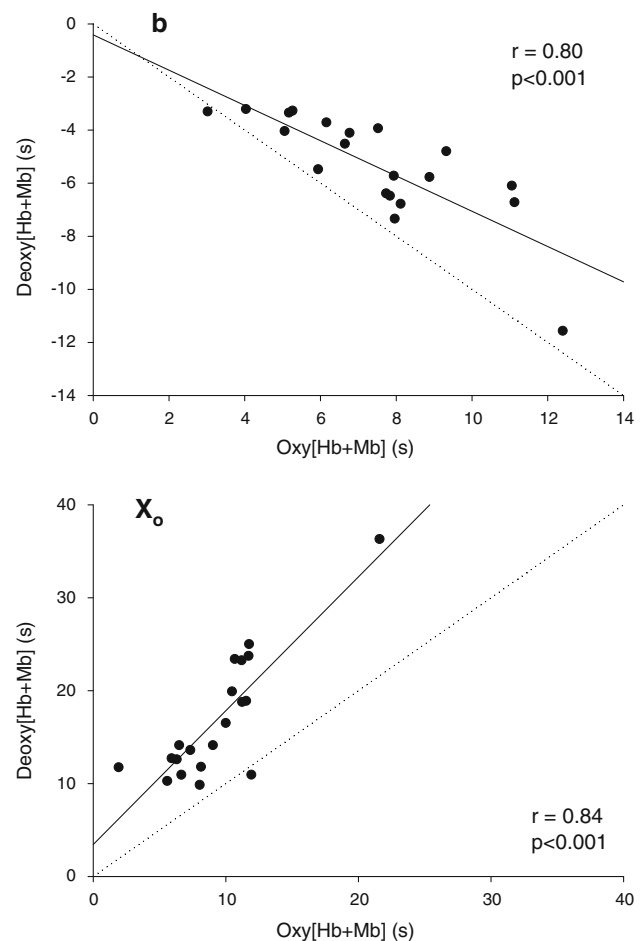
oxy[Hb+Mb] during PORH may involve additional unloading of oxygen during the early recovery phase of PORH to refill tissue Mb stores utilized during the ischemic period. Tran et al. (1999) demonstrated that during cuff ischemia of the lower leg, Mb desaturation by NMR reached a maximum value by  $\sim 4.5$  min. Given that Mb content of human skeletal muscle is greater than previously thought (Marcinek et al. 2007; Davis 2009), 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. (2004).

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



**Fig. 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

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. (2001) 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 (Bakker et al. 2009). Evaluation of the impact of diseases which affect microvascular circulation must await future studies.



**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

## 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 O<sub>2</sub> from Hb during the early phase of PORH to restore tissue MbO<sub>2</sub> stores.

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