**Impact of supine exercise on muscle deoxygenation kinetics heterogeneity: Mechanistic insights into slow pulmonary oxygen uptake dynamics**

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**New & Noteworthy**

* We show that supine exercise causes a greater degree of muscle deoxygenation in both deep and superficial muscle and increases the spatial heterogeneity of muscle deoxygenation.
* This study therefore suggests that any O2 delivery gradient towards deep versus superficial muscle is insufficient to mitigate impairments in oxidative function in response to reduced whole-muscle O2 delivery. More heterogeneous muscle deoxygenation is associated with slower O2 kinetics.

**Abstract**

Oxygen uptake (O2) kinetics are slowed in the supine (S) position due to impaired muscle O2 delivery (O2), however, these conclusions are predicated on single-site measurements in superficial muscle using continuous-wave near-infrared spectroscopy (NIRS). This study aimed to determine the impact of body position (i.e. upright [U] vs. S) on deep and superficial muscle deoxygenation (deoxy[heme]) using time-resolved (TR-) NIRS, and how these relate to slowed pulmonary O2 kinetics. 17 healthy men completed constant power tests during 1) S heavy intensity exercise; and 2) U exercise at the same absolute work rate, with a subset of 10 completing additional tests at the same relative work rate as S. Pulmonary O2 was measured breath-by-breathand, deoxy- and total[heme] were resolved via TR-NIRS in the superficial and deep *vastus lateralis* and superficial *rectus femoris*. The fundamental phase O2 time constantwas increased during S compared to U (S: 36±10 vs. U: 27±8 s, *P*<0.001). The deoxy[heme] amplitude (S: 25-28 vs. U: 13-18 µM, *P*<0.05) and total[heme] amplitude (S: 17-20 vs. U: 9-16µM, *P*<0.05) were greater in S compared to U and were consistent for the same absolute (above data) and relative work rates (*n*=10, all *P*<0.05). The greater deoxy- and total[heme] amplitudes in S vs. U supports that reduced perfusive O2 in S, even within deep muscle, necessitated a greater reliance on fractional O2 extraction and diffusive O2. The slower O2 kinetics in S vs. U demonstrates that, ultimately, these adaptations were insufficient to prevent impairments in whole-body oxidative metabolism.

**Key words:** Time-resolved near-infrared spectroscopy, oxygen delivery, muscle deoxygenation, oxidative metabolism

**INTRODUCTION**

In young, healthy individuals, it is generally considered that muscle and pulmonary O2 uptake (O2) kinetics are not constrained by muscle O2 delivery (O2) (54, 57), however this issue remains somewhat contentious (47). This ongoing controversy may, in part, be related to the fact that many previous investigations reaching the conclusion that O2 does not limit O2 kinetics have relied on “bulk” measurements of O2 (i.e. conduit artery blood flow, mixed venous effluent sampling) and/or single-site, superficial measurements of muscle deoxygenation (as deoxy[heme]) via continuous-wave near-infrared spectroscopy (CW-NIRS). However, in recent years, our group has shown that substantial spatial and temporal heterogeneity exists with respect to the muscle deoxy[heme] kinetic responses to constant work-rate and ramp incremental exercise (8, 9, 39, 40, 51), and that different muscles among the exercising muscle mass may rely on fundamentally different O2 transport and utilization strategies (37, 38, 51). Consequently, it remains likely that in a given individual, O2 transport may be limiting in some muscle regions and not others at any given time during the exercise transient. Hence, previous conclusions that O2 delivery was not limiting O2 kinetics lacked the granularity to determine whether this was true across the entirety of the muscle mass. Indeed, poorer spatial matching of O2-to-O2 would likely slow O2 kinetics in some muscle regions (29, 42), however, a lower degree of muscle deoxygenation heterogeneity has previously been associated with both slower (8) and unchanged O2 kinetics (39). Hence, the effect that regional heterogeneity in the O2/O2 ratio exerts on whole-body O2 kinetics, if any, remains unclear.

A useful intervention with which to manipulate O2 to assess regional control of O2 transport/utilization, and its subsequent impact on whole-body O2 kinetics, is supine exercise. Supine exercise impairs muscle perfusion pressure and slows O2 kinetics due to the loss of the hydrostatic gradient effect, effects which increase the fundamental phase time constant of O2 kinetics (O2; i.e. O2 kinetics become slower) (21, 36, 45). Indeed, a greater muscle deoxy[heme] amplitude (15) and a tendency for faster deoxy[heme] kinetics (36) have been noted in the supine position previously. However, previous comparisons of upright vs. supine exercise have used single-site measurements of CW-NIRS (15, 36), which conceals a substantial degree of deoxygenation heterogeneity (and hence, differences in O2/O2 matching) across the exercising muscle mass (20, 38–42, 49–51) and does not permit the quantification of absolute values of deoxy[heme] or total[heme]. A further limitation of previous studies comparing upright vs. supine exercise is that comparisons were made at the same absolute work rate, and thus the relative exercise intensity differed between modes (36, 43). Differences in muscle recruitment patterns between body positions due to differences in relative exercise intensity, rather than differences in O2 *per se*, therefore, may have been responsible for the differences in the speed of the O2 kinetics between postures. Indeed, previous comparisons of upright vs. supine cycling did not include measures of muscle recruitment (e.g. electromyography; EMG, (21, 36, 43), which could confound comparisons of muscle deoxygenation between body positions (9, 39). Therefore, the extent to which differences in pulmonary O2 kinetics between upright and supine exercise reflects differences in intramuscular O2-O2 matching, differing muscle fiber recruitment regimens between exercise modes, or inaccuracies related to CW-NIRS instruments (19, 41), remains unclear. Moreover, the influence of body position on the regional heterogeneity of muscle deoxygenation itself is presently unknown.

Specifically, deep muscle, with its higher O2/O2 (37–39, 51) and type I fibre proportion (35) compared to superficial muscle, might be considered more likely to sustain its local O2/O2 in the face of impaired whole-muscle O2 delivery. In contrast, the *rectus femoris*, which appears to evince characteristics more typical of muscles comprised predominantly of type II fibres when compared to the *vastus lateralis* (49), might display a relatively greater impairment in O2/O2 in response to compromised whole-muscle O2 delivery. Hence, investigating how impaired whole-muscle O2 delivery influences discrete muscular regions has the potential to provide a considerable amount of new information on regional control of muscle O2 transport and utilization. Such information would be highly valuable for the design of novel therapeutic interventions aimed at enhancing exercise tolerance in both healthy individuals and patient populations.

The present investigation compared pulmonary O2 kinetics, muscle deoxy[heme] kinetics, total[heme] responses (in the VLd, superficial VL[VLs] and *rectus femoris* [RFs]) and muscle EMG patterns (RFs and VLs) between supine and upright cycling at the same absolute and relative work rates. We hypothesized that in the face of slower pulmonary O2 kinetics during supine exercise, 1) superficial muscle deoxy[heme] kinetics would increase with greater amplitude and faster kinetics (i.e. reflecting a lower O2/O2), 2) total[heme] would be lower (i.e. reflecting lower orthostatic pressure), 3) this would promote a greater regional heterogeneity of muscle deoxygenation, but 4) deep muscle would be unaffected by the performance of supine exercise. Finally, we also hypothesized that 5) differences between upright and supine exercise observed at the same absolute work rate would persist for equivalent relative work rates, and when accounting for muscle activation patterns. Realization of the latter hypothesis would vindicate reduced O2 as responsible for slower O2 kinetics during supine exercise *per se*, rather than altered muscle fiber recruitment profiles.

**METHODS**

This study was conducted in two parts. Part 1 compared O2 kinetics, muscle deoxy[heme] and total[heme] in superficial muscle (VLs and RFs) between upright and supine exercise at the same absolute work rate in seventeen healthy male subjects. Part 2 utilised a subset of ten participants to compare O2 kinetics, muscle deoxy[heme] and total[heme] in superficial (VLs and RFs) and deep muscle (VLd) and muscle activation patterns (integrated EMG [iEMG] of the VLs and RFs) between upright and supine exercise at the same relative work rate (i.e. Δ40% in both modes).

*Participants.* In Part 1, seventeen healthy male participants (age: 24 ± 5 yr; height 175 ± 6 cm; weight 67 ± 9 kg; upright cycling O2 peak 50 ± 9 mL.kg-1.min-1) took part, whereas in Part 2 a subset of ten participants representative of the initial group (age: 23 ± 5 yr; height 175 ± 7 cm; weight 70 ± 12 kg; upright cycling O2 peak 49 ± 6 mL.kg-1.min-1) volunteered to take part. All participants provided written informed consent. The experiment was approved by the Human Subjects Committee of Kobe Design University (approval number 2019-3) and conformed to the Declaration of Helsinki, with the exception of registration in a database. Participants were instructed to avoid alcohol and strenuous exercise 24 h prior to each visit, not to consume caffeine on the same day as a scheduled laboratory visit and to arrive at least 3 h postprandial. Each test was scheduled at the same time of day ± 2 h.

*Experimental Overview.* All tests took place in a temperature-controlled laboratory that was maintained at 25 ± 1 °Cand 50 ± 10% humidity. Each participant visited the laboratory between 6-8 times over a 3–5-week period. All exercise tests were conducted using an electronically braked cycle ergometer (75XL-III; Combi, Tokyo, Japan). Saddle and handlebar height were recorded at the first test and replicated during all subsequent tests. A custom-built metal frame with an adjustable chair was attached to the back of the ergometer, on which participants lay flat during the supine exercise tests to enable supine cycling. The distance from the crank shaft to the shoulder was recorded at the first visit and replicated during all subsequent visits. Handles were available to grip during the supine exercise tests to prevent rear movements when forces were applied to the pedals. Throughout all exercise tests, cadence was strictly maintained at 60 rpm using an audible metronome. All tests were preceded by 2-min quiet rest on the ergometer and 4-min baseline cycling at 20 W. The order of upright and supine exercise tests was randomized.

Participants performed ramp incremental tests in the upright and supine positions on separate days to determine each mode-specific O2 peak and gas exchange threshold (GET). Each test consisted of a ramped, linear increase in work rate of 20 W/min until the participant could no longer maintain the required cadence despite strong verbal encouragement. Task failure was defined as the point at which cadence dropped below 55 rpm. Ventilatory and gas exchange variables were measured continuously breath-by-breath throughout each test. O2 peak was defined as the highest 20 s value recorded throughout the test. The GET and mean response time (MRT) were determined as previously described (7, 21).

Following the determination of the GET and O2 peak and on separate days, constant power exercise tests were performed for 6-min in both the upright and supine body positions. Comparisons between body positions were made at the same absolute and relative (Δ40%, i.e. 40% of the difference between O2 peak and the GET) work rates. For comparisons at the same absolute work rate, constant power tests were conducted within the heavy/severe domains. The range of target intensities were selected such that participants produced physiological responses typical of heavy/severe exercise in both upright and supine exercise. This range was determined as Δ10%-Δ60%, with the precise intensity chosen for each participant depending upon the participant’s capacity to sustain the exercise intensity without undue discomfort. In all cases, comparisons between body positions were made at the same absolute work rate. Participants performed two to three transitions at each intensity over a 3-5-week period. Only one heavy exercise transition was performed on each day.

*Measurements*

*Pulmonary O2.* Pulmonary gas exchange/ventilation were measured breath-by-breath throughout all tests using a hot-wire flowmeter (model AE-300S; Minato-Medical, Osaka, Japan) and gas analyser (model AE-300S; Minato-Medical, Osaka, Japan) as previously described (38, 39).

*Time-resolved near-infrared spectroscopy.* Continuous non-invasive measurements of absolute deoxy[heme], oxy[heme] and total[heme] (i.e. deoxy[heme] + oxy[heme]) in the RFs and VLs were made using two TRS-NIRS devices (TRS-20; Hamamatsu Photonics K.K., Hamamatsu, Japan), and a high-power TRS-NIRS device (TRS-20D; Hamamatsu Photonics K.K., Hamamatsu, Japan) was used to measure the same variables in the VLd in Part 2 only. The optodes for superficial muscles (interoptode spacing 3 cm) were placed on the distal sites of the VL and RF parallel to the major axis of the thigh. For deep muscle, the interoptode spacing was 6 cm and the optodes were placed on the proximal site of the VL muscle. The measurement principles and algorithms employed by the equipment (41, 48), as well as the specific measurement procedures used in our laboratory (1, 20, 37–39, 49–51) have been reviewed in detail elsewhere. Adipose tissue thickness (ATT) was measured at each muscle site using B-mode ultrasound (Logiq 400; GE-Yokogawa Medical Systems, Tokyo, Japan). To quantify the influence of ATT on NIRS signals, we employed the correction factor of Bowen et al. (8) with separate correction factors used for each muscle (12).

*Surface electromyography.* Surface electromyography (EMG) was measured using electrodes (Bluesensor T-00-S; Ambu, Ballerup; Denmark) attached to three separate bipolar EMG sensors connected to a multichannel data acquisition system (MP100; Biopac Systems, Goleta, CA) through an amplifier (Polyam 4; NIHON SANKETU, Osaka, Japan) to estimate muscle activation patterns near the TRS-NIRS optode sites of the VLs and RFs in Part 2, as previously described (39, 49). At the beginning of each visit, participants performed three repetitions of maximal voluntary contractions (MVCs) for 7-s each by extending their leg against an immovable bar while seated upright on a chair. Participants rested for 3 minutes before performing each subsequent MVC. The integrated EMG (iEMG) of the individual muscles was normalized to the highest 1-s iEMG value observed during the 7-s contraction producing the highest MVC observed during that visit, and expressed as a percentage of MVC.

*Data analysis*

Breath-by-breath O2 were edited to remove aberrant values (i.e. >4 SD outside local 5-breath mean). The edited O2 and deoxy[heme] data from the constant power exercise tests were linearly interpolated (1 s), time-aligned and ensemble-averaged across each transition for each subject (data treatment and analysis procedures have been described in full elsewhere, (21–25). A monoexponential model with time delay was then fitted to the data, as follows:

1. *(t)* =*Y*(*b*) + *AY* \* (1 – e – (*t* – TDY/ τY))

Where *Y(t)* is the value of the independent variable at any time *t*, (*b*) is the baseline value measured over the final 30 s of baseline cycling, *AY*is the amplitude of increase in *Y* above baseline, TDY is the time delay, and τY is the time constant of the response. For O2 data, the model was constrained to exclude the slow component and hence isolate the fundamental phase. Briefly, the onset of the O2 slow component was identified using purpose-designed programming in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), which iteratively fits a monoexponential function to the O2 data, starting at 60 s until the window encompasses the entire response. The estimated O2 values for each fitting window are then plotted against time, with the onset of the slow component determined as the point at which O2 consistently deviated from a previously “flat” profile. The amplitude of the O2 slow component was determined by calculating the difference between end-exercise O2 (i.e. mean O2 over the final 30 s of exercise) and O2 (*b*) + *AV*O2.

For the deoxy[heme] data, the TDdeoxy[heme] was determined as the first datum that was 1 SD above the mean baseline value from the final 30 s of baseline cycling, and the fitting window was constrained to the onset of the O2 slow component. The amplitude of the deoxy[heme] slow component was calculated by subtracting the absolute deoxy[heme] response (i.e. deoxy[heme]*(b)* + *A*deoxy[heme]) from the average value of deoxy[heme] during the final 30 s of exercise. Values for total[heme] were measured from the mean of the last 30 s of baseline pedalling and the 30 s immediately before 3- and 6-min of exercise. The changes in total[heme] between time points (i.e. amplitudes at 3- and 6-min) were also calculated. The amplitudes of deoxy- and total[heme] normalized to iEMG (i.e. μM/%MVC) were also determined from the mean of the 30 s prior to 3 and 6-min of exercise. Finally, as a measure of point-by-point inter-site heterogeneity of the deoxy- and total[heme] responses, root mean squared error (RMSE) for each subject at a given time was calculated as follows:

RMSE *(t)* = [Σ(*X*i - *X*ave )2 / n]

Where *X*i, *X*ave, and n are individual responses at each site, the mean of the three site values, and the number of sites (i.e. 3), respectively.

*Statistics*

In Part 1, all O2 kinetics parameters were compared between supine and upright exercise using paired t-tests. The NIRS parameters were compared using repeated measures analyses of variance (ANOVA) across body position (upright and supine matched absolute work rates), muscle (VLd, VLs, and RFs), and time (baseline, 3- and 6-min). In Part 2, O2, NIRS and iEMG parameters were compared using repeated measures ANOVA for either condition (supine, upright matched absolute work rate, upright matched relative work rate), muscle (VLd, VLs, and RFs), and time (baseline, 3- and 6-min). Where significant differences were found, Holm-Sidak adjusted post-hoc comparisons were used to locate these differences. Effect sizes (ES: using Cohen’s *d* and η2p) were also calculated. Statistical software (SigmaPlot 13.0, Systat Software, San Jose, CA) was used for all statistical analyses; figures were produced using GraphPad Prism (ver. 7.02, GraphPad software, San Diego, USA). Data are presented as means ± SD. Significance was declared when *P* < 0.05.

**RESULTS**

*Absolute work rate comparisons*

*O2 kinetics at the same absolute work rate*. Exercise was conducted at 174 ± 21 W, with O2 reaching Δ29 ± 28% in the upright position and Δ54 ± 20% in the supine position during the final 30 s of exercise. O2 (*n* = 17, *d* = 1.09, *P* < 0.001) was greater in the supine compared to the upright body positions (Figure 1*A*, Table 1).

*Muscle deoxy- and total[heme] responses at the same absolute work rate.* The time delay before the onset of muscle deoxygenation (TDdeoxy[heme])was shorter in the supine compared to the upright position at all muscle sites (superficial muscle, *n* = 17, η2p = 0.52, *P* = 0.031, Table 2, Figure 2; deep muscle, *n* = 10, η2p = 0.68, *P* = 0.037, Table 4, Figure 6). The fundamental phase amplitude of the deoxy[heme] (*A*deoxy[heme]) response was greater during supine compared to upright exercise for all muscle sites (superficial muscle, η2p = 0.81, *P* < 0.001; deep muscle, η2p = 0.68, *P* = 0.010); the fundamental phase time constant of muscle deoxygenation (τdeoxy[heme]) was also greater in supine compared with upright exercise (superficial muscle, η2p = 0.60, *P* = 0.004; deep muscle, η2p = 0.60, *P* < 0.001).

Baseline total[heme] was lower in supine versus upright exercise for all muscle sites (superficial muscle, η2p = 0.37, *P* = 0.002, Figure 3, Table 3; deep muscle, η2p = 0.96, *P* = 0.039, Figure 7, Table 5). The amplitude of the total[heme] response was greater in the supine position in the RFs and VLd (RFs: n = 17, η2p = 0.30, *P* = 0.018; VLd: n = 10, η2p = 0.28, *P* = 0.024), but end-exercise total[heme] did not differ between postures for any muscle (superficial muscle, η2p = 0.05, *P* = 0.34; deep muscle, η2p = 0.001, *P* = 0.90). The heterogeneity of both deoxy[heme] (η2p = 0.03, *P* = 0.91, Figure 4, Panel *A*) and total[heme] (η2p = 0.06, *P* = 0.66, Figure 4, Panel *B*) did not differ between the upright and supine positions.

*Muscle deoxy[heme] and total[heme] responses normalized to iEMG at the same absolute work rate.* iEMG normalized to MVC (%MVC) was greater in the supine compared to the upright position (*n* = 10, η2p = 0.44, P = 0.030, Figure 5*A-B*), however, the differences in deoxy[heme] amplitudes between supine and upright exercise remained when normalized by iEMG (η2p = 0.57, *P* = 0.026, Figure 5*C-D*). The differences in total[heme] amplitude were abolished when normalized by iEMG (η2p = 0.24, *P* = 0.211, Figure 5*E-F*).

*Relative work rate comparisons*

*O2 kinetics at the same relative work rate*. Exercise was conducted at 195 ± 23 W in the upright position and 159 ± 19 W in the supine position (*n* = 10), permitting close matching relative to O2 peak between body positions (O2 during final 30 s of exercise, supine: Δ43 ± 10% upright: Δ42 ± 13%, *d* = 0.20, *P* = 0.56). O2 was greater for supine compared with upright exercise (*n* = 10, *d* = 1.02, *P* = 0.011, Table 1, Figure 1*B*).

*Muscle deoxy- and total[heme] responses at the same relative work rate.* Some subjects did not demonstrate a clear exponential deoxy[heme] response profile in the upright position in VLd, hence kinetic data for VLd are omitted (Table 4). TDdeoxy[heme] was shorter in supine compared with upright exercise (η2p = 0.55, *P* = 0.006). The fundamental phase amplitude of deoxy[heme] was greater in the supine position for each muscle site (η2p = 0.81, *P* = 0.006), whereas τdeoxy[heme] (η2p = 0.01, *P* = 0.699) did not differ between body positions. In the supine compared to the upright position, baseline total[heme] was lower (η2p = 0.19, *P* = 0.048, Figure 7, Table 5), whereas the amplitude of the total[heme] response at 3-min (η2p = 0.59, *P* = 0.002) was greater for VLd and RFs and at 6-min (η2p = 0.41, *P* = 0.008) for all muscle sites. End-exercise total[heme] did not differ between body positions (η2p = 0.06, *P* = 0.56). The heterogeneity of total[heme] (Figure 4, Panel *B*, η2p = 0.03, *P* = 0.91) did not differ between positions, whereas the heterogeneity of the deoxy[heme] response was greater in the supine position (Figure 4, Panel *A*, η2p = 0.20, *P* = 0.002).

*Muscle deoxy[heme] and total[heme responses normalized to iEMG at the same relative work rate.* iEMG normalized to %MVC was greater in the upright compared to the supine position for the VLs only (η2p = 0.49, *P* = 0.017, Figure 5*A*). The differences in the deoxy[heme] amplitudes between supine and upright exercise at the same relative work rate remained when normalized for iEMG (η2p = 0.56, *P* = 0.025, Figure 5*C-D*), however, the differences in total[heme] amplitude were abolished when normalized for iEMG (η2p = 0.12, *P* = 0.209, Figure 5*E-F*).

**DISCUSSION**

For constant work rate exercise performed in the supine versus upright position our principal findings across the on-transient were that supine exercise: 1) resulted in a shorter time delay, greater amplitude, and greater fundamental phase time constant of muscle deoxy[heme] kinetics; 2) a lower baseline total[heme] and a greater amplitude of total[heme] in response to exercise; 3) an increased spatial heterogeneity of muscle deoxy[heme] responses across superficial and deep muscles; such that these effects culminated in 4) slower pulmonary (and presumably, muscle) O2 kinetics in the supine position when compared to upright exercise at both the same absolute and relative work rates. However, in disagreement with our hypotheses, the effects of the supine position on deoxy- and total[heme] were also apparent in deep muscle (i.e. VLd). Hence, the results of the present study demonstrate that greater heterogeneity in the O2/O2 ratio is associated with slower pulmonary O2 kinetics, and that any apparent advantage that deep muscle possesses over superficial muscle with respect to O2/O2 is insufficient to mitigate impairments in whole-muscle O2.

*Comparisons with previous work.* Previous studies have compared upright and supine exercise at the same absolute work rate, but participants were exercising in different exercise intensity domains (36, 43). The recruitment of type II fibers increases disproportionately during exercise above vs. below critical power (11). These fibers possess inherently slower O2 kinetics and a lower pressure head for O2 delivery (5, 13, 34, 56) and are thus more likely to lie to the left of their individual O2 delivery-tipping points (54). It is therefore feasible that these previously observed differences between body positions arose due to the performance of exercise above critical power during supine but not upright exercise, which may have resulted in the recruitment of a greater proportion of less oxidative type II fibers. Comparisons between upright and supine exercise at the same relative exercise intensity have yielded conflicting findings; DiMenna et al. (15) demonstrated no differences in O2 between postures during moderate-intensity exercise whereas Goulding et al. (21) found a greater O2 in the supine position during heavy-intensity exercise, albeit in different groups of participants. To avoid the confounding influence of differences in exercise intensity affecting our comparisons between postures, in the present study we elected to compare pulmonary O2 kinetics at the same absolute and relative work rates in the same subjects. Consistent with our hypotheses, we found that O2 was greater in the supine compared to the upright body position at both the same absolute and relative work rates. This finding suggests that, at least for supra-GET exercise, supine exercise pushes young, healthy, physically active individuals to the left of their “whole-body” O2 delivery tipping point (54), irrespective of differences in the relative exercise intensity.

*Mechanistic bases for slower O2 kinetics during supine exercise.* We found a reduced time delay before the exponential rise in deoxy[heme] at the onset of exercise (TDdeoxy[heme]) in the supine position, indicating that O2 began to outstrip O2 at an earlier time point during the transition when compared to upright exercise. Given the slower O2 kinetics observed in the supine position, this finding likely represents reduced O2 during the early phase of the exercise transition.We also observed an increased amplitude of the fundamental phase deoxy[heme] response to exercise in the supine compared to the upright position at both the same absolute and relative work rates across all muscle sites measured. This suggests a reduced O2 and thus, a greater reliance on fractional O2 extraction to satisfy a given increase in O2 (Figures 2 & 6, Tables 2 & 4). Such a notion is supported by the finding of slower muscle blood flow kinetics in the supine position (45) and with the arm above versus below heart level (33, 59), as well as the finding of reduced total[heme] (i.e., blood volume) during baseline cycling in the present study. The slower pulmonary O2 kinetics observed in the supine position indicate that the increases in fractional O2 extraction were insufficient to mitigate the reductions inO2 brought about during exercise in the supine posture.

We have previously shown that when normalized by muscle activation (i.e. iEMG), differences in deoxy[heme] amplitudes between both exercise modes (39, 49) and muscles (9) tend to disappear. Hence, valid comparison of absolute values of deoxy[heme] between body positions is only achievable with reference to the muscle activation profiles in each position. However, the deoxy[heme] amplitude was greater in the supine position compared to the upright position at both the same absolute and relative work rates even when normalized by iEMG. This finding therefore convincingly demonstrates that differences in muscle deoxygenation between upright and supine exercise are due to reduced perfusive O2 *per se*, rather than differences in muscle activation patterns. Thus, it is likely that the reduced convective O2 in the supine position caused fractional O2 extraction to become uncoupled from muscle recruitment, enabling far greater O2 extraction to be achieved in this position. A greater reliance on fractional O2 extraction to achieve a given O2 in the supine position would exacerbate contraction-induced reductions in microvascular *P*O2, possibly down to limiting values across the transient (i.e. a *"*critical*"* *P*O2, 3, 4, 6) when compared to the upright position (5, 46). The challenges for achieving a given blood-myocyte O2 flux in supine exercise would be exacerbated by the lower baseline total[heme] observed in the supine position. This measurement reflects a reduced blood volume (i.e. microvascular hematocrit, fewer RBCs per capillary) due to the loss of orthostatic pressure that is normally present in the upright position. As RBC number within those flowing capillaries adjacent to the contracting myocytes helps determine the O2 diffusing capacity (DmO2, rev. 18 ,27) this would also act to impair blood-myocyte O2 flux. These combined effects, in turn, would lower intracellular *P*O2 and constrain intramyocyte energetics, resulting in a slowing of muscle and pulmonary O2 kinetics.

A novel finding of the present study was an increased spatial heterogeneity of deoxy[heme] (as indicated by greater RMSE) during supine compared to upright exercise at the same relative work rate (Figure 4). The observation that spatial heterogeneity of deoxy[heme] did not differ between supine and upright exercise at the same absolute work rate suggests that spatial heterogeneity decreased with increasing work rate in the upright position. This finding seems somewhat counterintuitive given that blood flow heterogeneity appears to increase with increasing work rate (11, 44, 55), but might feasibly be explained by more homogenous muscle activation at higher work rates (9, 49). Indeed, at greater relative exercise intensities during ramp exercise, the iEMG and deoxy[heme] profiles of discrete muscles tend to converge, thereby reducing inter-muscular heterogeneity (9, 49). Moreover, this finding suggests that the relationship between deoxygenation heterogeneity and relative exercise intensity is steeper in the supine position, i.e., a given relative exercise intensity engenders a greater degree of heterogeneity in the supine compared to the upright position. The greater degree of heterogeneity in the supine position is likely representative of impaired regional O2/O2 matching, an effect which likely contributed to the slower O2 kinetics observed in this position. Hence, these data may represent the first evidence that a greater degree of inter/intramuscular heterogeneity of skeletal muscle deoxygenation is associated with slower O2 kinetics. Moreover, on the basis of these findings, an even spatial distribution of local O2/O2 ratios appears to be optimal in terms of optimizing whole-body O2 kinetics (42).

In the present investigation, we hypothesized that muscle deoxygenation and total[heme] responses in deep muscle would not be impacted by supine compared to upright exercise. This hypothesis was based on the observations that, when compared to superficial muscles, deep muscles receive far greater rates of blood flow at rest and during exercise (28, 29, 44, 52), operate at a higher O2/O2 (37, 38, 51), and are comprised of a greater proportion of type I fibres (14, 35) which in turn, demonstrate less pronounced falls in microvascular *P*O2 at the onset of contractions when compared to less oxidative muscle (5, 46). In contrast with our hypothesis, however, the impairments in muscle deoxygenation and total[heme] responses induced by supine exercise in superficial muscle were also present in deep muscle. These findings thus imply that any advantages that deep muscle possesses over superficial muscle with respect to O2/O2 appear to be insufficient to preserve oxidative function in the presence of whole-body impairments in O2 delivery, such as that brought about by supine exercise. Hence, our data reveal that exercise in the supine position pushed all muscle sites to the left of their microvascular O2 “tipping points” (54).

*Slower muscle deoxygenation kinetics during supine exercise.* Muscle deoxy[heme] kinetics were slower in the supine versus upright positions when exercise was conducted at the same absolute work rate in the present study. This finding was unexpected because slower muscle deoxygenation kinetics are typical in situations where *m* is relatively greater than the prevailing muscle O2 (5, 30, 32, 46). However, the lower baseline total[heme], shorter TDdeoxy[heme], and greater deoxy[heme] amplitude show that O2was clearly reduced in the supine position in the present study. It has previously been demonstrated that changes in blood volume also contribute to the deoxy[heme] signal derived from NIRS (1). One possibility, therefore, is that a slower adaptation of blood volume secondary to slower blood flow kinetics (45) contributed to the slower muscle deoxy[heme] kinetics observed in the supine position herein. However, neither the time course of muscle total[heme] nor regional muscle blood flow kinetics were determined in the present study, hence it is not possible to verify this hypothesis. Alternatively, slower deoxygenation kinetics in the supine position might reflect a relatively greater contribution of myoglobin deoxygenation to the overall TRS-NIRS-derived deoxy[heme] signal. For instance, the lower *P*50 of myoglobin compared to hemoglobin (myoglobin: 3-5 mmHg, hemoglobin: ~26 mmHg, 10) would mean that myoglobin occupies a “flatter” portion of its O2-dissociation curve for a given capillary *P*O2. As capillary *P*O2 approaches its nadir during the transition, hemoglobin will undergo a relatively greater degree of desaturation compared to myoglobin. Once hemoglobin saturation reaches its nadir, the primary contributor to changes in the NIRS-derived deoxy[heme] signal would be myoglobin, which would occupy a relatively higher portion of its saturation curve and thus manifest slower deoxygenation kinetics. It is not presently possible to distinguish between these two candidate mechanisms. Clearly, further work is required to reveal the mechanistic bases for the slower muscle deoxy[heme] kinetics during supine exercise.

*Effect of posture diffusive O2 transport.* A further novel finding of the present study was the increased amplitude of the total[heme] response to exercise in the supine versus upright positions (Figures 3 & 7, Tables 3 & 5). According to Fick’s Law of diffusion, O2 = DmO2 (PcapO2 – PmitoO2), where DmO2 is the diffusing capacity of the muscle, and PcapO2 and PmitoO2 are the capillary and mitochondrial O2 pressures, respectively. The DmO2 term is primarily determined by the aggregate number of red blood cells within capillaries adjacent to the myocytes at a given time (18, 27), and hence, changes in total[heme] are considered broadly representative of changes in DmO2 (2, 26). Therefore, exercise in the supine position necessitated a relatively greater reliance on changes in diffusive (as opposed to perfusive) O2 transport to satisfy a given O2 when compared to the upright position. These findings demonstrate that the enhanced fractional O2 extraction observed in response to reduced muscle convective O2 in the supine position is, at least partially, due to increases in diffusive O2 conductance. These findings thus add to a growing body of evidence that diffusive O2 conductance is an important regulated variable within the microcirculation and increases thereof are a primary method by which blood-to-myocyte O2 flux increases at the onset of exercise (16, 17, 31). The mechanism by which greater changes in DmO2 were achieved in the supine position is not possible to determine from the results of the present study. However, a possible candidate might include increased longitudinal recruitment along capillaries during supine exercise, a process that could have been facilitated by increased fractional O2 extraction which would increase the effective surface area for O2 diffusion along the length of the capillaries (31, 53). However, it is important to point out that once changes in total[heme] were normalized by iEMG, differences between postures were abolished. This suggests that changes in diffusive O2 transport between postures were primarily driven by differences in muscle activation.

*Limitations.* The depth penetration of NIR-light has been estimated as one-half of the distance between the emitter and receiver optodes, or approximately 3 cm for deep muscle in the present study. However, the pathlength estimated in this manner represents the overall average of an infinite number of possible pathlengths that may be travelled by photons as they pass from source to detector. Hence, some of the NIRS signal derived from the VLd in the present study likely includes measurements from both deep and superficial regions, as photons must pass through superficial regions to reach the deeper tissues. Hence, using this approach, it is not possible to subtract out the influence of superficial tissue to obtain information solely from deeper tissue. Notwithstanding these points, that a greater proportion of the NIRS signal from our high-power TRS-NIRS device is derived from deep tissue has been demonstrated previously by studies using optical phantoms with known optical characteristics (37), and the observation that deep tissue exhibits markedly altered muscle deoxygenation profiles in response to constant work rate (37–39) and ramp (51) exercise. Hence, we are confident that in the present study, our findings in the VLd in the supine position represent actual changes in muscle deoxygenation within the deeper muscle tissue.

*Conclusion.* This investigation suggests that exercise performed in the supine position results in profound impairments in microvascular O2 delivery to exercising muscle. Specifically, baseline total[heme] and TDdeoxy[heme] were reduced, whereas the fundamental phase deoxy[heme] amplitude was greater during supine exercise, supporting the presence of impaired muscle perfusion and, therefore, forcing a greater reliance on fractional O2 extraction to achieve a given metabolic rate. Surprisingly, and contrary to our hypothesis, these impairments also occurred in deep muscle, indicating that any apparent advantage that deep muscle possesses over superficial muscle with respect to O2/O2 is insufficient to defend oxidative function in response to impairments in whole-muscle O2. Further, the regional heterogeneity of muscle deoxy[heme] was increased during supine exercise, indicative that, at least in this case, greater O2/O2 heterogeneity is associated with slower pulmonary O2 kinetics. Moreover, the amplitude of the total[heme] response was increased during supine exercise, providing evidence that supine-induced perturbations to perfusive O2 delivery were partially offset by compensatory adaptations in diffusive O2 transport to achieve a given O2. That O2 was greater in the supine position (i.e. the rate of increase in O2 was slower) suggests that these adaptations to impaired muscle O2 in the supine position were ultimately insufficient to prevent impaired O2 kinetics.

**COMPETING INTERESTS**

The authors declare that there is no conflict of interest associated with this manuscript.

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**AUTHOR CONTRIBUTIONS**

RPG, DO, SM, DCP, TJB, and SK were responsible for the conception and design of the work. RPG, DO, SK, NK, and TL were responsible for data acquisition and analysis, whereas all authors were responsible for interpretation of the data. RPG drafted the work and all authors revised it critically for important intellectual content, approved the final version to be published, and agree to be accountable for all aspects of the work.

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**FIGURE LEGENDS**

**Figure 1.** Group mean responses of pulmonary oxygen uptake (O2) to cycle exercise in the supine and upright body positions at the same absolute (*n* = 17, panel *A*) and relative work rates (*n* = 10, panel *B*). White circles = supine exercise, black circles = upright exercise. Dashed black line indicates onset of exercise. Error bars omitted for clarity. Note the slower O2 responses in the supine position.

**Figure 2.** Group mean responses for muscle deoxygenated [heme] (deoxy[heme]) in the supine (white circles) and upright (black circles) body positions at the same absolute work rate in the superficial *vastus laterali*s (VLs, panel *A*), and superficial *rectus femoris* (RFs, panel *B*) (*n* = 17). Dashed line indicates onset of exercise. Error bars omitted for clarity. Note the far greater amplitude of muscle deoxy[heme] and slower kinetics in both muscles in the supine position.

**Figure 3.** Group mean responses for muscle deoxygenated + oxygenated [heme] (total[heme]) in the supine (white circles) and upright (black circles) body positions at the same absolute work rate in the superficial *vastus laterali*s (VLs, panel *A*) and superficial *rectus femoris* (RFs, panel *B*) (*n* = 17). Dashed line indicates onset of exercise. Error bars omitted for clarity. Note the lower baseline and greater amplitude of changes in muscle total[heme] in both muscles in the supine position.

**Figure 4.** Group mean responses for point-by-point root mean square error (RMSE) changes (i.e., inter-site heterogeneity across the quadriceps muscles) of muscle deoxygenated [heme] (deoxy[heme], Panel *A*) and deoxygenated + oxygenated [heme] (total[heme], Panel *B*) in the supine position (white circles), and the upright position at the same absolute (black circles) and relative (black triangles) work rates (*n* = 10). Dashed line indicates onset of exercise, error bars indicate standard deviations. \* a significant difference between supine and upright at the same relative work rate (*P* < 0.05).

**Figure 5.** *Panels A-B*: Group mean and individual responses during the last 30 seconds of exercise for the superficial *vastus lateralis* (VLs, left column) and *rectus femoris* (RFs, right column) during supine and upright exercise at the same absolute and relative work rates (*n* = 10) for muscle activity (as integrated electromyography; iEMG) (panels A and B), deoxy[heme] normalized to iEMG (i.e. μM/%maximum voluntary contraction [MVC%]) (Panel *C* and *D*), and total[heme] normalized to iEMG (Panel *E* and *F*). + different from the supine position, # different from the upright position at the same absolute work rate (*P* < 0.05).

**Figure 6.** Group mean responses for muscle deoxygenated [heme] (deoxy[heme]) in the supine (white circles) and upright body positions at the same absolute (black circles) and relative (black triangles) work rates in the deep *vastus lateralis* (VLd, Panel *A*), the superficial *vastus laterali*s (VLs, Panel *B*), and the superficial *rectus femoris* (RFs, Panel *C*) (*n* = 10). Dashed line indicates onset of exercise. Error bars omitted for clarity. Note the greater amplitude of muscle deoxy[heme] in each muscle in the supine position.

**Figure 7.** Group mean responses for muscle deoxygenated + oxygenated [heme] (total[heme]) in the supine (white circles) and upright body positions at the same absolute (black circles) and relative (black triangles) work rates in the deep *vastus lateralis* (VLd, Panel *A*), the superficial *vastus laterali*s (VLs, Panel *B*), and the superficial *rectus femoris* (RFs, Panel *C*) (*n* = 10). Group mean relative changes in total[heme] from the baseline value (i.e. Δtotal[heme] [μM]) are displayed in the inset of each panel to illustrate the transient phase of the response. Dashed black line indicates onset of exercise. Error bars omitted for clarity. Note the lower baseline and greater amplitude of changes in muscle total[heme] in each muscle in the supine position.