

RESEARCH ARTICLE

Effect of priming exercise and body position on pulmonary oxygen uptake and muscle deoxygenation kinetics during cycle exercise

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¹Applied Physiology Laboratory, Kobe Design University, Kobe, Japan; ²Japan Society for Promotion of Science, Tokyo, Japan; ³School of Health Sciences, Liverpool Hope University, Liverpool, United Kingdom; ⁴Osaka International University, Moriguchi, Japan; ⁵Department of Anatomy and Physiology and Department of Kinesiology, Kansas State University, Manhattan, Kansas; and ⁶Applied Physiology Laboratory, Kobe University, Kobe, Japan

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Goulding RP, Marwood S, Okushima D, Poole DC, Barstow TJ, Lei TH, Kondo N, Koga S. Effect of priming exercise and body position on pulmonary oxygen uptake and muscle deoxygenation kinetics during cycle exercise. *J Appl Physiol* 129: 810–822, 2020. First published August 6, 2020; doi:10.1152/japplphysiol.00478.2020.—We hypothesized that the performance of prior heavy exercise would speed pulmonary oxygen uptake ($\dot{V}\text{O}_2$) kinetics (i.e., as described by the time constant, $\tau_{\dot{V}\text{O}_2}$) and reduce the amplitude of muscle deoxygenation (deoxygen[heme]) kinetics in the supine (S) but not upright (U) body position. Seventeen healthy men completed heavy-intensity constant-work rate exercise tests in S and U consisting of two bouts of 6-min cycling separated by 6-min cycling at 20 W. Pulmonary $\dot{V}\text{O}_2$ was measured breath by breath; total and deoxygen[heme] were determined via time-resolved near-infrared spectroscopy (NIRS) at three muscle sites. Priming exercise reduced $\tau_{\dot{V}\text{O}_2}$ in S (*bout 1*: 36 ± 10 vs. *bout 2*: 28 ± 10 s, $P < 0.05$) but not U (*bout 1*: 27 ± 8 s vs. *bout 2*: 25 ± 7 s, $P > 0.05$). Deoxygen[heme] amplitude was increased after priming in S (*bout 1*: $25\text{--}28 \mu\text{M}$ vs. *bout 2*: $30\text{--}35 \mu\text{M}$, $P < 0.05$) and U (*bout 1*: $13\text{--}18 \mu\text{M}$ vs. *bout 2*: $17\text{--}25 \mu\text{M}$, $P > 0.05$), whereas baseline total[heme] was enhanced in S (*bout 1*: $110\text{--}179 \mu\text{M}$ vs. *bout 2*: $121\text{--}193 \mu\text{M}$, $P < 0.05$) and U (*bout 1*: $123\text{--}186 \mu\text{M}$ vs. *bout 2*: $137\text{--}197 \mu\text{M}$, $P < 0.05$). Priming exercise increased total[heme] in both S and U, likely indicating enhanced diffusive O_2 delivery. However, the observation that after priming the amplitude of the deoxygen[heme] response was increased in S suggests that the reduction in $\tau_{\dot{V}\text{O}_2}$ subsequent to priming was related to a combination of both enhanced intracellular O_2 utilization and increased O_2 delivery.

NEW & NOTEWORTHY Here we show that oxygen uptake ($\dot{V}\text{O}_2$) kinetics are slower in the supine compared with upright body position, an effect that is associated with an increased amplitude of skeletal muscle deoxygenation in the supine position. After priming in the supine position, the amplitude of muscle deoxygenation remained markedly elevated above that observed during upright exercise. Hence, the priming effect cannot be solely attributed to enhanced O_2 delivery, and enhancements to intracellular O_2 utilization must also be contributory.

near-infrared spectroscopy; oxygen delivery; oxygen uptake kinetics; oxygen utilization; priming exercise

INTRODUCTION

The primary limiting factors governing the rate of increase in muscle and pulmonary oxygen uptake ($\dot{V}\text{O}_2$) at the onset of exercise have traditionally been ascribed to either 1) an insufficiency of O_2 delivery ($\dot{Q}\text{O}_2$) to the exercising musculature (57) or 2) an intrinsic inertia of the intracellular oxidative metabolic pathways (29). That this rigid dichotomy persists may be due, in part, to limitations associated with methodological approaches, such as venous/mixed venous effluent sampling, single-site superficial measurements of muscle deoxygenation (deoxygen[heme]) using continuous-wave near-infrared spectroscopy (CW-NIRS), and a range of exercise modes and interparticipant differences in training status and/or fitness.

An intervention that has frequently been used to investigate the control mechanisms of $\dot{V}\text{O}_2$ kinetics is priming exercise (12, 22, 52). Briefly, when a bout of exercise is performed above the lactate threshold, the $\dot{V}\text{O}_2$ response to any subsequent exercise performed differs markedly from that of the first bout (22). Specifically for upright exercise, in the second bout the time constant of fundamental phase pulmonary $\dot{V}\text{O}_2$ kinetics ($\tau_{\dot{V}\text{O}_2}$) is typically unaltered (12, although see 56), whereas the amplitude of the fundamental phase $\dot{V}\text{O}_2$ kinetics is increased (8) and the amplitude of the $\dot{V}\text{O}_2$ slow component is reduced (12). Resolving the mechanisms underpinning this prior exercise effect is thus pivotal to understanding the control mechanisms of $\dot{V}\text{O}_2$ kinetics at exercise onset. These mechanisms remain poorly understood, however, because priming exercise upregulates multiple steps in the O_2 transport and utilization pathways (15). As such, an increase in bulk (i.e., conduit artery) (50) and local $\dot{Q}\text{O}_2$ (35), a right-shifted oxyhemoglobin dissociation curve enhancing diffusive O_2 transport (22), altered muscle recruitment patterns (7), and decreased intracellular metabolic inertia (15, 21, 31, 36) have all been implicated in the priming exercise effect. Despite this complexity, some have argued that the sole mechanism responsible for the prior exercise effect is enhanced microvascular $\dot{Q}\text{O}_2$ during the second bout (55, 57, 68).

An intervention that has been used in an attempt to isolate the $\dot{Q}\text{O}_2$ -related effects of priming exercise is supine exercise (24, 39). Exercise performed in the supine position results in a loss of muscle perfusion pressure that typically increases $\tau_{\dot{V}\text{O}_2}$ (i.e., slows $\dot{V}\text{O}_2$ kinetics) (19, 47, 54), and priming exercise has been shown to restore $\tau_{\dot{V}\text{O}_2}$ to values typically observed in the

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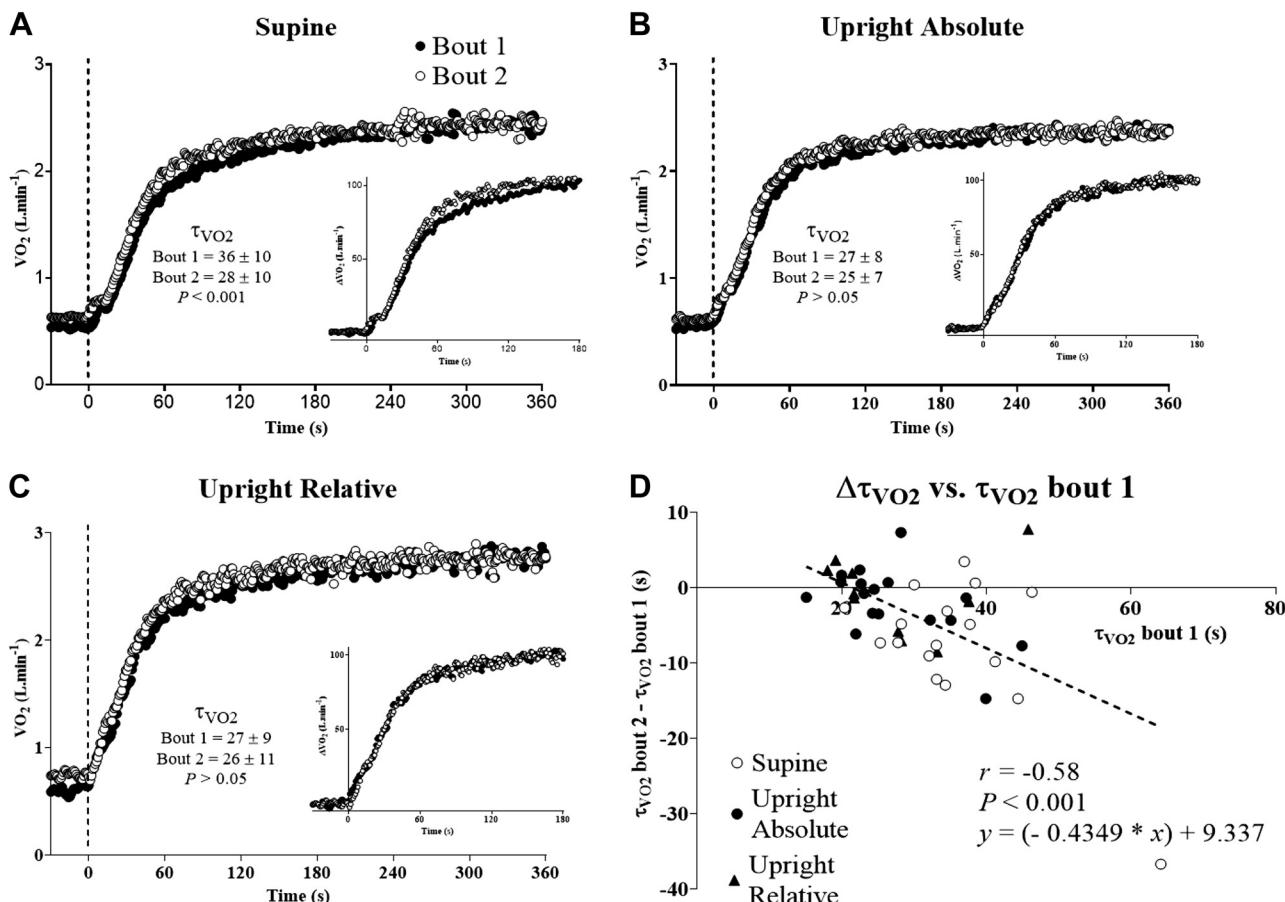


Fig. 1. Group mean responses of pulmonary oxygen uptake ($\dot{V}O_2$) to cycle exercise in the supine position ($n = 17$ participants, A), the upright body position at the same absolute work rate ($n = 17$ participants, B), and the upright position at the same relative work rate ($n = 10$ participants, C). Inset for each panel displays the relative changes over the transient phase of the response. Dashed black line indicates onset of exercise. Error bars omitted for clarity. D: relationship between $\dot{V}O_2$ time constant ($\tau_{\dot{V}O_2}$) in bout 1 and changes in $\tau_{\dot{V}O_2}$ between bout 1 and bout 2 (i.e., bout 2 – bout 1, $P < 0.001$). Open circles, supine position; black circles, upright position at the same absolute work rate; black triangles, upright position at the same relative work rate.

upright position (24, 39). Interventions that enhance muscle \dot{Q}_{O_2} can speed $\dot{V}O_2$ kinetics in situations where \dot{Q}_{O_2} is impaired (27, 37, 38, 49, 53), and priming exercise performed in the upright position (i.e., where bulk \dot{Q}_{O_2} is not considered limit-

ing; 62) does not typically alter $\tau_{\dot{V}O_2}$ (12, 24, 39). Hence, the priming-induced speeding of $\dot{V}O_2$ kinetics observed in the supine position has been taken as evidence that increased \dot{Q}_{O_2} at the onset of the second exercise bout is primarily responsible

Table 1. Pulmonary oxygen uptake kinetics during upright and supine cycle exercise at the same absolute and relative work rates in bout 1 and bout 2

Variable	Upright					
	Supine ($n = 17$)		Absolute ($n = 17$)		Relative ($n = 10$)	
	Bout 1	Bout 2	Bout 1	Bout 2	Bout 1	Bout 2
Relative intensity, $\Delta\%$	54 ± 20	52 ± 20	29 ± 28*	34 ± 33*	42 ± 13	43 ± 11
$\dot{V}O_2$ baseline, L/min	0.55 ± 0.09	0.63 ± 0.09*	0.56 ± 0.11	0.62 ± 0.09*	0.62 ± 0.05	0.75 ± 0.06**†
TD $\dot{V}O_2$, s	14 ± 4	15 ± 6	12 ± 6	13 ± 4	10 ± 4	12 ± 2
$\tau_{\dot{V}O_2}$, s	36 ± 10	28 ± 10*	27 ± 8*	25 ± 7	27 ± 9*	26 ± 11
$A\dot{V}O_2$, L/min	1.69 ± 0.28	1.68 ± 0.26	1.65 ± 0.22	1.67 ± 0.33	1.89 ± 0.28**†	1.87 ± 0.30**†
$\dot{V}O_2$ baseline + $A\dot{V}O_2$, L/min	2.24 ± 0.25	2.31 ± 0.23*	2.21 ± 0.21	2.29 ± 0.31*	2.51 ± 0.29**†	2.62 ± 0.31**†
End-ex $\dot{V}O_2$, L/min	2.44 ± 0.28	2.45 ± 0.25	2.38 ± 0.34*	2.37 ± 0.33*	2.77 ± 0.34**†	2.76 ± 0.33**†
$\dot{V}O_2$ slow component, L/min	0.21 ± 0.11	0.14 ± 0.09*	0.17 ± 0.27*	0.08 ± 0.07**	0.26 ± 0.10**†	0.14 ± 0.06**†

For upright matched relative work rate, data are for $n = 10$ participants; thus these comparisons were conducted against the same 10 participants only in the supine and upright matched absolute work rate conditions. The data for the supine and upright matched absolute work rate comparison for $n = 10$ participants are not shown. $A\dot{V}O_2$, fundamental amplitude; End-ex $\dot{V}O_2$, end-exercise oxygen uptake ($\dot{V}O_2$); TD $\dot{V}O_2$, fundamental time delay; $\tau_{\dot{V}O_2}$, fundamental time constant.

*Significantly different from bout 1 within same posture; #significantly different from supine within same bout number; †significantly different from upright matched absolute work rate within same bout number ($P < 0.05$).

for this effect (24, 39). However, as noted above, priming exercise upregulates multiple steps in the O_2 transport and utilization pathways, and therefore it is difficult to ascribe these effects to a single factor.

We have recently demonstrated that exercise in the supine position increases reliance on fractional O_2 extraction compared with upright exercise (23), consistent with previous studies utilizing CW-NIRS (17) and strongly indicating impaired Q_{O_2} in this position. Hence, if the primary mechanism responsible for the priming exercise-induced speeding of $\dot{V}O_2$ kinetics in the supine position is solely the enhanced Q_{O_2} , then a return of fractional O_2 extraction to values similar to those seen during upright exercise would be expected. Demonstration of such would provide strong evidence in favor of Q_{O_2} being the sole factor determining the priming-induced speeding of the $\dot{V}O_2$ kinetics in the supine position.

Time-resolved (TR)-NIRS enables the determination of absolute values of total and deoxy[heme] and therefore provides a more informative measure of fractional O_2 extraction compared with conventional CW-NIRS (3, 41, 45, 46). Moreover, the ability to sample superficial and deep muscles (which rely on fundamentally different O_2 transport strategies; 41–43, 60, 61) with TR-NIRS provides greater insight into the mechanisms underpinning changes in O_2 transport and utilization afforded by the respective interventions of supine and priming exercise. Furthermore, we have recently shown that normalizing muscle deoxy[heme] by electromyography (EMG) provides a measure of fractional O_2 extraction that is independent of differences in muscle activation between body positions or modes of exercise (23, 43). Hence, the purpose of the present study was to determine the influence of priming exercise on $\dot{V}O_2$ kinetics in the supine and upright positions, using TR-NIRS and EMG to noninvasively determine changes in muscle activation, Q_{O_2} , and O_2 utilization brought about by priming exercise in both positions. Our specific hypotheses were that, in the face of a greater $\tau_{\dot{V}O_2}$ and deoxy[heme] amplitude in the supine compared with upright position in the first bout of exercise (17, 23, 24, 39), 1) priming exercise would result in enhanced microvascular Q_{O_2} (inferred via TR-NIRS) in both body positions and 2) priming exercise would reduce $\tau_{\dot{V}O_2}$ and the deoxy[heme] amplitude in the supine but not upright positions. Comparisons were conducted at the same absolute and relative work rates to circumvent the confounding influence of differences in relative intensities between body postures.

METHODS

This study was conducted in two parts. *Part 1* compared $\dot{V}O_2$ kinetics and muscle deoxy[heme] and total[heme] in superficial muscle [vastus lateralis (VL)s and rectus femoris (RF)s] between primed and unprimed upright and supine exercise at the same absolute work rate in 17 healthy male subjects. *Part 2* utilized a subset of 10 participants to compare $\dot{V}O_2$ kinetics and muscle deoxy[heme] and total[heme] in superficial (VLs and RFs) and deep (VLD) muscle and muscle activation patterns (EMG of the VLs and RFs) between upright and supine exercise at the same relative work rate (i.e., $\Delta 40\%$ in both modes). This design was chosen to ensure that differences observed between postures were not merely a result of a greater relative exercise intensity in the supine posture during comparisons at the same absolute work rate or confounded by differences in absolute work rate during comparisons at the same relative work rate.

Participants

Seventeen healthy male participants (*part 1*: age: 24 ± 5 yr; height 175 ± 6 cm; weight 67 ± 9 kg; upright cycling $\dot{V}O_{2\text{peak}}$ 50 ± 9 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), inclusive of a subset of 10 participants (*part 2*: age:

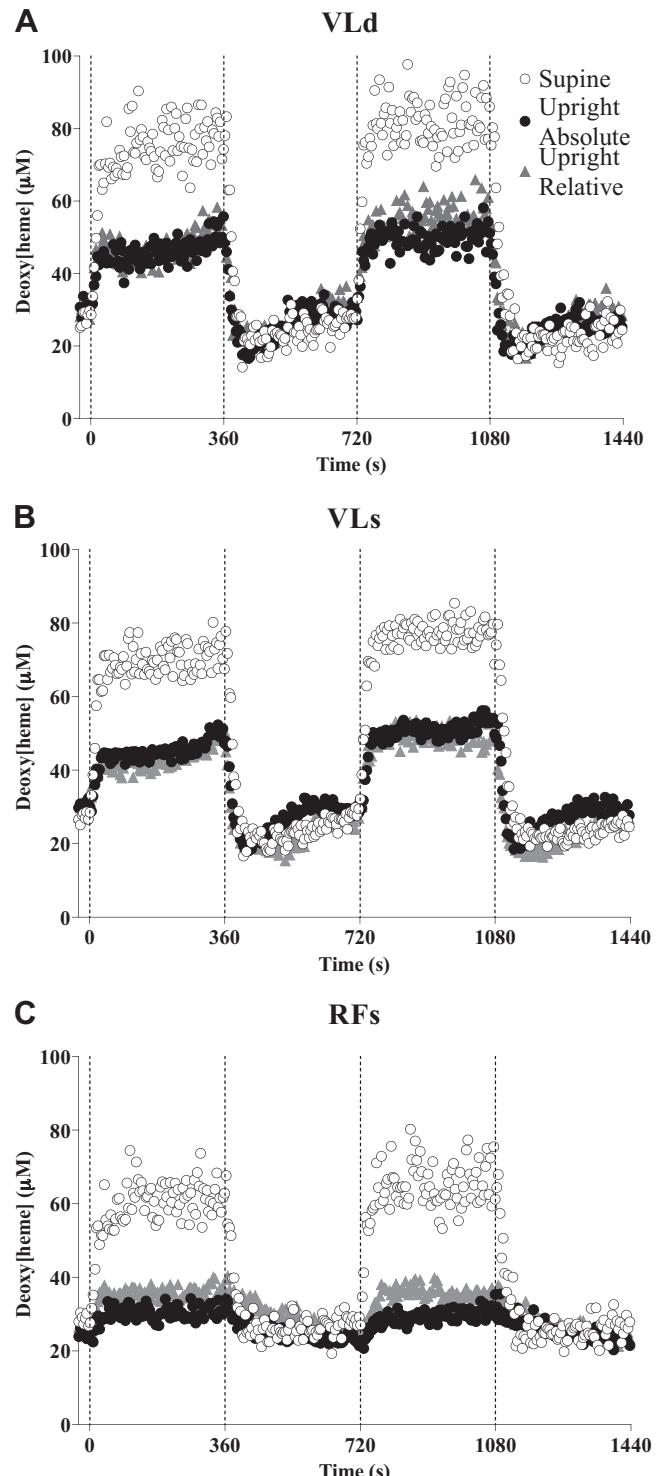


Fig. 2. Muscle deoxy[heme] responses in the supine position and the upright body position at the same absolute and relative work rates in the deep vastus lateralis (VLD, A), the superficial vastus lateralis (VLs, B), and the superficial rectus femoris (RFs, C) in a representative participant. Dashed black lines indicate onsets and offsets of exercise, respectively.

23 ± 5 yr; height 175 ± 7 cm; weight 70 ± 12 kg; upright cycling $\dot{V}O_{2\text{peak}}$ 49 ± 6 mL·kg⁻¹·min⁻¹) volunteered to take part, providing written informed consent. The experiment was approved by the Human Subjects Committee of Kobe Design University 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 before 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°C and 50 ± 10% humidity. Each participant visited the laboratory between six and eight times over a 3- to 5-wk period. All exercise tests were conducted with 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 with 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 $\dot{V}O_{2\text{peak}}$ 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. $\dot{V}O_{2\text{peak}}$ was defined as the highest 20-s value recorded throughout the test. The GET and mean response time (MRT) were determined as previously described (5, 24).

After the determination of $\dot{V}O_{2\text{peak}}$ and GET, and on separate days, constant-power exercise tests were performed in both the upright and supine body positions at the same absolute and relative ($\Delta 40\%$, i.e., 40% of the difference between $\dot{V}O_{2\text{peak}}$ 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 was selected such that participants produced physiological responses typical of heavy/severe exercise in both upright and supine exercise. This range was determined as $\Delta 10\%$ – $\Delta 60\%$, with the precise intensity chosen for each participant depending upon the participant's capacity to sustain the exercise intensity without undue discomfort. For each subject, comparisons between body positions were made at

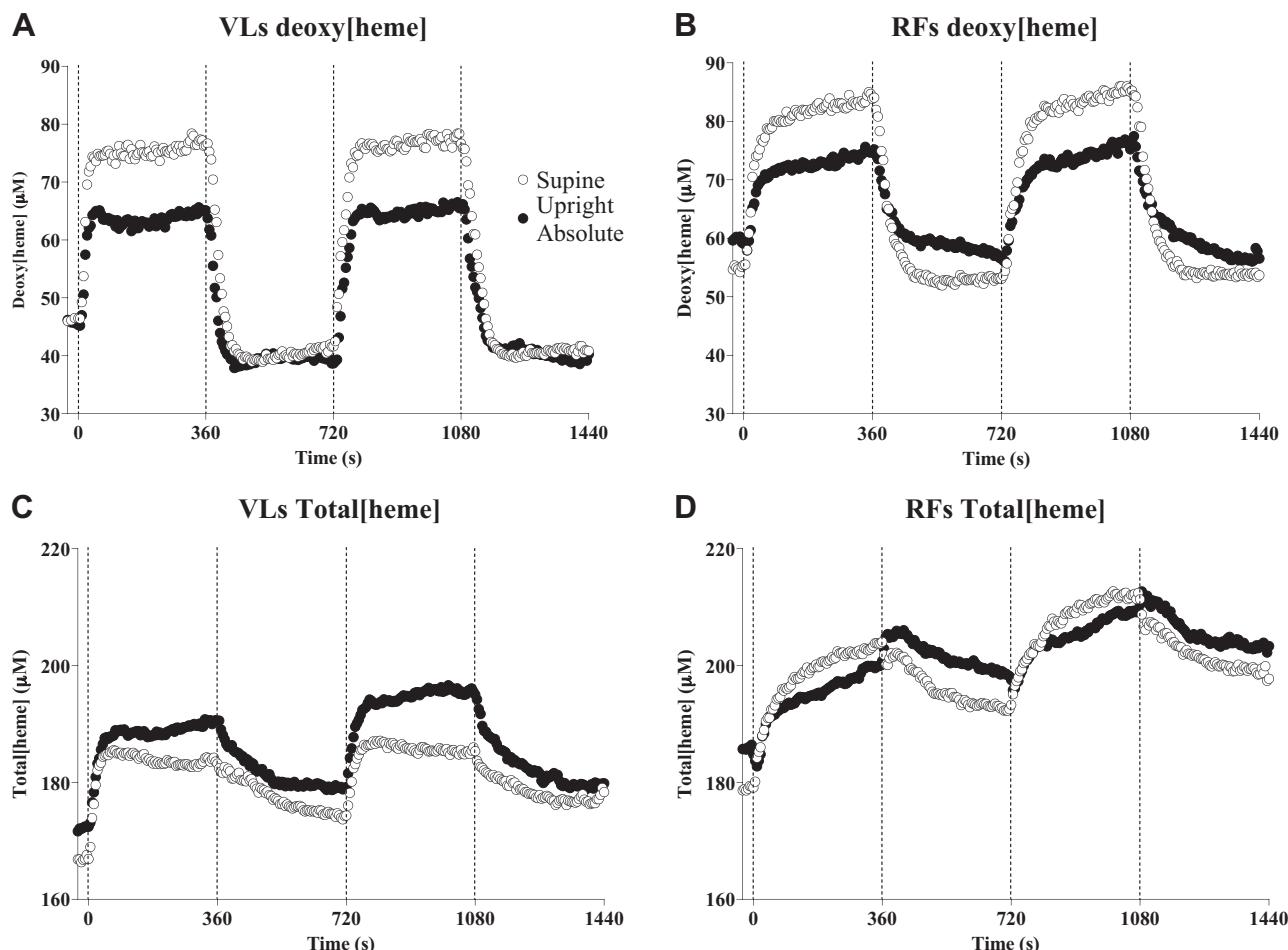


Fig. 3. Group mean responses for muscle deoxy[heme] (A and B) and total[heme] (C and D) in the supine position and the upright body position at the same absolute work rates in the superficial vastus lateralis (VLs, A and C) and the superficial rectus femoris (RFs, B and D) ($n = 17$ participants). Dashed black lines indicate onsets and offsets of exercise. Error bars omitted for clarity.

Table 2. Kinetic parameters of muscle deoxy[heme] responses during supine and upright cycle exercise at the same absolute work rate for VLs and RFs ($n = 17$) and VLD ($n = 10$) in bout 1 and bout 2

	Supine		Upright	
	Bout 1	Bout 2	Bout 1	Bout 2
Baseline, μM				
VLs	47 ± 29	41 ± 30*	46 ± 11	39 ± 10*
RFs	55 ± 42†	53 ± 43*†	60 ± 34†	57 ± 34*†
VLD	32 ± 14	28 ± 12	38 ± 19	36 ± 17
TD, s				
VLs	10 ± 4	13 ± 12	14 ± 7#	16 ± 12#
RFs	10 ± 5	11 ± 15	20 ± 9#†	21 ± 15#
VLD	13 ± 7	23 ± 8*	16 ± 7#	21 ± 13*
$\tau_{deoxy[heme]}, s$				
VLs	12 ± 6	16 ± 10*	8 ± 4#	13 ± 5**#
RFs	18 ± 8†	30 ± 15*†	11 ± 5#†	25 ± 11**#†
VLD	22 ± 11	18 ± 6	9 ± 5#	19 ± 12*
$A_{deoxy[heme]}, \mu M$				
VLs	28 ± 14	35 ± 16*	18 ± 9#	25 ± 14**#
RFs	25 ± 12†	30 ± 16*†	13 ± 8#†	17 ± 8**#†
VLD	31 ± 33	34 ± 35	16 ± 23#	19 ± 23#
End-exercise deoxy[heme], μM				
VLs	75 ± 38	77 ± 38*	65 ± 17	66 ± 18*
RFs	84 ± 51	85 ± 51*	75 ± 36	76 ± 37*
VLD	66 ± 47	67 ± 47	54 ± 40	57 ± 42
Deoxy[heme] SC, μM				
VLs	0.55 ± 5.32	0.71 ± 3.32	0.76 ± 6.45	1.54 ± 4.25
RFs	3.85 ± 4.01†	1.40 ± 4.80	4.03 ± 7.34†	2.50 ± 3.75
VLD	2.57 ± 7.52	4.35 ± 5.95	1.02 ± 7.03	2.69 ± 7.79

Deep vastus lateralis (VLD) data are for $n = 10$ participants; thus these comparisons were conducted against the same 10 participants only in the supine and upright matched absolute work rate conditions. $A_{deoxy[heme]}$, fundamental amplitude; baseline, average value over final 30 s of baseline period; deoxy[heme], muscle deoxygenated heme concentration; end-exercise, average value over final 30 s of exercise; RFs, superficial rectus femoris; SC, magnitude of the slow component; TD, fundamental time delay; VLs, superficial vastus lateralis; $\tau_{deoxy[heme]}$, fundamental time constant. *Significantly different from *bout 1* within same muscle; #significantly different from supine within same bout number; †significantly different from VLs within same bout number and posture ($P < 0.05$).

Table 3. Muscle total[heme] responses during supine and upright cycle exercise at the same absolute work rate for the VLs and RFs ($n = 17$) and VLD ($n = 10$) in bouts 1 and 2

	Supine		Upright	
	Bout 1	Bout 2	Bout 1	Bout 2
Baseline, μM				
VLs	166 ± 36	173 ± 35*	170 ± 35	177 ± 36*
RFs	179 ± 58	193 ± 59*†	186 ± 56#†	197 ± 56**#†
VLD	110 ± 53	121 ± 61*	123 ± 63#	137 ± 71*
Amplitude at 3 min, μM				
VLs	17 ± 8	12 ± 7*	16 ± 8	15 ± 8
RFs	20 ± 10†	19 ± 10*†	9 ± 9#†	7 ± 8#†
VLD	20 ± 22	14 ± 14	7 ± 14#	6 ± 9#
Amplitude at 6 min, μM				
VLs	17 ± 10	11 ± 8*	19 ± 8	17 ± 9#
RFs	25 ± 10†	22 ± 9*†	14 ± 9#	11 ± 9#†
VLD	21 ± 20	15 ± 15	10 ± 14#	13 ± 15
End-exercise, μM				
VLs	183 ± 52	184 ± 40*	189 ± 54	194 ± 41*
RFs	204 ± 66†	215 ± 66*†	200 ± 61†	208 ± 62*†
VLD	130 ± 69	136 ± 72*	137 ± 75	140 ± 83*
$\Delta A_{Amplitude}$ (6, 3), μM				
VLs	-0.18 ± 6.29	-0.80 ± 3.28	2.71 ± 5.62#	0.75 ± 3.13#
RFs	4.14 ± 3.08†	3.16 ± 2.40*†	5.06 ± 3.64#†	3.91 ± 3.66#†
VLD	1.15 ± 3.28	1.46 ± 3.28	2.74 ± 2.55#	6.95 ± 9.43#

Deep vastus lateralis (VLD) data are for $n = 10$ participants; thus these comparisons were conducted against the same 10 participants only in the supine and upright matched absolute work rate conditions (data not shown). Amplitude at 3 min, change in total[heme] between baseline and 3 min; amplitude at 6 min, change in total[heme] between baseline and 6 min; baseline, average value over final 30 s of baseline period; end-exercise, average value over final 30 s of exercise; RFs, superficial rectus femoris; total[heme], muscle total heme concentration; VLs, superficial vastus lateralis; $\Delta A_{Amplitude}$ (6, 3), change in total[heme] between 3 and 6 min. *Significantly different from *bout 1* within same muscle; #significantly different from supine within same bout number; †significantly different from VLs within same bout number and posture ($P < 0.05$).

the same absolute work rate. For each constant-power test, participants performed two 6-min bouts of exercise at the desired power output, separated by 6 min of cycling at 20 W. Participants repeated this protocol two or three times in each body position over a 3- to 4-wk period.

Measurements

Pulmonary $\dot{V}O_2$. Pulmonary gas exchange/ventilation was measured breath by breath throughout all tests with a hot-wire flowmeter (model AE-300S; Minato-Medical, Osaka, Japan) and a gas analyzer (model AE-300S; Minato-Medical, Osaka, Japan) as previously described (42, 43).

Time-resolved near-infrared spectroscopy. Continuous noninvasive measurements of absolute deoxy[heme], oxy[heme], and total[heme] (i.e., deoxy[heme] + oxy[heme]) in the RFs and VLs were made with two TRS-NIRS devices (TRS-20; Hamamatsu Photonics K.K., Hamamatsu, Japan), whereas a high-power TRS-NIRS device (TRS-20D; Hamamatsu Photonics K.K., Hamamatsu, Japan) was used to measure the same variables in the VLd. The optodes for superficial muscles 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 (44, 45, 58), as well as the specific measurement procedures used in our laboratory (1, 20, 41–43, 59–61), have been reviewed in detail elsewhere. Adipose tissue thickness (ATT) was measured at each muscle site with 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. (6), with separate correction factors used for each muscle (14).

Surface electromyography. Surface EMG was measured with 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 SANKEI, Osaka, Japan) to estimate muscle activation patterns near the TRS-NIRS optode sites of the VLs and RFs in part 2, as previously described (43, 59). 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 min 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 $\dot{V}O_2$ was edited to remove aberrant values (i.e., >4 SD outside local 5-breath mean). The edited $\dot{V}O_2$ and deoxy[heme] data from the constant-power exercise tests were linearly interpolated (1 s); identical bouts were time-aligned and ensemble-averaged across each transition for each subject [data treatment and analysis procedures have been described in full elsewhere (23–28)]. A monoexponential model with time delay was then fitted to the data, as follows:

$$Y_{(t)} = Y_{(b)} + A_Y \times (1 - e^{-(t-TD_Y\tau_Y)}) \quad (1)$$

where $Y_{(t)}$ is the value of the independent variable at any time t , $Y_{(b)}$ is the baseline value measured over the final 30 s of baseline cycling, A_Y is the amplitude of increase in Y above baseline, TD_Y is the time delay, and τ_Y is the time constant of the response. For $\dot{V}O_2$ data, the model was constrained to exclude the slow component and hence isolate the fundamental phase. Briefly, the onset of the $\dot{V}O_2$ slow component was identified with purpose-designed programming in Microsoft Excel (Microsoft Corporation, Redmond, WA), which it-

eratively fits a monoexponential function to the $\dot{V}O_2$ data, starting at 60 s until the window encompasses the entire response. The estimated $\tau_{\dot{V}O_2}$ values for each fitting window are then plotted against time, with the onset of the slow component determined as the point at which $\tau_{\dot{V}O_2}$ consistently deviated from a previously “flat” profile. The full data

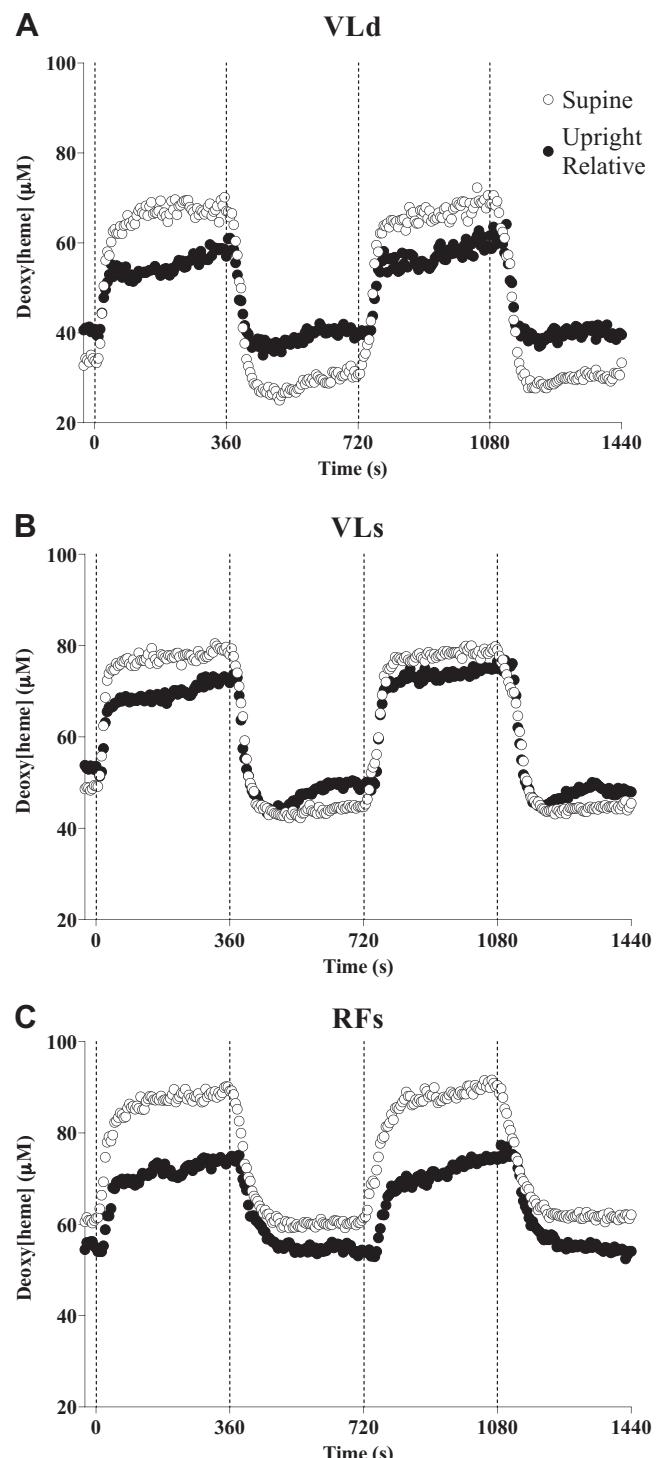


Fig. 4. Group mean responses for muscle deoxy[heme] in the supine position and the upright body position at the same relative work rates in the deep vastus lateralis (VLd, A), the superficial vastus lateralis (VLs, B), and the superficial rectus femoris (RFs, C) ($n = 10$ participants). Dashed black lines indicate onsets and offsets of exercise. Error bars omitted for clarity.

treatment and analysis procedures have been described in full elsewhere (23–28). The amplitude of the $\dot{V}O_2$ slow component was determined by calculating the difference between end-exercise $\dot{V}O_2$ (i.e., mean $\dot{V}O_2$ over the final 30 s of exercise) and $\dot{V}O_{2(b)} + A_{\dot{V}O_2}$.

$TD_{deoxy[heme]}$ was determined as the first datum that was 1 SD above the mean value from the final 30 s of baseline cycling, and the fitting window was constrained up to the onset of the $\dot{V}O_2$ slow component. The amplitude of the deoxy[heme] slow component was calculated by subtracting the average value of deoxy[heme] during the final 30 s of exercise from the absolute deoxy[heme] response (i.e., $deoxy[heme]_{(b)} + A_{deoxy[heme]}$). Values for total[heme] were measured from the mean of the last 30 s of baseline pedaling and the 30 s immediately before 3 and 6 min of exercise. The changes in total[heme] between time points (i.e., amplitude) were also calculated. The amplitudes of deoxy[heme] normalized to iEMG (i.e., $\Delta\mu M/\Delta\%MVC$) were also determined from the mean of the 30 s before 3 and 6 min of exercise. These particular time points (i.e., baseline, 3 min, and 6 min) were chosen to facilitate comparisons at a time point that approximated the end of the fundamental phase and emergence of the slow component for $\dot{V}O_2$ (i.e., 3 min) and at exercise termination (i.e., 6 min).

Statistics

Data were compared with two-way repeated-measures analysis of variance (ANOVA) across condition (supine, upright matched absolute work rate, upright matched relative work rate), bout number (*bout 1*, *bout 2*), muscle (VLd, VLs, and RFs), and time (baseline, 180 s, 360 s). Where significant differences were found, Holm–Sidak-adjusted post hoc comparisons were used to locate these differences. Partial eta squared (η_p^2) was also calculated as a measure of effect size. Pearson's product-moment correlation coefficient was used to explore

relationships between variables of interest. Normality was checked with the Shapiro–Wilk test; the assumption of sphericity was checked with Mauchly's test. Where the assumption of sphericity was violated, the Greenhouse–Geisser correction factor was used to adjust the degrees of freedom. Statistical software (SigmaPlot 13.0; Systat Software, San Jose, CA) was used for all statistical analyses; figures were produced with GraphPad Prism (version 7.02; GraphPad software, San Diego, CA). Data are presented as means \pm SD. Significance was declared when $P < 0.05$.

RESULTS

$\dot{V}O_2$ Kinetics at the Same Absolute Work Rate

$\tau_{\dot{V}O_2}$ was greater in the supine compared with the upright position in *bout 1*, whereas $\tau_{\dot{V}O_2}$ was reduced by priming exercise in the supine position only ($n = 17$, bout \times condition interaction: $\eta_p^2 = 0.56$, $F_{1,16} = 6.78$, $P = 0.019$; Fig. 1, Table 1). As a result, $\tau_{\dot{V}O_2}$ did not differ between supine and upright exercise in *bout 2* (Fig. 1, Table 1).

Muscle Deoxy and Total[Heme] Responses at the Same Absolute Work Rate in Superficial Muscle

The amplitude of the deoxy[heme] response was greater for both muscle sites (i.e., VLs, RFs) in the supine compared with the upright position in *bout 1* (both $P < 0.001$). Priming exercise reduced baseline deoxy[heme] ($n = 17$, VLs: $\eta_p^2 = 0.89$, $F_{1,16} = 22.58$, $P < 0.001$; RFs: $\eta_p^2 = 0.76$, $F_{1,16} = 7.74$, $P = 0.013$); consequently, the amplitude of the deoxy[heme]

Table 4. Kinetics parameters of the muscle deoxy[heme] responses during supine and upright cycle exercise at the same relative work rates for both deep and superficial muscle in bouts 1 and 2 ($n = 10$)

	Supine		Upright	
	Bout 1	Bout 2	Bout 1	Bout 2
Baseline, μM				
VLd	32 \pm 14	28 \pm 12	40 \pm 20	40 \pm 20
VLs	49 \pm 38	45 \pm 39*	53 \pm 20	49 \pm 19*
RFs	61 \pm 55‡	61 \pm 56‡	55 \pm 25‡	54 \pm 22‡
TD, s				
VLd	13 \pm 7	23 \pm 8*	—	—
VLs	10 \pm 6	17 \pm 14*	16 \pm 5#	32 \pm 6**§
RFs	11 \pm 6	19 \pm 17*	20 \pm 7#	32 \pm 11*
$\tau_{deoxy[heme]}$, s				
VLd	22 \pm 11	18 \pm 6	—	—
VLs	13 \pm 7	22 \pm 17*	11 \pm 7	16 \pm 10*
RFs	20 \pm 10	30 \pm 17*†‡	24 \pm 16	26 \pm 13*†
$A_{deoxy[heme]}$, μM				
VLd	31 \pm 33	34 \pm 35	13 \pm 15#	17 \pm 20#
VLs	27 \pm 16	33 \pm 20*	15 \pm 7#	24 \pm 13**§
RFs	25 \pm 14	27 \pm 15	17 \pm 10#§	17 \pm 13#§
End-exercise deoxy[heme], μM				
VLd	66 \pm 47	67 \pm 47	58 \pm 35	60 \pm 37
VLs	77 \pm 50	79 \pm 50	73 \pm 26	75 \pm 27
RFs	89 \pm 67	91 \pm 66	74 \pm 34#	75 \pm 33
Deoxy[heme] SC, μM				
VLd	2.57 \pm 7.52	4.35 \pm 5.95	—	—
VLs	0.35 \pm 5.61	0.99 \pm 3.46	4.26 \pm 3.65	0.40 \pm 8.24
RFs	3.17 \pm 2.51	2.49 \pm 2.67	2.15 \pm 7.80	5.47 \pm 16.54

$A_{deoxy[heme]}$, fundamental amplitude; baseline, average value over final 30 s of baseline period; deoxy[heme], muscle deoxygenated [heme] concentration; end-exercise, average value over final 30 s of exercise; RFs, superficial rectus femoris; SC, magnitude of the slow component; TD, fundamental time delay; VLd, deep vastus lateralis; VLs, superficial vastus lateralis; $\tau_{deoxy[heme]}$, fundamental time constant; —, a low signal-to-noise ratio precluded confident kinetics modeling at the matched relative work rate in the upright position. See text for more details. *Significantly different from *bout 1* within same muscle; #significantly different from supine within same bout number; †significantly different from VLs within same bout number and posture; ‡significantly different from VLd within same bout number and posture; §significantly different from upright absolute matched work rate within same bout number ($P < 0.05$).

response was increased with priming exercise ($n = 17$, VLs: $\eta_p^2 = 0.86$, $F_{1,16} = 40.69$, $P < 0.001$; RFs: $\eta_p^2 = 0.60$, $F_{1,16} = 12.25$, $P = 0.003$) in both positions (Figs. 2 and 3, Table 2). $\tau_{\text{deoxy[heme]}}$ ($n = 17$, VLs: $\eta_p^2 = 0.49$, $F_{1,16} = 8.31$, $P = 0.011$; RFs: $\eta_p^2 = 0.66$, $F_{1,16} = 33.42$, $P < 0.001$) was greater after priming exercise in both body positions (Figs. 2 and 3, Table 2). Priming exercise increased baseline and end-exercise total[heme] for both muscle sites in both positions (all $P < 0.05$) and reduced the amplitude of the total[heme] response in both muscles in the supine position only ($n = 17$, VLs: $\eta_p^2 = 0.28$, $F_{1,16} = 6.30$, $P = 0.023$; RFs: $\eta_p^2 = 0.52$, $F_{1,16} = 17.28$, $P < 0.001$; Fig. 3, Table 3).

Muscle Deoxy and Total[Heme] Responses at the Same Absolute Work Rate in Deep Muscle

Priming exercise increased $TD_{\text{deoxy[heme]}}$ in both body positions in the VLD ($n = 10$, $\eta_p^2 = 0.53$, $F_{1,9} = 33.46$, $P < 0.001$), whereas $\tau_{\text{deoxy[heme]}}$ was greater after prior exercise in the upright position only ($n = 10$, $\eta_p^2 = 0.54$, $F_{1,9} = 10.53$, $P < 0.001$; Table 2). The deoxy[heme] amplitude was greater in the supine position in both *bouts* 1 and 2 when compared with the upright position ($n = 10$, $\eta_p^2 = 0.87$, $F_{1,9} = 4.46$, $P = 0.027$). Priming exercise also increased baseline and end-exercise total[heme] compared with *bout* 1 in both positions in the VLD (both $P < 0.05$).

$\dot{V}O_2$ Kinetics at the Same Relative Work Rate

$\tau_{\dot{V}O_2}$ was greater in the supine compared with the upright position in *bout* 1, whereas $\tau_{\dot{V}O_2}$ was reduced by priming exercise in the supine position only ($n = 10$, bout \times condition interaction, $\eta_p^2 = 0.36$, $F_{1,9} = 5.57$, $P = 0.040$; Fig. 1, Table 1). As a result, $\tau_{\dot{V}O_2}$ did not differ between supine and upright exercise in *bout* 2 (Fig. 1, Table 1).

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 kinetics data for VLD are omitted. Priming exercise reduced baseline deoxy[heme] ($n = 10$, $\eta_p^2 = 0.61$, $F_{1,9} = 8.30$, $P = 0.018$) and increased the deoxy[heme] amplitude ($n = 10$, $\eta_p^2 = 0.69$, $F_{1,9} = 22.35$, $P = 0.001$) in both body positions for the VLs only (Fig. 4, Table 4). Irrespective of bout number and muscle site, the deoxy[heme] amplitude was greater in the supine versus upright position (all $P < 0.05$; Table 4). $TD_{\text{deoxy[heme]}}$ and $\tau_{\text{deoxy[heme]}}$ were both greater after priming exercise in both body positions in the VLs ($n = 10$, $TD_{\text{deoxy[heme]}}: \eta_p^2 = 0.61$, $F_{1,9} = 35.07$, $P < 0.001$; $\tau_{\text{deoxy[heme]}}: \eta_p^2 = 0.38$, $F_{1,9} = 5.18$, $P = 0.049$) and RFs ($n = 10$, $TD_{\text{deoxy[heme]}}: \eta_p^2 = 0.56$, $F_{1,9} = 26.42$, $P < 0.001$, $\tau_{\text{deoxy[heme]}}: \eta_p^2 = 0.34$, $F_{1,9} = 13.75$, $P = 0.005$). Priming exercise elevated baseline and end-exercise total[heme] in both positions and at all muscle sites (all $P < 0.05$), whereas the total[heme] amplitude at 6 min was reduced with priming in the VLs in the supine position only ($n = 10$, $\eta_p^2 = 0.51$, $F_{2,18} = 9.40$, $P = 0.002$) and in the RFs in both positions ($n = 10$, $\eta_p^2 = 0.47$, $F_{1,9} = 15.51$, $P = 0.003$; Fig. 5, Table 5). $\Delta\text{Deoxy[heme]}/\Delta iEMG$ was greater in supine versus upright matched absolute and relative work rate exercise in *bouts* 1 and 2 ($n = 10$, VLs: $\eta_p^2 = 0.87$, $F_{2,9} = 8.02$, $P = 0.003$; RFs: $\eta_p^2 = 0.82$, $F_{2,9} = 17.13$, $P < 0.001$) and was increased after priming exercise

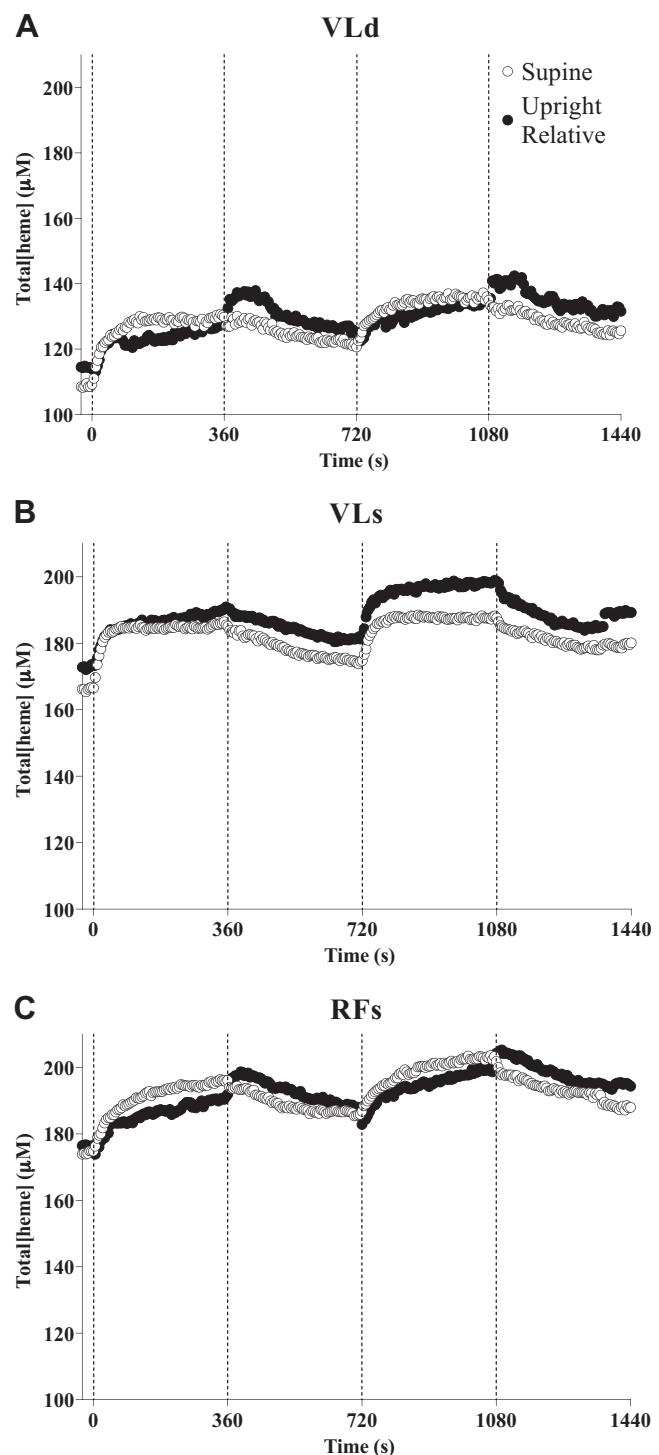


Fig. 5. Group mean responses for muscle total[heme] in the supine position and the upright body position at the same relative work rates in the deep vastus lateralis (VLD, A), the superficial vastus lateralis (VLs, B), and the superficial rectus femoris (RFs, C) ($n = 10$ participants). Dashed black lines indicate onsets and offsets of exercise. Error bars omitted for clarity.

$\Delta\text{Deoxy[heme]}/\Delta iEMG$ was greater in supine versus upright matched absolute and relative work rate exercise in *bouts* 1 and 2 ($n = 10$, VLs: $\eta_p^2 = 0.87$, $F_{2,9} = 8.02$, $P = 0.003$; RFs: $\eta_p^2 = 0.82$, $F_{2,9} = 17.13$, $P < 0.001$) and was increased after priming exercise

Table 5. Muscle total[heme] responses during supine and upright cycle exercise at the same relative work rates for both deep and superficial muscle in bouts 1 and 2 ($n = 10$)

	Supine		Upright	
	Bout 1	Bout 2	Bout 1	Bout 2
Baseline, μM				
VLD	110 \pm 53	121 \pm 61*	114 \pm 53	126 \pm 62*
VLS	166 \pm 47‡	174 \pm 48*‡	173 \pm 48‡	181 \pm 54*‡
RFs	174 \pm 76‡	186 \pm 80*‡	177 \pm 73‡	188 \pm 75*‡
Amplitude at 3 min, μM				
VLD	20 \pm 22	14 \pm 14	9 \pm 13#	5 \pm 11
VLS	18 \pm 9	14 \pm 7*	14 \pm 8‡	16 \pm 15‡
RFs	19 \pm 11	14 \pm 9	10 \pm 8#†	7 \pm 8#†
Amplitude at 6 min, μM				
VLD	21 \pm 20	15 \pm 15	13 \pm 15#	9 \pm 12
VLS	20 \pm 9	14 \pm 8*	17 \pm 7	17 \pm 7
RFs	22 \pm 10	16 \pm 11*	14 \pm 8#	11 \pm 8**
End-exercise, μM				
VLD	130 \pm 69	136 \pm 72*	127 \pm 66	135 \pm 70
VLS	186 \pm 52‡	188 \pm 52*‡	187 \pm 52‡	198 \pm 56*‡
RFs	196 \pm 85‡	202 \pm 85*‡	191 \pm 80‡	200 \pm 82*‡
Δ Amplitude (6, 3), μM				
VLD	1.15 \pm 3.28	1.46 \pm 3.28	4.27 \pm 4.22	4.35 \pm 4.15
VLS	1.30 \pm 5.66	-0.37 \pm 3.40‡	3.46 \pm 5.08	1.09 \pm 3.61‡
RFs	3.69 \pm 2.26	1.76 \pm 4.43†	4.08 \pm 4.28	4.34 \pm 5.20†

Amplitude at 3 min, change in total[heme] between baseline and 3 min; amplitude at 6 min, change in total[heme] between baseline and 6 min; baseline, average value over final 30 s of baseline period; end-exercise, average value over final 30 s of exercise; RFs, superficial rectus femoris; total[heme], muscle total heme concentration; VLD, deep vastus lateralis; VLS, superficial vastus lateralis; Δ Amplitude (6, 3), change in total[heme] between 3 and 6 min. *Significantly different from bout 1 within same muscle; #significantly different from supine within same bout number; †significantly different from VLS within same bout number and posture; ‡significantly different from VLD within same bout number and posture ($P < 0.05$).

in the VLS ($n = 10$, $\eta_p^2 = 0.67$, $F_{1,9} = 17.24$, $P = 0.002$; Fig. 6).

DISCUSSION

The principal findings of this study were that, in the face of a greater $\tau_{\dot{V}O_2}$ and deoxy[heme] amplitude in *bout 1* during supine compared with upright exercise, 1) across muscle sites priming exercise tended to reduce baseline deoxy[heme] but increase baseline total[heme], TD_{deoxy[heme]}, and $\tau_{deoxy[heme]}$ at the onset of *bout 2* in both body positions (i.e., indicating enhanced \dot{Q}_{O_2} in *bout 2*) and 2) priming exercise restored $\tau_{\dot{V}O_2}$ in the supine position to values similar to upright exercise but did not restore the deoxy[heme] amplitude to values not different from those observed in the upright position. *Finding 1* was consistent with our hypotheses. However, *finding 2* was not consistent with the hypothesis that priming exercise would reduce the deoxy[heme] amplitude in the supine but not upright position. Moreover, the greater deoxy[heme] amplitude in supine compared with upright exercise in *bout 2* persisted even when normalized for iEMG (i.e., Δ deoxy[heme]/ Δ iEMG). These findings challenge the notion that the effects of priming exercise on pulmonary $\dot{V}O_2$ kinetics are attributable solely to improved microvascular O_2 distribution (56, 57, 68) and suggest instead that enhancements to intracellular O_2 utilization are at least equivalently contributory.

Effect of Posture on $\dot{V}O_2$ Kinetics during Bout 1

$\tau_{\dot{V}O_2}$ was greater in supine versus upright exercise at both the same absolute and relative work rates in *bout 1*. This finding may be attributed to the loss of the “hydrostatic gradient effect” due to gravity in the supine position, thus lowering the pressure head for blood-to-myocyte O_2 diffusion and slowing the sub-

sequent rise in $\dot{V}O_2$ at exercise onset (17, 19, 37, 47). In support of this, we observed a lower baseline total[heme] in the supine position, indicating a reduced blood volume within the window of interrogation (16). TD_{deoxy[heme]} was reduced at all muscle sites in the supine position, indicating that $\Delta\dot{V}O_2$ began to exceed $\Delta\dot{Q}_{O_2}$ at an earlier time point during the exercise transition compared with upright exercise. Moreover, the deoxy[heme] amplitude and deoxy[heme]/iEMG were increased across all muscle sites in the supine versus upright position at both the same absolute and relative work rates. Hence, exercise in the supine position necessitated a greater reliance on fractional O_2 extraction to satisfy a given increment in metabolic rate. Collectively, therefore, the TR-NIRS data are consistent with the notion that pulmonary $\dot{V}O_2$ kinetics were slowed in the supine position because of impairments in \dot{Q}_{O_2} .

Effect of Posture and Priming on $\dot{V}O_2$ Kinetics

We found that priming exercise led to a reduction in $\tau_{\dot{V}O_2}$ in the supine but not upright position. Moreover, $\tau_{\dot{V}O_2}$ did not differ between upright and supine exercise after priming. This finding is in agreement with Goulding et al. (24) and Jones et al. (39), who both showed that prior heavy exercise reduces $\tau_{\dot{V}O_2}$ in the supine but not upright position. These findings align with the notion that $\tau_{\dot{V}O_2}$ is unaffected in most studies of priming exercise in the upright position in young healthy individuals (2, 7–12, 18, 20, 24, 39, 48, 52, 65–67, 71). The divergent responses to priming between upright and supine exercise are typically explained by the fact that in young healthy adults $\dot{V}O_2$ kinetics are not limited by \dot{Q}_{O_2} in the upright position (4, 28, 43, 48, 62, 63, 67, 70, 72) and hence $\tau_{\dot{V}O_2}$ is intransigent to the effects of priming exercise. Conversely, during supine exercise, where a reduced and sluggish

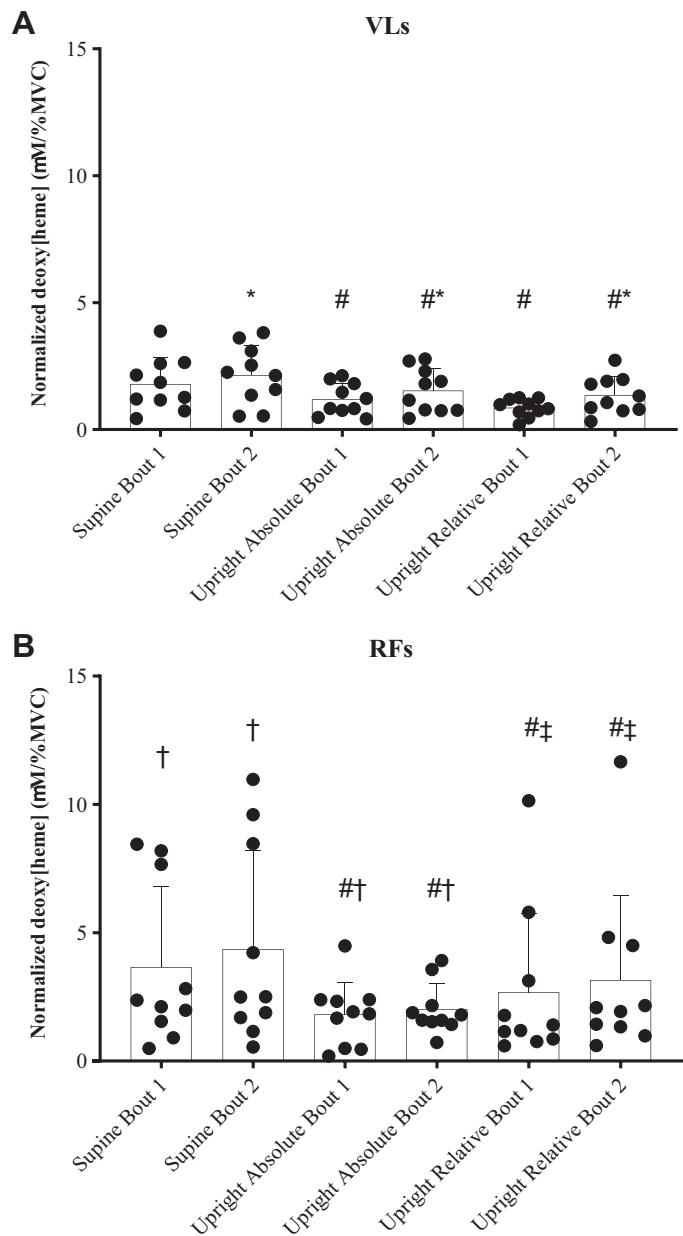


Fig. 6. Group mean amplitudes of deoxy[heme] normalized to integrated electromyography (iEMG) [i.e., $\mu\text{M}/\% \text{MVC}$] for the superficial vastus lateralis (VLs, A) and the superficial rectus femoris (RFs, B) in *bouts* 1 and 2. *Significantly different from *bout* 1 within same muscle; #significantly different from supine within same *bout* number; †significantly different from VLs within same *bout* number and posture; ‡significantly different from upright absolute matched work rate within same *bout* number ($P < 0.05$).

\dot{Q}_{O_2} response slows $\dot{V}O_2$ kinetics in the control condition, the \dot{Q}_{O_2} limitation is negated by the physiological effects of priming exercise (24, 27, 39). However, studies have demonstrated a reduction in $\tau_{\dot{V}O_2}$ following priming exercise in young, apparently healthy humans performing upright cycle exercise (15, 32, 56, 57, 68, 69). In these studies, individuals with slower $\dot{V}O_2$ kinetics evince a greater speeding of $\dot{V}O_2$ kinetics after priming exercise (32). Indeed, in the present study across both postures the reduction in $\tau_{\dot{V}O_2}$ following priming exercise was inversely linearly related to $\tau_{\dot{V}O_2}$ in *bout* 1 ($r = -0.58$;

Fig. 1D). Collectively, therefore, it seems that greater (i.e., slower) $\tau_{\dot{V}O_2}$ values in the control condition in any given posture increase the likelihood that $\tau_{\dot{V}O_2}$ will be reduced (i.e., faster) after priming.

Evidence for Enhanced O_2 Delivery

In both positions, total[heme] was greater at baseline and throughout *bout* 2 for all muscle sites, whereas baseline deoxy[heme] was reduced in superficial muscle. Hence, the first heavy bout of exercise resulted in postexercise hyperemia that persisted throughout *bout* 2, consistent with previous work (15, 20, 24, 32). The total[heme] amplitude was also reduced in superficial muscle in the supine position after priming, suggesting a greater reliance on convective rather than diffusive O_2 transport to satisfy their respective increases in metabolic rate. Furthermore, after priming exercise the $\Delta\text{deoxy[heme]}$ was greater in both positions and all muscle groups at the same relative work rate, whereas $\tau_{\text{deoxy[heme]}}$ was greater in both positions for all muscles except VLD in the supine position. In the canine hindlimb, muscle blood flow kinetics adapted more rapidly after a prior bout of contractions, suggesting greater vasodilation-induced \dot{Q}_{O_2} in the second bout of contractions (35), which could explain the overall slower rate of muscle deoxygenation after priming noted here. The present study thus demonstrates that priming exercise enhances local microvascular O_2 availability across the muscle mass and is supportive of the notion that changes thereof may have been contributory to the observed changes in the $\dot{V}O_2$ kinetics response following priming in both body positions. Moreover, the TR-NIRS data indicate that muscle \dot{Q}_{O_2} was enhanced in both postures after priming exercise but was only associated with faster $\dot{V}O_2$ kinetics in the supine position. This latter observation lends further credence to the notion that for young, healthy individuals performing upright cycle exercise \dot{Q}_{O_2} is not the rate-limiting factor for the speed of the $\dot{V}O_2$ kinetics (29, 63).

Evidence for Enhanced Intracellular O_2 Utilization

Despite clear evidence for enhanced microvascular O_2 availability after priming exercise in the supine posture, the deoxy[heme] amplitude remained substantially elevated above that observed during upright exercise. Additionally, $\Delta\text{deoxy[heme]}/\Delta\text{iEMG}$ remained greater in the supine position after priming and was increased in *bout* 2 for both positions in the VLs. Hence, compared with upright exercise, there was still a greater reliance on fractional O_2 extraction for a given muscle activation after priming exercise in the supine position. Furthermore, given the enhanced microvascular O_2 availability after priming exercise in the supine position, that deoxy[heme]/iEMG was increased in the VLs indicates that intracellular O_2 utilization must have been enhanced. The fact that the amplitude of fractional O_2 extraction (i.e., deoxy[heme]) remained markedly elevated above that in the upright position after priming indicates that priming did not fully redress the impairment in \dot{Q}_{O_2} induced by the supine position. Since $\tau_{\dot{V}O_2}$ did not differ between upright and supine exercise after priming, therefore, the enhanced intracellular O_2 utilization after priming must have contributed to the speeding of $\dot{V}O_2$ kinetics observed in the supine position. These findings are therefore in direct contradiction with the notion that the physiological

effects of priming exercise are brought about entirely via enhanced microvascular perfusion (56, 57, 68).

Evidence for Interaction between O_2 Delivery and Utilization in Determining $\dot{V}O_2$ Kinetics

The present findings do not allow elucidation of the relative contributions of \dot{Q}_{O_2} and O_2 utilization in determining the faster $\dot{V}O_2$ kinetics observed after priming in the supine position. They do, however, demonstrate a role for enhanced intracellular O_2 utilization in speeding $\dot{V}O_2$ kinetics in situations where $\dot{V}O_2$ kinetics are otherwise constrained primarily by \dot{Q}_{O_2} . For instance, the slower $\dot{V}O_2$ kinetics in the supine position are believed to be a consequence of reduced perfusion pressure (27, 39, 47, 54), and this notion is supported by evidence that $\dot{V}O_2$ kinetics are speeded by hyperoxia (27) and lower body negative pressure (37) in this position. Collectively, therefore, the present findings demonstrate that supine exercise places individuals in a situation where acute enhancements to both intracellular O_2 utilization and microvascular \dot{Q}_{O_2} will speed $\dot{V}O_2$ kinetics. Together, these observations suggest that limitations to the $\dot{V}O_2$ kinetics response may consist of an interaction between metabolic inertia and \dot{Q}_{O_2} rather than one or the other. Indeed, altered fractions of inspired O_2 change exercising intracellular P_{O_2} (64), in turn modulating phosphocreatine concentrations (33, 34, 51), which are known to regulate $\dot{V}O_2$ kinetics (29, 30). The precise nature of the regulation of $\dot{V}O_2$ kinetics by the dynamic balance between \dot{Q}_{O_2} and O_2 utilization mechanisms will thus require resolution in future research.

In contrast to the present study, Murias and colleagues (56, 57, 68) assert that the priming-induced reduction in $\tau_{\dot{V}O_2}$ is primarily related to improvements in microvascular \dot{Q}_{O_2} on the basis of concomitant reductions in the deoxy[heme]-to- $\dot{V}O_2$ ratio and the observation that priming exercise did not speed $\dot{V}O_2$ kinetics in hypoxia. Regarding the latter, to isolate the metabolic effects of priming exercise from those of \dot{Q}_{O_2} , the hypoxia-induced reduction in \dot{Q}_{O_2} is required to be precisely matched to the priming-induced increase in \dot{Q}_{O_2} ; this was not the case, however. Regarding the former, reliance on the deoxy[heme]-to- $\dot{V}O_2$ ratio to indicate changes in microvascular perfusion, the method assumes homogeneous $\dot{V}O_2$ across the exercising muscle mass, whereas in reality muscle perfusion (40), deoxygenation (44), and metabolism (13) are spatially heterogeneous during exercise. Hence, an under- or overshoot in the deoxy[heme]/ $\dot{V}O_2$ response could be caused by changes in the pulmonary $\dot{V}O_2$ response without any concomitant change in deoxy[heme] kinetics; in such a case the relevance to microvascular flow would be unclear. Finally, reliance on the temporal matching of relative changes in the responses of deoxy[heme] and $\dot{V}O_2$ is misleading because, as the present data show, slower muscle deoxygenation kinetics can coexist alongside a markedly elevated deoxygenation amplitude, the latter of which (in the case of the present study) most appropriately represents the state of O_2 availability after an intervention. Taken together, on the basis of the present data, the contention that microvascular O_2 distribution within the tissues is primarily responsible for the faster rate of adaptation of $\dot{V}O_2$ after heavy-intensity priming exercise (56, 57, 68) is no longer defensible.

Conclusions

The present study demonstrates that pulmonary $\dot{V}O_2$ kinetics are slowed during supine exercise because of primarily \dot{Q}_{O_2} -related impairments. Specifically, exercise in the supine position was initially characterized by a lower baseline total[heme], a shorter $TD_{deoxy[heme]}$, and a greater deoxy[heme] amplitude. Priming exercise improved muscle \dot{Q}_{O_2} in both body positions, evinced by a greater total[heme] throughout exercise and slower muscle deoxygenation kinetics. Accordingly, $\tau_{\dot{V}O_2}$ was reduced in the supine position after priming to values that were not different from upright exercise. Despite this, after priming in the supine position the deoxy[heme] amplitude remained markedly elevated above that observed during upright exercise. Hence, improvements in \dot{Q}_{O_2} in *separatum* are unable to account for the speeding of pulmonary $\dot{V}O_2$ kinetics following priming exercise in the supine position; therefore, enhancements to intracellular O_2 utilization must also have contributed. These findings challenge the notion that speeding of $\dot{V}O_2$ kinetics following priming exercise is exclusively due to improvements in microvascular O_2 distribution (56, 57, 68). Together, the present results support the notion that local control of $\dot{V}O_2$ kinetics may be more complex, related to an interaction between microvascular \dot{Q}_{O_2} and O_2 utilization as opposed to being “limited” by any one factor.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.P.G., S.M., D.O., D.C.P., and S.K. conceived and designed research; R.P.G., D.O., T.-H.L., N.K., and S.K. performed experiments; R.P.G. analyzed data; R.P.G., S.M., D.O., D.C.P., T.J.B., T.-H.L., and S.K. interpreted results of experiments; R.P.G. prepared figures; R.P.G. drafted manuscript; R.P.G., S.M., D.O., D.C.P., T.J.B., T.-H.L., N.K., and S.K. edited and revised manuscript; R.P.G., S.M., D.O., D.C.P., T.J.B., T.-H.L., N.K., and S.K. approved final version of manuscript.

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