**DISSOCIATION BETWEEN EXERCISE INTENSITY THRESHOLDS: MECHANISTIC INSIGHTS FROM SUPINE EXERCISE**

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**ABSTRACT**

**Introduction/purpose:** This study tested the hypothesis that the respiratory compensation point (RCP) and breakpoint in deoxygenated [heme] (deoxy[heme]*BP*, assessed via near-infrared spectroscopy (NIRS)) during ramp incremental exercise would occur at the same metabolic rate in the upright (U) and supine (S) body positions. **Methods:** Eleven healthy men completed ramp incremental exercise tests in U and S. Gas exchange was measured breath-by-breath and time-resolved-NIRS was used to measure deoxy[heme] in the vastus lateralis (VL) and rectus femoris (RF). **Results:** RCP (S: 2.56 ± 0.39, U: 2.86 ± 0.40 L.min-1, *P* = 0.02) differed from deoxy[heme]*BP* in the VL in U (3.10 ± 0.44 L.min-1, *P* = 0.002), but was not different in S in the VL (2.70 ± 0.50 L.min-1, *P* = 0.15). RCP was not different from the deoxy[heme]*BP* in the RF for either position (S: 2.34 ± 0.48 L.min-1, U: 2.76 ± 0.53 L.min-1, *P* > 0.05). However, the deoxy[heme]*BP* differed between muscles in both positions (*P* < 0.05), and changes in deoxy[heme]*BP* did not relate to delta RCP between positions (VL: *r* = 0.55, *P* = 0.080, RF: *r* = 0.26, *P* = 0.44). The deoxy[heme]*BP* was consistently preceded by a breakpoint in total[heme], and was, in turn, itself preceded by a breakpoint in muscle surface electromyography (EMG). **Conclusions:** RCP and the deoxy[heme]*BP* can be dissociated across muscles and different body positions and, therefore, do not represent the same underlying physiological phenomenon. The deoxy[heme]*BP* may, however, be mechanistically related to breakpoints in total[heme] and muscle activity.

Key words: Time-resolved near infrared spectroscopy; respiratory compensation point; deoxy- and total hemoglobin + myoglobin

**INTRODUCTION**

The physiological threshold separating “steady-state” from “non-steady state” exercise (i.e. reflecting the heavy-severe exercise intensity domain boundary) is crucial when normalizing exercise intensity. This boundary has variously been proposed to be best-characterized by the maximal lactate steady-state (MLSS) (1–3), critical power (CP) (4–8), the respiratory compensation point (RCP) (9), and the breakpoint in deoxygenated [heme] (deoxy[heme]*BP*) determined by near-infrared spectroscopy (NIRS) (3, 10–13). Of the potential candidates, RCP and deoxy[heme]*BP* are attractive because they can be determined with relative ease from a single ramp incremental exercise test. Moreover, it has been suggested that each of these thresholds (i.e., CP, MLSS, RCP, deoxy[heme]*BP*) reflects the occurrence of a common underlying physiological phenomenon (13), i.e., the heavy-severe boundary. A crucial test of this hypothesis is that the degree of agreement between any of these thresholds should remain following an intervention expected to alter both parameters.

During ramp incremental exercise, the RCP refers to the point at which ventilation (V̇E) increases disproportionately from carbon dioxide elimination (V̇CO2) causing arterial CO2 tension (PCO2) to fall (14). This typically occurs at ~75-85% V̇O2 max, although this can vary according to training status (14). The deoxy[heme]*BP*,on the other hand, represents the point during ramp incremental exercise at which deoxy[heme] plateaus. This point is typically identified by either sigmoidal (15, 16) or double-linear fitting (17) of deoxy[heme] versus time or power. The deoxy[heme]*BP* also occurs at ~75-85% V̇O2 max (17), and is typically measured in the superficial vastus lateralis (VL). In recent years it has been argued with increasing regularity that the RCP and deoxy[heme]*BP* reflect the heavy-severe boundary, and are thus caused by similar physiologic mechanisms (10–13, 18–22). Such arguments are typically made on the basis of observing strong correlations alongside no significant differences between RCP and deoxy[heme]*BP* during upright cycling, and they are often highly correlated (10–13, 18–22). However, it is erroneous to assume equivalence based upon the lack of a statistically significant difference (23–25). Indeed, in other investigations clear dissociations exist between these two variables (26, 27). Hence, evidence regarding the equivalence between RCP and deoxy[heme]*BP* is equivocal. Moreover, different quadriceps muscles (i.e., VL and rectus femoris, RF) display systematically different deoxy[heme] profiles during high-intensity cycle exercise with RF deoxy[heme] increasing more rapidly above its break point rather than plateauing like VL (28, 29). For a local threshold such as the deoxy[heme]*BP* to correspond to a whole-body threshold such as the RCP, the whole-body metabolic rate at which the deoxy[heme]*BP* occurs should not differ across different locomotor muscles.

Given the inconclusive evidence regarding whether RCP and the deoxy[heme]*BP* are equivalent, therefore, further investigations are required to determine whether the correspondence between the two variables remains following an intervention. Supine, as compared to upright, exercise impairs muscle perfusion pressure (30–34), and thus might be expected to alter both variables. Although the influence of supine exercise on the RCP has not been tested, the gas exchange threshold, V̇O2 max and maximal ventilation are reduced during supine compared to upright cycling (35, 36). Moreover, muscle deoxy[heme] is greatly increased in supine versus upright constant work rate (33, 34) and ramp incremental cycle exercise (37). Hence, supine exercise might be expected to differentially alter both the RCP and deoxy[heme]*BP*. The first purpose of this study, therefore, was to test the relationships between the RCP and deoxy[heme]*BP* during supine and upright cycle exercise.

Despite claims that the deoxy[heme]*BP* precisely reflects the heavy-severe boundary, the physiological mechanisms underpinning the deoxy[heme]*BP* are unclear. Boone et al. (26, 38) previously found that a plateau in total[heme] during ramp incremental exercise (i.e. the total[heme] *BP*) consistently preceded the deoxy[heme]*BP* in VL. It therefore remains possible that the total[heme]*BP* and deoxy[heme]*BP* are mechanistically related. Hence, to test this latter hypothesis, we determined the relationship between the total[heme]*BP* and the deoxy[heme]*BP* during upright and supine exercise.

We therefore tested the hypotheses that: 1*A*) the RCP and deoxy[heme]*BP* would occur at a common metabolic rate, 1*B*) would be highly correlated in both positions and muscles, and that 1*C*) changes in both variables between positions (i.e. ΔRCP and Δdeoxy[heme]*BP*) would be related in both muscles; and 2*A*) the total[heme]*BP*would occur prior to, and be strongly correlated with, the deoxy[heme]*BP* in both muscles and body positions, and 2*B*) that the slopes and magnitude (i.e., ΔμM) of changes in both variables would be correlated following their respective breakpoints.

**METHODS**

*Participants.* Eleven healthy male participants (age: 22 ± 4 yr; height 175 ± 7 cm; weight 69 ± 9 kg) who volunteered to take part provided written informed consent. The experiment was approved by the Human Subjects Committee of Kobe Design University and conformed to the Declaration of Helsinki, except for 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 for two experimental sessions. A familiarization session was also conducted in the supine position to familiarize participants with the unusual mode of cycling and minimize intra-individual variation in cycling gait throughout the test. All exercise tests were conducted using an electronically braked cycle ergometer (75XL-III; Combi, Tokyo, Japan). 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. Handles were available to grip during the supine exercise tests to prevent rear movements when forces were applied to the pedals. The setup was similar to that presented by Egaña et al. (39) (see Figure 1 therein). Distance from the iliac crest to the crank shaft was recorded in the first visit and replicated in the subsequent visit in the second posture. Throughout all exercise tests, cadence was strictly maintained at 60 rpm using an audible metronome. The order of upright and supine exercise tests was counterbalanced.

Participants performed ramp incremental exercise tests in the upright and supine positions on separate days. Each test was preceded by 2-min quiet rest on the ergometer and 4-min baseline cycling at 20 W. This was followed by 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 for longer than 5 s.

*Measurements*

*Pulmonary* $\dot{VO\_{2}}$Pulmonary gas exchange/ventilation were measured breath-by-breath throughout all tests using the same methods previously published in our laboratory (40, 41). The breath-by-breath gas exchange system (model AE-300S; Minato-Medical, Osaka, Japan) was calibrated according to the manufacturer’s instructions before each test. Participants breathed through a low-resistance mouthpiece containing a hot-wire flowmeter for measurement of inspiratory and expiratory flows and volumes. Inspired and expired gases were sampled continuously from the mouth, and O2 and CO2 fractional concentrations were measured by fast-responding paramagnetic and infrared analyzers, respectively. Gas volume and concentration signals were time aligned to account for the time lag between the signals to calculate $\dot{V}$O2 on a breath-by-breath basis. Alveolar gas exchange variables were calculated according to the algorithms published by Beaver et al. (42).

*Time-resolved near-infrared spectroscopy.* Continuous non-invasive measurements of absolute deoxy[heme], oxy[heme], 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). The optodes were placed on the distal sites of the VL and RF parallel to the major axis of the thigh, with an interoptode spacing of 3 cm. The measurement principles and algorithms employed by the equipment (43), as well as the specific measurement procedures used in our laboratory (40, 44–47) have been reviewed in detail elsewhere. Adipose tissue thickness (ATT) was measured at each muscle site during the first visit 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. (48) with separate correction factors used for each muscle (49). Briefly, we conducted a linear regression between ATT and total[heme] determined during a 2 min period of rest for each muscle. Subsequently, all measured NIRS values were corrected for an ATT of 0 mm.

*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, as previously described (37, 40, 41). At the beginning of each visit, participants were seated on an upright chair (i.e. a knee joint angle of 90o and a hip angle of 100o) and performed three repetitions of maximal voluntary contractions (MVCs) for 7 s each: extending their leg against a strap attached to a chain, which was in turn attached to a force transducer (T.K.K. 1269f, Takei Scientific Instruments Co., Niigata, Japan), amplifier system (T.K.K. 1268, Takei Scientific Instruments Co., Niigata, Japan) and multichannel data acquisition system (*see above*), that was hooked onto an immovable bar. The MVCs were performed to induce maximal activation of the knee extensor muscles for assessing the maximal EMG activity associated with the maximal recruitment of these muscles, which was then used to normalize the EMG response during ramp incremental cycling. Previously, Alkner et al. (50) reported a close-to-linear relationship between force and EMG signals. Moreover, the reproducibility of the iEMG signals during an MVC in the same subjects on separate days in our laboratory was previously shown to be excellent (intraclass correlation coefficient > 0.95) (41). 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 which produced the highest MVC observed during that visit (i.e. to ensure a true maximum value for each participant on any given day), and expressed as a percentage of MVC.

*Data analysis*

$\dot{V}$O2 peak was defined as the highest 20 s bin-averaged value recorded throughout the test. For young, healthy subjects, $\dot{V}$O2 peak derived from ramp incremental exercise testing has been shown to yield values not different from $\dot{V}$O2 max that are highly reproducible and protocol-independent (51), abrogating the need for a supramaximal verification trial (52). RCP was determined visually by experienced investigators as the $\dot{V}$O2 at which end-tidal *P*CO2 began to fall after a period of isocapnic buffering (i.e. stable end-tidal *P*CO2), corroborated by the second and first breakpoints in the $\dot{V}$*E*- and$\dot{V}$*E*/$\dot{V}$CO2-$\dot{V}$O2 relationships, respectively (53). The $\dot{V}$O2 mean response time (MRT) was determined by fitting the $\dot{V}$O2-time relationship with a linear regression, removing the first 120 s and final 180 s so as to isolate the linear portion of the relationship. The MRT was defined as the time between the beginning of the ramp incremental exercise test and the intersection between baseline $\dot{V}$O2 and backwards extrapolation of the regression line of the $\dot{V}$O2-time relationship. The $\dot{V}$O2 values associated with the power outputs at which the various NIRS thresholds (i.e., the deoxy[heme]*BP*, total[heme]*BP* and the iEMG*BP*) occurred in the present study were therefore calculated using by left-shifting the $\dot{V}$O2 data by the MRT.

Individual deoxy[heme], total[heme] and iEMG data were first linearly interpolated to 1 s intervals and plotted as a function of time, before conversion to power output. The profiles of deoxy[heme], total[heme] and iEMG were analyzed using a double-linear model (17). Data used in this analysis were chosen via visual inspection and included all points between the onset of the systematic rise in the variable of interest near the beginning of the test and the end of the test. Piecewise linear regression analysis was then applied and yielded two linear functions:

*y* = *m*1*x* + *c*1, for *x* < BP

*y* = *m*2*x* + *c*2, for *x* > BP

Where *m* represents the slope and *c* represents the intercept. Subsequently, a BP reflecting the intersection of these two linear functions could be determined by the lowest sum of squared errors. Figure 1 illustrates the methods of threshold determination that were applied in one representative subject.

*Statistical analysis.* Normality of the data was examined by the Kolmogorov-Smirnov Test. Two-way repeated-measures ANOVA were used to compare the average values for RCP, deoxy[heme]*BP*, total[heme]*BP* and iEMG*BP* between positions (upright and supine) and muscles (i.e. VL and RF). Significant effects were followed up with Holm-Sidak post-hoc tests. The relationships among each of the thresholds determined herein were assessed using Pearson’s product moment correlation coefficient. The degree of agreement between measures was assessed using Bland-Altman limits of agreement (LoA) analysis (54). 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**

*Comparisons between RCP and deoxy[heme]BP*

Peak values attained during the incremental ramp exercise test are reported in Table 1, whereas the values for exercise thresholds are presented in Table 2. The RCP and the deoxy[heme]*BP* in both the VL and the RF were reduced in the supine compared to the upright positions (Figures 1 and 2, all *P* < 0.05).

*Vastus Lateralis, VL*

The RCP was correlated with the deoxy[heme]*BP* in the VL in both the supine (*r* = 0.78, *P* = 0.004) and upright (*r* = 0.90, *P* < 0.001) positions, and also when data points were combined across positions (*r* = 0.86, *P* < 0.001, Figure 3*A*). RCP did not differ from the deoxy[heme]*BP* in the VL in the supine position (*P* = 0.15), however, the deoxy[heme]*BP* was greater than the RCP in the upright position (*P* = 0.002, Table 1). Across both positions the mean difference between the RCP and deoxy[heme]*BP* in the VL was 0.19 ± 0.25 L O2 .min-1, with 95% limits of agreement of 0.49 L.min-1 (+ 0.69, - 0.31 L O2.min-1, Figure 4*A*). However, ΔRCP and Δdeoxy[heme]*BP* (i.e., changes in both variables between positions) in the VL were not correlated (*r* = 0.55, *P* = 0.080, Figure 5*A*).

*Rectus Femoris, RF*

The RCP did not differ from the deoxy[heme]*BP* in the RF in either the supine (*P* = 0.10) or the upright (*P* = 0.44) positions (Table 1), and both variables were correlated across both positions (*r* = 0.67, *P* < 0.001, Figure 3*B*). Across positions the mean difference between the RCP and deoxy[heme]*BP* in the RF was – 0.16 L O2.min-1, with 95% LoA of 0.79 L O2.min-1 (+ 0.63, - 0.95 L O2.min-1, Figure 4*B*). The deoxy[heme]*BP* in the RF was lower than the deoxy[heme]*BP* in the VL in supine (*P* = 0.027) and upright (*P* = 0.023) positions (Table 1). However, ΔRCP and Δdeoxy[heme]*BP* in the RF were not correlated (*r* = 0.26, *P* = 0.44, Figure 5*B*).

*Comparisons between the deoxy[heme]BP and total[heme]BP.*

The total[heme]*BP* is displayed in Figure 2 for a representative participant in both positions for the VL (Figure 2C) and RF (Figure 2D). The total[heme]*BP* occurred at 2.64 ± 0.47 L O2.min-1 in the VL and 2.54 ± 0.57 L O2.min-1 in the RF in the upright position, and was reduced in both muscles in the supine position (VL: 2.42 ± 0.50 , RF: 1.97 ± 0.40 L O2.min-1, *P* = 0.006). The total[heme]*BP* was lower than the deoxy[heme]*BP* in both positions and both muscles (all *P* < 0.05), and occurred at a lower metabolic rate than the deoxy[heme]*BP* in 89% of individual cases (Figure 6A). With both positions and muscles combined, the total[heme]*BP* was correlated with the deoxy[heme]*BP* (*r* = 0.79, *P* < 0.001, Figure 6*A*), and Δtotal[heme]*BP* was correlated with Δdeoxy[heme]*BP* across both muscles (*r* = 0.66, *P* < 0.001, Figure 6*B*). The slope of the linear function describing the change in total[heme] following the total[heme]*BP* (i.e. “slope *m2*”) was correlated with slope *m2* of the deoxy[heme]response with both positions and muscles combined (*r* = 0.75, *P* < 0.001), as was the absolute magnitude of changes in both variables following their respective breakpoints when both positions and muscles and muscles were combined (*r* = 0.82, *P* < 0.001).

*Comparisons between the total[heme]BP* and the iEMG*BP*

The iEMG*BP* is displayed in Figure 2 for a representative participant in both positions for the VL (Figure 2E) and RF (Figure 2F). The iEMG*BP* occurred at 2.45 ± 0.62 L O2.min-1 in the VL and 2.34 ± 0.92 L O2.min-1 in the RF in the upright position, and was reduced in both muscles in the supine position (VL: 1.82 ± 0.51, RF: 1.83 ± 0.61 L O2.min-1, *P* = 0.009, Table 1). In both positions, the iEMG*BP* occurred at a lower metabolic rate than the total[heme]*BP* in the VL(*P* = 0.006), but there were no differences between the metabolic rates associated with the iEMG*BP* and the total[heme]*BP* in the RF (*P* = 0.42). With both positions and muscles combined, the iEMG*BP* was correlated with the total[heme]*BP* (*r* = 0.63, *P* < 0.001), and ΔiEMG*BP* was correlated with Δtotal[heme]*BP* across both muscles (*r* = 0.61, *P* = 0.003).

**DISCUSSION**

The principal findings of the present investigation were: the RCP was strongly correlated with the deoxy[heme]*BP* across both muscles and both positions, and these variables were not different in either position in the RF or in the supine position in the VL, partially consistent with hypotheses 1*A* & 1*B*. However, the RCP was significantly lower than the deoxy[heme]*BP* in the VL in the upright position and Δdeoxy[heme]*BP* did not correlate with ΔRCP across positions in either muscle, inconsistent with hypotheses 1*A* & 1*C*. Moreover, the wide LoA indicated a large degree of inter-individual variability in the degree of agreement between these two variables, and the deoxy[heme]*BP* was dissociated between the RF and VL in both the supine and upright positions. Hence, the findings of the present study suggest that RCP and the deoxy[heme]*BP* are not equivalent, do not represent a common mechanism, and thus, should not be used interchangeably.

In agreement with our second hypothesis; in both the RF and VL the total[heme]*BP* consistently preceded the deoxy[heme]*BP*, Δtotal[heme]*BP* was correlated with Δdeoxy[heme]*BP*, and the slopes of change in total[heme] and deoxy[heme] following their respective breakpoints were correlated. In turn, the iEMG*BP* preceded the total[heme]*BP* in the VL and occurred at a similar metabolic rate in the RF, and ΔiEMG*BP* was correlated with Δtotal[heme]*BP*. Hence, the results of the present study support the notion that deoxy[heme]*BP* is mechanistically related to the total[heme]*BP*, which in turn appears to be related to the iEMG*BP*.

*Comparison between the RCP and deoxy[heme]BP.*

To our knowledge Murias et al. (18, 19) first suggested that the RCP and deoxy[heme]*BP* represent the same underlying physiological phenomenon. Specifically, they demonstrated that the two variables were not significantly different and were highly correlated in a small sample of individuals. These findings were then confirmed by Fontana et al. (20), who demonstrated that these variables were not significantly different and highly correlated in a sample of 118 healthy men. Thereafter, Keir et al. (13) demonstrated that the metabolic rates associated with CP, the maximum lactate steady-state (MLSS), RCP and the deoxy[heme]*BP* were not significantly different. The authors interpreted these findings to indicate that each threshold shared a common underlying physiological mechanism that represents the boundary between heavy and severe exercise intensity domains. Since these earlier studies, multiple other studies have been published which have suggested that RCP and deoxy[heme]*BP* are equivalent, although exclusively from the same research group (10–12, 21, 22). However, the common theme underpinning each of these studies is that the inference of equivalence is made from the observation of no statistically significant difference, an approach that is based on a statistical misconception (23, 24, 55).

 The finding of a close correspondence between RCP and the deoxy[heme]*BP* is far from universal. Boone et al. (26) showed that the deoxy[heme]*BP* was slightly but systematically lower than the RCP. Conversely, Caen et al. (27) demonstrated that the deoxy[heme]*BP* was greater than RCP, and the ΔWR values associated with each variable were not related following a 6-week exercise training intervention. In the present study, we found that, under certain conditions (i.e. in the RF in both positions and the VL in the supine position), these two variables were not significantly different, and were also highly correlated. However, in a group of participants with heterogeneous aerobic capacities such as in the present study (i.e., 38 – 58 mL O2.kg-1.min-1 across both positions), physiological events that occur in the same proximity (i.e. CP, MLSS, RCP, deoxy[heme]*BP*) will tend to correlate with each other. Hence, determining whether they are mechanistically related or not is a matter that requires interventional experimental scrutiny.

Despite some evidence that these two variables were related in the present study, in the upright position the deoxy[heme]*BP* in the VL was significantly greater than the RCP. This shows a clear dissociation between these two variables under the conditions in which they are most typically measured (i.e. during upright cycle exercise in the superficial VL). Furthermore, we found no significant relationship between Δdeoxy[heme]*BP* and ΔRCP across positions in either the RF or VL, indicating a large degree of intra- and inter-individual variability in the responses of each threshold to changes in body position. Finally, the LoA between RCP and deoxy[heme]*BP* were rather wide in the present study: ± 0.49 in the VL and ± 0.79 L O2.min-1 in the RF. The study of Fontana et al. (20) represents the largest sample to compare the RCP and deoxy[heme]*BP* in the VL, and these authors reported similarly wide LoA between these two variables in that study (see Figure 3 therein). It is notable that those authors concluded that the deoxy[heme]*BP* could be used to detect the metabolic rate associated with the RCP, despite the wide LoA. Assuming a 95% LoA between RCP and deoxy[heme]*BP* for the VL of ~0.5 L O2.min-1 (present study, 11), however, would suggest that for any given individual there is a 95% probability that the difference between RCP and deoxy[heme]*BP* would be between ± 0.5 L O2.min-1 of the mean of both measurements (i.e. a 1 L O2.min-1-wide possible margin of error). Such a wide degree of statistical uncertainty thus clearly indicates that these variables are not equivalent, and highlights the fallacy of assuming such from the observation of no statistical difference.

The physiological rationale for why a whole-body measurement such as the RCP should closely correspond to a localized measure such as the deoxy[heme]*BP* is also unclear. Indeed, in the present investigation, despite a singular RCP for all participants, the deoxy[heme]*BP* occurred at significantly different metabolic rates in the RF versus the VL. It would thus seem unlikely that a threshold which occurs at distinctly different metabolic rates among the various locomotor muscles employed during cycling would hold any mechanistic relationship with a singular whole-body threshold. In contrast, Iannetta et al. (11) found that the deoxy[heme]*BP* occurred at the same metabolic rate in the VL, RF, and VM, and that each of these thresholds, in turn, occurred at the same metabolic rate as the RCP. The RCP reflects the work rate at which substantial hyperventilation with respect to $\dot{V}$CO2 ensues. The primary mechanisms underpinning the RCP are still highly debated, including carotid body stimulation by falling arterial pH (56), rising plasma [K+] (57, 58), increased central command (59), with a high likelihood that redundancy of these control mechanisms exists (60). Hence, Iannetta et al. (11) interpreted the association between the RCP and deoxy[heme]*BP* among muscles to indicate that above or near intensities associated with the RCP, the accumulation of hydrogen ions and other vasoactive substances would cause vasodilation that would lead to increased O2 delivery to areas of high metabolic demand. This would forestall any further increase in fractional O2 extraction, and thus could explain the plateau in deoxy[heme] that is typically observed in the VL and VM towards the end of ramp incremental exercise. What this interpretation cannot explain, however, is the increased slope of deoxy[heme] versus time/power following the deoxy[heme]*BP* in the RF observed in the present study and previously (11). Collectively, therefore, the results of the present study suggest that the physiological mechanism that was originally suggested to link the RCP and the deoxy[heme]*BP* (18) cannot account for the distinct profiles of deoxy[heme] following its breakpoint in discrete muscle groups (28), and hence, these two variables are not equivalent.

In the spirit of the great philosopher of science Karl Popper, the strength of a scientific theory comes from its ability to withstand repeated attempts at falsification. The instances in the literature where RCP and deoxy[heme]*BP* have been demonstrated to be highly correlated and not to differ statistically appear to demonstrate that these variables are equivalent (10–13, 18–22). However, as recently pointed out by Broxterman et al. (61), these “White Swans” cannot provide definitive proof that these two variables are equivalent, as the observation of a single “Black Swan” would prove the theory incorrect. Indeed, the results of the present study represent the latest in a series of “Black Swans” (26, 27), confirming that the RCP and deoxy[heme]*BP* are not equivalent and should not be used interchangeably. The studies in the literature that have concluded equivalence between the RCP and deoxy[heme]*BP* are thus excellent examples of Popper’s problem of induction, in that they have attempted to infer universal statements from singular ones (25). However, it is only via the deductive method of testing, that is, empirically testing hypotheses after they have been advanced, that we can determine the validity of hypotheses such as those pertaining to the equivalence between the RCP and deoxy[heme]*BP* (25).

*Comparison between the total[heme]BP and the deoxy[heme]BP.*

Boone et al. (26, 38) previously showed that above ~70% peak work rate during ramp incremental exercise, total[heme] either plateaued or declined in the VL. Moreover, this total[heme]*BP* consistently preceded the deoxy[heme]*BP* suggesting that the two breakpoints in each variable may be mechanistically related. The results of the present study are in substantial agreement with that of Boone et al. (26, 38). In the present study, the total[heme]*BP* occurred at a significantly lower metabolic rate than the deoxy[heme]*BP*, and before the deoxy[heme]*BP* in 89% of individual cases across muscles and body positions in the present study. Moreover, the present findings extend those of Boone et al. (26, 38) by demonstrating that a total[heme]*BP* also occurs during high-intensity ramp exercise in the RF muscle. However, much like the response observed in deoxy[heme] in this muscle in some participants at similar intensities, in the same participants the increase in total[heme] in the RF following its respective breakpoint was accelerated with respect to its initial rate of increase (e.g. Figure 2*B* & *D*). Finally, across both muscles and body positions, both the slopes and absolute magnitudes of changes in total[heme] and deoxy[heme] following their respective breakpoints (i.e., slope 2) were strongly correlated. This latter finding demonstrates that changes in deoxy[heme] during high-intensity exercise are strongly tied to the degree of change in total[heme]; with this relationship being strengthened by the observation that it occurs in both the positive (RF) and negative (RF and VL) directions. Hence, the present findings provide support for the possibility that the total[heme]*BP* and deoxy[heme]*BP* may be closely mechanistically related.

 Muscle O2 extraction (i.e. estimated noninvasively herein by deoxy[heme]) is described by the relationship between *D*O2 and muscle blood flow ($\dot{Q}$), where O2 extraction = (1 – e –*D*O2/*β*$\dot{Q}$), and *β* is the slope of the O2 dissociation curve in the physiologically relevant range (62). *D*O2 is primarily dependent upon the available surface area for diffusion and thus the number of RBCs in the capillaries adjacent to the contracting muscle fiber at any one time and, therefore, to microvascular hematocrit (63). Davis & Barstow (64) have suggested that, because muscle [myoglobin] presumably remains unchanged during exercise, changes in total[heme] determined via NIRS represent changes in microvascular [hematocrit] and thus *D*O2. Hence, in the present study in the VL, *D*O2 may have reached a ceiling above the total[heme]*BP* in the VL, which in turn would have caused muscle O2 extraction to plateau above the deoxy[heme]*BP*. In the RF in some participants, however, because muscle activation was relatively lower prior to the iEMG*BP*, the accelerated rise in muscle activity following the iEMG*BP* would have been unlikely to cause significant blood flow limitation but rather, may have facilitated further increases in total[heme]. Hence, above the total[heme]*BP* *D*O2 may have begun to increase more rapidly, driving an accelerated rate of muscle O2 extraction past the deoxy[heme]*BP*. The results of the present study therefore suggest that the breakpoints in deoxy[heme] that are typically observed during ramp incremental exercise are precipitated by breakpoints in total[heme] (i.e. *D*O2) occurring at slightly lower intensities. The factors that cause *D*O2 to plateau during high-intensity exercise are presently unclear, however it may be related to the higher intramuscular pressures generated at greater exercise intensities (65, 66), which could constrain RBC transit time and limit further O2 extraction in the VL. In the RF, on the other hand, muscle activation is far lower than the VL (29, 40). Hence, more pronounced increases in muscle activation in the RF following its iEMG*BP* (11) would facilitate accelerated rises in microvascular [hematocrit] and *D*O2, which in turn would enable a greater rate of muscle O2 extraction following the deoxy[heme]*BP*. This interpretation is supported by the observations in the present study that the iEMG*BP* preceded the total[heme]*BP* in the VL and occurred at a similar metabolic rate to the total[heme]*BP* in the RF, and that ΔiEMG*BP* was correlated with Δtotal[heme]*BP*. These latter findings suggest that breakpoints in local muscle activation may drive changes in total[heme] following the total[heme]*BP*. Future research should therefore utilize interventions which would be expected to alter the iEMG, deoxy- and total[heme]*BP*,to determine whether the agreement between their absolute values as metabolic rates and subsequent slopes and magnitudes of change persists.

*Limitations.* Muscle activity during MVCs was determined at a fixed knee joint angle of 90 degrees, and it has previously been demonstrated that RF and VL iEMG activity may differ at the same knee joint angle (67). However, in the present study, any error in the determination of the maximal activity of a given muscle was mitigated by ensuring that knee and hip joint angles were consistent across subjects and within subjects across visits. Another limitation that might be levelled at the present study is that critical power was not measured, and the vast preponderance of evidence indicates that critical power represents the most appropriate quantification of the heavy-severe domain boundary (5). Hence, whether either the RCP or the deoxy[heme]*BP* also reflectthis boundary cannot be directly determined from the present study. However, this was not the purpose of the present study. Rather, a large volume of publications have recently suggested that the RCP and the deoxy[heme]*BP* occur at the same metabolic rate (10–13, 18–22); our purpose was strictly to test this latter hypothesis. Regarding whether or not either RCP or the deoxy[heme]*BP* reflect the same physiological phenomenon as critical power, evidence is equivocal (3, 27). The finding in the present study that the deoxy[heme]*BP* occurs at different metabolic rates in different muscles in the same individuals is difficult to reconcile with the notion that it could be equivalent to a singular threshold such as the heavy-severe domain border. With respect to the RCP, this is also unlikely: RCP does not occur in some modes of exercise and species (61) and can be completely dissociated from the metabolic acidosis proposed to link RCP with the heavy-severe boundary (59, 68). Moreover, under certain conditions (e.g., hypoxia) the RCP can occur at the same metabolic rate as the GET (69). Hence, future research should test the equivalence of critical power, the RCP and deoxy[heme]*BP* before and after an intervention expected to alter each of these variables.

*Conclusion.* This study demonstrates that whilst the RCP and deoxy[heme]*BP* did appear to share a close association in certain instances herein, they were clearly dissociated during upright cycle exercise in the VL. The changes in the RCP were not related to the changes in the deoxy[heme]*BP* between upright and supine exercise in either the VL or the RF, and the two variables shared wide LoA. Moreover, the deoxy[heme]*BP* displayed a clear dissociation between the RF and VL in both the upright and supine positions. Hence, the results of the present study indicate that the deoxy[heme]*BP* and the RCP do not represent the same underlying physiological phenomenon and should not be used interchangeably. The total[heme]*BP*, on the other hand, consistently preceded the deoxy[heme]*BP* in both positions and muscles, and the slopes of changes in total- and deoxy[heme] were strongly related following their respective breakpoints in both the positive (i.e. RF) and negative (i.e. RF & VL) directions. Consequently, the present results suggest that the deoxy[heme]*BP* may occur subsequent to the attainment of a local threshold in *D*O2 during high-intensity exercise, which in turn might be related to an inability of the circulatory system to overcome the greater intramuscular pressures generated at such intensities in the VL, or proportionally greater recruitment at higher intensities in the RF.

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The authors hereby declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The authors also declare that there is no conflict of interest associated with this manuscript. The results of the present study do not constitute endorsement by the American Physiological Society.

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**AUTHOR CONTRIBUTIONS**

RPG was responsible for the conception of this work, whereas RPG and SK were responsible for the 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.

**DATA AVAILABILITY STATEMENT**

All data used in the present manuscript are available upon reasonable request from the first author.

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**FIGURE LEGENDS**

**Figure 1.** Determination of the respiratory compensation point (RCP) in the upright (black circles) and supine positions (clear circles) in a representative participant. The vertical dashed lines represent the $\dot{V}$O2 at which the RCP occurred. S = supine, U = upright.

**Figure 2.** Determination of the muscle deoxygenated[heme] (A & B), total[heme] (C & D) and integrated electromyography (E & F) breakpoints in the VL (A, C & E) and RF (B, D & F) in the upright (black circles) and supine positions (clear circles) in a representative participant. Red lines represented the modelled double-linear fits and the vertical dashed lines represent the $\dot{V}$O2 at which each breakpoint occurred. S = supine, U = upright.

**Figure 3**. Relationships between the metabolic rate ($\dot{V}$O2) at the respiratory compensation point (RCP) and the $\dot{V}$O2 at the muscle deoxygenated [heme] breakpoint (deoxy[heme]*BP*) in the vastus lateralis (VL; panel *A*) and the rectus femoris (RF; panel *B*). Solid black line indicates the regression line and dashed black lines represent the line of identity. Clear circles = supine exercise, black circles = upright exercise. Both relationships were significant (*P* < 0.001).

**Figure 4.** Bland-Altman limits of agreement (LoA) analysis showing the degree of agreement between the metabolic rates ($\dot{V}$O2) at the respiratory compensation point (RCP) and the muscle deoxygenated [heme] breakpoints (deoxy[heme] BP) in the vastus lateralis (VL; panel *A*) and the rectus femoris (RF; panel *B*). The difference between the two variables (i.e. deoxy[heme]*BP* – RCP) is plotted against the average of deoxy[heme]*BP* and RCP, with the mean bias indicated by the solid black line and the LoA denoted by the two dashed lines. Clear circles = supine exercise, black circles = upright exercise.

**Figure 5.** Relationships between the changes in the metabolic rate (∆$\dot{V}$O2) at the respiratory compensation point (Δ$\dot{V}$O2 RCP) and the ∆$\dot{V}$O2 at the muscle deoxygenated [heme] breakpoint (Δ$\dot{V}$O2 deoxy[heme]*BP*) between upright and supine exercise (i.e. upright – supine) in the vastus lateralis (VL; panel *A*) and the rectus femoris (RF; panel *B*). Neither relationship was significant (*P* > 0.05).

**Figure 6.** Panel A: relationship between the metabolic rates ($\dot{V}$O2) at the muscle deoxygenated [heme] breakpoint (deoxy[heme]*BP*) and total[heme] breakpoint (total[heme]*BP*) in the VL (circles) and RF (triangles) for supine (clear points) and upright (black points) exercise. Dashed black line represents the line of identity. Panel B: relationships between changes in the metabolic rates at the deoxy[heme] BP (Δ$\dot{V}$O2 deoxy[heme]*BP*) and total[heme]*BP* (Δ$\dot{V}$O2 total[heme]*BP*) between upright and supine exercise (i.e. upright – supine) in the VL (clear circles) and RF (black triangles). Solid lines indicate the regression lines and dashed lines represent the 95% confidence limits. Both relationships were significant (P < 0.001).