



## Original research

# The near-infrared spectroscopy-derived deoxygenated haemoglobin breaking-point is a repeatable measure that demarcates exercise intensity domains



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## ABSTRACT

**Objectives:** A breaking-point in the near-infrared spectroscopy (NIRS)-derived deoxygenated haemoglobin ([HHb]) profile towards the end of a ramp incremental (RI) cycling test has been associated to the respiratory compensation point (RCP). Despite the physiological value of this measure, its repeatability remains unknown. The aim was to examine the repeatability of the [HHb] breaking-point ([HHb]BP) and its association to RCP during a RI cycling test.

**Design:** A repeated measures design was performed on 11 males ( $30.5 \pm 8.4$  year;  $76.5 \pm 8.4$  kg) and 4 females ( $30.5 \pm 5.9$  year;  $61.9 \pm 4.4$  kg).

**Methods:** Gas exchange and NIRS [HHb] data were collected during RI tests performed on two different days separated by 48 h. The [HHb]BP and the RCP were determined and compared for each trial.

**Results:** The [HHb]BP and the respiratory compensation point (RCP) occurred at the same  $\dot{V}O_2$  in test 1 and test 2 ([HHb]BP:  $3.49 \pm 0.52$  L min<sup>-1</sup> test 1;  $3.48 \pm 0.45$  L min<sup>-1</sup> test 2; RCP:  $3.38 \pm 0.40$  L min<sup>-1</sup> test 1;  $3.38 \pm 0.44$  L min<sup>-1</sup> test 2) ( $P > 0.05$ ). The  $\dot{V}O_2$  associated with the [HHb]BP and the  $\dot{V}O_2$  at RCP were not significantly different from each other either in test 1 as well as in test 2 ( $P > 0.05$ ). Neither test 1 nor test 2 showed significant mean average error between the  $\dot{V}O_2$  at the [HHb]BP and RCP using Bland & Altman plots.

**Conclusions:** The [HHb]BP is a repeatable measure that consistently occurs towards the end of a RI test. The association between the [HHb]BP and the RCP reinforces the idea that these parameters may share similar mechanistic basis.

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## 1. Introduction

Near infrared spectroscopy (NIRS)-derived muscle oxygenation is widely measured during exercise to gain insight into the characteristics of muscle oxygen delivery and utilization during various exercise challenges and using different exercise modalities.<sup>1–3</sup> Recently, much attention has been directed towards the behaviour of the deoxygenated haemoglobin concentration ([HHb]) signal during ramp incremental (RI) cycling tests.<sup>4–6</sup> During RI exercise, the [HHb] signal generally increases in a linear-fashion, indicating a progressively greater reliance of the working muscle(s) on oxygen extraction to support the oxidative energy demand. However, towards the end of the RI test, the linear increase in the [HHb] profile exhibits a plateau-like response occurring during the final

minutes of exercise. Initially, the relationship between the increase in energy demand (work rate) and the [HHb] signal during a RI test was described, and modelled, as a sigmoidal profile<sup>7–9</sup> rather than a linear increase followed by a plateau response. However, Spencer et al.<sup>4</sup> first described the [HHb] profile during a RI test as a double linear function that offered a better quality fit than the sigmoidal, with a breaking-point parameter that specifically quantified the attenuation of the increase in the [HHb] and the onset of the plateau-like response. Later, the pulmonary oxygen uptake ( $\dot{V}O_2$ ) associated with this breaking-point was shown to correspond to that of the respiratory compensation point (RCP).<sup>5</sup> Various studies have also observed the occurrence of the [HHb] breaking-point ([HHb]BP) during RI cycling tests and corroborated its correspondence with RCP.<sup>10–12</sup> For this reason, the [HHb]BP has gained increased attention as an useful measure defining the boundary between the heavy and the severe exercise domains. Furthermore, Keir et al.<sup>13</sup> demonstrated that the [HHb]BP was also associated with the  $\dot{V}O_2$  corresponding with critical power and

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maximal lactate steady state in addition to RCP, suggesting that all these individual thresholds manifest from a similar metabolic phenomenon. In contrast, a more recent study found that  $\text{VO}_2$  associated with the [HHb]BP was slightly higher than that associated with RCP, suggesting that a sequence of events better describes the relationship between [HHb]BP and RCP rather than an equivalence.

Based on the aforementioned studies, different sets of data provided by different laboratories support the idea of a correspondence between the [HHb]BP during RI tests and the RCP. However, studies reporting the test-retest repeatability of the [HHb]BP and its association with RCP are lacking. In other words, it is critical to assess whether or not the [HHb]BP is a consistent phenomenon, that can be observed at the same metabolic rate in the same subject population in two separate testing sessions. This is also relevant as future studies need to examine whether or not the relationship between the [HHb]BP and the RCP is maintained following interventions such as exercise training or exposure to different environmental conditions (e.g., heat and hypoxia); however, this cannot be done without first establishing the repeatability of these measures under similar testing conditions. Therefore, the first aim of the present study was to evaluate, through a test-retest design, the repeatability of the [HHb]BP. We hypothesized that the [HHb]BP would occur at the same  $\text{VO}_2$  during two identical ramp incremental cycling tests performed on two separate days. The second aim was to evaluate the consistency of the correspondence between the [HHb]BP and RCP. It was hypothesized that the [HHb]BP and RCP would both occur at the same  $\text{VO}_2$ .

## 2. Methods

15 individuals [11 men:  $30.5 \pm 8.4$  year;  $76.5 \pm 8.4$  kg; body mass index (BMI) =  $23.6 \pm 3.0 \text{ Kg m}^{-2}$ ; 4 women:  $30.5 \pm 5.9$  year;  $61.9 \pm 4.4$  Kg; BMI =  $23.3 \pm 2.2 \text{ Kg m}^{-2}$ ] gave their written consent to participate in this study. Participants were well-trained individuals who engaged in structured endurance training programs. Ten of them were cyclists who trained 4–5 time/week with typical 2–3 h duration sessions. Five were either runners or triathletes with similar training frequency and duration of training as the cyclists. Importantly, even for runners and triathletes, cycling was a relevant component of their training schedule (2–3 time/week). They were non-smokers, non-obese and not undergoing any medical treatment that could affect their cardiovascular response to exercise. All procedures were approved by the Conjoint Health Research Ethics Board of the University of Calgary. Subjects came into the lab on two days separated by 48 h to perform a ramp incremental test (RI) on an electromagnetically braked cycle ergometer (Velotron, Dynafit Pro, Racer Mate, Seattle, WA, USA). Participants were already familiarized with the testing protocols and procedures as they routinely undergo testing in our laboratory for different purposes. In case the peak power output (PPO; watts) achieved in the second testing session was more than 10 W lower or higher than the first one, subjects were asked to report to the lab a third occasion to perform another RI. Only the two RI with the highest PPO were included in the analysis. The RI protocol consisted of a baseline of 4 min at 50 W, followed by a  $30 \text{ W min}^{-1}$  (1 W every 2 s) and  $25 \text{ W min}^{-1}$  (1 W every 2.4 s) increasing ramp for men and women, respectively as previously described.<sup>6</sup> Subjects were instructed to maintain a pedal rate between 75 and 85 revolutions per minute (RPM). The RI was stopped when the cycling cadence dropped below 65 RPM despite strong verbal encouragement, or due to volitional exhaustion. PPO was determined as the highest power output achieved at the cessation of the test. Subjects were not allowed to visualize the PO during the tests.

Breath-by-breath pulmonary gas exchanges were measured by a metabolic cart (Quark CPET, Cosmed, Rome, Italy). Briefly, inspired

and expired flow rates were measured continuously through a low dead space turbine which was calibrated beforehand with a syringe of a known volume. Inspired and expired gases were analysed for concentrations of  $\text{O}_2$  and  $\text{CO}_2$  after calibration with precision-analysed gas mixtures according to the manufacturer specifications.

Local muscle deoxygenation (HHb) signal was monitored and recorded by the NIRS (Oxiplex TS™, ISS, Champaign, USA). The NIRS probe was placed on the Vastus Lateralis belly approximately halfway between the trochanter and the knee joints after the skin area was shaved and wiped. The Vastus Lateralis is commonly examined<sup>9,10,12</sup> in the literature as this muscle is highly involved in cycling exercise. The probe was covered by an optically dense, black vinyl sheet to avoid the intrusion of external light, and secured in place by an elastic strap and by an elastic band to minimize movement. The apparatus was calibrated on each testing day after a warm-up of at least 30 min as per manufacturer recommendations. Data were stored online at an output frequency of 2 Hz, and reduced to 1-s bins for all subsequent analyses within the present study. Before removing the probe, the area was marked to ensure the consistency of the placement for the following visit.

Breath-by-breath  $\text{VO}_2$  data were individually analysed as previously described<sup>14</sup>: aberrant data points that were 3 SD from the local mean were removed and then linearly interpolated to 1 s intervals. The second by second data were then time aligned so that time “zero” represented the onset of exercise (onset of the RI). To account for the individual circulatory transit of  $\text{O}_2$  between muscle and lung, the mean response time (MRT) was calculated on an individual basis as previously described<sup>12</sup> using Origin software (Origin, Origin Lab, Northampton, MA). Briefly, a double linear model was fitted from baseline to the previously established gas exchange threshold (GET). The MRT corresponded to the time delay between the onset of the RI test (i.e., 240 s) and the intersection of the forward extrapolation of the baseline  $\text{VO}_2$  (slope constrained to “zero”) and backwards extrapolation of the linear  $\text{VO}_2$ -time relationship from GET.<sup>9</sup>

The respiratory compensation point (RCP) was determined by 3 independent exercise physiologists through visual inspection using standard ventilatory and gas exchange indices as previously described.<sup>15,16</sup> Briefly, RCP corresponded to the second disproportional increase (i.e., second breakpoint) in the  $\text{V}_E/\text{VO}_2$  relation, where end-tidal  $\text{PCO}_2$  began to fall after a period of isocapnic buffering. Further, the relation between  $\text{V}_E/\text{VCO}_2$   $\text{VO}_2$  was considered for confirmation of the RCP. In case of a disagreement of more than  $150 \text{ mL min}^{-1}$  in the result, physiologists would reevaluate together the profiles until a consensus was reached.  $\text{VO}_{2\text{peak}}$  was defined as the highest  $\text{VO}_2$  computed from a 20-s rolling average.

As previously described,<sup>4</sup> the [HHb] – time relationship was modeled with the following piece-wise ‘double-linear’ model:

$$f = \text{if}(x < BP, g(x), h(x))$$

$$g(x) = i_1 + (s_1 x)$$

$$i_2 = i_1 + (s_1 BP)$$

$$h(x) = i_2 + (s_2 (x - BP))$$

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where  $f$  is the double-linear function,  $x$  is time and  $y$  is [HHb],  $BP$  is the time coordinate corresponding to the interception of the two regression lines (i.e., the [HHb]BP),  $i_1$  and  $i_2$  are the intercepts of the first and second linear function respectively and  $s_1$  and  $s_2$  are the slopes. Model parameter estimates for each individual were determined by linear least-square regression analysis.

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