



Research Article

Increases in the incremental exercise mean response time across the steady state domain: Implications for exercise testing & prescription

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ABSTRACT

We hypothesized that slowed oxygen uptake ($\dot{V}O_2$) kinetics for exercise transitions to higher power outputs (PO) within the steady state (SS) domain would increase the mean response time (MRT) with increasing exercise intensity during incremental exercise. Fourteen highly trained cyclists (mean \pm standard deviation [SD]; age (39 ± 6) years [yr]; and $\dot{V}O_2$ peak = (61 ± 9) mL/kg/min) performed a maximal, ramp incremental cycling test and on separate days, four 6-min bouts of cycling at 30%, 45%, 65% & 75% of their incremental peak PO (Wpeak). SS trial data were used to calculate the MRT and verified by mono-exponential and linear curve fitting. When the ramp protocol attained the value from SS, the PO, in Watts (W), was converted to time (min) based on the ramp function W to quantify the incremental MRT (iMRT). Slope analyses for the $\dot{V}O_2$ responses of the SS versus incremental exercise data below the gas exchange threshold (GET) revealed a significant difference ($p = 0.003$; $[0.437 \pm 0.08]$ vs. $[0.382 \pm 0.05]$ L·min⁻¹). There was a significant difference between the 45% Wpeak steady state $\dot{V}O_2$ (ss $\dot{V}O_2$) ($[3.08 \pm 0.30]$ L·min⁻¹, respectively), and 30% Wpeak ss $\dot{V}O_2$ (2.26 ± 0.24) ($p < 0.0001$; $[3.61 \pm 0.80]$ vs. $[2.20 \pm 0.39]$ L·min⁻¹) and between the iMRT for 45% and 30% Wpeak ss $\dot{V}O_2$ values ($[50.58 \pm 36.85]$ s vs. $[32.20 \pm 43.28]$ s). These data indicate there is no single iMRT, which is consistent with slowed $\dot{V}O_2$ kinetics and an increasing $\dot{V}O_2$ deficit for higher exercise intensities within the SS domain.

1. Introduction

Historically, the definition of a mean response time (MRT) in the rate of oxygen consumption ($\dot{V}O_2$) during exercise was based on the time constant resulting from the mono-exponential increase in $\dot{V}O_2$ during an exercise transition from a lower to higher intensity within the steady state (SS) domain.¹ The mathematical definition of the MRT is that it equals 63% of the time required to reach steady state $\dot{V}O_2$ (ss $\dot{V}O_2$).² In recreationally active young men, Iannetta et al.¹ recently applied a novel correction technique for determining MRT during incremental exercise, where ss $\dot{V}O_2$ at 100 watt (W) was superimposed on the $\dot{V}O_2$ response to step transition incremental exercise at the same PO. The time difference between the linear fit of the incremental $\dot{V}O_2$ to the superimposed 100 W $\dot{V}O_2$ was defined as the MRT, which for clarity we define as the incremental MRT (iMRT). This work was important as it quantified the delay in the $\dot{V}O_2$ response during incremental exercise and provided further insight into the oxygen (O_2) deficit concept.

Iannetta's work also illustrated the importance of correctly quantifying the iMRT to accurately identify incremental $\dot{V}O_2$ parameters.¹ For instance, analysis of the $\dot{V}O_2$ -PO relationship requires that incremental $\dot{V}O_2$ data are properly aligned with PO as the iMRT of $\dot{V}O_2$ at ramp-onset temporarily misaligns $\dot{V}O_2$ gain ($\Delta \dot{V}O_2/\Delta PO$) such that the $\dot{V}O_2$ kinetics of incremental exercise are right-shifted relative to the corresponding PO³ until the gas exchange threshold (GET). Prior methodology to quantify the iMRT have included: 1) fitting a mono-exponential function⁴ using a nonlinear least-squares regression, and 2) applying a segmented linear regression model with the intersection of two regression lines to represent the time interval before the increase in $\dot{V}O_2$.¹ However, these methods demonstrated poor reproducibility in the calculation of iMRT due to the influence of small test-to-test differences in not only baseline $\dot{V}O_2$, but also changes in $\dot{V}O_2$ gain.⁵⁻⁷ By superimposing ss $\dot{V}O_2$ onto the ramp-PO relationship, Iannetta et al.¹ found not only greater reproducibility of iMRT in a test-retest design, but also reduced influence of baseline $\dot{V}O_2$ and changes in $\dot{V}O_2$ gain in comparison to prior fitting strategies.

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| Abbreviations | |
|----------------------------|--|
| CI | confidence interval |
| CO ₂ | carbon dioxide |
| CWR | constant work rate |
| ECG | electrocardiography |
| F | Female |
| GET | gas exchange threshold |
| GET%W _{peak} | gas exchange threshold expressed as a percentage (%) of peak Watts |
| HRMax | Maximum heart rate |
| iMRT | incremental MRT |
| LRS | linear regression slope |
| M | Male |
| Max | maximum |
| Min | minimum |
| MOE | margin of error |
| MRT | mean response time |
| NSS | non-steady state |
| PO | power output |
| RR | respiratory rate |
| S | slope |
| S _{1-CWR} | slope for constant work rate |
| S _{1-ramp} | slope for ramp oxygen uptake |
| SD | Standard Deviation |
| sEMG | muscle surface electromyography |
| SS | steady state |
| ss $\dot{V}O_2$ | steady state $\dot{V}O_2$ |
| tte | time to exhaustion |
| $\dot{V}CO_2$ | carbon dioxide output |
| $\dot{V}E$ | ventilation |
| $\dot{V}O_2$ | oxygen uptake |
| $\dot{V}O_2$ gain | delta (Δ) change in oxygen uptake ($\dot{V}O_2$) divided by the delta change in power output (PO) |
| $\dot{V}O_2$ peak | Maximum oxygen uptake |
| $\dot{V}O_2$ peak absolute | $\dot{V}O_2$ in L·min ⁻¹ |
| $\dot{V}O_2$ peak relative | $\dot{V}O_2$ in ml·min ⁻¹ ·kg ⁻¹ |
| W | Watts |
| W _{peak} | peak power output |

Although this novel method appears to improve the reproducibility of quantifying the iMRT, using a single ss $\dot{V}O_2$ -W condition to derive one value for the iMRT is inappropriate, since this approach assumes that the iMRT is constant across all SS exercise intensities. In fact, data from Keir et al.^{8,9} show that step incremental MRT increases curvilinearly in healthy young men, suggesting that research based on the method of Iannetta et al.¹ but for multiple SS intensities should yield similar results. However, besides the recent study of Iannetta et al.,¹ to the best of our knowledge, no investigative study has been undertaken to compare iMRT's for multiple submaximal constant load ss $\dot{V}O_2$'s below the GET.

While ss $\dot{V}O_2$ at 100 W is likely to be within the moderate-intensity domain and below the GET for trained athletes, further research is needed to conclude whether this PO may be too high to elicit a SS in sedentary and older individuals. In addition, given the 2–3 min time requirement to reach ss $\dot{V}O_2$,¹⁰ and the growing body of evidence identifying slowed $\dot{V}O_2$ kinetics during exercise transitions at the higher end of SS,^{8–15} we hypothesized that the iMRT is not constant across SS exercise intensities during ramp incremental exercise. Therefore, the purpose of this study was to compare estimates of the iMRT at multiple SS exercise intensities. These data will elucidate adjustment of the $\dot{V}O_2$ response to SS conditions during incremental exercise which has application to exercise prescription in healthy and clinical populations.

2. Materials & methods

2.1. Ethical approval

This research involved a within subjects repeated-measures, experimental design with informed consent obtained from each participant. The study was reviewed and approved by the University Human Research Ethics Committee (UHREC) (ethics number 4252).

2.2. Participants

Participants were moderate to highly trained cyclists who reported cycling endurance training of at least 45 min, three times per week. The inclusion criteria also included males aged between 18 and 45 years and females aged between 18 and 55 years who were free from musculoskeletal, cardio-pulmonary, and metabolic disease. Participants were excluded from the study if they were current smokers or had a history of smoking, has/or had a recent musculoskeletal injury (within the past 3

months), and any surgical procedures (within the past 3 months) that prevented exercise participation.

Twelve males and two females (See Table 1 for descriptive data) gave their written informed consent and volunteered to participate in the study. Recruitment took place within the staff and student population at an Australian, Queensland university and via online communication with local cycling groups. All potential participants were asked to complete an Exercise and Sports Science Australia: Adult Pre-Screening System¹⁶ tool to determine that they were in good physical health with no musculoskeletal disorders or risk factors for sedentary lifestyle diseases prior to study participation. Based on *a priori* power and sample size estimation analyses (GPower, v3.1.9.4, Universität Kiel, Germany) for the primary outcomes of $\dot{V}O_2$ peak, ss $\dot{V}O_2$, and iMRT ($f = 0.5$, power = 0.8, $\alpha = 0.05$) a sample size of 12 was required. However, the sample size was increased to 14 to allow for participant dropout.

2.3. Experimental protocol

The protocol involved four sessions of exercise testing held at the same time of day within subjects, with a minimum of 24 h between each session. All tests were conducted within the exercise physiology laboratory of the Faculty of Health Biomedical Research facility, Queensland University of Technology (QUT). The protocol included a familiarization session, a maximal $\dot{V}O_2$ test, four constant load submaximal $\leq 75\%$ of peak power output (W_{peak}) trials, and four constant load, non-steady state (NSS) trials. For the purposes of this study, the four constant load, NSS trials and muscle surface electromyography (sEMG) data are not included as they do not inform the aims of this paper and are being published elsewhere. Additionally, all SS trials preceded the NSS bouts and thus, were not influenced by prior heavy exercise.

To minimize subject fatigue and negative carry-over effects between trials, trials were separated evenly across all sessions with subjects completing the protocol in the following order: a familiarization session and maximal incremental exercise test on day 1, and multiple constant load submaximal bouts amounting to two on day 2, and one each on days 3 and 4. All tests were performed on an electronically braked cycle ergometer (Excalibur Sport, Corval Lode B.V., Lode Medical Technology, Groningen, the Netherlands) with gas-exchange measurements and sEMG data collected throughout. Subjects were instructed to refrain from strenuous exercise 24 h prior to the initial familiarization test and between sessions. Subjects were further instructed to refrain from consuming caffeine and alcohol 12 h prior and food, nutritional

Table 1Descriptive data for all subjects ($n = 14$). * Subject was removed from all mean comparison statistical analyses (see methods).

| Subject # | Age (yrs) | Gender (M or F) | Height (cm) | Weight (kg) | $\dot{V}O_2$ Peak - absolute (L·min ⁻¹) | $\dot{V}O_2$ Peak - relative (ml·min ⁻¹ ·kg ⁻¹) | GET (%) Wpeak | Wpeak (Watts) | HRmax (beats min ⁻¹) | Ramp function (Watts) |
|--------------------------------|-----------|-----------------|-------------|-------------|---|--|---------------|---------------|----------------------------------|-----------------------|
| 1 | 42 | M | 174.10 | 87.45 | 5.90 | 67.41 | 73.82 | 459.90 | 171 | 35 |
| 2 | 29 | M | 187.00 | 73.85 | 5.69 | 76.84 | 79.11 | 451.00 | 187 | 40 |
| 3 | 43 | M | 175.00 | 69.60 | 5.09 | 73.14 | 57.53 | 399.70 | 176 | 35 |
| 4 | 43 | M | 184.90 | 85.40 | 5.41 | 63.38 | 58.39 | 494.55 | 183 | 35 |
| 5 | 46 | F | 172.40 | 69.45 | 3.80 | 54.71 | 65.02 | 354.20 | 160 | 35 |
| 6 | 34 | M | 190.60 | 97.30 | 5.91 | 60.74 | 62.73 | 484.00 | 180 | 40 |
| 7 | 35 | M | 182.10 | 82.05 | 4.97 | 60.55 | 60.49 | 432.25 | 185 | 35 |
| 8* | 47 | F | 159.20 | 51.40 | 2.24 | 43.38 | 59.97 | 222.60 | 167 | 30 |
| 9 | 41 | M | 192.20 | 88.80 | 4.94 | 55.60 | 64.59 | 460.60 | 171 | 35 |
| 10 | 30 | M | 173.50 | 71.15 | 4.24 | 59.56 | 71.17 | 355.08 | 170 | 35 |
| 11 | 34 | M | 183.20 | 76.80 | 4.42 | 57.57 | 78.08 | 425.20 | 171 | 40 |
| 12 | 36 | M | 177.90 | 68.10 | 4.87 | 71.54 | 71.50 | 471.60 | 184 | 40 |
| 13 | 40 | M | 188.80 | 83.65 | 4.33 | 51.70 | 67.05 | 388.40 | 174 | 35 |
| 14 | 43 | M | 188.80 | 91.80 | 4.85 | 52.88 | 68.68 | 369.95 | 170 | 35 |
| Mean | 39 | | 181 | 78 | 4.76 | 60.64 | 67.01 | 412.07 | 175 | 36 |
| Standard Deviation (SD) | 6 | | 9 | 12 | 0.96 | 9.20 | 7.02 | 71.92 | 8 | 3 |

Note: F = Female; M = Male; $\dot{V}O_2$ Peak = Maximum oxygen uptake; GET = gas exchange threshold expressed as a percentage (%) of peak Watts; Wpeak, = maximum Watts at $\dot{V}O_2$ peak; HRmax = maximal heart rate.

supplements, and water at least 3 h prior to all exercise trials. However, all participants were encouraged to hydrate and consume a high carbohydrate meal prior to the 3-h cut-off period.

2.4. Familiarization session

Each participant's age (years), mass (kg) by digital scale (Ceca, Hamburg, Germany) (kg), height (cm) by stadiometer and resting heart rate (beats·min⁻¹) were recorded. Participants were fitted with a multiple one-way valve “Y” shaped mouthpiece that was supported by an acrylic head unit. Participants then cycled for 5 min at 100 W to determine the seat height (cm), handle-bar positioning (cm), preferred cycling cadence (rev·min⁻¹), and to allow necessary refinements to the size of the mouthpiece.

2.5. $\dot{V}O_2$ peak test

To collect heart rate data throughout the test and monitor cardiovascular events, participants were fitted with a 5-lead electrocardiography (ECG) configuration (Custo-Med™, Ottobrunn, Germany). For 2 min before the ramp protocol, resting expired gases were measured (to assess the quality of the calibration) followed by a 2 min warm-up at a workload equivalent to double the participant's 1-min ramp incrementation rate, followed by a near continuous ramp function to volitional exhaustion. The ramp protocol was determined by the participants' self-reported fitness level and with the intent to produce volitional exhaustion within 8 and 12 min,^{17–19} resulting in work rate increments between 25 and 40 W·min⁻¹ between subjects (See Table 1 for descriptive data). Participants then cycled at their predetermined cadence (within ± 5 rev·min⁻¹) until volitional exhaustion which was determined by the inability of the subject to maintain at or above 20 rev·min⁻¹ less than their predetermined target cadence, or by subject termination. Following the maximal incremental exercise test, subjects laid supine for 10 min to monitor adverse events and to facilitate recovery.

2.6. Incremental $\dot{V}O_2$ peak & Wpeak determination

$\dot{V}O_2$ peak was determined as the highest 7-breath average $\dot{V}O_2$ ^{20,21} and the subsequent inability of the participant to maintain at or above 20 rev·min⁻¹ less than their predetermined target cadence, or via participant termination. Secondary criteria to validate determination of $\dot{V}O_2$

peak were not implemented as prior work has suggested such criteria would now allow for a 30%–40% underestimation of true $\dot{V}O_2$ peak and/or the probability for test rejection where subjects had achieved a maximal $\dot{V}O_2$.²² Considering the high fitness level of our subjects and their familiarity with exhaustive cycling, a validation test was not necessary to confirm $\dot{V}O_2$ peak attainment as prior work has shown maximal incremental testing to yield highly reproducible $\dot{V}O_2$ peak values irrespective of the ramp protocol function, exercise test protocol, or participants' pacing strategy.^{22,23} Additionally, the necessity of verification testing, especially for highly trained populations, has been called into question given the concordance between the peak $\dot{V}O_2$ attained during ramp incremental testing and during the verification phase.²⁴

Wpeak was determined as expressed in Equation (1).

$$W_{peak} = (t_{te} - \text{resting time}) \times \text{ramp function} \quad (1)$$

Where t_{te} is the time to exhaustion (min) corresponding to the highest 7-breath average $\dot{V}O_2$, resting time is the total duration (min) of resting gas exchange data, and ramp function of each participant's pre-determined ramp work rate increment (W·min⁻¹) for the incremental $\dot{V}O_2$ peak test.

2.7. Submaximal constant load testing

Participants returned to the lab at least 24-h following the ramp incremental test to complete two submaximal constant load trials at 30% and 45% Wpeak with simultaneous gas-exchange collection. The submaximal constant load trials consisted of 2 min rest on the cycle ergometer followed by 2 min of unloaded cycling, and then 6 min of constant load cycling. Each submaximal constant load trial was separated by 10 min of supine rest. Participants returned to the lab on day three to complete one submaximal constant load trial at 65% of Wpeak. On the fourth day, participants completed the final submaximal constant load trial at 75% of Wpeak.

Whilst the GET, VT, or lactate threshold (LT) are regarded as the optimal approach to prescribing submaximal exercise intensities from ramp incremental exercise^{25,26} there are several reasons as to why the SS trials were prescribed using %Wpeak. Firstly, these thresholds are still not perfect in identifying the maximal steady state (MSS) and therefore, may not accurately delineate between SS versus NSS.²⁷ Irrespective of whether submaximal exercise prescription was based on fixed percentages of a maximum value (i.e., %Wpeak) or via physiological thresholds,

SS verification criteria were used (see 5.1 SS determination) to adequately delineate between SS versus NSS. Our use of multiple submaximal intensities from 30% to 75% of W_{peak} served to elucidate intensities both above and below the GET and thus, we could compare more than one SS value below the GET to test our hypothesis. Lastly, when comparing SS to NSS bouts, determination of exercise intensities based on a metabolic threshold measure was not relevant to this manuscript, nor was the delineation between exercise intensity domains given our methodology was used to ascertain which bouts were SS versus NSS.

3. Measurements

3.1. Pulmonary gas exchange

During the ramp incremental test, respiratory rate (RR), ventilation ($\dot{V}E$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide output ($\dot{V}CO_2$) were measured breath-by-breath using a fast response turbine flow transducer (Hans Rudolph-430, Van Nuys, CA, USA) connected to the inspired side of the mouthpiece. Expired gas analysis was acquired using a 3 L latex compliant and elastic mixing (allowing variable volume functionality determined by tidal volume) bag placed on the expired port of the mouthpiece, and mixed expired air was sampled continuously and pumped to rapid response O_2 and carbon dioxide (CO_2) gas analysers (AEI Technologies, Model S-3 A and Model CD-3H, Pittsburgh, PA, USA). All data were acquired using custom developed software (LabVIEW™, National Instruments, Austin, TX, USA) and commercial electronic acquisition devices (National Instruments, Austin, TX). The breath-by-breath system was calibrated prior to the ramp test using a 3 L syringe and commercial medical grade calibration gas (room air and then 17.2% O_2 , 4.13% CO_2). The methods used for indirect calorimetry have been validated and described elsewhere.^{28,29} Electrocardiography (ECG) was also collected to determine HR throughout the ramp-incremental test using a 5-lead ECG configuration (CASE, GE Healthcare, Waukesha, USA). The methods of ECG lead placement have been described elsewhere.²⁹

4. Data processing

4.1. Determination of the GET

Prior research has revealed the most accurate method for detection of the GET is the ventilatory equivalents method.³⁰ This method involves graphically comparing the change in the ventilatory equivalents for O_2 and CO_2 ($\dot{V}E\dot{V}O_2$ and $\dot{V}E\dot{V}CO_2$, respectively) throughout the ramp protocol. The detection of the first sustained increase in $\dot{V}E\dot{V}O_2$ while $\dot{V}E\dot{V}CO_2$ is stable identifies the GET. For detection of the GET, custom software (LabVIEW™, National Instruments, Austin, TX, USA) was used to apply three linear segments to the $\dot{V}E\dot{V}$ data. Linear segments were adjusted to the lowest residuals error, and the GET was determined as the time of the intersection between segment 1 (baseline response, slope ~ 0) and segment 2 (initial deviation from baseline), with detection requiring agreement (± 10 s) between two investigators.

4.2. Gas-exchange measurements

To remove variability from single breath data, the raw ramp-incremental $\dot{V}O_2$ data were processed using a 7 breath average³¹ applied using custom software (LabVIEW, V-12, National Instruments, Austin, TX).

4.3. $\dot{V}O_2$ slope analyses

The raw files from the exercise bouts were imported into a custom software program (LabVIEW, V-12, National Instruments, Austin, TX) for conversion to 7 breath averaged data. $\dot{V}O_2$ data were then graphed, and a

linear segment was applied to the data, commencing 1 min after the initial 2 min double the ramp incrementation rate, then extending to the GET. Linear segments were manually adjusted to the least residuals error, and the resulting ramp slopes were recorded.

4.4. $ss \dot{V}O_2$ and MRT

The method used to compute the iMRT was based on the method of Iannetta et al.¹ As $\dot{V}O_2$ response data were collected from four constant PO trials (30%, 45%, 65%, and 75% W_{peak}), the $\dot{V}O_2$ time constant and plateau $\dot{V}O_2$ value were determined from a mono-exponential curve fitting of the $\dot{V}O_2$ response profile, common to prior studies of the $\dot{V}O_2$ kinetics to increments in exercise intensity to SS³² and presented in Equation (1). Whether the mono-exponential $\dot{V}O_2$ profile attained SS was determined based on a linear regression slope (LRS) of the $\dot{V}O_2$ versus time data for the last 3-min of each bout, with the cut-off value for SS being $<0.0290 \text{ L}\cdot\text{min}^{-1}\cdot\text{min}^{-1}$ (see Results). Since not all constant PO trials induced SS, (please see Results), only trials that elicited SS data were used in the estimation of iMRT.

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss)1 - e^{-1(t-TD)/\tau} \quad (1)$$

Where $1 - e^{-1(t-TD)/\tau}$ is the exponential function describing the rate at which $\dot{V}O_2$ rises toward the plateau amplitude.³²

t = time; TD = time delay before the start of the exponential term; $\Delta\dot{V}O_2(t)$ = the value of $\dot{V}O_2$ at any time during the ramp test, and τ = time constant.

The $ss \dot{V}O_2$ data were then superimposed onto the ramp incremental exercise $\dot{V}O_2$ response data for each participant. For trials that met SS criteria, linear regression was performed on the multiple $ss \dot{V}O_2$ and time (min) data points to derive the slope of the $ss \dot{V}O_2$ responses. To reveal this methodology, data from two representative subjects are presented in Figs. 1 and 2. The time from the $ss \dot{V}O_2$ data was calculated from the W ramp function for the $ss \dot{V}O_2$. Linear regression was then applied to the pre-GET segment of the ramp incremental protocol, and the time for the $ss \dot{V}O_2$ to be attained in the ramp protocol was calculated using Equation (2).

$$\text{ramp time (min)} = (ss\dot{V}O_2 + Y_{int}) / \text{ramp}\dot{V}O_{2,slope} \quad (2)$$

The difference between the $ss \dot{V}O_2$ time value and the ramp time from Equation (2) was the iMRT.

5. Statistical analyses

5.1. SS determination

Simple LRS were applied to the $\dot{V}O_2$ ($\text{L}\cdot\text{min}^{-1}$) data below the GET (typically spanning 3.5–8 min) for all four constant exercise intensity bouts for all subjects (Table 1) (GraphPad Prism, v9). Using descriptive statistics, the maximum (max) and minimum (min) LRS, and the margin of error (MOE) from the 99% confidence intervals (CI) were calculated for each intensity bout as detailed in Table 2. The criteria for SS or NSS of each exercise intensity was determined by the max LRS of the 45% W_{peak} (i.e., if the remaining LRS was greater than the max LRS at 45% W_{peak} , the $\dot{V}O_2$ data for that subject's bout was considered NSS and if it was lower, the intensity for that subject was considered SS). The max LRS of the 45% W_{peak} bout was used as the deciding criterion for SS versus non-SS as this exercise bout elicited overall, the greatest LRS for $ss \dot{V}O_2$ compared to cycling at 30% W_{peak} (See Table 2).

The 99% CI MOE was not used to categorize SS because it often revealed low values for the NSS $\dot{V}O_2$ when the slow component $\dot{V}O_2$ response was linear. Thus, LRS were used as the final deciding criterion.

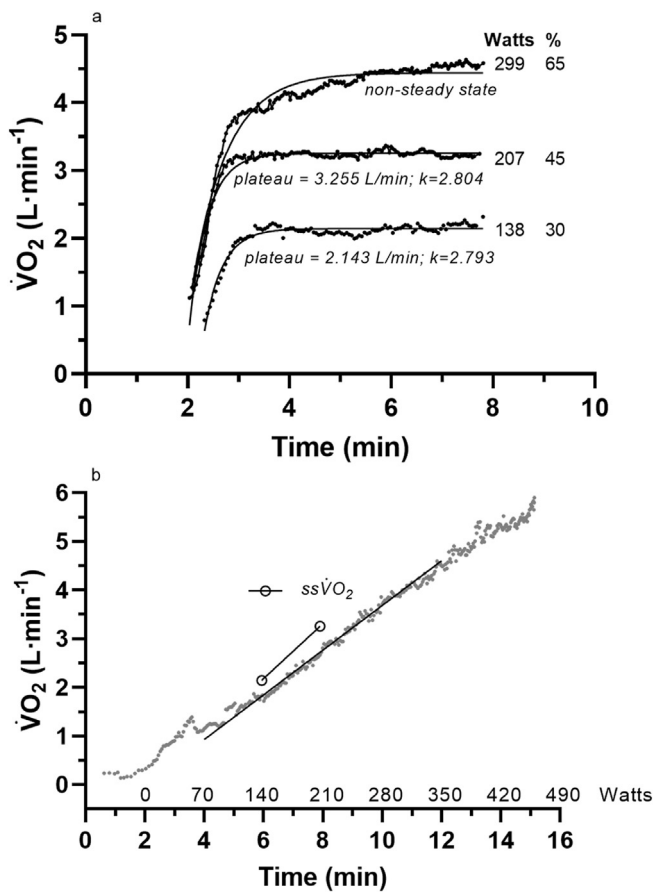


Fig. 1. An example of a) the oxygen uptake ($\dot{V}O_2$) data for two low to moderate intensity constant load exercise bouts, along with one non-steady state (NSS) bout for one representative subject (#1). The mono-exponential curve fitting is shown for each bout and exercise intensities are provided in the figure. b) The steady state $\dot{V}O_2$ (ss $\dot{V}O_2$) data superimposed to the ramp incremental $\dot{V}O_2$ data for this subject. See Figure three for individual ramp and ss $\dot{V}O_2$ -time slopes for all subjects ($n = 13$).

5.2. T-test analyses

A total of three paired t-tests were performed to compare: 1) the $\dot{V}O_2$ slope responses for SS versus the incremental ramp protocol, 2) differences between the 30% ss $\dot{V}O_2$ and 45% ss $\dot{V}O_2$ values below the GET, and lastly, 3) the iMRT's of the first (iMRT-30) and last (iMRT-45) pre-GET ss $\dot{V}O_2$ values. Due to the poor quality of data for subject eight, we could not fit a linear slope to $\dot{V}O_2$ data below the GET from the maximal incremental test. Therefore, these data were removed from statistical analyses, resulting in a sample size of 13 for the t-tests. All data met the normality assumption (Shapiro-Wilks), and statistical significance was set as $p < 0.05$.

6. Results

Descriptive characteristics of all subjects ($n = 14$) are presented in Table 1. Table 2 presents the LRS for the last 3 min of cycling at 30%, 45%, 65%, and 75% of W_{peak} . As explained in the Methods, the 99% CI, MOE and the LRS were used to decipher the attainment of SS. The LRS data were determined to be the most sensitive in detecting SS, and the cut-off value used to determine SS was from the 45% LRS slope ($< 0.0290 \text{ L}\cdot\text{min}^{-1}\cdot\text{min}^{-1}$). Based on these criteria, all subjects attained SS at 30% and 45% W_{peak} , yet only four achieved SS during cycling at 65% W_{peak} , and no subjects achieved SS at 75% W_{peak} . The ss $\dot{V}O_2$ ($\text{L}\cdot\text{min}^{-1}$) data at 30% and 45% W_{peak} were $[2.26 \pm 0.24]$ vs.

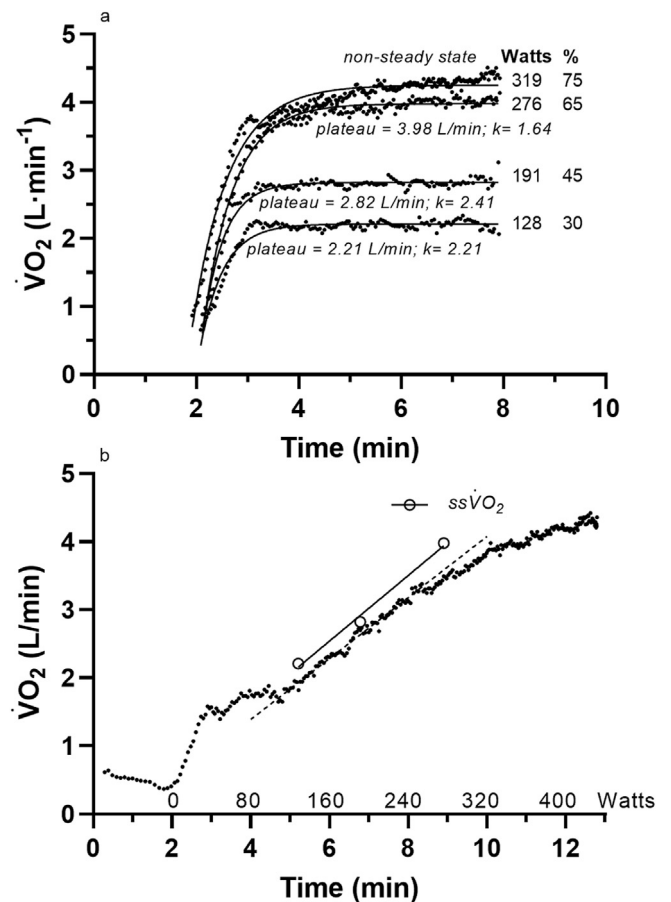


Fig. 2. An example of a) the oxygen uptake ($\dot{V}O_2$) data for three low to moderate intensity constant load exercise bouts, along with one non-steady state (NSS) bout for one representative subject (#11). The mono-exponential curve fitting is shown for each bout and exercise intensities are provided in the figure. b) The steady state $\dot{V}O_2$ (ss $\dot{V}O_2$) data superimposed to the ramp incremental $\dot{V}O_2$ data for this subject. See Figure three for individual ramp and ss $\dot{V}O_2$ -time slopes for all subjects ($n = 13$).

$[3.08 \pm 0.30] \text{ L}\cdot\text{min}^{-1}$ (mean \pm standard deviation [SD]), respectively ($t = 10.82$, $df = 12$, $p < 0.0001$).

Figs. 1 and 2 present data from participant #1 and #11 and highlight the data processing methods used in quantifying the MRT for the SS exercise bouts, and the linear regression of the $\dot{V}O_2$ data segment below the GET for the ramp incremental protocol. Both subjects demonstrate a greater $\dot{V}O_2$ regression slope from SS data compared to the $\dot{V}O_2$ segment below the GET, with SS slopes data presented in Table 2 and the ramp slopes in Fig. 3. The respective SS and ramp data for the $\dot{V}O_2$ time slopes for these representative subjects were 0.5645 vs. 0.4404 and 0.4478 vs. 0.3931 $\text{L}\cdot\text{min}^{-1}\cdot\text{min}^{-1}$.

Establishing the difference between the slopes of the $\dot{V}O_2$ responses for the SS and ramp incremental protocol were important to decipher prior to analyzing the data for iMRT. Fig. 3a presents the individual data for the slopes resulting from the linear regression fit of the ss $\dot{V}O_2$ data and the linear regression of the $\dot{V}O_2$ data across intensities below the GET. Fig. 3b compares the mean \pm SD between the two mean slopes of these variables, revealing a significantly greater slope for ss $\dot{V}O_2$ versus incremental $\dot{V}O_2$ ($p = 0.003$).

Our results comparing the SS to ramp incremental exercise $\dot{V}O_2$ slopes informs the analyses for iMRT. For example, the significantly greater ss $\dot{V}O_2$ slope implies that the iMRT would follow a similar pattern. Fig. 4 presents the individual data for the iMRT at 30% and 45% W_{peak} (solid circles) and the resulting linear regression (solid line). Open

Table 2

The linear regression slopes (LRS) for the last 3 min of four constant load exercise intensity bouts at 30%, 45%, 65%, & 75% of peak power output (W_{peak}) for all subjects ($n = 14$). Also presented are data of the margin of error (MOE) for the 99% confidence intervals (CI) of the mean of oxygen uptake ($\dot{V}O_2$) in $L \cdot min^{-1}$.

| Subject | 30% LRS | 30% MOE | SS vs. NSS | 45% LRS | 45% MOE | SS vs. NSS | 65% LRS | 65% MOE | SS vs. NSS | 75% LRS | 75% MOE | SS vs. NSS |
|---------|---------|---------|------------|---------|---------|------------|---------|---------|------------|---------|---------|------------|
| 1 | 0.0213 | 0.0169 | SS | 0.0006 | 0.0100 | SS | 0.1405 | 0.0423 | NSS | 0.1854 | 0.0550 | NSS |
| 2 | 0.0088 | 0.0255 | SS | -0.0132 | 0.0274 | SS | 0.1401 | 0.0474 | NSS | 0.1971 | 0.0538 | NSS |
| 3 | -0.0060 | 0.0226 | SS | 0.0130 | 0.0226 | SS | 0.1291 | 0.0368 | NSS | 0.1567 | 0.0404 | NSS |
| 4 | -0.0070 | 0.0224 | SS | 0.0290 | 0.0337 | SS | 0.0592 | 0.0278 | NSS | 0.1530 | 0.0449 | NSS |
| 5 | -0.2200 | 0.0142 | SS | -0.0019 | 0.0103 | SS | 0.0413 | 0.0144 | NSS | 0.0951 | 0.0258 | NSS |
| 6 | -0.0109 | 0.0302 | SS | 0.0018 | 0.0300 | SS | 0.0897 | 0.0322 | SS | 0.1205 | 0.0356 | NSS |
| 7 | -0.3470 | 0.0258 | SS | -0.0261 | 0.0237 | SS | 0.0283 | 0.0146 | SS | 0.1556 | 0.0461 | NSS |
| 8* | -0.0343 | 0.0250 | SS | 0.0106 | 0.0172 | SS | 0.0063 | 0.0121 | SS | 0.0797 | 0.0278 | NSS |
| 9 | -0.0126 | 0.0192 | SS | 0.0219 | 0.0238 | SS | 0.0554 | 0.0265 | NSS | 0.1519 | 0.0587 | NSS |
| 10 | -0.0370 | 0.0280 | SS | -0.0107 | 0.0224 | SS | 0.0272 | 0.0181 | SS | 0.0895 | 0.0277 | NSS |
| 11 | 0.0071 | 0.0142 | SS | 0.0143 | 0.0132 | SS | 0.0770 | 0.0270 | NSS | 0.1226 | 0.0323 | NSS |
| 12 | 0.0241 | 0.0245 | SS | 0.0100 | 0.0291 | SS | 0.1172 | 0.0378 | NSS | 0.1105 | 0.0300 | NSS |
| 13 | -0.0307 | 0.0217 | SS | 0.0111 | 0.0126 | SS | 0.0667 | 0.0215 | NSS | 0.1514 | 0.0428 | NSS |
| 14 | -0.0145 | 0.0175 | SS | -0.0064 | 0.0198 | SS | 0.1189 | 0.0385 | NSS | 0.1262 | 0.0367 | NSS |
| Max | 0.0241 | 0.0302 | | 0.0290 | 0.0337 | | 0.1405 | 0.0474 | | 0.1971 | 0.0587 | |
| Min | -0.3470 | 0.0142 | | -0.0261 | 0.0100 | | 0.0063 | 0.0121 | | 0.0797 | 0.0258 | |

Note: *Subject was removed from all mean comparison statistical analyses (see methods). See methods for criteria for classifying steady state (SS) versus non-steady state (NSS). LRS = Linear regression slope; W_{peak} = peak power output; MOE = margin of error; CI = confidence interval. For determination of exercise intensities from W_{peak} please see section 2.6 - Incremental $\dot{V}O_2$ peak and W_{peak} determination.

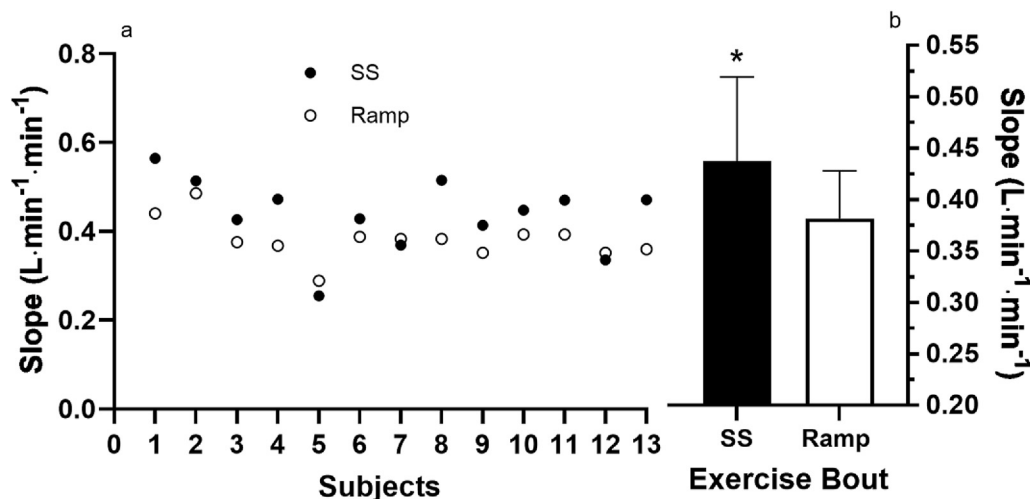


Fig. 3. Comparison of oxygen uptake ($\dot{V}O_2$) slopes ($L \cdot min^{-1} \cdot min^{-1}$) from the multiple steady state $\dot{V}O_2$ (ss $\dot{V}O_2$) data points vs. the ramp incremental exercise protocols for a) all subjects ($n = 13$) and b) Mean \pm Standard Deviation (SD) results. Significant difference * $p = 0.003$.

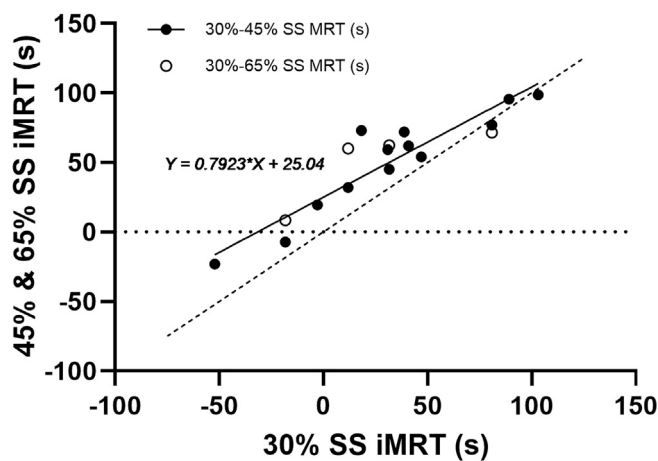


Fig. 4. Linear regression of the incremental mean response time (iMRT) for steady state $\dot{V}O_2$ (ss $\dot{V}O_2$) of the 30% versus 45% peak workload (W_{peak}) for all subjects ($n = 13$). Data for the 65% peak workload (W_{peak}) bouts for four subjects are also shown (open circles) to document the similarity of this data.

circles represent the subjects that attained SS and therefore had an iMRT calculation at 65% W_{peak} . The dashed line represents the line of identity to document the extent to which the iMRT results are higher for the 45% W_{peak} exercise bouts.

Fig. 5a presents the iMRT for each of the ss $\dot{V}O_2$ data across the multiple trials of each subject. As previously explained, subjects differed in the number of the four constant load bouts that exhibited SS. Since all subjects attained ss $\dot{V}O_2$ at 30% and 45% W_{peak} , these two means were analyzed by paired t -test. The paired t -test results for the iMRT at 30% versus 45% ss $\dot{V}O_2$ trials revealed a $t = -3.921$, $df = 12$, $p = 0.002$ (two-sided analysis). Mean \pm SD data for this analysis are presented in Fig. 5b.

7. Discussion

The iMRT has been calculated using fitting-based methods that led to poor test-to-test reproducibility.^{33–36} To prescribe constant-intensity exercise from ramp-incremental exercise, it is imperative that the iMRT is correctly quantified to prevent overestimations of the $\dot{V}O_2$ -work-rate relationship. A novel method was proposed by Iannetta et al.¹ which reported greater validity and reproducibility when calculating the iMRT in a test-retest design. The limitation of this method in favor of prior

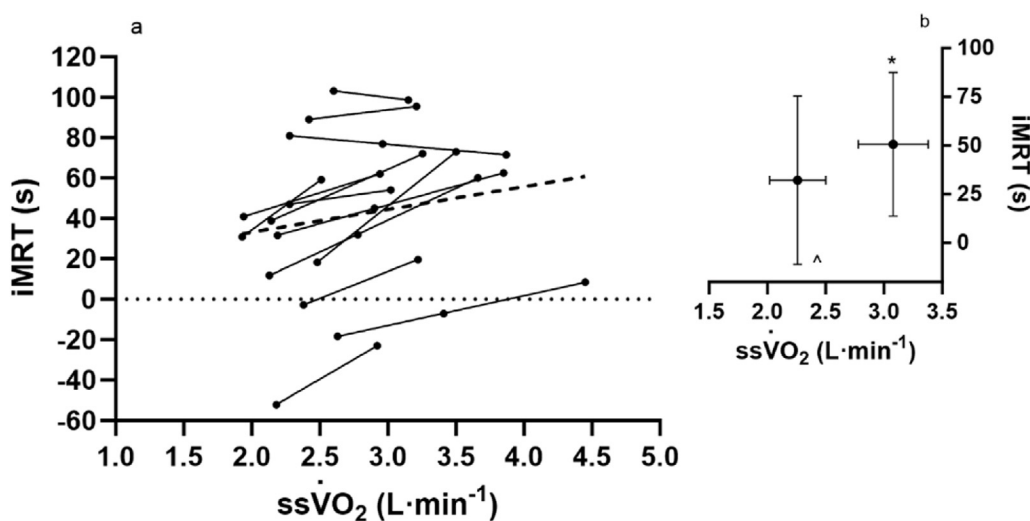


Fig. 5. a) Individual subject data ($n = 13$) for the incremental mean response time (iMRT), in seconds, for each documented steady state $\dot{V}O_2$ (ss $\dot{V}O_2$) exercise bout. Of the 13 subjects 9 had two steady state (SS) bouts (30% & 45% SS) and four had three (also 65% SS). b) Mean \pm Standard Deviation (SD) data for the mean response time (MRT)'s for 30% vs 45% of peak workload (W_{peak}) ss $\dot{V}O_2$. See methods and results for statistical analyses. Significant difference * $p = 0.002$ for MRT & \wedge $p < 0.0001$ for ss $\dot{V}O_2$.

fitting-based methods is that it requires only one ss $\dot{V}O_2$ to W condition to derive one value for the iMRT. This method assumes that the iMRT is constant across all SS exercise intensities and in turn, the slope of the ss $\dot{V}O_2$ -time responses is similar to the $\dot{V}O_2$ -time (we converted W to time based on the individualized ramp function) incremental exercise ramp function.

In the current study, ss $\dot{V}O_2$ values were measured from multiple constant load low to moderate intensity exercise bouts as well as from incremental exercise. Our results demonstrate a significantly greater $\dot{V}O_2$ -time slope for ss $\dot{V}O_2$ data vs. incremental $\dot{V}O_2$ data (Fig. 2). In addition, the iMRT was significantly greater for higher SS exercise intensities (Figs. 3, 4 and 5a and b), which is consistent with the findings of Wilcox et al.³⁵ during step-transition SS exercise. The iMRT was also variable across the SS domain, a finding consistent with the known increase in the time constant for exercise transitions to SS of increasing exercise intensities¹⁵ and the attendant increasing $\dot{V}O_2$ deficit of incremental exercise, regardless of how small the work rate increments (e.g., ramp protocols). Overall, these results provide new insight to the understanding of $\dot{V}O_2$ kinetics during incremental exercise.

7.1. Comparison to prior research

Contrary to prior observations³⁶ and in support of our hypothesis, our findings revealed a significant difference between the slope of ss $\dot{V}O_2$ versus the slope of incremental $\dot{V}O_2$ pre-GET (Figs. 1–3; Table 2). Prior research by Caen et al.³⁶ observed that regardless of the ramp function used (15 or 30 $W \cdot \text{min}^{-1}$) there was no significant difference between the slope (S) of constant work rate (CWR) (S_{1-CWR}) versus the S for ramp $\dot{V}O_2$ (S_{1-ramp}) within the moderate intensity domain. However, these results need to be interpreted with caution as the initial data point of both their CWR slope (Fig. 1) and ramp slope was from CWR exercise at 50 W. Inclusion of the 50 W baseline $\dot{V}O_2$ data would attenuate the slope function for the CWR condition which consequently led to the non-significant difference between the slope of CWR versus ramp exercise (type 2 error). In contrast, we only used $\dot{V}O_2$ data for our linear regression segments below the GET that occurred during the ramp function of the incremental protocol.

7.2. Methods used to quantify the iMRT

There is no doubt that the methodological advances to the calculation of the iMRT has been warranted¹; however, various differences in exercise protocols and equations used complicate true comparison of the validity of the past and present methodologies. The current method of

superimposing ss $\dot{V}O_2$ data onto the $\dot{V}O_2$ -PO relationship yields vastly different iMRT's.^{36,37} The most consistent iMRT's calculated utilizing this method are from Iannetta et al.,¹ with a iMRT equal to (26 ± 11) s, and by De Almeida Azevedo et al.³⁸ equal to (24 ± 10) s, with both studies employing a 30 $W \cdot \text{min}^{-1}$ ramp protocol) Yet, using the same methodology and ramp protocol (30 $W \cdot \text{min}^{-1}$) in two different studies, Caen et al.^{36,37} calculated larger iMRT's. In a 2020 study by Caen et al.³⁶ the calculated iMRT of males was (42 ± 15) s and in a later study, (42 ± 8) s for females and (52 ± 6) s for males.³⁷

Multiple factors influence the iMRT including warm-up PO, $\dot{V}O_2$ in response to the warm-up, and the amplitude of the $\dot{V}O_2$ change (as gain = $\Delta \dot{V}O_2 / \Delta PO$). The training level and $\dot{V}O_2$ peak of subjects are also important, and thus relative exercise intensities (rather than absolute) are important to consider to optimize exercise prescription. The inclusion of the warm-up intensity equal to 50 W in the methodology used by Caen et al.,^{36,37} in addition to the magnitude of this initial workload, may have contributed to the larger iMRT reported in these studies. Additionally, the influence of differing $\dot{V}O_2$ baselines and $\dot{V}O_2$ gain appears to contribute to poor reproducibility of the iMRT.¹ Consequently, we employed relative and therefore individualized exercise intensities for our constant load bouts and the initial 2 min of the incremental protocol.

It is also challenging to compare iMRT's across prior studies when the definition of the iMRT differed. The previously accepted and most common definition of the iMRT as it relates to translating the PO of incremental exercise to CWR conditions is that it "... incorporates both the transit delay for deoxygenated blood from the working muscles to be expressed at the lungs and the kinetics component of muscle $\dot{V}O_2$ as it adjusts to the increase in adenosine triphosphate demand imposed by the ramp".¹ This definition alone suggests that the iMRT reflects a progressive increasing $\dot{V}O_2$ deficit and denotes that even within the SS domain, the iMRT would increase. However, Keir et al.³ used the methodological criteria of Boone & Bourgois⁵ to calculate the iMRT. This methodology quantified the iMRT as the time between the onset of exercise and the increase in $\dot{V}O_2$. This definition alone represents a completely different iMRT to what is presented by Iannetta et al.,¹ though it has been used as a component of computing non-linear $\dot{V}O_2$ kinetics for exercise transitions to SS^{10,11,32} and additionally, would be highly influenced by the response time delay (sensitivity) of the metabolic cart.

When compared to traditional methods of iMRT calculation, the novel approach by Iannetta et al.¹ of superimposing the ss $\dot{V}O_2$ from a 100-Watt step-incremental protocol onto the $\dot{V}O_2$ -PO relationship, increased the reproducibility of calculating the iMRT. Despite this improvement, this calculation is limited by the measurement and use of only one iMRT

across the SS domain.

7.3. Variability of the iMRT

Based on our results, there is marked individual variability and a systematic increase in the iMRT pre-GET which refutes the method of Iannetta et al.¹ Yet, similar observations of this variability are not widely reported in prior research regardless of the prior fitting methods that were used to derive the iMRT. For example, despite utilizing the same method as Iannetta, Caen et al.³⁶ calculated an iMRT that was almost 20 s greater (± 15 s) than reported by Iannetta et al.,¹ despite the fact the ramp protocol ($30 \text{ W}\cdot\text{min}^{-1}$) and ssW (100) condition were identical. However, large deviations in iMRTs can be impacted by a multi-faceted influence of individual subject differences (e.g., fitness level, age, motor unit expression, etc). However, this variability between studies has been largely ignored, as have studies identifying between-subject variability in iMRT calculation.

Fig. 5b shows that the mean iMRT was (32.74 ± 43.41)s and (49.70 ± 36.43)s at 30% and 45% Wpeak. These intensities were above 100 W (124 W and 185 W, see Table 1), indicating the high fitness status of the subjects and the questionable low intensity used to calculate the iMRT in prior research.^{1,36,37} The ss $\dot{V}O_2$'s from our results were also relative to each subject's Wpeak as calculated from their maximal ramp incremental test (see Methods). In contrast, the 100 W ss $\dot{V}O_2$ used in recent studies is a different relative exercise intensity for each subject, inducing different levels of metabolic strain in each participant.

In addition, recent research has observed that the iMRT significantly increases above the moderate intensity domain³⁶ and as such, initiated concerns for how to translate ramp incremental $\dot{V}O_2$ within the heavy to severe domain for CWR exercise. Strategies to resolve this variation involve extending the prior methodology of Iannetta et al.¹ to correct for large variations in the iMRT above the moderate intensity domain.³⁶ However, the rationale for extending the calculations for the iMRT above the moderate intensity domain due to ss $\dot{V}O_2$ dependence of the method may be inappropriate, in addition to the unknown influences of motor unit recruitment,⁴ the O_2 cost of ventilation³⁹ and additional variables such as cycling efficiency⁴ that could increase, or decrease, the gain of whole body $\dot{V}O_2$ compared to values pre-GET and as such, complicate interpretations of whole body $\dot{V}O_2$ as a reflection of the exercised musculature.

7.4. Interpreting the value of the individual differences in the iMRT

Overall, the significant differences in the ss $\dot{V}O_2$ versus incremental $\dot{V}O_2$ time slopes (Fig. 3b) and the iMRT between-subjects' (Fig. 5a) and of the mean data (Fig. 5b) warrant a re-evaluation of the methodology used by which to calculate the iMRT. Moreover, there is value in how we interpret these individual differences. In research, where variability is presented in experimental results, we typically try to minimize or ignore between-subject differences, but there is potential for considerable metabolic and physiological inquiry to understand why such responses (of iMRT) can differ.

7.5. Implications for exercise testing & prescription

Recent work suggests that applying a MRT correction to the $\dot{V}O_2$ -PO relationship of ramp incremental exercise is imperative in preventing the under and/or over estimation of work rates (WR) of constant load exercise prescribed from incremental tests.¹ However, our results provide new insights and suggest the use of one 100 W SS exercise condition is not sufficient in determining the most accurate iMRT correction and that, there exists significant variability in the MRT's between multiple SS W conditions below the GET. Considering the importance of commonly deriving accurate constant load WR's from maximal incremental tests for both clinical and elite population exercise prescription, it is crucial that

the estimation of the W at a given $\dot{V}O_2$ during incremental exercise is precise. Given the results of this study and the uncertainty of calculating an accurate iMRT using one 100 W SS condition, further research is required to compare the multiple methodologies for calculating the MRT and assess the validity of such methods against multiple SS W conditions.

8. Conclusion

Several concerns have been raised regarding the use of the novel approach of Iannetta et al.¹ to derive the iMRT. Our results show that overlaying multiple ss $\dot{V}O_2$ data to the $\dot{V}O_2$ -PO (converted to time) relationship within the moderate intensity domain reveals significant increases in the iMRT. Given the variability of the iMRT between-subjects' with differing exercise intensities, it is reasonable to ascertain that the iMRT is not a constant and should therefore be reflected as such in future calculations of the iMRT. Nevertheless, more research is needed to investigate the iMRT during different exercise conditions (modes, intensities, durations, etc.) and elucidate between-subjects' variations in this outcome.

Submission statement

All authors have read and agree with the manuscript content and while this manuscript is being reviewed for this journal, the manuscript will not be submitted elsewhere for review and publication.

Ethical approval statement

This research involved repeated-measures, experimental design that was approved by the University Human Research Ethics Committee (UHREC) (ethics number 4252). All participants gave their written informed consent and volunteered to participate.

CRediT authorship contribution statement

Bridgette G.J. O'Malley: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Robert A. Robergs:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Todd A. Astorino:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

No author of this manuscript has direct or indirect interests that are in direct conflict with the conduction of the study.

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