Identifying exercise intensity "thresholds": Implications for metabolic responses, performance, and exercise intensity prescription.

Iannetta, Danilo

http://hdl.handle.net/1880/110852
doctoral thesis

University of Calgary graduate students retain copyright ownership and moral rights for their thesis. You may use this material in any way that is permitted by the Copyright Act or through licensing that has been assigned to the document. For uses that are not allowable under copyright legislation or licensing, you are required to seek permission.

Downloaded from PRISM: https://prism.ucalgary.ca
UNIVERSITY OF CALGARY

Identifying exercise intensity “thresholds”: Implications for metabolic responses, performance, and exercise intensity prescription.

by

Danilo Iannetta

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

GRADUATE PROGRAM IN KINESIOLOGY

CALGARY, ALBERTA

AUGUST, 2019

© Danilo Iannetta 2019
ABSTRACT

The exercise intensity spectrum, from rest to maximal oxygen uptake (\(\dot{V}O_2\text{max}\)), can be partitioned into three domains of intensity: moderate, heavy, and severe. These domains are demarcated by the lactate threshold (LT) (moderate-to-heavy) and critical power (CP) or maximal lactate steady-state (MLSS) (heavy-to-severe), with the respiratory compensation point (RCP) of the ramp-incremental exercise also being proposed as a marker of the heavy-to-severe boundary of exercise intensity. Although the physiological concepts underpinning these thresholds are well established, methodological issues associated with their determination may lead to inaccuracies and contrasting interpretations regarding their equivalence. The general purpose of this thesis was to find solutions to some of the issues associated with the determination of these “thresholds” and demonstrate why their accurate determination is fundamental in exercise physiology. Using a variety of exercise protocols it was demonstrated that: i) current methods to compute the mean response time (MRT) of \(\dot{V}O_2\) during ramp-exercise are inaccurate – the novel method proposed was valid and more reproducible than these methods; ii) exercising slightly above MLSS, although characterized in this study by a stable \(\dot{V}O_2\) response, disproportionally impaired maximal exercise capacity; iii) if the \(\dot{V}O_2\) dynamics during ramp-incremental exercise are carefully considered, the work rates at RCP and CP/MLSS are not different – refuting the idea that the RCP is not a valid surrogate of the heavy-to-severe boundary of exercise intensity; iv) current methods to prescribe exercise intensity based on fixed-percentage of maximum values (e.g., \(\dot{V}O_2\text{max}\)) do not provide an accurate procedure by which to control exercise intensity. Collectively, these findings provide solutions/explanations to some of the issues related to the correct identification of these exercise thresholds and suggest that their correct identification is of extreme importance when interpreting their physiological implications and to guarantee an accurate exercise intensity prescription.
PREFACE

This thesis includes versions of the following manuscripts that are either published, or in revision, or ready for submission:


ACKNOWLEDGMENTS

I would like to thank my supervisor, Dr Juan Murias. Thanks for taking me as your (first) PhD student. I had no idea what I was getting into. Now that we have made it to this point I can say that I would do it again.

To Dr. Guillaume Millet who also took a chance on me (twice). Thank you.

I would like to thank Dr. Daniel Keir, for your invaluable help.

I also want to thank all of my colleagues (friends) who during all these years, from my undergraduate to my PhD, contributed to my academic achievements. Amongst these, Felipe Mattioni Maturana and Dr. Federico Fontana deserve a special thanks. Thank you for helping me any time I was in need – I feel I have received more from you than I could ever give you in return.

Thanks Calaine – for always being next to me.

Finally, I want to thank my family. My wonderful parents, Fabio e Teresa, and my special sister, Rachele. Without you, I could not have made it.
Table of Contents

ABSTRACT ................................................................................................................................. ii
PREFACE ................................................................................................................................. iii
ACKNOWLEDGMENTS .............................................................................................................. iv
LIST OF TABLES ........................................................................................................................ vii
LIST OF FIGURES ..................................................................................................................... viii
LIST OF ABBREVIATIONS ......................................................................................................... x

CHAPTER I ..................................................................................................................................... 1
Introduction .................................................................................................................................. 1
   Hypothesis ............................................................................................................................... 2
   Thesis outline ........................................................................................................................... 3

CHAPTER II .................................................................................................................................. 4
Exercise intensity “thresholds” ...................................................................................................... 4
Exercise intensity domains .......................................................................................................... 5
Control-system of VO₂ kinetics ................................................................................................... 6
   Moderate-intensity domain ...................................................................................................... 7
   Heavy-intensity domain ........................................................................................................... 8
   Severe-intensity domain ......................................................................................................... 9
Markers of exercise intensity separating the heavy and the severe domains. Physiological basis, correspondence, and methodological issues....................................................... 10
   CP and MLSS ......................................................................................................................... 10
   Methodological issues ........................................................................................................... 12
   RCP ....................................................................................................................................... 15
Similar VO₂ but different work rate between RCP and CP/MLSS ............................................ 16
   VO₂ dynamics during ramp-exercise ...................................................................................... 16
   Correcting the VO₂ data for the time-delay at ramp-onset .................................................... 18
Exercise intensity “thresholds” and implications for exercise intensity prescription ............... 19
| CHAPTER III: A simple method to quantify the $\dot{V}O_2$ mean response time of ramp-incremental exercise | 23 |
| Introduction | 23 |
| Methods | 25 |
| Results | 30 |
| Discussion | 35 |
| CHAPTER IV: Metabolic and performance-related consequences of exercising at and slightly above MLSS | 40 |
| Introduction | 40 |
| Methods | 42 |
| Results | 51 |
| Discussion | 58 |
| CHAPTER V: Establishing the $\dot{V}O_2$ versus constant-work rate relationship from ramp-incremental exercise: Simple strategies for an unsolved problem | 66 |
| Introduction | 66 |
| Methods | 68 |
| Results | 74 |
| Discussion | 82 |
| CHAPTER VI: A critical evaluation of current methods for exercise prescription in women and men | 87 |
| Introduction | 87 |
| Methods | 89 |
| Results | 94 |
| Discussion | 100 |
| CHAPTER VII: Overall summary, discussion, and future directions | 106 |
| Important findings and future directions | 111 |
| REFERENCES | 114 |
| APPENDIX I: Permissions | 137 |
CHAPTER III: A simple method to quantify the \( \dot{V}O_2 \) mean response time of ramp-incremental exercise

Table 3.1. Mean parameter estimates of MRT using different methods ........32

CHAPTER IV: Metabolic and performance-related consequences of exercising at and slightly above MLSS

Table 4.1. Peak gas exchange, ventilatory, heart rate, and blood lactate concentration values recorded during ramp incremental and time-to-exhaustion tests ..............................................................51

Table 4.2. Comparison among baseline and peak values in the [HHb] signal (µM) in the vastus lateralis (VL) and rectus femoris (RF) muscles during MLSS (MLSS\(_p\)), 10 W above MLSS (MLSS\(_{p+10}\)), and subsequent time-to-exhaustion (TTE) trial..56

Table 4.3. Comparison among baseline and peak values in tot[HB] signal (µM) in the vastus lateralis (VL) and rectus femoris (RF) muscles during MLSS (MLSS\(_p\)), 10 W above MLSS (MLSS\(_{p+10}\)), and subsequent time-to-exhaustion (TTE) trials..56

CHAPTER V: Establishing the \( \dot{V}O_2 \) versus constant-work rate relationship from ramp-incremental exercise: Simple strategies for an unsolved problem

Table 5.1. Peak and submaximal parameters and indices of aerobic performance during different ramp-protocols.................................................................75

Table 5.2. Comparison on the basis of work rate between MMSS and RCP (corrected by MRT\(_{SS}\)) and between CP and RCP (corrected by MRT\(_{LIN}\)) .........80

CHAPTER VI: A critical evaluation of current methods for exercise prescription in women and men

Table 6.1. Participants’ physical characteristics and physiological performance variables measured during the cardiopulmonary incremental exercise test ....94

Table 6.2. Participants’ absolute and relative values of LT and MLSS on the basis of \( \dot{V}O_2 \), WR, and HR (in absolute and relative values) .........................96
LIST OF FIGURES

CHAPTER II: Similar \( \dot{V}O_2 \) but different work rate between RCP and CP/MLSS

Figure 2.1. \( \dot{V}O_2 \) response at the onset of ramp-exercise (30 W\( \cdot \)min\(^{-1} \)) fitted with “mono-exponential” and “double-linear” functions…………………………….20

CHAPTER III: A simple method to quantify the \( \dot{V}O_2 \) mean response time of ramp-incremental exercise

Figure 3.1. Schematic of the experimental protocol……………………………………26

Figure 3.2. \( \dot{V}O_2 \) response profile of a representative participant from the step-transitions and ramp-incremental exercise protocols………………………………29

Figure 3.3. Regression plots and individual variability between test 1 and test 2 of the MRT determined by linear model (MRT\(_{\text{LIN}}\)) non-linear model (\( \tau' \)), and novel method proposed in the current study (MRT\(_{\text{SS}}\))……………………………33

Figure 3.4. Comparison of MRT values determined for a representative individual by linear model (MRT\(_{\text{LIN}}\)), non-linear model (\( \tau' \)), and novel method proposed in the current study (MRT\(_{\text{SS}}\)) during ramp-incremental test 1 and 2 …………………34

CHAPTER IV: Metabolic and performance-related consequences of exercising at and slightly above MLSS

Figure 4.1. Time-to-exhaustion performance recorded at baseline (TTE\(_b\)), after exercising at MLSS\(_{p}\) (TTE\(_{\text{MLSSp}}\)), and MLSS\(_{p+10}\) (TTE\(_{\text{MLSSp+10}}\))……………………………52

Figure 4.2. Group mean data (with SD bars) display physiological responses during 30-min constant-PO trials at MLSS\(_{p}\) (open circles) and MLSS\(_{p+10}\) (grey circles)……………………………………………………………………………………………………………………………………………………………………54

Figure 4.3. Group mean (with SD bars) data displaying NIRS-derived local deoxygenated haemoglobin ([HHb]) and total haemoglobin (tot[Hb]) (panels C and D) signals as well as EMG signal during 30-min constant-PO trials at MLSS\(_{p}\) and MLSS\(_{p+10}\) (right panels) for VL (open circles) and RF (grey circles)………………57

CHAPTER V: Establishing the \( \dot{V}O_2 \) versus constant-work rate relationship from ramp-incremental exercise: Simple strategies for an unsolved problem

Figure 5.1. \( \dot{V}O_2 \) functional gain as function of work rate during each ramp protocol. LT (lactate threshold) and MMSS (maximal metabolic steady-state) demarcate the moderate, heavy, and severe intensity domains………………………………………76

Figure 5.2. MRT of \( \dot{V}O_2 \) in response to each ramp-protocol…………………77
Figure 5.3. Gas-exchange and ventilatory profiles during 5 (5-s average data) and 25 W·min⁻¹ ramp-protocols, power-duration relationship for critical power (CP) estimation, and \( \dot{V}O_2 \) response during 30-min constant-load trial at CP in a representative subject........................................................................................................79

Figure 5.4. \( \dot{V}O_2 \) (L·min⁻¹) and work rate (W) at the lactate threshold (LT) and respiratory compensation point (RCP). Panel C and D show the correlation and bias (with limits of agreement), respectively, between the work rate at RCP during the 5 W·min⁻¹ ramp-protocol (RCP) and MMSS.................................................................81

CHAPTER VI: A critical evaluation of current methods for exercise prescription in women and men

Figure 6.1. Distribution of women and men on the basis of absolute and relative \( \dot{V}O_2_{\text{max}} \)......................................................................................................................95

Figure 6.2. Distribution of individuals in the moderate (M), heavy (H) and severe (S) domains at discrete %\( \dot{V}O_2_{\text{max}} \), %WR\text{peak}, and %HR\text{max}......................................................98

Figure 6.3. Classification plot displaying the observed individual’s classification, for both women and men, in different exercise intensity domains (moderate (M), heavy (H) and severe (S)) at discrete %\( \dot{V}O_2_{\text{max}} \), %WR\text{peak}, and %HR\text{max} with respect to ACSM guidelines (represented by vertical lines).................................99
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>[HHb]BP</td>
<td>deoxygenated hemoglobin concentration breakpoint</td>
</tr>
<tr>
<td>[HHb]</td>
<td>deoxygenated hemoglobin concentration</td>
</tr>
<tr>
<td>[La^-]b</td>
<td>blood lactate concentration</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AMPK</td>
<td>adenosine monophosphate-activated protein kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>bpm</td>
<td>beats per minute</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>calcium</td>
</tr>
<tr>
<td>CCC</td>
<td>Lin’s concordance coefficient</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CP</td>
<td>critical power</td>
</tr>
<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyography</td>
</tr>
<tr>
<td>f/B</td>
<td>breathing frequency</td>
</tr>
<tr>
<td>G</td>
<td>gain</td>
</tr>
<tr>
<td>H^+</td>
<td>hydrogen</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>HR_{max}</td>
<td>maximal heart rate</td>
</tr>
<tr>
<td>K^+</td>
<td>potassium</td>
</tr>
<tr>
<td>LT</td>
<td>lactate threshold</td>
</tr>
<tr>
<td>MLSS</td>
<td>maximal lactate steady-state</td>
</tr>
<tr>
<td>MMSS</td>
<td>maximal metabolic steady-state</td>
</tr>
<tr>
<td>MRT</td>
<td>mean response time</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;lin&lt;/sub&gt;</td>
<td>mean response time from linear regression models</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;ss&lt;/sub&gt;</td>
<td>mean response time from steady-state moderate transition</td>
</tr>
<tr>
<td>NIRS</td>
<td>near-infrared spectroscopy</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>oxygen</td>
</tr>
<tr>
<td>PCr</td>
<td>phosphocreatine</td>
</tr>
<tr>
<td>P&lt;sub&gt;ET&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>end-tidal partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Peroxisome proliferator-activated receptor-γ coactivator 1α</td>
</tr>
<tr>
<td>P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>inorganic phosphate</td>
</tr>
<tr>
<td>PO</td>
<td>power output</td>
</tr>
<tr>
<td>PO&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>peak power output</td>
</tr>
<tr>
<td>Q&lt;sub&gt;m&lt;/sub&gt;</td>
<td>muscle blood flow</td>
</tr>
<tr>
<td>RCP</td>
<td>respiratory compensation point</td>
</tr>
<tr>
<td>RER</td>
<td>respiratory exchange ratio</td>
</tr>
<tr>
<td>RF</td>
<td>rectus femoris</td>
</tr>
<tr>
<td>RI</td>
<td>ramp-incremental</td>
</tr>
<tr>
<td>RMS</td>
<td>root mean square</td>
</tr>
<tr>
<td>RMSE</td>
<td>root-mean square error</td>
</tr>
<tr>
<td>RPE</td>
<td>rating of perceived exertion</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SD</td>
<td>standard-deviation</td>
</tr>
<tr>
<td>TD</td>
<td>time-delay</td>
</tr>
<tr>
<td>tot[Hb]</td>
<td>total-hemoglobin concentration</td>
</tr>
<tr>
<td>TTE</td>
<td>time-to-exhaustion</td>
</tr>
<tr>
<td>V̇CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>rate of carbon dioxide production</td>
</tr>
<tr>
<td>V̇E</td>
<td>minute-ventilation measured on exhaling</td>
</tr>
<tr>
<td>VL</td>
<td>vastus lateralis</td>
</tr>
<tr>
<td>V̇O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>rate of oxygen uptake</td>
</tr>
<tr>
<td>V̇O&lt;sub&gt;2&lt;/sub&gt;max</td>
<td>maximal rate of oxygen uptake</td>
</tr>
</tbody>
</table>
\[ \dot{\text{VO}}_{2\text{peak}} \] peak rate of oxygen uptake

\[ \dot{\text{VO}}_{2\text{ss}} \] rate of oxygen uptake at steady-state

W watts

W' curvature constant of the power-duration relationship

\[ \text{WR}_{\text{peak}} \] work rate peak

\[ \tau \] time-constant

\[ \tau' \] time-constant of ramp-incremental test

\[ \chi^2 \] chi-squared
CHAPTER I

Introduction

Exercise intensity has a prominent role in shaping metabolic responses and training adaptations. Although it may appear to develop as a linear phenomenon (as a function of maximal \( \text{O}_2 \) uptake (\( \text{\textit{V}}\text{O}_{2\text{max}} \)), exercise intensity is dependent on the human body’s responsiveness to adjust to changes in adenosine triphosphate (ATP) demand and on its capacity to sustain this demand while maintaining steady-state metabolic responses.

The intensity spectrum can be partitioned into three domains (\textit{moderate, heavy, severe}) within which the achievement of steady-state metabolic responses is rapid or delayed (\textit{moderate} versus \textit{heavy}) and whether it is attainable or unattainable (\textit{heavy} versus \textit{severe}). These domains are demarcated by discrete physiological markers, or thresholds: the lactate threshold (LT) and the critical power (CP)/maximal lactate steady-state (MLSS).

Although the concepts underpinning these thresholds are well established, there are uncertainties regarding how to accurately identify their corresponding work rate (such as in the case of LT), whether or not some may be considered equivalent (e.g., CP versus MLSS), and whether or not other indices (e.g., the respiratory compensation point (RCP)) can be used as valid surrogates. Thus, the general purpose of this thesis is to explore the issues associated with the determination of these “thresholds” to give perspective on why their accurate identification is fundamental in exercise physiology.

The specific purposes of this thesis were:

1. To investigate the validity and the repeatability of a novel method to quantify the \( \text{\textit{V}}\text{O}_2 \) mean response time (MRT) of ramp-incremental exercise.
2. To determine the metabolic responses and the performance-related consequences of exercising slightly above MLSS.

3. To determine the $\dot{V}O_2$ MRT in response to different ramp-protocols and verify whether or not applying a correct left-shifting of the ramp-$\dot{V}O_2$ data would guarantee consistent work rates at LT across the different protocols.

4. To explore whether the variability between RCP and CP/MLSS may be reduced, or abolished, when correctly accounting for the $\dot{V}O_2$ dynamics during ramp-incremental exercise.

5. To quantify the magnitude of the concordance between the fixed-percentage approach for exercise intensity prescriptions and the exercise intensity domain framework.

Hypotheses

This thesis specifically addresses the following hypotheses:

1. That a novel method not relying on fitting models would be valid and more reproducible than previous methods when quantifying the $\dot{V}O_2$ MRT of ramp-exercise.

2. That sustaining exercise slightly above MLSS would result in the inability of maintaining metabolic stability and disproportionate reduction in maximal performance capacity.

3. That $\dot{V}O_2$ kinetic dynamics during ramp-exercise would determine the magnitude of the dissociation between ramp-$\dot{V}O_2$ and constant-$\dot{V}O_2$ at a given work rate.

4. That applying a correct left-shifting of the ramp-$\dot{V}O_2$ data guarantees that the work rate at LT is constant across ramp-protocols differing in slope.

5. That RCP and CP/MLSS occur at a similar work rate when the $\dot{V}O_2$ dynamics during ramp-exercise are appropriately considered.
6. That the fixed-percentage approach for exercise intensity prescription will result in heterogeneous distributions of the individuals within the exercise intensity domain schema.

**Thesis outline**

This thesis is comprised by 7 chapters (including the present introductory chapter).

- Chapter II includes a review of the relevant scientific literature on the topic and highlights some of the issues that this current thesis addresses.
- Chapter III evaluates the validity and repeatability of a novel approach for characterizing the VO$_2$ MRT in response to ramp-exercise.
- Chapter IV investigates the metabolic- and performance-related consequences of exercising slightly above MLSS.
- Chapter V investigates the issues associated with the characterization of the VO$_2$ response during ramp-exercise and their implications for “thresholds” identification and comparison.
- Chapter VI explores the concordance between the fixed-percentage approach for exercise intensity prescriptions and the exercise intensity domain framework.
- Chapter VII summarizes important findings of this thesis, states general conclusions, and proposes future directions.
CHAPTER II

Exercise intensity “thresholds”

From the early work of Owles (1930), it became apparent that when exercising above certain levels of intensity, the increased metabolic rate would cause an increase in the concentration of metabolites in the blood linked to the higher metabolic activity. This was possibly the first piece of scientific evidence linking exercise intensity with the concept of a “critical metabolic level”, or “threshold” as it was later defined. Since then, the interest on this topic has grown exponentially, leading to many important discoveries as well as divergent findings. To rephrase Svedhal and MacIntosh from their review paper (2003), “few concepts in the field of exercise science have generated such debate as that of…exercise intensity thresholds”.

The concept of “exercise intensity threshold” typically refers to the existence of an intensity of exercise (\(\dot{V}O_2\) or work rate) that demarcates the onset of alterations and/or disproportional physiological changes that indicate challenges to the metabolic homeostasis within the active tissues. Over the years, several studies have investigated the occurrence of these thresholds, primarily examining i) the physiological mechanisms underpinning their manifestation as exercise intensity increases, ii) accurate ways for their identification, and iii) the physiological and performance-related consequences of exercising at intensities surrounding these thresholds (e.g., above versus below). The great interest on this topic was – and currently is – due to the fact that the mechanisms responsible for the manifestation of these exercise intensity thresholds directly relate to the mechanisms governing exercise tolerance and adaptive responses to exercise, in healthy and clinical populations.
Exercise intensity domains

Although in exercise physiology numerous terms exist to define the concept of thresholds (Binder et al., 2008), in the last 30 years the exercise intensity domain schema proposed by Whipp and colleagues (Whipp & Mahler, 1980) has proven to be a fundamental model explaining metabolic responses and exercise tolerance. This schema identifies ranges of intensities (i.e., domains) within which metabolic responses (e.g., $\dot{V}O_2$ and blood lactate concentration [La$^-$]) to exercise have common characteristics (Rossiter, 2011). These include the moderate, heavy, and severe intensity domains which are commonly demarcated by the lactate threshold (LT) and critical power (CP) or maximal lactate steady-state (MLSS). This schema is based on the body’s capacity to increase – more or less rapidly – and to stabilize – over time – metabolic activity in response to changes in ATP requirement. The direct advantage of this framework over other developed threshold-based models, is that metabolic responses and tolerable duration of exercise become dependent on a set of domain-specific mechanisms (Black et al., 2017). Additionally, this framework allows the standardization of the metabolic-stress when individuals exercise at a common percentage of LT and/or CP/MLSS, which is an important pre-requisite when evaluating adaptive responses to exercise (DiMenna & Jones, 2009).

The different $\dot{V}O_2$ kinetics in response to exercise are key features of the intensity domain schema (Poole & Jones, 2012). Thus, the understanding of the controls and the implications of these dynamics is of critical importance. In the following sections, the $\dot{V}O_2$ kinetics (in relation to system-control and to the different intensity domains) will be discussed.
Control-system of $\dot{V}O_2$ kinetics

Since the work of Hill and colleagues (1924) and subsequently Henry and colleagues (1951), the linearity of the $\dot{V}O_2$ response to changes in metabolic demand has constituted an important assumption upon which exercise testing and prescription has been based. In general, a “linear” control system obeys the principle of superposition which implies that $i$) a given input produces a predictable output, $ii$) two separate inputs produce an outcome that equals the sum of the two inputs, $iii$) on- and off- responses to a given input are symmetric. In the context of $\dot{V}O_2$ kinetics, this would imply that the parameters of the control system ($\dot{V}O_2$ functional gain: $G = \Delta \dot{V}O_2/\Delta$ work rate) and the time-constant ($\tau'$) are invariant and independent of the magnitude of the change in work rate, and that on- and off-$\dot{V}O_2$ kinetics are symmetric (Wilcox et al., 2016). This notion (i.e., linearity of the $\dot{V}O_2$ control), although being somehow reinforced over the years by the “seemingly” linear behavior of the $\dot{V}O_2$ response during ramp-incremental exercise (Whipp et al., 1981), has been progressively replaced by the evidence that the features of the $\dot{V}O_2$ response are better described to adhere to a “non-linear” dynamic control system (Rossiter, 2011). Support for this non-linear control of $\dot{V}O_2$ resides in the observation that: $i$) $G$ and $\tau'$ become progressively greater during exercise transitions to increasing work rates (Henry & DeMoor, 1956; Whipp & Wasserman, 1972; Barstow & Mole, 1991; Poole et al., 1991; Casaburi et al., 2017); $ii$) $G$ and $\tau$ are dependent on the baseline work rate (Wilkerson et al., 2004; DiMenna et al., 2010; Bowen et al., 2011; Spencer et al., 2011; Keir et al., 2016c, 2016a; Diamond et al., 2017); $iii$) on- and off-$\dot{V}O_2$ kinetics are not symmetric (Paterson & Whipp, 1991; Özyener et al., 2001). Evidence for this non-linear dynamics can be appreciated when considering the $\dot{V}O_2$ response within each domain.
**Moderate-intensity domain**

The moderate-intensity domain encompasses the range of metabolic rates from rest to LT (~50-60% of VO2max). Constant-load square-wave exercise transitions within this exercise intensity domain elicit a VO2 response that is well characterized by a mono-exponential equation (Rossiter, 2011):

$$\Delta \dot{V}O_2(t) = \Delta \dot{V}O_{2ss} \cdot (1 - e^{-(t-TD)/\tau})$$

where $\Delta \dot{V}O_{2ss}$ is the steady-state increment in $\dot{V}O_2$, $\tau$ is the time constant of the exponential, and TD the time delay between exercise onset and the start of the rise in $\dot{V}O_2$. This mono-exponential function characterizes the “fundamental phase” (or Phase II) of the $\dot{V}O_2$ kinetics response at the level of the mouth. Within this domain, if exercise transitions are initiated from the same baseline work rate, G and $\tau'$ are not affected by the magnitude of the change in work rate (Keir et al., 2016c). Therefore, under these circumstances, the $\dot{V}O_2$ control system responds linearly to changes in work rate (Keir et al., 2016c). G is typically $\leq 10$ ml·min$^{-1}$·W$^{-1}$, and time to reach $\dot{V}O_2$ steady steady-state in this domain can be between 1 and 2 min in healthy young and older trained individuals (George et al., 2018) but can be significantly longer (3-4 min) in sedentary age-matched individuals (Grey et al., 2015) or in clinical populations (Nery et al., 1982; Sietsema et al., 1994). The main implication of exercising in this domain is that the reliance on substrate level phosphorylation is constrained to the brief on-transient adjustment of $\dot{V}O_2$ (until attainment of steady-state $\dot{V}O_2$) (Whipp et al., 2005), with the exercise being fueled largely by degradation of fatty acids once steady-state $\dot{V}O_2$ is attained. Therefore, exercise in this domain can be sustained for very long time and results in small accumulation of fatigue (Cannon et al., 2011), unless protracted until “exhaustion” (Black et al., 2017).
Heavy-intensity domain

The heavy-intensity domain encompasses the range of intensities between LT and CP/MLSS. Within this domain, the attainment of VO₂ steady-state is delayed (by as much as 15 min) (Poole et al., 1988) and the overall VO₂ response deviates from that predicted from the moderate-intensity domain (Barstow & Molé, 1991; Paterson & Whipp, 1991). The reason for this “deviation” from linearity is attributed to the increased O₂ cost (i.e., G ≥ 10 ml·min⁻¹·W⁻¹) of exercising above LT. This additional and slowly developing O₂ cost has been termed “slow-component” of VO₂ and, although considerable debate exists upon its etiology (Jones et al., 2011; Korzeniewski & Zoladz, 2015; Grassi et al., 2015; O’Connell et al., 2017), it is deemed to originate within the working muscles (Poole et al., 1991) as a consequences of i) a greater ATP requirement for a given force output (at the site of muscle contraction) (Rossiter et al., 2002; Cannon et al., 2014), and/or for a given unit of energy produced (at the mitochondrial level) (Layec et al., 2012); ii) additional recruitment of motor units during the task as fatigue accumulates (Shinohara & Moritani, 1992; Krstrup et al., 2004); iii) recruitment of type II fibers which are less efficient (and kinetically slower) than the muscle fibers recruited at the onset of the exercise (Krupstrup et al., 2004, 2008; Zoladz et al., 2008; Vanhatalo et al., 2011). Overall, the delayed attainment of steady-state increases the reliance on substrate level phosphorylation, which combined with an overall augmented metabolic cost of the exercise conspire to increase the accumulation of fatigue and reduce the tolerable exercise duration in this domain compared to the moderate domain (Black et al., 2017). The characterization of the VO₂ responses during transitions within the heavy domain requires more complex mathematical models that consider the additional time delay and the exponential phase of the VO₂ slow-component (Barstow & Molé, 1991; Rossiter, 2011). However, given the difficulty of discerning accurately the onset of the slow-component within the VO₂
kinetics response (Poole & Jones, 2012) and the fact that its characterization implies the sole action of a single metabolic compartment from its onset (Whipp et al., 2002), it would be more appropriate to “simply” characterize and describe the \( \dot{V}O_2 \) slow-component as a progressive metabolic inefficiency that develops with a slow kinetics (Rossiter, 2011).

**Severe-intensity domain**

The severe-intensity domain encompasses the range of intensities from CP/MLSS to \( \dot{V}O_{2\text{max}} \). Depending on the proximity of the work rate being sustained >CP/MLSS, \( \dot{V}O_2 \) will either rise rapidly to maximal values (without discernible \( \dot{V}O_2 \) slow component) or develop a slow-component that, if sustained sufficiently, project to \( \dot{V}O_{2\text{max}} \) (Poole & Jones, 2012). In this latter case, the rate at which the slow-component develops will predict the tolerable duration of exercise within the severe domain (Jones et al., 2010). In this context, tolerable durations of exercise >CP/MLSS are constrained within the limits of the hyperbolic relationship that exists between exercise intensity (in this case power) and duration (power-duration relationship) (Poole et al., 2016) (the CP model is further discussed later in this thesis). Given that, by definition, \( \dot{V}O_2 \) does not attain steady-state in this domain, from exercise onset to exhaustion there is considerable anaerobic contribution to the energy turnover (di Prampero & Ferretti, 1999) leading to an accelerated accumulation of byproducts, such as hydrogen ions (H\(^+\)), adenosine diphosphate (ADP), and inorganic phosphate [Pi], which are linked to the development of peripheral fatigue and exercise intolerance (Cannon et al., 2011; Burnley et al., 2012; Keir et al., 2016b). Interestingly, the magnitude of peripheral fatigue during time-to-exhaustion trials performed in the severe domain is similar regardless of the duration of the task (Burnley et al., 2012; Black et al., 2017; Schäfer et al., 2019), demonstrating that the mechanisms responsible for the failure of muscle contraction in this domain may be similar.
Markers of exercise intensity separating the heavy and the severe domains. Physiological basis, correspondence, and methodological issues.

While there is agreement on the fact that LT separates the moderate from the heavy domain (Wasserman et al., 1973; Rossiter, 2011) (although some inaccuracies may exist when assigning a correct work rate to LT), there is considerable debate on whether more than one marker of exercise intensity could identify the boundary between the heavy and the severe domain. In this context, Keir et al. (2015) have found that CP, MLSS, and the respiratory compensation point (RCP) occurred at the same \( \dot{V}O_2 \). This finding led to the interpretation that, by occurring at a common \( \dot{V}O_2 \), these different indices could all represent the “critical metabolic rate” demarcating the heavy-to-severe boundary of exercise intensity. However, given that each of them could arise from different underlying mechanisms, and the fact that their corresponding work rate may be variable, it is uncertain whether they can demarcate the same physiological event. In this context, the reasons of this variability may originate from issues related to the protocol and approaches used for their determination. In the following sub-sections, constant-work rate (CP and MLSS) and ramp-exercise (RCP) derived indices will be reviewed.

**CP and MLSS**

CP is represented by the asymptote of the hyperbolic relationship between severe work rates (W) and the duration (s or min) for which they can be sustained (Jones et al., 2010). Although CP was originally described to represent the highest work rate sustainable without accumulation of fatigue (Monod & Scherrer, 1965) (a description that is evidently inaccurate), a more physiologically established definition describes CP as the upper boundary at which measures of metabolic activity (e.g., \( \dot{V}O_2 \) and \([La^-]_b\)) can attain steady-state (Poole et al., 2016; Jones & Vanhatalo, 2017). Thus, CP is typically depicted to represent a “metabolic rate” at which oxidative
sources of energy account for all the ATP turnover and at which the rates of lactate production and clearance are matched, resulting in no net accumulation of lactate in the blood (Poole et al., 2016). Given these definitions, it is no surprise that some of the physiological determinants of CP are the proportion of type I fibers (Vanhatalo et al., 2016) and indices of capillarization (Mitchell et al., 2018). The physiological implications of exercising <CP versus >CP have been extensively described. In addition to the characteristic VO₂ responses and the reduced exercise tolerance previously reviewed, exercising >CP leads to progressive rise in intracellular [Cr], [La⁻]b, plasma [K⁺] and decrease in intracellular [PCr] and pH (Vanhatalo et al., 2016; Black et al., 2017).

An important feature of the power-duration relationship is the curvature of the hyperbola (W′) which describes the amount of work that can be performed >CP. Given that during exercise <CP the ATP turnover should be sustained exclusively by means of oxidative sources of energy, W′ is typically depicted as a constant anaerobic work capacity that can be utilized when exercising >CP (Morton, 2006). However, it has been demonstrated that the magnitude of the W′ is dependent on the interplay between both substrate-level and oxidative phosphorylation and by the speed of adjustment of VO₂ during severe exercise (Vanhatalo et al., 2010). From this perspective, evaluating W′ following exercise carried <CP may provide mechanistic insights into to the mechanisms of fatigue during exercise >CP (Clark et al., 2018, 2019).

Similar to what CP is deemed to represent, MLSS identifies the highest work rate at which there is an equilibrium between lactate production and clearance (Svedahl & Macintosh, 2003). It is typically assessed by performing a series (3-5) of constant-work rate trials of 30 min of duration (Heck et al., 1985), and the highest constant-work rate trial eliciting a delta [La⁻]b between the 10th and the 30th min of ≤1 mM corresponds to MLSS (Beneke, 2003a). Given that the rates of lactate appearance and oxidation are correlated with [La⁻]b (Brooks, 1986), the rationale
of the MLSS concept is that when the increase in [La\(^{-}\)]\(_{b}\) is stabilized over time, then oxidative processes account for the entire energy turnover during the exercise (Heck \textit{et al.}, 1985; Svedahl & MacIntosh, 2003). Contrary, when lactate accumulates within the blood, the energy requirement exceeds the capacity of the oxidative system to provide all the energy required, with this amount that is proportional to the accumulation of lactate (di Prampero & Ferretti, 1999). In other words, during exercise above MLSS there is a greater reliance on glycolysis leading to disproportional lactate formation that overloads the buffering mechanisms within the active muscles and other body compartments. For this reason, MLSS has been closely related to the concept of anaerobic threshold – intended as the point at which the anaerobic metabolism provides a significant contribution to the energy turnover (Svedahl & MacIntosh, 2003).

\textit{Methodological issues}

Conceptually, CP and MLSS share similar definitions and are described to essentially demarcate the same physiological event (Jones \textit{et al.}, 2010, 2011; Keir \textit{et al.}, 2015; Grassi \textit{et al.}, 2015; Poole \textit{et al.}, 2016; Jones & Vanhatalo, 2017). In reality, however, the work rate corresponding to CP and MLSS has been found to vary. In this context it is important to critically examine the approaches used for their determination.

The gold-standard method for establishing an individual’s power-duration relationship and estimate CP is to perform a series of time-to-exhaustion trials (ideally four-to-five) within the severe domain (Poole \textit{et al.}, 1988; Morton \textit{et al.}, 1990; Mattioni Maturana \textit{et al.}, 2018) lasting between 2 and 20 min (Morton, 2006; Mattioni Maturana \textit{et al.}, 2018). There exists several mathematical models that can be used to fit the power-duration relationship, including the 3-parameter exponential (Hopkins \textit{et al.}, 1989), the 2- (Hill, 1993) and 3-parameter (Hugh Morton, 1996) hyperbolic, the linear work-time (Moritani \textit{et al.}, 1981), and the linear work inverse-of-time
(Murgatroyd et al., 2014) models. The selection of any of these models depends on the number of time-to-exhaustion trials performed whereas the acceptance of the CP estimate from any of these models depends on the “quality” of the model fit (Mattioni Maturana et al., 2018). A degree of “measurement error” in the estimation of CP is, however, intrinsic to the method and may originate from the fact that this model heavily relies on exhaustive effort from participants. In this context, between-day variability in time-to-exhaustion trials has been reported to be in the range of ~2 to ~30% (McLellan et al., 1995), with this likely influencing the accuracy of the CP estimation. These limitations may be exacerbated when, to avoid the somewhat lengthy procedures of the gold-standard approach, fewer time-to-exhaustion trials and/or alternative methods for estimation of CP are used. Indeed, the risk of this choice is to incur in larger errors in the estimate of CP, as, for example, reducing the number of time-to-exhaustion trials may not guarantee an adequate distribution of durations leading to under- or over-predictions of CP (Bishop et al., 1998; Mattioni Maturana et al., 2018). From this perspective, it is acknowledged that the typical error of CP is about 5% (e.g., 10 W for an estimated CP of 200 W), which partly explains the considerable inter-individual variability of time-to-exhaustion at CP (Housh et al., 1989; McLellan & Cheung, 1992; Brickley et al., 2002).

Although the concept of MLSS is well established (Billat et al., 2003), it can be argued whether this methodology can identify accurately the work rate separating the heavy and the severe domains. Indeed, MLSS carries an intrinsic measurement error that equals the delta work rate used between the determination trials. Given that the actual MLSS lays within this measurement error, MLSS may underestimate the true boundary between the heavy and severe domain by as much as the delta change in work rate from one trial to another. Furthermore, although it has been demonstrated that steady-state [La']b is typically achieved within the first 10 min of the MLSS trial
(Beneke, 2003b), a slower rise of [La\textsuperscript{−}]\textsubscript{b} within this timeframe, or a delayed achievement of [La\textsuperscript{−}]\textsubscript{b} steady-state may facilitate the delta [La\textsuperscript{−}]\textsubscript{b} to exceed 1mM at the end of the 30-min trial, leading to the conclusion that the tested work rate is above MLSS and, thus, potentially underestimating the true MLSS. In this context, it must be acknowledged that the combination of biological variation in [La\textsuperscript{−}]\textsubscript{b} and analytical error of current instruments produce a variability in the [La\textsuperscript{−}]\textsubscript{b} measurements that can range between ~10 and 50% (Saunders et al., 2004; Bonaventura et al., 2015) which has the potential to jeopardize the accurate identification of MLSS.

Considering these limitations, it is not surprising that the correspondence between CP and MLSS is variable and that in most instances the work rate at CP has been reported to be slightly higher than that at MLSS. For example, Pringle et al. (2002) found that CP was 20 W higher than MLSS, with CP estimated by fitting the linear inverse-time model (using three-to-four time-to-exhaustion trials) and MLSS determined by using delta work rates of ~19 W. Dekerle et al. (2003) reported a difference between CP and MLSS of 30 W, with CP estimated fitting the linear model (using four time-to-exhaustion trials) and MLSS determined using delta work rates eliciting a difference of 5% of \(\dot{V}O_2\text{max}\). Mattioni Maturana et al. (2016) found an average difference between CP and MLSS of 20 W, with CP estimated fitting the 2-parameter hyperbolic model (using five time-to-exhaustion trials) and MLSS determined with delta work rates of 10 W. In contrast to these previous observations, however, Keir et al. (2015) found similar work rates at CP and MLSS (the average difference was 3 W), with CP estimated by fitting the three-parameter hyperbolic model (using four-five time-to-exhaustion trials) and MLSS determined by using 10 W delta work rates.

Collectively, these observations would suggest that the work rate at MLSS tends to be lower than that at CP. However, given the different methods used for CP estimation and MLSS determination amongst the studies, it is difficult to establish whether this difference is due to a
consistent bias (i.e., CP overestimates or, vice versa, MLSS underestimates the true “maximal metabolic steady-state) or due to errors associated to particular models and/or protocols adopted for their determination.

**RCP**

The RCP demarcates the onset of the disproportional increase in minute ventilation with respect to CO$_2$ output ($\dot{V}_{\text{CO}_2}$) and of the systematic fall in end-tidal partial pressure of CO$_2$ ($P_{ET\text{CO}_2}$) after a period of isocapnia (Whipp et al., 1989). Although the control of ventilation (and of the hyperpnoea in particular) is complex, this hyperventilatory response is thought to be initiated to partially compensate the increased $[H^+]$ in the blood (Whipp et al., 1989). Although, the exact source of $H^+$ is highly controversial (Robergs, 2004; Böning & Maassen, 2008; Vinnakota & Kushmerick, 2011), it is plausible that $H^+$ may originate from i) intermediates reactions during glycolysis, and/or the instantaneous dissociation of lactic acid in lactate ($La^-\text{ and } H^+$ at the end of glycolysis. Since glycolytic rate is accelerated during high intensity exercise, it is possible that when the rate of $H^+$ and lactate formation exceeds the capacity of intracellular buffering mechanisms (e.g., mitochondria, Cr), $H^+$ along with lactate are released (through $H^+$ and lactate symporters) into the blood stream (Robergs, 2004). This rapid rise in $[H^+]$ (and $[La^-]_b$) alters blood pH and gives additional stimulus for an near-immediate reflex-increase in ventilation that results in the fall in $P_{ET\text{CO}_2}$ (Stringer et al., 1992; Meyer et al., 2004; Wasserman et al., 2011).

The RCP is a phenomenon unique to the ramp-exercise (Whipp et al., 1989) that occurs at discrete $\dot{V}_{O_2}$ values (Scheuermann & Kowalchuk, 1998; Leo et al., 2017). Different interventions can alter the characteristics of the respiratory compensation, such as the manipulation of the inspired fraction of O$_2$ (which would modify the sensitivity of the carotid bodies) (Rausch et al., 1991) and the use of slow/fast ramp-protocols (e.g., $<10$ or $>60$ W·min$^{-1}$). In this context, it
has been suggested that slow ramp-slopes elicit greater compensatory hyperventilation to the ongoing acidosis (Scheuermann & Kowalchuk, 1998) while fast ramp-slope elicits delayed and/or attenuated compensatory hyperventilation resulting in a blunted removal of CO₂ stores (Ward & Whipp, 1992). Although unclear, the mechanisms underpinning these different patterns may be related to altered chemoreceptors sensitivity and/or pH response kinetics when the work rate is incremented rapidly (Wasserman & Whipp, 1983). Regardless, although the magnitude of compensation is influenced by the protocol of choice, the “dynamics” of the gas-exchange and ventilatory profiles are unaltered, with the RCP occurring at a consistent \( \dot{V}O_2 \) (Scheuermann & Kowalchuk, 1998).

**Similar \( \dot{V}O_2 \) but different work rate between RCP and CP/MLSS**

In spite of the fact that the \( \dot{V}O_2 \) at the RCP and CP/MLSS is similar (Dekerle et al., 2003; Keir et al., 2015; Leo et al., 2017; Inglis et al., 2019), the correspondence of these demarcation points is more variable when expressed in terms of work rate (Keir et al., 2016a; Leo et al., 2017; Inglis et al., 2019). In fact, the work rate at the RCP is generally greater than that at CP/MLSS. Putative reasons explaining this dichotomy (similar \( \dot{V}O_2 \) but different work rate) may reside within the different dynamics of \( \dot{V}O_2 \) in response to ramp- vs constant-work rate exercise.

\( \dot{V}O_2 \) dynamics during ramp-exercise.

The \( \dot{V}O_2 \) increase during ramp exercise is typically characterized by i) a time-delay at the onset of the ramp reflecting the muscle-to-lung “transit-time” and the kinetic component of the response (typically referred to as mean response time (MRT) (Linnarsson, 1974)), and ii) a subsequent “seemingly” linear increase until attainment of \( VO_{2\text{max}} \).
The \( \dot{V}O_2 \) increase during ramp-exercise can be well characterized by the integration of the mono-exponential equation used to describe the \( \dot{V}O_2 \) in response to step-transitions within the moderate domain (Swanson & Hughson, 1988):

\[
\Delta \dot{V}O_{2(0)} = \Delta \dot{V}O_{2SS} \cdot [t - \tau' \cdot (1 - e^{-\frac{t}{\tau'}})]
\]

where \( \Delta \dot{V}O_{2SS} \) is the increment above the baseline required for work rate at the time \( t \) and \( \tau' \) is the effective time constant of the response (s). The \( \tau' \) parameter reflects both the transit time and the kinetic component of the response, which is the time required for the \( \dot{V}O_2 \) to express its linear increase. An alternative approach to quantify the “transit-time” delay and the kinetic component of the \( \dot{V}O_2 \) at the onset of the ramp-exercise is the “back extrapolation” approach (Boone & Bourgois, 2012):

\[
f = \begin{cases} 
\text{g}(t), & \text{if } (t < \text{MRT}_{\text{LIN}}) \\
\text{h}(t), & \text{else}
\end{cases}
\]

\[
g(t) = i_1 + m_1 t; \ i_2 = i_1 + m_1 t; \ h(t) = i_2 + m_2 t - \text{MRT}_{\text{LIN}}
\]

where \( f \) is the “double-linear” function, \( t \) is time and \( g \) and \( h \) are \( \dot{V}O_2 \), \( \text{MRT}_{\text{LIN}} \) (i.e., mean response time using the linear regression model) is the time corresponding to the intersection of the two regression lines, \( i_1 \) and \( i_2 \) are the intercepts of the first and second linear function, respectively, and \( m_1 \) and \( m_2 \) are the slopes. The \( m_1 \) parameter is fixed at “zero” and thus \( i_1 \) gives baseline \( \dot{V}O_2 \). Similarly to the \( \tau' \) parameter, the \( \text{MRT}_{\text{LIN}} \) is representative of the time it takes for the \( \dot{V}O_2 \) to begin its linear increase. Overall, the transit-time delay and the kinetic component misaligns the \( \dot{V}O_2 \)-to-work rate relationship during the ramp-exercise (Boone & Bourgois, 2012; Keir et al., 2018a). In other words, the \( \dot{V}O_2 \) is “right-shifted” from its corresponding work rate.

After this initial time-delay, the \( \dot{V}O_2 \) manifests the typical linear increase. Although this could support the notion that \( \dot{V}O_2 \) is governed by a linear-control system, in reality this linear increase may be the result of the conflation of increasing \( G \) and \( \tau' \) throughout the ramp-exercise.
In other words, the typical dynamics of \( \dot{V}O_2 \) observed across the intensity spectrum in response to step-transitions are obscured during ramp-exercise, such that the slowly developing inefficiency (increasing G) and progressively slower kinetics (increasing \( \tau' \)) by balancing each other could produce a “seemingly” linear increase in \( \dot{V}O_2 \) (Rossiter, 2011). This has been recently confirmed by Wilcox et al. (2016) and Keir et al. (2016a) who, by modeling varying G and \( \tau' \) measured during step-transition protocols, demonstrated that the linear increase in \( \dot{V}O_2 \) during ramp-incremental exercise is the product of these two parameters at any point during the ramp-exercise. In this scenario, the progressively increasing inefficiency (hidden) and slower kinetics (evident) result in the \( \dot{V}O_2 \) increasingly lagging the change in work rate which produces a “dissociation” between the \( \dot{V}O_2 \) and the work rate during ramp-exercise.

Therefore, the action of the transit-time delay and the kinetic component (included in the \( \tau' \) and MRT\(_{\text{LIN}} \) parameters) and the progressively increasing dissociation during the ramp-exercise between \( \dot{V}O_2 \) and work rate (result of the increased inefficiency and slower kinetics) combine to inflate the work rate at any \( \dot{V}O_2 \) during the ramp-exercise. This may explain why, although similar in terms of \( \dot{V}O_2 \), the RCP and CP/MLSS differ in terms of work rate (Dekerle et al., 2003; Keir et al., 2015; Leo et al., 2017; Caen et al., 2018; Inglis et al., 2019).

Correcting the \( \dot{V}O_2 \) data for the time-delay at ramp-onset

In order to partly attenuate the distortions within the \( \dot{V}O_2 \)-to-work rate relationship during ramp-exercise, the ramp-\( \dot{V}O_2 \) can be left-shifted by the time (or W) corresponding to \( \tau' \) or MRT\(_{\text{LIN}} \) parameters, which generally equal to ~20-40 s during a 30 W·min\(^{-1}\) ramp-exercise (Fontana et al., 2015). Given that up to LT the \( \dot{V}O_2 \) responds linearly, this practice should theoretically guarantee the alignment between \( \dot{V}O_2 \) and its corresponding work rate in the moderate-intensity domain. However, although both \( \tau' \) and MRT\(_{\text{LIN}} \) parameters have been used quite extensively, their
reliability has been criticized (Hughson & Inman, 1986; Markovitz et al., 2004; Boone & Bourgois, 2012, 2017). Specifically, Hughson et al. (1986b) found that the $\tau'$ parameter is highly affected by variations in $G$, whereas Boone et al. (2008) found that the $MRT_{LIN}$ is affected by variations in pre-ramp baseline $\dot{V}O_2$. Additional issues may be related to the slope of the ramp-protocol of choice, as slower-ramp protocols (<20 W·min$^{-1}$) tend to lengthen both $\tau'$ and $MRT_{LIN}$ compared to faster ramp-protocols (>20 W·min$^{-1}$) (Boone et al., 2008a). These highly variable dynamics of $\tau'$ and $MRT_{LIN}$ parameters are displayed in Figure 2.1. To note is that $\tau'$ and $MRT_{LIN}$ may not necessarily result in the same estimate and that both demonstrate high susceptibility to slight changes in pre-ramp baseline $\dot{V}O_2$ and $G$. Thus, considering these issues and the fact that particularly at baseline and at ramp-onset the $\dot{V}O_2$ response may be characterized by a high noise-to-signal ratio (Keir et al., 2014), *left-shifting* the ramp-$\dot{V}O_2$ data by $\tau'$ or MRT may not solve the issue of time-alignment of the ramp-$\dot{V}O_2$.

**Exercise intensity “thresholds” and implications for exercise intensity prescription.**

The identification of the exercise intensity “thresholds” carries important implications for a correct exercise prescription. As previously discussed, exercise intensity is not a linear function of $\dot{V}O_{2\text{max}}$, rather its characteristics depend on whether (or not) metabolic responses (e.g., $\dot{V}O_2$ and \([\text{La}^-]_b\)) can attain steady-state. Considering that LT and CP/MLSS occur at different percentages of maximum values (e.g., $\dot{V}O_{2\text{max}}$), it would seem appropriate to normalize the exercise intensity based on these physiological markers. This practice guarantees the “normalization” of the exercise intensity stimulus across the individuals allowing the correct evaluation at both the molecular and systemic levels of the adaptive responses to exercise. Surprisingly, although these concepts are well established (e.g., CP/MLSS) and the shortcomings of percentage of maximum-
Figure 2.1. $\dot{V}O_2$ response at the onset of ramp-exercise (30 W·min$^{-1}$) fitted with “mono-exponential” (upper panels) and “double-linear” (lower-panels) functions. Note that i) for the same dataset $\tau'$ and MRT$_{LIN}$ can be different, ii) both models are extremely influenced by slight variations in baseline (middle panels) and functional gain (right panels) of $\dot{V}O_2$, and iii) $\tau'$ and MRT$_{LIN}$ are fitted over $\dot{V}O_2$ versus time and $\dot{V}O_2$ versus work rate, respectively. Since work rate is analogous to time during a ramp-test, MRT$_{LIN}$ is subsequently converted to time (s).
based approaches are well demonstrated (Meyer et al., 1999; DiMenna & Jones, 2009; Scharha-Rosenberger et al., 2010; Lansley E. et al., 2011; Rossiter, 2011; Poole & Jones, 2012), the exercise intensity domain framework has been underutilized when prescribing exercise intensity, particularly in research settings.

Normalization of the exercise intensity based on parameters or variables obtained during incremental tests to exhaustion has been the gold-standard approach adopted for exercise intensity prescription. Percentage of $\dot{\text{VO}}_2\text{max}$ or other “maximum” variables, such as $HR_{\text{max}}$ and peak work rate, have been extensively used to assess training responsiveness across individuals and to compare the efficacy of different exercise regimens. Several studies, such as those examining the role of genetic traits on training adaptations or comparing the effectiveness of different exercise regimens, utilized intensity prescriptions based on percentage of maximum values. The typical exercise intensity prescription in these studies would range between 60% and 75% of $\dot{\text{VO}}_2\text{max}$; a range of intensity that could potentially span across the three domains of intensity within a group of individuals. Indeed, it has been demonstrated that within this range, physiological responses and tolerable durations are highly variable when exercising at a common %$\dot{\text{VO}}_2\text{max}$ (Scharhag-Rosenberger et al., 2010). While some could reach steady-state $\dot{\text{VO}}_2$ quite rapidly and sustain the task for relatively long time (e.g., >40 min), others could tolerate the same common %$\dot{\text{VO}}_2\text{max}$ for a much shorter time (i.e., <15min) (Scharhag-Rosenberger et al., 2010).

Exercise intensity has been attributed an important role in mediating skeletal muscle adaptations following exercise interventions (Bishop et al., 2014; Granata et al., 2016; MacInnis & Gibala, 2017). First of all, exercise intensity determines the preferred substrate for ATP turnover (Romijn et al., 1993). Linked to this, metabolic stress is proportional to exercise intensity and the activation of metabolic signals is greater when exercising at higher intensities compared to lower
intensities (MacInnis & Gibala, 2017). For example, intracellular [La⁻], [Cr], [ADP], [AMPK] increase with increase in exercise intensity (Wojtaszewski et al., 2000; van Loon et al., 2004). In addition to altering the energy state of the muscle cell, the elevated concentration of some of these molecules is associated with greater expression of proteins (PGC-1α) involved in mitochondrial biogenesis (Bishop et al., 2014). In this context, the heterogeneous metabolic stimuli occurring at common percentages of ŶO₂max could explain the high variability in training responsiveness typically reported (Bouchard et al., 1999; Ross et al., 2015).
CHAPTER III
A simple method to quantify the \( \dot{V}O_2 \) mean response time of ramp-incremental exercise

Introduction

The ramp-incremental test has become a gold-standard protocol in research and clinical settings to quantify key parameters of the aerobic system (e.g., maximal \( \dot{V}O_2 \) (\( \dot{V}O_2_{\text{max}} \)), lactate threshold (LT)) that are needed to accurately prescribe exercise and to assess cardiorespiratory health. In response to ramp-exercise, O\(_2\) uptake (\( \dot{V}O_2 \)) measured at the mouth increases linearly with power output after an initial time interval, known as mean response time (MRT). The MRT 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 ATP demand imposed by the ramp (Boone & Bourgois, 2012; Keir et al., 2018a). This delayed adjustment in \( \dot{V}O_2 \) at ramp-onset temporally misaligns the \( \dot{V}O_2 \)-to-power output relationship such that the \( \dot{V}O_2 \) is right-shifted relative to its corresponding power output for all intensities up to the LT; thereafter the \( \dot{V}O_2 \) slow component – which is not accounted for in the MRT – further dissociates this relationship (Keir et al., 2016a, 2018a).

To determine the MRT two methods have been used: 1) a segmented linear regression model, with the intersection of two regression lines (e.g., baseline and slope of \( \dot{V}O_2 \) vs time) representing the time interval before the systematic rise of \( \dot{V}O_2 \) begins, and 2) a mono-exponential function, with a single time constant (\( \tau' \)) that describes the time it takes for \( \dot{V}O_2 \) to conform to the linear increase in power output. When the ramp-incremental test was first introduced by Whipp and colleagues in 1981 (Whipp et al., 1981), it was proposed that MRT (referred therein as \( \tau' \)) could be determined from a single test. However, even small test-to-test differences in both pre-ramp \( \dot{V}O_2 \) baseline and the amplitude of the \( \dot{V}O_2 \) change (as gain \( \Delta\dot{V}O_2/\Delta\text{PO} \)) can contribute to poor reproducibility of
MRT using either the linear and the non-linear regression models (Hughson & Inman, 1986; Markovitz et al., 2004; Boone & Bourgois, 2012). In addition, the MRT can be artificially lengthened if the pre-ramp baseline power output is too low (Boone et al., 2008b). For instance, compared to higher baseline power outputs (e.g., ≥40 W) cycling at very low power outputs (e.g., ≤20 W) engenders a greater ΔVO₂/ΔPO (less efficient), presumably due to increased internal work (Francescato et al., 1995; Neptune & Kautz, 2001) and/or underestimation of power outputs by the cycle ergometers within this range of resistance. As a result, when ramp-exercise is initiated from low power outputs, this relative “inefficiency” progressively disappears during the initial portion of the ramp (e.g., from 20 W to 40 W) such that no appreciable change in VO₂ is detected during this period causing an artificial prolongation of the MRT (Boone et al., 2008b).

When using ramp-incremental tests to prescribe constant-intensity exercise it is imperative that the MRT be quantified and accounted for (Keir et al., 2018a, 2018b). Neglecting to do so leads to an overestimation of the power output for a target VO₂ and hinders the ability to determine the power output associated with LT – a key marker of intensity domain-specific exercise (Black et al., 2017). However, as previously mentioned, current approaches for determining the MRT lack reproducibility and thus a more precise method is warranted. In this perspective, we considered that, unlike ramp-changes in power output, step-changes to a given moderate-intensity constant-power output allow time for VO₂ to reach its new steady-state. With this premise, if the steady-state VO₂ is superimposed on the ramp-VO₂-to-power output relationship and used to retrieve (by linear interpolation) the corresponding power output, the discrepancy between the constant-power output and the ramp-identified power output should be equivalent to the MRT (since power output is analogous to time during ramp-exercise). Based on this logic, the purpose of this study was to test the validity and the reproducibility of this novel approach for determining the MRT of VO₂.
during ramp-incremental exercise. We hypothesized that this new method would be valid and exhibit a more reproducible estimation of MRT compared to traditional linear and non-linear regression modelling.

**Methods**

*Participants*

Twelve recreationally-active young men (mean ± standard deviation: age 30 ± 10 years; body mass 78 ± 10 kg; height 179 ± 7 cm) gave their written informed consent and volunteered to participate in the study. All procedures were approved by the Conjoint Health Research Ethics Board of the University of Calgary. Participants were free of any medical conditions and were not taking any medications that might alter their cardiopulmonary and metabolic responses to exercise.

*Methodological approach for determining MRT with the novel method*

Ramp-incremental exercise was preceded by a moderate-intensity step-transition protocol. With this procedure, the steady-state $\dot{V}O_2$ at a single moderate-intensity power output could be determined before the ramp $\dot{V}O_2$ vs power output relationship was established. The difference between the constant-power output and the ramp-power output associated with the steady-state $\dot{V}O_2$ from constant-power output exercise would correspond to the MRT.

*Experimental protocol*

During two laboratory visits separated by 48 hours, participants performed the following exercise protocol on a cycle ergometer (Velotron, Dynafit Pro, Racer Mate, Seattle, WA) (Figure 3.1): three consecutive step-transitions to a constant, moderate-intensity power output followed by a ramp-incremental test to the limit of tolerance. The three step-transitions consisted of 6 min of pedaling at 20 W followed by 6 min at 100 W and the ramp-incremental protocol consisted of a 30 W/min
increase in power output that was preceded by a 4-min baseline at 50 W. Two minutes of rest separated the last step-transition from the pre-ramp baseline. During the constant-power output step-transition trials, the pre-ramp baseline, and the first portion of the ramp-incremental test (up to 150 W), participants were asked to maintain a cycling cadence of 70 rpm. Above this intensity, they were free to cycle at their preferred cadence in a range between 70 and 85 rpm. For each participant the position of the handlebar and the seat were recorded during the first visit and reset to the same value for the subsequent visit.

Figure 3.1. Schematic of the experimental protocol. The protocol began with three step-transitions from 20 W to 100 W with each step of 6 min of duration. These were followed by a 2-min recovery and the ramp-exercise protocol beginning from a baseline of 50 W and increasing by 30 W·min⁻¹.
Measurements

Breath-by-breath ventilatory and gas-exchange variables were measured continuously during the entire exercise protocol using a metabolic cart (CPET, Cosmed, Rome, Italy). Briefly, inspired and expired volume rates were measured by a low-dead-space turbine after being calibrated with a syringe of known volume (3 L). Fractional concentrations of inspired and expired O\textsubscript{2} and CO\textsubscript{2} for each breath were assessed by gas analyzers that were calibrated prior to each test using a gas mixture of known concentration.

Data analyses

The raw gas exchange data from the ramp-incremental test were used to determine LT. LT was identified by visual inspection of the \(\dot{V}O_2\)-to-\(\dot{V}CO_2\) relationship in combination with equivalents and end-tidal pressures of O\textsubscript{2} and CO\textsubscript{2} (Beaver et al., 1986). Then, the \(\dot{V}O_2\) data were filtered by removing aberrant data points that lay outside 3 standard deviations of the local mean (derived from the linear and mono-exponential fitting models). Data for each step-transition and ramp-exercise were then interpolated to 1 s intervals for subsequent analysis. \(\dot{V}O_2\max\) was defined as the highest \(\dot{V}O_2\) computed from a 20 s rolling average. Peak power output was the greatest power output value achieved at the end of the ramp-incremental test. The power output associated with LT was retrieved by linearly interpolating the \(\dot{V}O_2\) vs power output relationship after the \(\dot{V}O_2\) was \textit{left-shifted} by a time-interval corresponding to the MRT calculated with the novel method (\textit{see below for description of this method}).

Step-transitions. For each testing session, the breath-by-breath \(\dot{V}O_2\) data from the three step-transitions were time-aligned such that time zero corresponded to the onset of the transition (100 W). Then, these were ensemble-averaged to yield a single averaged response for each subject. The average \(\dot{V}O_2\) of the last 2 min was calculated and used to retrieve the MRT during the ramp-
incremental exercise (see point 3 below). To identify with the greatest level of confidence the steady-state $\dot{V}O_2$ at 100 W the ensemble-averaged data from the three step-transitions were considered, although post-hoc analysis revealed that this value was not statistically different from the steady-state $\dot{V}O_2$ from a single step-transition trial.

Ramp-incremental. The $\dot{V}O_2$ data for each ramp trial were time-aligned such that time zero corresponded to the onset of the ramp, linearly interpolated on a second-by-second basis. Next, three different approaches were used to fit the ramp data and compute the MRT:

1) A piecewise equation that included two linear segments (Boone & Bourgois, 2012):

$$ f = \begin{cases} g(t), & \text{if } (t < \text{MRT}) \\ h(t), & \text{else} \end{cases} $$

where $f$ is the piecewise function, $t$ is time and $g$ and $h$ are $\dot{V}O_2$, MRT is the time corresponding to the intersection of the two regression lines ($\text{MRT}_{\text{LIN}}$), $i_1$ and $i_2$ are the intercepts of the first and second linear function, respectively, and $s_1$ and $s_2$ are the slopes. The $s_1$ parameter was fixed at “zero” (Keir et al., 2018a). The two linear segments were fitted from the last 3 min of the pre-ramp baseline ($t = -180$ s) to the previously established LT (Boone et al., 2008b).

2) a mono-exponential function using a nonlinear least-squares regression (Whipp et al., 1981):

$$ \dot{V}O_2(t) = \dot{V}O_{2\text{BSL}} + \Delta\dot{V}O_{2\text{ss}} \cdot (t - \tau' [1 - e^{-t/\tau'}]) $$

where $\dot{V}O_2(t)$ is the value of $\dot{V}O_2$ at any time during the ramp, $\dot{V}O_{2\text{BSL}}$ is the baseline ramp value, $\Delta\dot{V}O_{2\text{ss}}$ is the increment above $\dot{V}O_{2\text{BSL}}$ required for the power output at time $t$, and $\tau'$ is the effective time constant of the response. Accordingly to previous studies (Whipp et al., 1981; Keir et al., 2016a) the fitting window was constrained from the onset ($t = 0$) to the end of the ramp-exercise.

3) A linear regression was used to fit the $\dot{V}O_2$ response (vs power output) from the onset of its systematic rise (that was visually determined) to the previously identified LT. Subsequently, the
steady-state \( \dot{V}O_2 \) value from the moderate step-transition trials was superimposed on the \( \dot{V}O_2 \) vs power output relationship. The difference between: \( i) \) the power output corresponding to the abscissa of intersection between \( \dot{V}O_2 \) and the (ramp) linear fit of \( \dot{V}O_2 \) vs PO, and \( ii) \) 100 W, was then converted to time to yield MRT\(_{SS} \). For example, if the \( \dot{V}O_2 \) derived from the step-transition intersected the ramp-\( \dot{V}O_2 \) at a power output of 112 W, the 12 W difference between the constant-power output step-transition (i.e., 100 W) and the ramp-power output is equivalent to a MRT of 24 s (i.e., 1 W equals 2 seconds for a 30 W\( \cdot \)min\(^{-1} \) ramp (Figure 3.2)).

![Figure 3.2. \( \dot{V}O_2 \) response profile of a representative participant from the step-transitions and ramp-incremental exercise protocols. To determine the MRT, the averaged steady-state \( \dot{V}O_2 \) at 100 W was superimposed on the \( \dot{V}O_2 \) vs power output relationship of the ramp-incremental exercise. The difference in power output between the steady-state \( \dot{V}O_2 \) and the ramp-\( \dot{V}O_2 \) at the point of intersection with the linear fit was converted to time (s) to retrieve the MRT.](image)

These three fitting strategies were performed on the individual ramp-incremental trials and on the ensemble-average response. The gain \( \Delta \dot{V}O_2/\Delta \text{PO} (G_{\text{ramp}}) \) (i.e., amplitude of the \( \dot{V}O_2 \) increase) for the linear and non-linear models was calculated with respect to time (\( \Delta \dot{V}O_2/\Delta \text{time} \) (mL\( \cdot \)min\(^{-1} \)\( \cdot \)s\(^{-1} \))) and expressed as \( \Delta \dot{V}O_2/\Delta \text{PO} \) (mL\( \cdot \)min\(^{-1} \)\( \cdot \)W\(^{-1} \)).
The model parameters were estimated by least-squares nonlinear (mono-exponential) and linear regression (segmented) using customized functions of a commercially available statistical software package (Origin, OriginLab Corp., Northampton, MA).

**Statistical analyses**

Data are presented as mean ± standard deviation. Where appropriate the coefficients of variation (CV) were calculated. A paired t-test was used to compare aerobic fitness indices (\(\dot{V}O_2\text{max}, \text{LT}\)) between test 1 and test 2. A one-way repeated measure analysis of variance (ANOVA) was used to compare within and between differences in the three methods for MRT calculation for test 1 and test 2. The Pearson’s coefficient was used to assess the level of correlation between test 1 and test 2. A one-way ANOVA was also used to compare the baseline and steady-state \(\dot{V}O_2\) responses during the step-transition trials (raw and 1 s interpolated data). A multiple-linear regression analysis was performed to evaluate how changes from test-to-test in \(\dot{V}O_2\) baseline and \(\Delta\dot{V}O_2/\Delta\text{PO}\) (independent variables) influenced the derived MRT (dependent variable) from each of the three methods. Where appropriate a Bonferroni’s post hoc test was used. Statistical significance was set at an \(\alpha\) level < 0.05.

**Results**

There were no differences between test 1 and 2 for \(\dot{V}O_2\text{max}\) (4.00 ± 0.47 L\(\cdot\)min\(^{-1}\), and 4.05 ± 0.48 L\(\cdot\)min\(^{-1}\), respectively) and peak power output (394 ± 55 W, and 396 ± 50 W, respectively) (\(P > 0.05\)). The \(\dot{V}O_2\) at the estimated LT was 2.15 ± 0.23 L\(\cdot\)min\(^{-1}\) and 2.12 ± 0.20 L\(\cdot\)min\(^{-1}\) for test 1 and test 2, respectively (\(P > 0.05\)). These values corresponded to 141 ± 22 W (test 1) and 139 ± 19 W (test 2) (range = 105 – 185 W) (\(P > 0.05\)). Overall, for the moderate step-transition trials, participants exercised at 73 ± 11 % (range: 54 – 95%) of the power output associated to LT. The average \(\dot{V}O_2\) during the step-transition protocol was 1.00 ± 0.17 L\(\cdot\)min\(^{-1}\) for the baseline at 20 W,
and 1.74 ± 0.15 L·min⁻¹ for the steady-state at 100 W. The \(\dot{V}O_2\) during the step-transition trial was not different between tests 1 and 2.

Table 3.1 displays the mean parameter estimates of MRT from the three methods examined. There were no differences between and within these methods for test 1 and test 2 \((P > 0.05)\). However, MRT\(_{SS}\) provided the most reproducible values, as the intra-individual coefficients of variation of the MRT\(_{LIN}\) and \(\tau\) (56 ± 57\% and 58 ± 32\%) were greater than that of MRT\(_{SS}\) (15 ± 9\%) \((P < 0.05)\). Figure 3.3 shows the regression plots of the MRT\(_{LIN}\) \((panel A)\), \(\tau\) \((panel C)\), and MRT\(_{SS}\) \((panel E)\) between test 1 and test 2 and the Pearson’s correlation coefficients. The variability of the three different methods is exemplified in Figure 3.3 \((panels B, D, F)\). Figure 3.4 displays the \(\dot{V}O_2\) profiles from ramp-exercise 1 \((top)\) and 2 \((bottom)\) of a representative subject for each model fit with its respective residuals.
<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Ensemble-averaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRT\textsubscript{LIN}, (s)</td>
<td>28 ± 16</td>
<td>26 ± 15</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>b\textsubscript{slin}, (L\textperiodcentered min\textsuperscript{-1})</td>
<td>1.26 ± 0.15</td>
<td>1.28 ± 0.15</td>
<td>1.27 ± 0.14</td>
</tr>
<tr>
<td>m</td>
<td>0.01 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>G\textsubscript{ramp}, (ml\textperiodcentered min\textsuperscript{-1}\textperiodcentered W\textsuperscript{-1})</td>
<td>9.4 ± 1.6</td>
<td>9.7 ± 1.6</td>
<td>9.6 ± 1.5</td>
</tr>
<tr>
<td>SEE, (s)</td>
<td>14</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>τ' (s)</td>
<td>27 ± 12</td>
<td>21 ± 12</td>
<td>41 ± 24</td>
</tr>
<tr>
<td>b\textsubscript{slin}, (L\textperiodcentered min\textsuperscript{-1})</td>
<td>1.28 ± 0.14</td>
<td>1.25 ± 0.17</td>
<td>1.29 ± 0.16</td>
</tr>
<tr>
<td>m</td>
<td>0.005 ± 0.000</td>
<td>0.005 ± 0.000</td>
<td>0.005 ± 0.000</td>
</tr>
<tr>
<td>G\textsubscript{ramp}, (ml\textperiodcentered min\textsuperscript{-1}\textperiodcentered W\textsuperscript{-1})</td>
<td>9.0 ± 0.6</td>
<td>9.3 ± 0.7</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td>SEE, (s)</td>
<td>11</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>MRT\textsubscript{SS} (s)</td>
<td>26 ± 11</td>
<td>27 ± 7</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>m</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>b</td>
<td>0.62 ± 0.17</td>
<td>0.66 ± 0.27</td>
<td>0.66 ± 0.18</td>
</tr>
<tr>
<td>SEE, (s)</td>
<td>8</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Data presented are mean values ± SD (n = 12). “Ensemble-averaged” refers to the time-aligned ensemble-average of the two ramp-incremental tests. MRT\textsubscript{ LIN} (s) represents the mean response time computed from a piecewise equation that included two linear segments with the point of intersection representing the time-interval before the systematic increase in VO\textsubscript{2}. τ' represents the ramp time-constant (s) from the mono-exponential fit. MRT\textsubscript{ SS} represents the mean response time (s) computed from the discrepancy in power output between the step-transition and ramp-exercise protocols identified by the steady-state VO\textsubscript{2} at 100 W. b\textsubscript{ slin} = baseline VO\textsubscript{2}; m = slope of VO\textsubscript{2} increase; G\textsubscript{ ramp} = VO\textsubscript{2} functional gain; b = intercept. SEE = standard error of the estimate based off the ensemble-average ramp-tests.
Figure 3.3. Regression plots and individual variability between test 1 and test 2 of the MRT determined by linear model (MRT\textsubscript{LIN}) (A, B), non-linear model (τ') (C, D), and novel method proposed in the current study (MRT\textsubscript{SS}) (E, F).
Figure 3.4. Comparison of MRT values determined for a representative individual by linear model (MRT$_{LIN}$) (A, B), non-linear model ($\tau'$) (C, D), and novel method proposed in the current study (MRT$_{SS}$) during ramp-incremental test 1 (upper panels) and 2 (bottom panels).
From test-to-test, the intra-individual CVs of the $\dot{V}O_2$ pre-ramp baseline derived from the linear and non-linear models were 4.3 ± 3.4% and 5.0 ± 3.9%, respectively. The intra-individual CVs of the $\Delta \dot{V}O_2/\Delta \text{PO}$ from the linear and non-linear models were 10.3 ± 10.2% and 3.8 ± 3.2%, respectively. The multiple linear regression analysis showed that, overall, the changes from test-to-test in $\dot{V}O_2$ baseline and $\Delta \dot{V}O_2/\Delta \text{PO}$ were associated to changes in $\text{MRT}_{\text{LIN}} [(F(2,9) = 7.214, \ P < 0.05); \ R^2 = 0.785]$ and $\tau^\prime [(F(2,9) = 16.928, \ P < 0.001); \ R^2 = 0.889)]$, but not in $\text{MRT}_{\text{SS}} [(F(2,9) = 1.011, \ P > 0.05); \ R^2 = 0.428)]$. Specifically, for the linear model, the changes in $\text{MRT}_{\text{LIN}}$ were correlated to the changes in $\Delta \dot{V}O_2/\Delta \text{PO} (\beta = 7.796, \ P < 0.05)$ but not $\dot{V}O_2$ baseline (\(\beta = 36.735, \ P > 0.05\)) whereas, for the non-linear model, the changes in $\tau^\prime$ were correlated to the changes in $\dot{V}O_2$ baseline (\(\beta = 139.836, \ P < 0.01\)) but not $\Delta \dot{V}O_2/\Delta \text{PO} (\beta = 8.964, \ P > 0.05)$.  

**Discussion**

The MRT of ramp-incremental exercise, representing the time-interval before $\dot{V}O_2$ conforms to the linear increase in power output, is an important parameter to consider when using ramp test for constant-intensity exercise selection. Two fitting-based methods have been used to quantify this time-interval, yet neither demonstrates high test-to-test reproducibility (Hughson & Inman, 1986; Markovitz et al., 2004; Boone & Bourgois, 2012). The purpose of this study was to measure the reproducibility of a novel approach for determining the MRT compared to traditional fitting strategies. The novel method consisted of determining the steady-state $\dot{V}O_2$ corresponding to a bout of moderate-intensity constant-power output exercise performed prior to ramp-exercise and comparing the ramp-derived power output associated with that $\dot{V}O_2$ to the constant-power output that elicited that $\dot{V}O_2$. The difference between these power outputs was converted to time to retrieve the time-interval corresponding to MRT. In our test-retest design, this method produced
similar mean values to those of the other established fitting-based approaches but was more reproducible for determining the MRT.

The MRT has generally been quantified using linear regression and/or mono-exponential models (Rossiter, 2011; Keir et al., 2018a). In accordance with previous observations (Hughson & Inman, 1986; Markovitz et al., 2004; Boone & Bourgois, 2012), our findings identified critical limitations in terms of accuracy and reproducibility when quantifying the MRT with these methods. By comparison, the method developed herein demonstrated a higher between-test correlation coefficient (0.87; P<0.05) and an intra-individual variation that was three times smaller than MRT_LIN and τ. Furthermore, although the mean MRT values were similar across the three methods (~25 s), the between-subject variation was smaller using the novel method, which yielded MRT values that were “physiologically plausible” in all instances (i.e., > 15 s). Contrarily, for example, MRT_LIN and τ produced values that were – or were close to – 0 s in six cases, which is unlikely to be accurate because this would imply that muscle VO₂ increases immediately at the onset of the ramp (with no kinetic component) and that this increase is immediately evident at the level of the lungs. Interestingly, this large between-subject variation in MRT values derived from the linear and non-linear models persisted even after the two ramp-exercises were ensemble-average (a procedure suggested by previous studies to increase the signal-to-noise ratio and improve the confidence of the estimation (Boone & Bourgois, 2012)).

Changes in VO₂ baseline and ΔVO₂/ΔPO have previously been shown to impact the estimation of MRT (Hughson & Inman, 1986). In accordance with these findings, we showed that ~80% of the variability of the MRT derived from the linear regression and mono-exponential models could be attributed to test-to-test changes in VO₂ baseline and ΔVO₂/ΔPO. Specifically, MRT_LIN was highly affected by test-to-test variation in ΔVO₂/ΔPO whereas τ was influenced by variations in baseline
\( \dot{V}O_2 \). This is an important limitation as both baseline and \( \Delta \dot{V}O_2/\Delta PO \) of the \( \dot{V}O_2 \) derived from these methods were highly variable (CV = ~5-10\%). This variability may result from the small number of data points to which these models are fitted, thus even minimal data fluctuations from test-to-test can reduce their accuracy and reproducibility.

On the contrary, our novel method to determine the MRT showed a high level of reproducibility that may be attributed to the reasons that: 1) the method does not rely on fitting models, which are highly influenced by breath-by-breath noise, and by pre-ramp baseline and \( \Delta \dot{V}O_2/\Delta PO \) variations; 2) the “intersection” of the steady-state \( \dot{V}O_2 \) (from the step-transition) with the ramp-\( \dot{V}O_2 \) occurs at a point where the latter has already fully developed its linear increase, thus minimizing the issues related to \( \dot{V}O_2 \) fluctuations; 3) the \( \dot{V}O_2 \) response during the constant-power output step-transitions (100 W) is highly reproducible (CV = ~2\%), which could be related to the large number of data points (i.e., breaths) that are averaged together and to a greater consistency of the ventilatory and leg-muscle recruitment patterns associated with cycling at higher power outputs (within the moderate-intensity domain).

An important aspect to consider is that our novel method necessitates that ramp-exercise is preceded by a step-transition protocol. Given that part of this study was to test its accuracy, to ensure a high confidence in the steady-state \( \dot{V}O_2 \) measure at 100 W, three 6 min moderate exercise bouts were performed. Although these additional bouts lengthened the protocol by ~30 minutes, post-hoc analysis revealed that a single 6-minute step-transition is sufficient to determine with accuracy the steady-state \( \dot{V}O_2 \) at 100 W. Therefore, we propose that one step-transition prior to a ramp-incremental protocol would be sufficient to accurately quantify the MRT. This strategy would extend the total exercise time by only ~10 min.
A requisite of the proposed method is that the step-transition protocol must be set to a constant-power output that is below the LT and within the moderate-intensity domain where the $\Delta\dot{V}O_2/\Delta P_O$ is relatively constant and not affected by the $\dot{V}O_2$ slow component (Barstow & Molé, 1991; Paterson & Whipp, 1991; Spencer et al., 2011; Keir et al., 2016c). Because the aerobic parameters of the individuals participating in the current study were known from previous testing, we were confident that all participants would be exercising within the moderate-intensity domain at 100 W. Recognizing that the power output associated with LT is usually not known *a priori*, selecting a power output for the step-transition protocol that is below LT but high enough to elicit a $\dot{V}O_2$ steady-state that is appreciably above baseline and within the portion of the $\dot{V}O_2$ ramp response that is increasing linearly with power output might be challenging. Based on previous studies from our group (Spencer et al., 2013; Keir et al., 2014; George et al., 2018), we suggest that a power output of approximately 100 W would, in most cases, fulfill these requirements in recreationally-active and trained individuals. In contrast, however, this power output (i.e., 100 W) might be too high in sedentary young and older individuals; thus, a lower power output for the step-transition protocol concomitantly with a lower power output for the pre-ramp baseline (e.g., < 50 W) might be optimal. In this perspective, some basic pre-test screening may be of help to predict an adequate intensity based on an individual’s level of fitness (Pogliaghi et al., 2014). For instance, the pre-ramp baseline could be set as low as 20 W and the moderate step-transition could be performed at 50 W or lower. Provided the $\dot{V}O_2$ steady-state from the step-transition is greater than the ramp $\dot{V}O_2$ baseline, the MRT calculation using our novel approach should still demonstrate a high level of precision. Overall, this strategy will guarantee the linearity of the $\dot{V}O_2$ response during the step-transition phase as well as an increase in the number of data points in the ramp-exercise portion within the moderate-intensity domain.
Conclusions

In summary, the present study tested the validity and the reproducibility of a novel method to calculate the MRT of the \( \dot{V}O_2 \) during ramp-incremental exercise. Our findings demonstrated that this method is more reproducible in a test-retest design and less variable among all the individuals compared to traditional model-based methods because it is not influenced by variations in the pre-ramp baseline and \( \Delta \dot{V}O_2/\Delta PO \). For these reasons, we propose that this approach provides an accurate quantification of the MRT and suggest its use to quantify the time interval by which ramp-incremental \( \dot{V}O_2 \) data need to be left-shifted in relation to power output.
CHAPTER IV

Metabolic and performance-related consequences of exercising at and slightly above MLSS

Introduction

The maximal lactate steady-state (MLSS) represents the highest intensity of exercise at which lactate production and utilization/removal are in equilibrium, so that blood lactate concentration ([La\(^-\)\(_b\)]) remains stable (Billat et al., 2003). As a progressive acceleration of the glycolytic rate leading to [La\(^-\)\(_b\)] accumulation occurs when constant-load exercise is performed above this intensity, MLSS, by definition, experimentally demarcates the upper boundary at which the aerobic metabolism solely accounts for the overall energy expenditure and metabolic responses are elevated but stabilized (Billat et al., 2003). For these reasons MLSS is often defined as the physiological landmark separating the heavy from the severe exercise intensity domain.

The acute responses of exercising in these two different domains have been described quite extensively (Poole et al., 1988; Jones et al., 2008; Vanhatalo et al., 2016; Black et al., 2017), with the critical power (CP) concept used in these studies as the paradigm to separate the heavy from the severe-intensity domain, which, similarly to MLSS, is supposed to identify the “critical intensity” of exercise (i.e., the highest) sustained exclusively by oxidative sources of energy (Jones et al., 2010; Keir et al., 2015; Grassi et al., 2015; Poole et al., 2016). However, as the estimation of this intensity through the CP model can be subject to errors, it could be possible that in some circumstances the highest and true metabolic steady-state might be under- or over-predicted. For instance, CP presents a typical error that is usually reported to be within a 5% range (Poole et al., 2016) which might arise from biases associated with the protocols and models employed (Bishop et al., 1998; Mattioni Maturana et al., 2018). For example, Pringle et al. (2002), and more recently Mattioni Maturana et al. (2016), observed that the power output (PO) at CP was on average ~20
W higher than that at MLSS. Considering these potential differences, it might be possible that the metabolic disturbances associated with exercising in the severe-intensity domain have been only described in the literature at intensities greatly exceeding MLSS. Thus, it is unknown if and to what extent a very small increase in PO above MLSS (but within the reported typical error assigned to CP) would alter physiological responses and consequent maximal exercise performance.

In relation to the physiological responses associated with the upper limit of sustainable exercise (i.e., heavy-to-severe boundary), a growing amount of evidence has shown that, during ramp-incremental cycling exercise to exhaustion, following a rather linear increase from the onset of exercise, the NIRS-derived deoxygenated haemoglobin [HHb] signal of the vastus lateralis (VL) muscle displays a breakpoint ([HHb]BP) that occurs at ~75-85% of \( \dot{V}O_2\)peak (Spencer et al., 2012; Murias et al., 2013; Okushima et al., 2015; Boone et al., 2016a; Iannetta et al., 2017a, 2017b; Inglis et al., 2017). This leads to a plateau-like response in the [HHb] signal, with controlling mechanisms for this “apparent” upper limit still being a subject of debate (Okushima et al., 2015; Inglis et al., 2017). The [HHb]BP has been linked to a metabolic rate similar to MLSS and CP (Keir et al., 2015), and is also seen (albeit with a different profile) in the rectus femoris (RF) muscle (Iannetta et al., 2017b). These distinctive responses in the [HHb] profiles are thought to reflect the changes in the dynamic balance between local muscle blood flow (\( \dot{Q}_m \)) and \( \dot{V}O_2 \) (\( \dot{V}O_2m \)) (\( \dot{Q}_m\)-to-\( \dot{V}O_2m \) ratio) that results from alterations within the intra and extracellular environments as well as from variations in muscle fibres recruitment patterns that occur during incremental exercise (McDonough et al., 2005; Copp et al., 2010; Chin et al., 2011; Iannetta et al., 2017b). However, during constant-load exercise when the intensity is sustained at or slightly above the PO associated with MLSS, the behaviour of the [HHb] response is unknown. Given the physiological alterations observed during exercise in the severe-intensity domain, it might be possible that local changes in
the $\dot{Q}_m$-to-$\dot{V}O_2m$ ratio may affect local $O_2$ extraction. Thus, characterization of the behaviour of the [HHb] signal during prolonged constant-load exercise at, and slightly above the intensity associated with MLSS is warranted.

Therefore, the aim of this study was to i) evaluate the metabolic and performance-related consequences of exercising at an intensity slightly exceeding MLSS, and ii) characterize the behaviour of the local muscle oxygen extraction ([HHb]) signal in the VL and RF muscles during constant-load exercise. We tested the hypothesis that prolonged exercise sustained only $10\ W\ (\sim 3\%-5\%)$ above MLSS would result in a significant increase in physiological variables linked to metabolic activity (i.e., $[La^-]$ and $\dot{V}O_2$) which would lead to a greater decrease in time-to-exhaustion performance compared to exercise sustained at the PO associated with MLSS. Furthermore, given the similar $\dot{V}O_2$ associated with the plateau of the [HHb] signal of the VL muscle (during ramp-incremental exercise) and MLSS, it was hypothesized that during constant-PO exercise the [HHb] signal of the VL muscle would achieve its peak values during constant-load exercise at the intensity corresponding to MLSS, with additional increments above this intensity only possible in the RF muscle.

Methods

Participants

Eleven recreationally-active men (31±10 yrs; 78.4±10.5 kg), who indicated during a preliminary interview that they routinely performed endurance training at least three times per week, voluntarily participated in the study. Participants were non-smokers, non-obese and not undergoing any medical treatment that could affect their cardiovascular responses to exercise. Participants were made aware of all testing procedures as well as the risks and benefits of participating in the study, and they all signed an informed consent. All procedures were approved.
by the Conjoint Health Research Ethics Board of the University of Calgary, in compliance with the Declaration of Helsinki.

**Procedures**

All participants completed the following testing procedures on an electromagnetically braked cycle ergometer (Velotron; RacerMate, Seattle, WA) within a 2-week period: *i) One ramp-incremental tests to exhaustion* *ii) two to four, 30-min constant-PO rides for determination of the PO associated with MLSS (MLSS\(p\)) and 10 W above MLSS (MLSS\(p+10\)) immediately followed by time-to-exhaustion trials (i.e., TTE\(_{MLSS\(p\)}\) and TTE\(_{MLSS\(p+10\)}\)), and *iii) a baseline time-to-exhaustion trial (i.e., not preceded by exercise) (TTE\(_b\)).* All tests were carried in an environmentally controlled room (temperature: 19-20° C; humidity 50-60%) at a similar time of the day (the time could vary by ±30 min). Prior to each exercise testing session, all participants were instructed to avoid consumption of food and caffeinated beverages for at least 2 and 8 hours, respectively, and to abstain from vigorous physical activity for 24 hours. A minimum of 48 hours and a maximum of 72 hours of rest was allowed between each visit. During each session participants were blinded to the PO and the elapsed time, but received visual feedback on their pedal cadence.

*Ramp-incremental test.* A ramp-incremental exercise test to the limit of tolerance was performed to determine \(\dot{V}O_{2\text{peak}}\), the lactate threshold (LT), the respiratory compensation point (RCP), maximal heart rate (HR\(_{\text{max}}\)), peak PO (PO\(_{\text{peak}}\)), and the near-infrared spectroscopy (NIRS)-derived HHb-breakpoint ([HHb]BP) of the VL muscle. After a 4-min period of cycling at 50 W, the PO was increased in a ramp-like manner by 30 W\(\cdot\)min\(^{-1}\) (1 W every 2 s). Participants were instructed to cycle at their preferred cadence in a range between 75 and 95 rpm. The ramp-incremental test was stopped when participants failed to maintain the targeted cadence by 10 rpm for more than ten consecutive seconds despite strong verbal encouragement, or when volitional exhaustion ensued.
**MLSS determination trials.** On successive appointments, participants performed constant-PO tests of 30 min of duration. MLSS\textsubscript{p} corresponded to the highest PO that resulted in a difference in [La\textsuperscript{-}]\textsubscript{b} of less than 1 mmol\textsuperscript{-1}\textsubscript{L} between the 10\textsuperscript{th} and 30\textsuperscript{th} min of exercise (Beneke, 2003\textit{b}). Each trial was initiated with a 4-min baseline cycling at 80 W after which the PO was instantaneously increased to a predetermined value. For all the constant-PO trials participants were asked to cycle at the preferred cadence established during the previous ramp-incremental test. To determine the resistance of the first constant-PO trial, participants were asked to self-select a load that could correspond to MLSS (Mattoni Maturana et al., 2017), from a set of proposed loads derived from a mathematical model for MLSS estimation recently developed in our laboratory (Iannetta et al., 2018\textit{a}). Briefly, this mathematical model, by using the respiratory compensation point (RCP) (expressed in W\textsuperscript{-}Kg\textsuperscript{-1}), \(\dot{V}O_{2\text{peak}}\) (expressed in ml\textsuperscript{-}Kg\textsuperscript{-1}\cdot\text{min}\textsuperscript{-1}), and body weight (Kg) as predictive variables, can provide an estimate with a high degree of accuracy the PO associated with MLSS. This model allowed us to notably reduce the number of laboratory visits necessary to determine MLSS\textsubscript{p}. Regardless, the constant-PO rides were repeated as long as the criteria for MLSS\textsubscript{p} and MLSS\textsubscript{p+10} identification were satisfied. In this regard, depending on whether the [La\textsuperscript{-}]\textsubscript{b} response from the first 30-min constant-PO test was greater, or less (or equal) than 1 mmol\textsuperscript{-1}\textsubscript{L}, the PO for the subsequent constant-PO ride was either decreased or increased by 10 W, respectively. Five subjects exercised at MLSS\textsubscript{p+10} during the first determination trial, whereas the remaining six at MLSS\textsubscript{p}, with this leading to a homogeneous distribution of the rides at and above MLSS\textsubscript{p}. [La\textsuperscript{-}]\textsubscript{b} was assessed during baseline (within the first 3-min) and every 5 min after the PO was increased. Furthermore, at the 10\textsuperscript{th} and 30\textsuperscript{th} min [Lac]\textsubscript{b} was taken in duplicate and the average of the two measures was used for subsequent analysis.
**Time-to-exhaustion trials.** At the end of each 30-min constant-PO test and after a three-minute recovery period while cycling at 50 W, participants performed a time-to-exhaustion trial with the resistance set at 80% of PO\text{peak} previously recorded at the end of the ramp-incremental exercise. Participants were asked to maintain the same cadence as during the trials for MLSS determination. The test was continued until volitional exhaustion. A time-to-exhaustion trial preceded by a 4-min of baseline cycling (80 W) was also performed on a separate occasion at the end of the experimental protocol. The percentage of PO\text{peak} during the time-to-exhaustion trials was selected to elicit exhaustion within approximately 5-6 min in the condition not preceded by the 30 min ride. Immediately after each time-to-exhaustion trial, a sample of capillary blood was taken for [La\text{\textsuperscript{-}}\text{b}] assessment.

**Data collection**

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to measure breath-by-breath gas exchanges. The turbine flowmeter was calibrated using a syringe of known volume (3 L). Gas analyzers were calibrated using a gas mixture of known concentration (16% O\textsubscript{2}; 5% CO\textsubscript{2}; balance N\textsubscript{2}), as per the manufacturer recommendations. Heart rate (HR) was recorded using radiotelemetry (SP0180 Polar Transmitter; Polar Electro, Inc., Kempele, Finland) and [La\text{\textsuperscript{-}}\text{b}] was measured with a portable lactate analyzer (Lactate Scout, SensLab GmbH, Leipzig, Germany). Briefly, after wiping the finger with an alcohol swab and performing a pin-prick, a 2 µL capillary sample of whole blood was collected and immediately analyzed for determination of [La\text{\textsuperscript{-}}\text{b}].

A two-channel NIRS system (Oxiplex TS\textsuperscript{TM}, ISS, Champaign, IL) was used in our study to monitor local [HHb] and total-haemoglobin (\text{tot[Hb]}) signals. Each NIRS probe was composed of eight laser diodes operating at two wavelengths (\(\lambda = 690\) and 828 nm, four at each wavelength), which were pulsed in rapid succession, and a photomultiplier tube. The lightweight plastic NIRS probes
(connected to laser diodes and a photomultiplier tube by optical fibres) consisted of two parallel rows of light-emitting fibres and one detector fibre bundle; the source–detector separations for this probe were 2.0, 2.5, 3.0 and 3.5 cm for both wavelengths. The two NIRS probes were placed on the belly of the VL muscle (midpoint between the greater trochanter of the femur and the knee joint) and of the distal portion of the RF muscle of the right leg. The areas of placement were carefully prepared by shaving hair and gently wiping the skin. Double-sided tape as well as an elastic bandage were used to secure in place the probes. An optically dense, black vinyl sheet was used to cover the probes to avoid the intrusion of external light. The apparatus was calibrated on each testing day after a warm-up of at least 30 min, as per the 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. The areas of probes’ placement in VL and RF were marked and recorded to ensure consistency for the following visits.

A multi-channel surface electromyography system (Delsys Inc, Boston, MA) was used for monitoring muscle activity (i.e., EMG) at a sampling rate of 1000 Hz. Two bipolar surface electrodes (41 × 20 × 5 mm) (DE-2.1, Delsys Inc. Boston, MA) were placed on the belly of the VL and RF muscles in close proximity (longitudinally) of the NIRS probes after the skin area was carefully prepared. Excessive hair was shaved and the area of placement was gently abraded and cleaned with alcohol swab to reduce skin impedance. Electrodes were secured in place by bi-adhesive and surgical tape, and were connected to an EMG amplifier which was connected to the acquisition apparatus (Power Lab, ADInstruments, Bella Vista, Australia) linked to a computer software (LabChart 8, ADInstruments, Bella Vista, Australia). Probe placement was recorded to ensure consistency between visits.

Data analyses
**Ventilatory and gas exchange data.** Two experimenters independently inspected the ramp-incremental test performed by each participant to identify LT and RCP from the gas exchange and ventilatory variables that were plotted against \( \dot{V}O_2 \). In the circumstance of a disagreement of more than 100 ml\cdot min\(^{-1}\), the experimenters re-evaluated together the profiles to form a consensus. LT was determined as the point at which \( \dot{V}CO_2 \) began to increase out of proportion in relation to \( \dot{V}O_2 \), coincidental with a systematic rise in the \( \dot{V}E/\dot{V}CO_2 \) relation and end-tidal PO\(_2\), whereas the ventilatory equivalent of \( \dot{V}CO_2 \) (\( \dot{V}E/\dot{V}CO_2 \)) and end-tidal PCO\(_2\) were stable (Beaver *et al.*, 1986). RCP was identified as the point at which end-tidal PCO\(_2\) began to fall after a period of isocapnic buffering. This point was confirmed by examining the \( \dot{V}E/\dot{V}CO_2 \) and \( \dot{V}E/\dot{V}O_2 \) relationships as well as by identifying the second breakpoint in the \( \dot{V}E/\dot{V}O_2 \) relation (Whipp *et al.*, 1989). The highest \( \dot{V}O_2 \) value computed from a 30-s rolling average during the ramp-incremental test was considered as \( \dot{V}O_{2\text{peak}} \). The same strategy was employed to determine \( \dot{V}O_{2\text{peak}} \) during each time-to-exhaustion trial. PO\(_{\text{peak}}\) was the highest PO value recorded at the end of the ramp-incremental test for each participant. The \( \dot{V}O_2 \), \( \dot{V}E \), frequency of breathing (fB), and heart rate (HR), at MLSS\(_p\) and MLSS\(_{p+10}\) at the 10\(^{th}\) and 30\(^{th}\) minutes were calculated as the average of two minutes data surrounding the 10\(^{th}\) minute (9\(^{th}\) – 11\(^{th}\) min) and the last two minutes, respectively, of the 30-min constant-PO exercise. Baseline \( \dot{V}O_2 \) and the average of the last 10 min of the \( \dot{V}O_2 \) response during the 30-min constant-PO exercise was used to compute the functional gain of the \( \dot{V}O_2 \) during each MLSS trial determination.

The \( \dot{V}O_2 \) mean response time of the ramp-incremental exercise test was determined as previously suggested (Boone *et al.*, 2008b). Briefly, \( \dot{V}O_2 \) vs time was plotted and time-aligned such that time “zero” corresponded to the onset of the ramp portion of the test. A preliminary linear fit of the \( \dot{V}O_2 \) data during both the baseline and the ramp-incremental portion of the test was used to identify and
remove data points lying ± 3 SD from the local mean. Afterwards, processed $\dot{V}O_2$ data were linearly interpolated to 1 s intervals and the best fit of a double-linear function applied from the last 2 min of the baseline to the previously established LT using non-linear least squares regression procedures (Origin, Origin Lab, Northampton, USA). The slope of the first segment of the double-linear was fixed at “0” and the breakpoint reflected the mean response time. The $\dot{V}O_2$ data were subsequently *left-shifted* by the individual-specific mean response time and the $\dot{V}O_2$ as well as the PO corresponding to RCP were identified.

*Adipose tissue thickness correction of [HHb] and tot[Hb] signals.* Both the [HHb] and tot[Hb] signals were analysed after accounting for the adipose tissue thickness of each of the muscles investigated. Briefly, a Harpenden skin caliper (Baty Int., West Sussex, UK) was used to measure the adipose tissue thickness (mm) in the areas of NIRS probe placements. The same investigator took measurements in duplicate and the average of the two was used. In case of a discrepancy of more than 0.4 mm, a third measurement was taken and the average of the closest two was used. Subsequently, a linear regression analysis of the relationship between the adipose tissue thickness and resting tot[Hb] was calculated and the measured [HHb] and tot[Hb] data were corrected to a common adipose tissue thickness of 0 mm.

*HHb* during ramp incremental test. The [HHb] data recorded during the ramp-incremental test on the VL muscle were plotted against time and modeled with the following piece-wise “double-linear” fit, as previously described (Spencer *et al.*, 2012):

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

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

$$i_2 = i_1 + (s_1 \cdot BP)$$
\[ h(x) = i_2 + (s_2 \cdot (x - BP)) \]

fit \( f \) to \( y \),

where \( f \) is the double-linear function, \( x \) is time and \( y \) is \([\text{HHb}] \), \( BP \) is the time coordinate corresponding to the interception of the two regression lines (i.e., \([\text{HHb}]_{BP}\)). \( i_1 \) 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 participant were determined by linear least-square regression analysis. A preliminary fit was used to identify and delete aberrant data that were ±3 SD from the local mean. The double linear fit was used from the onset of the systematic increase in the \([\text{HHb}] \) signal until the last data point corresponding to the end of the test. A linear interpolation was then used to retrieve the \( \dot{\text{VO}}_2 \) (\textit{left-shifted} to account for the mean response time) and \( \text{PO} \) values corresponding to the \([\text{HHb}]_{BP}\). Finally, the slope of change in the \([\text{HHb}] \) signal in the RF muscle was determined from linear regression in two distinctive portions of the response: below and above the visually determined point at which the \([\text{HHb}] \) would systematically change its slope (Okushima \textit{et al.,} 2016). The slopes of increase/decrease in the signals and the values of \( \dot{\text{VO}}_2 \) and \( \text{PO} \) associated with the \([\text{HHb}]_{BP}\) for each individual from both ramp-incremental tests were averaged together.

\textit{Surface electromyography.} The EMG data recorded during the 30-min constant-PO exercise and subsequent time-to-exhaustion trials was amplified, band-pass filtered (5 – 500 Hz), rectified, and computed as 1-s root mean square (RMS) amplitude. Subsequently the processed EMG data were normalized to the averaged last two minutes of the baseline cycling at 80 W. This normalization strategy was chosen as it is representative of the actual dynamic muscular patterns during cycling.
[HHb], tot[Hb], and EMG data during 30-min constant-PO and time-to-exhaustion trials. The [HHb], tot[Hb], and EMG data were plotted against time and the following bin-averaging strategy was employed at the time points of interest for successive statistical analysis: bsln1: last two minutes of the 4-min baseline cycling at 80 W; 10th min: two minutes surrounding the 10th minute mark; 30th min: last two minutes of the 30-min constant-PO exercise; bsln2: last two minutes of the 3-min recovery period while cycling at 50 W; TTE: last 30 seconds of the time-to-exhaustion trial.

Rating of Perceived Exertion. A 0-10 rating of perceived exertion (RPE) scale was used to monitor perceptual responses to exercise. The scale was displayed to the participants during baseline and every five minutes during the rides for MLSS determination and immediately at the end of every time-to-exhaustion tests.

Statistical analyses

Data are presented as means ± SD. All statistics were performed using SPSS version 23 (SPSS, IBM, Chicago, IL). A repeated-measures ANOVA was used to compare i) the \( \dot{V}O_2 \) values corresponding to MLSS_p, RCP, and [HHb]BP; ii) the time duration of each time-to-exhaustion trial performed iii) the peak ventilatory, gas exchange, [La\(^{-}\)]_b, and RPE values recorded at end of the ramp-incremental test and at the end of each time-to-exhaustion trial; iv) changes in \( \dot{V}O_2 \), \( \dot{V}E \), HR, [La\(^{-}\)]_b, [HHb], tot[Hb], and EMG variables from min 10 to min 30 during the 30-min constant-PO trials and time-to-exhaustion trials; v) \( \dot{V}O_2 \), \( \dot{V}E \), fB, HR, [La\(^{-}\)]_b, RER, and RPE responses between MLSS_p and MLSS_p+10. Where appropriate, a Bonferroni post hoc was applied. Statistical significance was accepted at \( \alpha < 0.05 \).
Results

The average PO<sub>peak</sub> at the end of the ramp-incremental test was 396±55 W. The average 80% of PO<sub>peak</sub> used for all the time-to-exhaustion trials was 317±43 W. Peak physiological responses measured at the end of ramp-incremental, TTE<sub>MLSSp</sub>, TTE<sub>MLSSp+10</sub> and TTE<sub>b</sub> tests are shown in Table 4.1. The VO<sub>2peak</sub> recorded during TTE<sub>MLSSp+10</sub> was approximately 7% lower than the VO<sub>2peak</sub> values recorded during the other TTE and ramp-incremental tests (P<0.05). A lower peak ventilatory response (~10%) was also found at the end of the TTE<sub>MLSSp+10</sub> compared to the other exercise conditions (P<0.05). The durations of TTE<sub>b</sub>, TTE<sub>MLSSp</sub>, and TTE<sub>MLSSp+10</sub> were 349±78 s, 220±75 s, and 125±40 s, respectively. The percent decreases in TTE<sub>MLSSp</sub> and TTE<sub>MLSSp+10</sub> tests compared to TTE<sub>b</sub> were 37.3±16.4 % and 64.6±6.3 %, respectively (P<0.05) (Figure 4.1).

Table 4.1. Peak gas exchange, ventilatory, heart rate, and blood lactate concentration values recorded during ramp incremental and time-to-exhaustion tests.

<table>
<thead>
<tr>
<th>variable</th>
<th>Ramp-incremental test</th>
<th>TTE&lt;sub&gt;MLSSp&lt;/sub&gt;</th>
<th>TTE&lt;sub&gt;MLSSp+10&lt;/sub&gt;</th>
<th>TTE&lt;sub&gt;b&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO&lt;sub&gt;2p&lt;/sub&gt; (L·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bsln</td>
<td>1.28±0.15</td>
<td>1.65±0.29</td>
<td>1.65±0.20</td>
<td>1.56±0.13</td>
</tr>
<tr>
<td>peak</td>
<td>4.01±0.57</td>
<td>4.03±0.53</td>
<td>3.78±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.07±0.48</td>
</tr>
<tr>
<td>RER</td>
<td>1.20±0.07</td>
<td>1.13±0.07</td>
<td>1.07±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13±0.04</td>
</tr>
<tr>
<td>VE (L·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>181±17</td>
<td>183±22</td>
<td>167±28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178±24</td>
</tr>
<tr>
<td>fB (breath·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>64.5±7.2</td>
<td>67.9±7.5</td>
<td>62.4±9.4</td>
<td>62.3±9.0</td>
</tr>
<tr>
<td>HR&lt;sub&gt;peak&lt;/sub&gt; (bpm)</td>
<td>181±9</td>
<td>179±9</td>
<td>176±8</td>
<td>176±8</td>
</tr>
<tr>
<td>[La&lt;sub&gt;b&lt;/sub&gt;] (m·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>11.7±1.9</td>
<td>11.7±2.4</td>
<td>11.0±2.5</td>
<td>12.5±2.2</td>
</tr>
<tr>
<td>RPE</td>
<td>9.5±0.4</td>
<td>9.5±0.7</td>
<td>9.8±0.4</td>
<td>9.5±0.7</td>
</tr>
</tbody>
</table>

Values are mean values (± SD). VO<sub>2p</sub> bsln: pre-exercise baseline values for VO<sub>2p</sub>. VO<sub>2p</sub> peak: peak values recorded at the end of each test. RER: respiratory-exchange-ratio. VE: minute ventilation. fB: frequency of breathing. HR<sub>peak</sub>: peak heart rate. [La<sub>b</sub>]: blood lactate concentration.

<sup>a</sup>Difference from all other conditions.
Figure 4.1. Time-to-exhaustion performance recorded at baseline (TTE<sub>b</sub>), after exercising at MLSS<sub>p</sub> (TTE<sub>MLSS<sub>p</sub></sub>), and MLSS<sub>p+10</sub> (TTE<sub>MLSS<sub>p+10</sub></sub>) (Panel A). \( \dot{V}O_2 \)peak during the ramp-incremental and the various time-to-exhaustion tests. * different than TTE<sub>b</sub>; § different than TTE<sub>MLSS<sub>p</sub></sub>

The \( \dot{V}O_2 \) corresponding to RCP (3.29±0.48 L·min<sup>-1</sup> or 79.2±4.0% of \( \dot{V}O_{2\max} \)), MLSS<sub>p</sub> (3.31±0.53 L·min<sup>-1</sup> or 79.5±5.3% of \( \dot{V}O_{\max} \)) and [HHb]<sub>BP</sub> of the VL muscle (3.44±0.5 L·min<sup>-1</sup> or 84.5±8.3% of \( \dot{V}O_{2\peak} \)) were not different (\( P>0.05 \)). The PO at RCP, MLSS<sub>p</sub>, and [HHb]<sub>BP</sub> were 270±48 W (68±5% of PO<sub>peak</sub>), 231±49 W (58±5% of PO<sub>peak</sub>), and 292±46 (74±6% of PO<sub>peak</sub>), respectively, and were different from each other (\( P<0.05 \)). Baseline and peak values for the [HHb] signal from the ramp-incremental test were 28.1±11.2 and 43.6±20.1 for the VL muscle, and 18.2±6.3 and 29.7±11.2 for the RF muscle, respectively. The slopes of the linear regression in the [HHb] signal before and after the [HHb]<sub>BP</sub> of the VL muscle during the ramp-incremental test were 0.032±0.017 and 0.010±0.013, respectively (\( P<0.05 \)). In the RF muscle these slopes in the [HHb] signal, calculated below and above ~80% of the \( \dot{V}O_{2\peak} \), were 0.012±0.011 and 0.043±0.038, respectively (\( P<0.05 \)).
Figure 4.2 shows $\dot{V}O_2$, $V_E$, fB, HR, $[La^-]_b$, and RPE responses during the 30-min constant-PO trials at MLSS$_p$ and MLSS$_{p+10}$. The $\dot{V}O_2$ response stabilized during both MLSS$_p$ (min 10, 3.27±0.58 L·min$^{-1}$; min 30, 3.30±0.51 L·min$^{-1}$; $P>0.05$) and MLSS$_{p+10}$ trials (min 10, 3.41±0.56 L·min$^{-1}$; min 30, 3.46±0.52 L·min$^{-1}$) ($P>0.05$), despite a more pronounced upward drift during this latter condition. The $\dot{V}O_2$ functional gain was lower during MLSS$_p$ (10.1±1.2 ml·W$^{-1}$) than during MLSS$_{p+10}$ (10.6±1.2 ml·W$^{-1}$) ($P<0.05$). $V_E$ increased from min 10 (102±18 L·min$^{-1}$) to min 30 (108±20 L·min$^{-1}$; +5.8%) during MLSS$_p$ ($P<0.05$) and it increased to a greater extent during MLSS$_{p+10}$ (min 10, 115±22 L·min$^{-1}$; min 30, 131±25 L·min$^{-1}$; +13.8%; $P<0.05$). fB during MLSS$_p$ was 39.1±7.7 breath·min$^{-1}$ at min 10 and 42.5±8.1 breath·min$^{-1}$ at min 30; during MLSS$_{p+10}$ fB was 42.7±8.0 breath·min$^{-1}$ at min 10 and 50.8±11.9 breath·min$^{-1}$ at min 30. The magnitude of change of fB was greater during MLSS$_{p+10}$ (+18.9±7.5%) than during MLSS$_p$ (+8.7±3.2%). HR rose significantly from min 10 to min 30 in both conditions [min 10, 156±9 bpm; min 30, 162±9 bpm (+3.8%) during MLSS$_p$ ($P<0.05$); min 10, 162±9 bpm; min 30, 169±9 bpm (4.3%) during MLSS$_{p+10}$ ($P<0.05$)], and it was higher during MLSS$_{p+10}$ compared to MLSS$_p$ ($P<0.05$). $[La^-]_b$ values were higher during MLSS$_{p+10}$ compared to MLSS$_p$. During MLSS$_p$, $[La^-]_b$ was 5.1±1.8 mMol·L$^{-1}$ at min 10 and 5.6±1.7 mMol·L$^{-1}$ at min 30 ($\Delta=0.5±0.3$ mMol·L$^{-1}$). During MLSS$_{p+10}$, $[La^-]_b$ rose from 6.2±1.9 mMol·L$^{-1}$ at min 10 to 7.9±2.3 mMol·L$^{-1}$ at min 30 ($\Delta=1.7±1.1$ mMol·L$^{-1}$) ($P<0.05$). The delta change in $[La^-]_b$ from min 10 to min 30 was greater during MLSS$_{p+10}$ compared to MLSS$_p$ ($P<0.05$). RER values during MLSS$_{p+10}$ were greater than during MLSS$_p$ (0.97±0.05 vs. 0.95±0.04 respectively, $P<0.05$). RPE values were consistently higher throughout the exercise at MLSS$_{p+10}$ compared to MLSS$_p$ ($P<0.05$).
**Figure 4.2.** Group mean data (with SD bars) displaying $\dot{V}O_2$ (A) ventilation ($\dot{V}_E$) (B), frequency of breathing ($fB$) (C), heart rate (D), blood lactate concentration ($[La ]_b$) (E), and Rating of Perceived Exertion (RPE) (F) during 30-min constant-PO trials at MLSS$_p$ (open circles) and MLSS$_{p+10}$ (grey circles). Refer to results section for statistical significances.
Table 4.2 and Table 4.3 summarize the values of [HHb] and $tot[Hb]$, respectively, for each time point of interest during the constant-PO tests in the VL and RF muscles. Figure 4.3 displays the group mean profile for [HHb], $tot[Hb]$, and EMG (RMS) for VL and RF muscles during MLSS$_p$, MLSS$_{p+10}$, and the subsequent time-to-exhaustion trials. The [HHb] signal in the VL muscle was stable during MLSS$_p$ as well as during MLSS$_{p+10}$ and no further increase in the [HHb] signal was observed during the time-to-exhaustion trials performed immediately after each condition ($P>0.05$). The [HHb] signal was stable in the RF muscle throughout the trials performed at MLSS$_p$ but progressively increased during the trial at MLSS$_{p+10}$ so that the values were greater at 30 min compared to 10 min ($P<0.05$). Furthermore, during the TTE performed following the MLSS$_{p+10}$ ride, the [HHb] signal in the RF muscle rose above the levels achieved during the previous 30-min constant-PO trials ($P<0.05$). The $tot[Hb]$ values remained unchanged in the VL muscle at min 10 and min 30 during both MLSS$_p$ and MLSS$_{p+10}$ trials as well as during the TTE trials that followed each of those tests ($P>0.05$). On the other hand, the $tot[Hb]$ values progressively increased in the RF muscle in both the MLSS$_p$ and MLSS$_{p+10}$ trials from min 10 to min 30 ($P<0.05$), with a further significant increase observed during TTE$_{MLSS_p}$ and TTE$_{MLSS_{p+10}}$ ($P<0.05$). The baseline RMS values for VL and RF during MLSS$_p$ were 0.032±0.016 and 0.090±0.086, respectively. The baseline RMS values for VL and RF during MLSS$_{p+10}$ were 0.031±0.021 and 0.089±0.083, respectively. The EMG signal of the VL muscle increased progressively from min 10 to min 30 during MLSS$_p$ (from 85±73% to 132±159%) and MLSS$_{p+10}$ (from 180±126% to 224±187%) ($P<0.05$). The increases in the EMG signal of VL during the TTE$_{MLSS_p}$ and TTE$_{MLSS_{p+10}}$ from baseline were 276±159% and 282±240%, respectively ($P<0.05$). In the RF muscle, the EMG signal was stable during MLSS$_p$ (min 10, 111±99%; min 30, 98±153%) but progressively rose during MLSS$_{p+10}$ (min 10, 76±111%; min 30, 108±237%, ($P<0.05$)).
TTEMLSSp+10, the EMG signal of RF increased to 337±355% and 253±321% from baseline, respectively (P<0.05).

**Table 4.2. Comparison among baseline and peak values in the \[HHb\] signal (µM) in the vastus lateralis (VL) and rectus femoris (RF) muscles during MLSS (MLSSp), 10 W above MLSS (MLSSp+10), and subsequent time-to-exhaustion (TTE) trials.**

<table>
<thead>
<tr>
<th>muscle</th>
<th>condition</th>
<th>bsln 1</th>
<th>min 10</th>
<th>min 30</th>
<th>bsln 2</th>
<th>TTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td>MLSSp</td>
<td>30.5±10.7</td>
<td>39.6±13.6</td>
<td>39.6±13.2</td>
<td>29.3±10.5</td>
<td>39.9±13.5</td>
</tr>
<tr>
<td></td>
<td>MLSSp+10</td>
<td>29.1±11.1</td>
<td>38.8±15.0</td>
<td>39.6±15.1</td>
<td>26.9±10.3</td>
<td>40.3±16.1</td>
</tr>
<tr>
<td>RF</td>
<td>MLSSp</td>
<td>21.6±9.1</td>
<td>27.7±11.0</td>
<td>28.4±12.2</td>
<td>21.3±7.2</td>
<td>32.5±15.9</td>
</tr>
<tr>
<td></td>
<td>MLSSp+10</td>
<td>23.3±9.8</td>
<td>30.3±13.5</td>
<td>31.4±14.0</td>
<td>22.5±9.8</td>
<td>34.3±16.0</td>
</tr>
</tbody>
</table>

Data presented are mean values (± SD) at the time points of interest. Statistical differences were calculated within the same muscle across time during the two different exercise conditions (MLSSp and MLSSp+10).
a Difference from bsln 1 and bsln 2.
b Difference from bsln 1.
c Difference from 10 min.
d Difference from 10 min and 30 min.
(P<0.05)

**Table 4.3. Comparison among baseline and peak values in tot[Hb] signal (µM) in the vastus lateralis (VL) and rectus femoris (RF) muscles during MLSS (MLSSp), 10 W above MLSS (MLSSp+10), and subsequent time-to-exhaustion (TTE) trials.**

<table>
<thead>
<tr>
<th>muscle</th>
<th>condition</th>
<th>bsln 1</th>
<th>min 10</th>
<th>min 30</th>
<th>bsln 2</th>
<th>TTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td>MLSSp</td>
<td>91.9±26.0</td>
<td>102.3±28.2</td>
<td>103.0±29.6</td>
<td>106.6±32.0</td>
<td>102.1±30.3</td>
</tr>
<tr>
<td></td>
<td>MLSSp+10</td>
<td>89.5±28.3</td>
<td>98.8±32.1</td>
<td>101.6±31.6</td>
<td>104.9±31.7</td>
<td>100.5±32.3</td>
</tr>
<tr>
<td>RF</td>
<td>MLSSp</td>
<td>72.7±24.9</td>
<td>78.7±26.2</td>
<td>82.2±27.3</td>
<td>86.8±25.4</td>
<td>81.4±25.2</td>
</tr>
<tr>
<td></td>
<td>MLSSp+10</td>
<td>69.6±22.7</td>
<td>74.3±24.6</td>
<td>76.1±24.4</td>
<td>78.8±22.4</td>
<td>75.5±22.2</td>
</tr>
</tbody>
</table>

Data presented are mean values (± SD) at the time points of interest. Statistical differences were calculated within the same muscle across time during the two different exercise conditions (MLSSp and MLSSp+10).
a Difference from bsln 1 and bsln 2.
b Difference from bsln 1.
c Difference from 10 min.
(P<0.05)
Figure 4.3. Group mean (with SD bars) data displaying NIRS-derived local deoxygenated haemoglobin ([HHb]) (panels A and B) and total haemoglobin ([tHb]) (panels C and D) signals as well as EMG (panels E and F) signal during 30-min constant-PO trials at MLSS$_p$ (left panels) and MLSS$_{p+10}$ (right panels) for VL (open circles) and RF (grey circles). Refer to Table 2, Table 3, and results section for statistical significances.
**Discussion**

The novel findings of the study were that: 1) the greater increases in physiological responses while exercising only 10 W (≈100 ml·min⁻¹) above MLSS remarkably reduced time-to-exhaustion performance by approximately 50%; 2) participants achieved lower $\dot{V}O_2$peak values during the TTE\textsubscript{MLSSp+10} compared to those recorded during the TTE\textsubscript{MLSSp}, TTE\textsubscript{b}, and ramp-incremental test; 3) the [HHb] signal in the VL muscle achieved its peak amplitude at the intensity of exercise associated with MLSS, with no further increases during MLSS\textsubscript{p+10} or any of the subsequent time-to-exhaustion trials; 4) the behaviour of the [HHb] signal in the RF muscle markedly differed from that of the VL muscle, as it rose progressively when exercising at MLSS\textsubscript{p+10} and increased further during the following time-to-exhaustion trial.

*Physiological implications of exercising at a PO associated with and slightly above MLSS*

A stable $\dot{V}O_2$ response was observed during MLSS\textsubscript{p} and, contrary to our hypothesis, also during MLSS\textsubscript{p+10}. However, when exercising at MLSS\textsubscript{p+10} a progressive accumulation of blood lactate and a greater ventilatory response as well as greater rating of perceived effort occurred. An accumulation of lactate in the blood that cannot be stabilized is indicative of an accelerated glycolytic flux, as well as an inability for lactate clearance to match lactate production (Stainsby, 1986). Concomitantly with the accumulation of lactate, a greater ventilatory response when exercising at MLSS\textsubscript{p+10} was also observed. Multiple factors might have contributed to this exacerbated response, such as augmented metabolic acidosis, body temperature, respiratory muscle fatigue, and perception of effort (Poole et al., 1988; Hayashi et al., 2006; Marcora et al., 2008; Taylor & Romer, 2008). The greater increase in $\dot{V}_E$, along with the accumulation of lactate, seems to be a defining characteristic of exercising slightly above MLSS (compared to “at” MLSS).
The stability in the $\dot{V}O_2$ response while exercising at $MLSS_{p+10}$ is in contrast with a previous finding that showed a progressive rise in $\dot{V}O_2$ throughout the constant-PO trial above MLSS (Pringle & Jones, 2002). The reason for these contrasting results may lie on the different delta PO adopted. Indeed, while the present study employed a 10 W difference between the exercise trials for MLSS determination, the other investigation used on average a much larger increase (19 W). Increments greater than 10 W may induce non-stable $\dot{V}O_2$ responses when exercising above MLSS and may not provide sufficient sensitivity for identifying the highest PO associated with a stable lactate response (i.e., MLSS). In this perspective, these findings highlight that, despite the metabolic perturbations (e.g., lactate accumulation, greater increase in $\dot{V}E$) and greater functional gain (~0.5 ml·W$^{-1}$), $\dot{V}O_2$ can still appear to stabilize if the increment in metabolic demand above MLSS is relatively small. This emphasizes the fact that a stable $\dot{V}O_2$ response is not necessarily reflective of the overall metabolic steady-state during exercise at this intensity. This suggestion is supported by a previous study that demonstrated that a 30-min constant-PO exercise performed at the PO associated with MLSS altered pH and blood ammonia concentration, in spite of stable $\dot{V}O_2$, $\dot{V}E$, and $[La^-]_b$ responses (Baron et al., 2003). As suggested by these authors, MLSS may represent the highest intensity of exercise at which, despite some ongoing physiological changes, compensatory mechanisms may still be able to preserve overall metabolic steady-state. This also implies that if a careful “validation” of the individual critical intensity during dynamic exercise (i.e., cycling, running) is required, this should be performed by monitoring $[La^-]_b$ in response to small changes in sustained PO.

The progressive loss of metabolic steady-state and increased fatigue accumulation is also evidenced by the increased EMG activity of VL during $MLSS_p$ and $MLSS_{p+10}$. On the other hand, the EMG activity of the RF was stable during $MLSS_p$ but progressively increased during
Further increases were then observed in both muscles during the time-to-exhaustion trials. The increased central motor drive (firing rate and/or motor unit recruitment) observed during the 30-min constant-PO exercises may reflect the necessity to compensate for the progressive failure of peripheral contractile capacity as motor units fatigue (Decorte et al., 2012). In the case of the RF, it may also signify a greater need to support the action of the prime mover muscle (i.e., VL). The RF is a bi-articular muscle and a relatively greater activation of this muscle would be particularly needed to enhance hip flexion during the recovery phase of the pedal cycle and the generation of forward force during the early phase of the knee extension (Jorge & Hull, 1986).

*Performance implications of exercising at a PO associated with and slightly above MLSS*

The consequences of exercising at and slightly above MLSS on exercise performance capacity are highlighted by the reduction in the time-to-exhaustion tests. According to the traditional view, any exercise carried out at an intensity equivalent to or lower than that supposedly eliciting the highest but stable metabolic responses (i.e., MLSS and/or CP) can be sustained indefinitely without fatigue accumulation (Monod & Scherrer, 1965). However, the present study demonstrates that exercising for 30 minutes at MLSS, with elevated but stable metabolic responses (i.e., [La\(^-\)\(_b\)] and \(\dot{V}O_2\)), results in a significant reduction in subsequent exercise performance. This is in agreement with a recent study showing that exercising for 2 h in the heavy-intensity domain (at an intensity even lower than the one used in the present study) reduced subsequent work capacity carried out in the severe-intensity domain (Clark et al., 2018).

In addition to this, the present study found a disproportionate decrement in time-to-exhaustion performance (~50%) after exercising at MLSS\(_{p+10}\) which was accompanied by a decrease in \(\dot{V}O_2\)\(_{\text{peak}}\) values (~7%) observed during the TTE\(_{\text{MLSS}_{p+10}}\). This reduction in performance highlights the “non-linear” nature of the decrease in maximal exercise capacity when sustaining work in the
severe-intensity domain which may be caused by a significantly greater decrease of glycogen stores and/or a disproportionate build-up of metabolites. Regarding the lower $\dot{V}O_2$ values recorded, although a decreased maximal capacity to deliver and/or utilize O$_2$ cannot be excluded, it can be possible that in some subjects ($n=4$) the premature exhaustion, that occurred within 1.5 min, could have limited the full expression of the $\dot{V}O_2$ kinetics, thus truncating the $\dot{V}O_2$ response before the achievement of peak values. Furthermore, the contribution of the higher perception of effort after exercising at MLSS$_{p+10}$ must be also considered when explaining this greater reduction of performance. Indeed, RPE values were consistently higher during exercise at MLSS$_{p+10}$ compared to MLSS$_p$. Thus, a higher perception of effort at the onset of the TTE$_{MLSSp+10}$ may have contributed to a more rapid achievement of maximal RPE and an earlier termination of the exercise (i.e., task disengagement). This effort-based decision to disengage from exhaustive exercise prematurely is postulated to occur when individuals, due to a “higher-than-normal” perception of effort, are no longer willing to exert effort (Marcora et al., 2008; Pageaux & Lepers, 2016).

After exercising in the severe-intensity domain at a PO substantially above MLSS a reduction in performance is to be expected. The findings from the present study are notable as they demonstrate that even a very small increase in PO (+10 W, ~3-5%) above MLSS, reflected in a ~100 ml$\cdot$min$^{-1}$ increase in $\dot{V}O_2$, results in a progressive rise in [La$^-$]$_b$ and disproportionately impairs subsequent exercise performance. This is an important observation, as most methods utilized to estimate the PO associated with MLSS, and similar thresholds or critical intensities (supposedly eliciting stable [La$^-$]$_b$ and $\dot{V}O_2$ responses) generally have an error in their estimate that is greater than 10 W (Pringle & Jones, 2002; Mattoni Maturana et al., 2016). Where an accurate determination of the heavy-to-severe-intensity boundary of exercise is required (e.g., for exercise prescription), the PO
value estimated from any model should be carefully confirmed against validated protocols and physiological criteria to minimise unintended responses.

*Muscle deoxygenation during constant-PO and time-to-exhaustion trials in the VL and RF muscles*

The exercise design adopted in the current study allowed, for the first time, for the characterization of the [HHb] profiles at exercise intensities surrounding MLSS. In accordance to our hypothesis, these findings experimentally demonstrated what previous investigations describing the [HHb] signal during ramp-incremental test had speculated (Keir et al., 2015; Iannetta et al., 2017a): that is, the achievement of peak values in the [HHb] signal of the VL muscle at a metabolic rate corresponding to MLSS, that is independent from the exercise protocol adopted (i.e., ramp-incremental versus constant-PO exercise), and the existence of a potential for further increase in this signal in the RF muscle beyond this intensity of exercise.

Overall, these data corroborate previous evidence highlighting the diverse relative contribution of each of the muscles (or muscle portions) engaged in the exercise task to the whole-body arteriovenous O$_2$ difference (Chin et al., 2011; Okushima et al., 2015, 2016; Iannetta et al., 2017b) which arises from the heterogeneity in motor unit recruitment patterns (Chin et al., 2011; Iannetta et al., 2017b), fibre-type expression (McDonough et al., 2005), and the resulting vascular dynamic controls (McDonough et al., 2005; Copp et al., 2010) that characterize the active muscles. What is interesting is that the greater muscle activity in the VL muscle (i.e., greater EMG signal) during MLSS$_{p+10}$ and subsequent time-to-exhaustion trials was not accompanied by an increase in the [HHb] signal (in contrast to the RF muscle). It could be expected that the additional recruitment of muscle fibres would be accompanied by a greater fractional O$_2$ extraction, reflected in a rise in the [HHb] signal. However, this was clearly not the case in the VL muscle. These differences
between the VL and RF [HHb] signals resemble those observed in this and previous studies (Chin et al., 2011; Iannetta et al., 2017b) when exercising above the RCP during ramp-incremental test.

The physiological mechanisms underlying these different patterns of behaviour of the VL and RF muscles in regulating the delivery and utilization of O₂ are a current topic of debate (Chin et al., 2011; Murias et al., 2013; Spencer et al., 2014; Okushima et al., 2015; Boone et al., 2016b; Inglis et al., 2017; Iannetta et al., 2018c). What is important in relation to the findings of the present study is that these behaviour patterns might be reflective of the metabolic changes occurring when transitioning from the heavy to the severe exercise intensity domain. In this perspective, these may be representative not only of divergent local capacities to regulate the Qₘₐₓ-to-VO₂m ratio, but they may also be indicative of the fact that some muscle areas (i.e., VL vs RF) may contribute more than others to the rise in metabolites linked to the progressive acceleration of the glycolytic flux and fatigue accumulation when exercising in the severe-intensity domain.

**Limitations**

The present study used the PO corresponding to MLSS to separate the heavy from the severe-intensity domain. Although MLSS experimentally determines the highest PO associated with elevated but “stable” metabolic responses (e.g., stable [La⁻¹]b), it is important to acknowledge that this “method” also has a measurement error. For example, in the current study the measurement error could have been as high as 9 W (given the 10 W delta we used), leading to an underestimation of the PO corresponding with MLSS. Considering this, it is possible that in some circumstances the increase in PO above the “true” MLSS (during the MLSS_p+10 condition) could have been even less than 10 W. However, this observation strengthens the interpretation of the findings of the present study.
It is important to acknowledge that TTEb was always performed as the last testing session. Although we cannot exclude that this choice exacerbated the differences in time-to-exhaustion performance (e.g., due to a possible greater motivation of the participants), however, considering that i) the rides at MLSSp and MLSS_{p+10} were evenly distributed with marked differences recorded, ii) the \( \dot{V}O_{2\text{peak}} \) values were similar between TTE_{MLSSp} and TTE_{b}, and iii) also the RPE values recorded at the end of the time-to-exhaustion trials were similar across the tested conditions, we believe that this factor marginally contributed to the observed differences.

Another aspect to consider is the interpretation of the EMG data. Although we attempted to reduce the variability of the EMG signal by normalizing it against baseline cycling at 80 W (Torres-Peralta et al., 2014; Iannetta et al., 2017b), it is important to acknowledge that this variability remained quite large. Furthermore, we cannot ascertain whether an increase in the EMG signal is truly reflective of differences in muscle fibres recruitment (e.g., greater number of muscle fibres recruited reflected in a greater force production). Therefore, the EMG findings should be interpreted with some caution.

**Perspective**

The novelty of the present study is that we used a very small increase in PO (10 W) above the MLSS to evaluate how such small increments in PO affect metabolic responses and consequent performance capacity. We found that exercising for 30 min slightly above the PO at MLSS disproportionally reduces subsequent exercise performance. The findings from this study have important implications for exercise prescription and they suggest that the adoption of stringent methods is required when establishing the heavy-to-severe exercise intensity boundary with accuracy. Furthermore, the different dynamics in the behavior of the [HHb] signal confirms that the regulation of the delivery and utilization of O\(_2\) is different across the quadriceps muscles, and
may reveal that metabolic perturbations of exercising in the severe-intensity domain may be
greater in some muscles compared to others. However, further investigations will be needed to
clarify the specific influence of these heterogeneities when exercising above MLSS.
CHAPTER V

Establishing the V̇O₂ versus constant-work rate relationship from ramp-incremental exercise: Simple strategies for an unsolved problem.

Introduction

With step-transitions to work rates within the moderate (below the lactate threshold (LT)), heavy (above LT but below the “maximal metabolic steady-state” (MMSS)), and severe (above MMSS) exercise intensity domains, the phase II time constant (τ) of O₂ uptake (V̇O₂) and total gain (G; i.e., ΔV̇O₂/Δwork rate) of the V̇O₂ response become progressively greater, until steady-state V̇O₂ can no longer be attained (i.e., within the severe-intensity domain) (Poole & Jones, 2012). With ramp-incremental exercise, these intensity domain-specific V̇O₂ response characteristics (varying τ and G) yield a “linear” rise in V̇O₂ (Rossiter, 2011; Wilcox et al., 2016; Keir et al., 2016a). Knowledge of this phenomenon is important because V̇O₂ is the gold standard of aerobic exercise intensity and ramp protocols are the primary tool used to determine a work rate that will elicit a target V̇O₂ (Whipp et al., 1981). However, it is often assumed that any specified V̇O₂ can accurately be targeted selecting a work rate by interpolating the ramp-V̇O₂ response (DiMenna & Jones, 2009). This supposition, of course, neglects that V̇O₂ lags increasingly the changes in work rate during ramp-exercise, particularly at intensities exceeding LT, and that when performed constantly, any work rate will inevitably elicit a greater V̇O₂ than expected based on the ramp V̇O₂ response (Keir et al., 2018a). Interestingly, ramp-V̇O₂ responses computed from varying τ and G have predicted that compared to faster ramp-protocols (i.e., 30 W·min⁻¹), slow ramp-protocols (i.e., ≤15 W·min⁻¹) would reduce the “gap” between steady-state and ramp-V̇O₂ at any given work rate (Wilcox et al., 2016; Keir et al., 2016a). However, empirical data supporting these hypotheses over a wide range of ramp-protocols are still needed.
Irrespective of ramp-slope, another factor to consider when establishing the \( \dot{V}O_2 \)-to-constant-work rate relationship are the \( \dot{V}O_2 \) kinetics at the onset of ramp-exercise (Boone & Bourgois, 2012). After ramp-onset, the muscle-mouth circulatory transit-time and the muscle \( \dot{V}O_2 \) kinetics delay the beginning of the linear rise in \( \dot{V}O_2 \), such that \( \dot{V}O_2 \) is “right-shifted”, or offset, from its corresponding work rate (Keir et al., 2018a). To nullify the effect of this time delay, ramp-\( \dot{V}O_2 \) data are typically aligned with their corresponding work rate by computing and correcting for the mean response time (MRT) (Boone & Bourgois, 2012; Fontana et al., 2015). For 30-W·min\(^{-1}\) ramp-protocols, we have recently shown that the MRT is best calculated by measuring prior to ramp-exercise the steady-state \( \dot{V}O_2 \) during a 6-min bout of moderate exercise which, projected on the ramp-\( \dot{V}O_2 \)-to-work rate relationship, provides an accurate estimate of time (s) (or W) by which to left-shift the \( \dot{V}O_2 \) data (Iannetta et al., 2019). Given that \( G \) in the moderate domain should be greater in response to slower ramp-protocols (Scheuermann et al., 2002; Wilcox et al., 2016; Keir et al., 2016a), it is hypothesized that the difference in seconds between the ramp and constant-load \( \dot{V}O_2 \) (i.e., the MRT) would be reduced progressively from faster to slower ramp-protocols; necessitating less of a correction to achieve the same work rate at LT across different ramp-slopes.

Finally, a disadvantage of traditional ramp-protocols that is yet to be overcome is that \( \dot{V}O_2 \) and work rate associated with MMSS cannot be identified (Keir et al., 2018a). The \( \dot{V}O_2 \) at the respiratory compensation point (RCP) is consistently similar to that at MMSS (Scheuermann & Kowalchuk, 1998; Keir et al., 2015; Iannetta et al., 2018a, 2018b) but the high variability in work rate between these two markers has led to the interpretation that the RCP is not a suitable surrogate (Dekerle et al., 2003; Broxterman et al., 2015; Leo et al., 2017; Caen et al., 2018). This interpretation, however, does not consider that the work rate at RCP is a function of the characteristics of the ramp-slope (Scheuermann & Kowalchuk, 1998; Leo et al., 2017; Keir et al.,
If MMSS and RCP represent the same physiological event, then strategies that: 

1) provide sufficient time for the VO₂ control system to develop its kinetics during ramp-exercise and; 

2) apply accurate left-shifting of the VO₂ data should result in similar work rates at MMSS and RCP that are less variable than previously reported (Leo et al., 2017).

Therefore, the present study characterized the VO₂ response to five ramp-protocols of varying slope (5, 10, 15, 25, and 30 W·min⁻¹ increments). Thereafter, the discrepancy between constant-work rate and ramp-work rate at a given moderate VO₂ (i.e., MRT) was determined to identify and compare between ramp-protocols the work rates corresponding to LT and RCP. It was hypothesized that the dissociation between VO₂ and work-rate during ramp-exercise would be explained by the characteristics of work rate increase (i.e., faster ramp-slopes would result in greater dissociations between the VO₂ response and its corresponding work rate) and would be reduced, or even abolished, with slower ramp-protocols. Based on this idea, it was also hypothesized that the work rate at RCP and MMSS would be the same when using the slowest ramp-protocols.

Methods

Participants

Eleven recreationally-trained participants (6 men and 5 women; mean±SD, age=28±7; height=174±9; weight=66±8) completed the study. None of the participants were undergoing any medical treatment that could potentially alter their cardiorespiratory and metabolic responses to exercise. All participant signed an informed consent and all procedures were approved by the Conjoint Health Research Ethics Board of the University of Calgary in compliance with the latest version of the declaration of Helsinki.
Experimental protocol

The study encompassed three phases: *i*) five ramp-incremental tests to exhaustion; *ii*) four-five time-to-exhaustion trials for critical power (CP) estimation; *iii*) two-three constant-load trials to measure/confirm the physiological variables elicited by the work rate at the estimated CP. All testing sessions were performed on an electromagnetically-braked cycle ergometer (Velotron, RacerMate, Seattle, USA). Participants performed the tests on separate days, with 6±1 day-interval between the ramp-tests, and a minimum of 48h and a maximum of 72h interval between the time-to-exhaustions and between the constant-load trials. All testing sessions were performed at the same time of the day (±30 min). The preferred cadence was self-selected by the participants during their first laboratory visit, recorded, and maintained the same for subsequent visits. During both ramp- and time-to-exhaustions tests, the tests were stopped when participants could no longer maintain the self-selected cadence by 10 rpm for more than 10 s, or at volitional exhaustion despite strong verbal encouragement. In all conditions, participants were blinded to the work rate and elapsed time but received visual feedback on their cadence.

*Ramp-tests.* The ramp-slopes were 5, 10, 15, 25, and 30 W⋅min⁻¹ increment-rates and were initiated from a 50 W baseline cycling of 4 min. The 25 W⋅min⁻¹ ramp was always performed first; thereafter, the remaining ramp-tests were performed in a randomized order. Each ramp-test was preceded by a step-transition protocol within the moderate-intensity domain. This transition, which consisted of 6 min at 20 W followed by 6 min at 100 W, served the purpose of calculating the ramp-MRT of V̇O₂, as previously described (Iannetta *et al.*, 2019) (see also *Data analysis* section).

*Time-to-exhaustion trials.* For the estimation of CP through the power-duration relationship, the work-rates for the four-five time-to-exhaustion trials were selected to elicit exhaustion times between approximately 1.5-20 min with the objective of generating an even distribution curve.
between the trials (Morton, 2006; Mattioni Maturana et al., 2018). With this logic, percent work rate ranging from 70% to 110% of the peak work rate achieved during the 25 W·min\(^{-1}\) were chosen (Mattioni Maturana et al., 2016). The order of these trials was randomized, and each consisted of a 4-min baseline cycling at 50 W, which was followed by the square-wave transition to the predetermined work rate.

**Constant-load trials.** Participants performed additional constant-load trials of 30 min of duration at CP, or to exhaustion (which ever occurred earlier), to measure steady-state physiological responses (stability of \(\dot{V}O_2\) and blood lactate concentration ([La\(^-\)\(_b\)]), such that attainment of true maximal metabolic steady-state could be confirmed. In circumstances where participants could not complete the task (n=1), or \(\dot{V}O_2\) and/or [La\(^-\)\(_b\)] were unstable (n=2), another trial was performed at a lower work rate (-10 W). Vice versa, if \(\dot{V}O_2\) and [La\(^-\)\(_b\)] were stable at CP the following ride was performed at higher work rate (+10 W). A steady-state was established as no further appreciable change in \(\dot{V}O_2\) (\(\leq 100 \text{ ml} \cdot \text{min}^{-1}\)) (Keir et al., 2014) after 15 min within the trial in concomitance with stable [La\(^-\)\(_b\)] responses (\(\leq 1 \text{ mM}\)) (Heck et al., 1985). We anticipate that on average there was no difference between estimated work rate at CP and the “confirmed” work rate corresponding to stable metabolic responses (MMSS) (see Results section). [La\(^-\)\(_b\)] measurements were taken at baseline and every five minutes after the work rate was instantaneously increased to the predetermined work rate. At “sensitive” time-points (10, 15, 30 min), [La\(^-\)\(_b\)] measures were taken in triplicate and the average of the two closest measures was used for further analysis.

**Data collection**

Gas exchange and ventilatory variables were measured breath-by-breath with a metabolic cart (CPET, Cosmed, Rome, Italy). The system consisted of a low dead space turbine as well as \(O_2\) and carbon dioxide (CO\(_2\)) gas analyzers; these were calibrated with a syringe of known volume (3 L)
and a gas-mixture of known concentration (16% O₂; 5% CO₂; balance N₂), respectively. Capillary blood samples were drawn from a finger-prick and immediately analyzed for \([\text{La}^-]_b\) (Biosen C-Line, EKF Diagnostics, Barleben, Germany).

Data analyses

CP and \(W'\) parameters were estimated using the three-parameter hyperbolic model using non-linear least squares regression analysis (Morton, 2006), as follow:

\[
t = \frac{W'}{(P - CP)} + \frac{W'}{(CP - P_{\text{max}})}
\]

where \(t\) is time to exhaustion (s), \(W'\) is the anaerobic work capacity in joules, CP is the critical power in watts, and \(P_{\text{max}}\) is the maximal “instantaneous” power. The “goodness of fit” for the hyperbolic model was determined as the 95% confidence interval for CP. \(\dot{V}O_2\) responses during the constant-load 30-min trials at CP were averaged into 2 minutes bins so that responses across time could be examined. The \(\dot{V}O_2\) corresponding to MMSS was calculated as the average of the last 5 min of the 30-min trial.

The raw ventilatory and gas exchange data for each participant were scrutinized by two experts to identify the \(\dot{V}O_2\) corresponding to LT and RCP. In the circumstances of a disagreement of more than 100 ml·min⁻¹, the experimenters re-evaluated together the profiles until an agreement was reached. Briefly, LT corresponded to the point at which \(\dot{V}CO_2\) began to increase out of proportion in relation to \(\dot{V}O_2\), coincidental with a systematic rise in the \(\dot{V}E/\dot{V}O_2\) relation and end-tidal PO₂, whereas the ventilatory equivalent of \(\dot{V}CO_2\) (\(\dot{V}E/\dot{V}CO_2\)) and end-tidal PCO₂ were stable (Beaver et al., 1986). RCP was identified as the point corresponding to the systemic fall in end-tidal PCO₂ after a period of isocapnic buffering (Whipp et al., 1989). This point was confirmed by examining
the \( \dot{V}_E/\dot{V}CO_2 \) and \( \dot{V}_E/\dot{V}O_2 \) relationships as well as by identifying the second breakpoint in the \( \dot{V}_E \)-to-\( \dot{V}O_2 \) relation.

\( \dot{V}O_2 \) data during the moderate step-transition, ramp, time-to-exhaustion, and constant-load trials were edited on an individual basis by removing aberrant data that lay 3 SD from the local mean. Subsequently, all trials were interpolated on a second-by-second basis and time aligned.

The MRT of \( \dot{V}O_2 \) for each ramp-exercise trial was calculated, as previously described (Iannetta et al., 2019). Briefly, a linear fit was used to fit the ramp-\( \dot{V}O_2 \) data from the onset of its systematic increase up to the previously established LT. Then, the average \( \dot{V}O_2 \) of the last two minutes of the moderate step-transition was used to linearly extrapolate the corresponding work rate during the ramp-exercise. The difference in watts between the work rate of the moderate step-transition and the extrapolated ramp-work rate at the common \( \dot{V}O_2 \) corresponded to the MRT<sub>SS</sub> and was used to align the ramp-\( \dot{V}O_2 \) with the corresponding ramp-work rate (Iannetta et al., 2019). Thereafter, the work rates at LT and RCP during each ramp-protocol (RCP<sub>5</sub>, RCP<sub>10</sub>, RCP<sub>15</sub>, RCP<sub>25</sub>, RCP<sub>30</sub>) were linearly extrapolated from the aligned \( \dot{V}O_2 \)-to-work-rate profiles.

Additionally, the MRT was also calculated with the commonly used “back extrapolation” technique (Boone et al., 2008a). Briefly, a piecewise equation that included two linear segments was used:

\[
\begin{align*}
f &= \text{if } (t < \text{MRT}) \text{ use } g(t); \quad g(t) = i_1 + s_1t; \quad i_2 = i_1 + s_1t; \quad h(t) = i_2 + s_2t - \text{MRT} \\
\end{align*}
\]

where \( f \) is the piecewise function, \( t \) is time and \( g \) and \( h \) are \( \dot{V}O_2 \), MRT is the time corresponding to the intersection of the two regression lines (MRT<sub>LIN</sub>), \( i_1 \) and \( i_2 \) are the intercepts of the first and second linear function, respectively, and \( s_1 \) and \( s_2 \) are the slopes. The \( s_1 \) parameter was fixed at
“zero”. The two linear segments were fitted from the last 3 min of the pre-ramp baseline (t = -180 s) to the previously established LT (Keir et al., 2018a).

After averaging the \( \dot{V}O_2 \) data in response to the different ramp-protocols in 5 s bins, each \( \dot{V}O_2 \) profile was partitioned in three domains of intensity demarcated by the previously established \( \dot{V}O_2 \) at LT and MMSS. Thereafter, G during each ramp-exercise was calculated in the moderate (G\text{MOD}) (below LT), in the heavy (G\text{HVY}) (between LT and MMSS), and in the severe (G\text{SVR}) (above MMSS) domains and expressed as ml·min\(^{-1}\)·W\(^{-1}\). A linear fit of the \( \dot{V}O_2 \) responses as function of work rate was used to retrieve G in each domain. In the moderate-intensity domain, the linear fit was initiated after the lag phase of the \( \dot{V}O_2 \), whereas in the severe domain the linear fit was terminated before the (potential) plateau in \( \dot{V}O_2 \). All data editing and fitting was performed with customized functions of a commercially available statistical software package (Origin, OriginLab Corp., Northampton, MA).

**Statistical analyses**

Data are presented as mean±SD. Repeated-measures ANOVA were performed to detect possible differences in response to the different ramp-protocols for: peak work rate, \( \dot{V}O_2\text{max} \), G (during the same ramp-protocol across the different domains as well as across the ramp-protocols for the same domain), MRT\text{SS}, MRT\text{LIN}, LT and RCP (with either one approach for left-shifting). Repeated-measures ANOVA were also performed to compare the \( \dot{V}O_2 \) at the RCP values and at MMSS, as well as to compare the work rate at the RCP, MMSS, and CP. Bland-Altman plots were performed to calculate the agreement (e.g., bias and limits of agreement) between markers of intensity. Pearson’s index, root-mean square error (RMSE), and Lin’s concordance coefficient (CCC) were used to calculate correlation, typical error, and concordance, respectively, between the intensity markers. As previously described (Leo et al., 2017), the CCC was interpreted as follow: almost
perfect agreement = CCC > 0.99, substantial agreement = 0.95 > CCC < 0.99, moderate agreement = 0.90 > CCC < 0.95, and poor agreement = CCC < 0.90 (McBride, 2005). Statistical significance was set at a α value of <0.05. Where appropriate a post-hoc analysis was performed. All statistics were performed using SPSS version 23 (SPSS, IBM, Chicago, IL).

**Results**

While peak work rate progressively increased (P<0.05), VO\(_{2}\)\(_{\text{max}}\) was not different with increasing ramp-slopes (P>0.05) (Table 1). G was progressively smaller with increasing ramp-slopes regardless of the exercise intensity domain (for locus of significant differences see Table 5.1). Figure 5.1 displays the average G for each ramp-protocol modelled within each intensity domain as function of work rate.

The MRT\(_{\text{SS}}\) of VO\(_{2}\) expressed in s and W (computed from the moderate step-transitions preceding the ramp-protocols) in response to 5, 10, 15, 25, and 30 W·min\(^{-1}\) ramp-tests was 7±6, 14±9, 21±12, 24±10, 29±11 s, and 1±1, 2±1, 5±3, 10±4, 15±6 W, respectively, and these were all different from each other (P<0.05) (Figure 5.2). The MRT\(_{\text{LIN}}\) of VO\(_{2}\) expressed in s and W (computed from the “back-extrapolation” approach) in response to 5, 10, 15, 25, and 30 W·min\(^{-1}\) ramp-tests was 38±44, 69±50, 42±23, 18±14, 21±11 s, and these were not different from each other (P>0.05). When expressed on the basis of work rate MRT\(_{\text{LIN}}\) values (from the slowest to the fastest ramp-slope) were 3±4, 12±8, 11±6, 8±6, 11±5 W. The MRT\(_{\text{LIN}}\) from the 5 W·min\(^{-1}\) ramp was different from all the others (P<0.05), with the remaining values not different from each other (P<0.05).

LT and RCP (on the basis of both VO\(_{2}\) and work rate) are shown in Table 5.1. There was no difference across the ramp-exercise protocols for the VO\(_{2}\) at either LT or at RCP (P>0.05).
Table 5.1. Peak and submaximal parameters and indices of aerobic performance during different ramp-protocols.

<table>
<thead>
<tr>
<th>Ramp-slope (W·min⁻¹)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>25</th>
<th>30</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Work rate peak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( W )</td>
<td>262±55 $</td>
<td>291±59 $</td>
<td>310±63 $</td>
<td>340±66 $</td>
<td>353±69 $</td>
<td>-</td>
</tr>
<tr>
<td>( \dot{V}O_2_{\text{max}} ) ( L\cdot\text{min}^{-1} )</td>
<td>3.35±0.68</td>
<td>3.44±0.67</td>
<td>3.44±0.69</td>
<td>3.44±0.74</td>
<td>3.44±0.72</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>([\text{La}^-]_b) ( mM )</td>
<td>10.6±2.3</td>
<td>11.2±1.9</td>
<td>11.9±2.4</td>
<td>12.4±2.7</td>
<td>12.5±2.0</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td><strong>LT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( L\cdot\text{min}^{-1} )</td>
<td>2.10±0.36</td>
<td>2.08±0.33</td>
<td>2.09±0.35</td>
<td>2.10±0.33</td>
<td>2.10±0.36</td>
<td>0.03±0.02</td>
</tr>
<tr>
<td>( W )</td>
<td>147±27</td>
<td>150±31</td>
<td>150±34</td>
<td>155±29</td>
<td>152±33</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td><strong>RCP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( L\cdot\text{min}^{-1} )</td>
<td>2.83±0.65</td>
<td>2.84±0.59</td>
<td>2.82±0.61</td>
<td>2.86±0.60</td>
<td>2.86±0.61</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>( W )</td>
<td>212±54 $</td>
<td>221±53 $</td>
<td>231±55 $</td>
<td>242±56 $</td>
<td>247±58 $</td>
<td>-</td>
</tr>
<tr>
<td>( G_{\text{MOD}} )</td>
<td>10.0±1.1 \text{a}</td>
<td>9.9±0.6 \text{b}</td>
<td>10.1±0.9 \text{c}</td>
<td>9.0±0.7 \text{d}</td>
<td>8.9±1.3 \text{b,d}</td>
<td>-</td>
</tr>
<tr>
<td>( G_{\text{HVY}} )</td>
<td>11.2±1.2 \text{a}</td>
<td>10.6±1.6</td>
<td>9.2±1.2 \text{*}</td>
<td>8.8±0.9 \text{*,#}</td>
<td>8.3±1.4 \text{c,#}</td>
<td>-</td>
</tr>
<tr>
<td>( G_{\text{SVR}} )</td>
<td>15.0±3.9 \text{a}</td>
<td>11.8±1.9</td>
<td>9.2±1.7 \text{†}</td>
<td>7.6±2.0 \text{†}</td>
<td>6.6±1.5 \text{†,&amp;}</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. LT: lactate threshold. RCP: respiratory compensation point.

\( G_{\text{MOD}} \): \( \dot{V}O_2 \) gain in the moderate domain. \( G_{\text{HVY}} \): \( \dot{V}O_2 \) gain in the heavy domain. \( G_{\text{SVR}} \): \( \dot{V}O_2 \) gain in the severe domain.

\$ Denotes significant differences across all ramp-protocols

\text{a} Denotes significant differences within the same domain

\text{b} Denotes significant difference between \( G_{\text{MOD}} \) and \( G_{\text{SVR}} \)

\text{c} Denotes significant difference between \( G_{\text{HVY}} \) and \( G_{\text{SVR}} \)

\text{*} Denotes significant differences from 10 and 15 W·min⁻¹ ramp

\text{†} Denotes significant differences from 5 and 10 W·min⁻¹ ramp

\text{*} Denotes significant differences from 5 W·min⁻¹ ramp

\text{#} Denotes significant differences from 10 W·min⁻¹ ramp

\text{&} Denotes significant differences from 15 W·min⁻¹ ramp
Figure 5.1. $\dot{V}O_2$ functional gain as function of work rate during each ramp protocol. LT (lactate threshold) and MMSS (maximal metabolic steady-state) demarcate the moderate, heavy, and severe-intensity domains. To note is the “upward” and “downward” curvilinear increases occurring with slower and faster ramp-protocols, respectively.
Figure 5.2. MRT of \( \dot{V}O_2 \) in response to each ramp-protocol.

LT and RCP (on the basis of both \( \dot{V}O_2 \) and work rate) are shown in Table 5.1. There was no difference across the ramp-exercise protocols for the \( \dot{V}O_2 \) at either LT or at RCP (\( P>0.05 \)). However, in response to the different ramp-slopes and after left-shifting the ramp-\( \dot{V}O_2 \) data (with MRT\(_{SS}\)), whereas the work rate associated with LT was not different (\( P>0.05 \)), the work rate at RCP progressively increased with faster ramp-slopes (\( P<0.05 \)) (Figure 5.3, panels A, B). When using MRT\(_{LIN}\) for left-shifting the ramp-\( \dot{V}O_2 \), the work rate at LT was greater during the 25 and 30 W·min\(^{-1}\) compared to the other ramp-protocols (\( P<0.05 \)).

Time-to-exhaustion during the severe domain trials for CP estimation were on average (from the shortest to the longest): 1.8±0.8, 3.5±1.5, 6.7±1.2, 11.6±1.5, 16.2±2.1 min (the longest trial was required in seven participants), and corresponded on average to: 110±3, 93±3, 82±10, 75±5, and 71±5% peak work rate of the 25 W·min\(^{-1}\) ramp. The group mean average of the CP and \( W' \) parameter estimates from the hyperbolic model were 214±59 W and 32.3±12.5 kJ, respectively.
The $R^2$ and the SEE of the estimate were 0.99±0.01 and 6.3±5.0 W, respectively. In three participants, metabolic stability was possible at work rates greater than the one predicted by CP. One participant could not complete the 30-min trial at CP. In this participant, and in two others who were able to complete the trial, end-trial $\dot{V}O_2$ was within 95\% of $\dot{V}O_{2\text{max}}$ and $[\text{La}^-]_b$ values were similar to those recorded at the end of the ramp-tests. In the remaining five participants, the work rate at the predicted CP conformed to MMSS. There was no difference between the work rate at CP and the confirmed work rate at MMSS (215±55 W), which corresponded to 63±5\% of peak work rate recorded at the end of the 25 W·min$^{-1}$ ramp. The group average of $\dot{V}O_2$ at the end of the 30-min trial at MMSS was 2.90±0.67 (L·min$^{-1}$) which corresponded to 81.9±5.9\% of $\dot{V}O_{2\text{max}}$, and was not different from any of the $\dot{V}O_2$ values at RCP measured during the different ramp-protocols (range bias = 0.03-0.07 L·min$^{-1}$; $P>0.05$) and highly correlated to the $\dot{V}O_2$ values at RCP (range $r = 0.987-0.990$; $P<0.05$). Group average $[\text{La}^-]_b$ values at the end of the 30-min trial at MMSS was 5.6±2.0 mM. End-trials group average $\dot{V}O_2$ and $[\text{La}^-]_b$ values at 10 W above MMSS were 3.17±0.69 (L·min$^{-1}$), which corresponded to 91.9±4.9\% of $\dot{V}O_{2\text{max}}$, and 8.2±2.4 mM, respectively. Figure 5.3 depicts the profiles of end tidal pressure of CO$_2$ (mmHg) and minute-ventilation (L·min$^{-1}$) during 5 and 25 W·min$^{-1}$ ramp-test (panels A and C), power-duration relationship for CP estimation (panel B), and $\dot{V}O_2$ response during the constant-load trial at the estimated CP for a representative subject (panel D).
Figure 5.3. Gas-exchange and ventilatory profiles during 5 (5-s average data) and 25 W·min⁻¹ ramp-protocols (panels A and C), power-duration relationship for critical power (CP) estimation, and \( \dot{VO}_2 \) response during 30-min constant-load trial at CP in a representative subject.

Table 5.2 displays the comparison between the work rate at MMSS and at CP and RCP from each ramp-protocol. The agreement was nearly perfect between MMSS and RCP₅ (\( P>0.05 \)). This comparison was characterized by small bias, limits of agreement, and RMSE, and high concordance (Figure 5.4 panel C and D). With faster ramp-slopes, this agreement worsened and the work rate at MMSS was lower than RCP in all circumstances (\( P<0.05 \)). The comparison between estimated CP and RCP showed that both RCP₅ and RCP₁₀ are not different from estimated CP (\( P>0.05 \)). However, these comparisons were characterized by large limits of agreement and
RMSE. With increasing ramp-slopes, then, the agreement between estimated CP and RCP worsened with significant differences detected between the two indices ($P<0.05$).

Table 5.2. *Comparison on the basis of work rate between MMSS and RCP (corrected by MRT$_{SS}$) and between CP and RCP (corrected by MRT$_{LIN}$).*

<table>
<thead>
<tr>
<th>Ramp-slope (W·min$^{-1}$)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMSS vs RCP (corrected by MRT$_{SS}$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bias (W)</td>
<td>-3</td>
<td>6</td>
<td>16</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>r</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>RMSE (W)</td>
<td>6</td>
<td>11</td>
<td>19</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>CCC</td>
<td>0.99</td>
<td>0.98</td>
<td>0.94</td>
<td>0.87</td>
<td>0.83</td>
</tr>
<tr>
<td>(0.97-1.00)</td>
<td>(0.92 - 0.99)</td>
<td>(0.83 - 0.98)</td>
<td>(0.69 – 0.95)</td>
<td>(0.62 – 0.93)</td>
<td></td>
</tr>
</tbody>
</table>

| **CP vs RCP (corrected by MRT$_{LIN}$)** |     |     |     |     |     |
| Bias (W)                   | -6  | -4  | 10  | 29  | 35  |
| (-36 – 23)                 | (-46 – 39) | (-23 – 43) | (-7 – 65) | (0 – 71) |
| r                          | 0.97| 0.93| 0.96| 0.95| 0.95|
| RMSE (W)                   | 16  | 21  | 19  | 34  | 39  |
| CCC                       | 0.96| 0.92| 0.94| 0.83| 0.80|
| (0.86 – 0.99)              | (0.75 – 0.98) | (0.81 – 0.98) | (0.59 – 0.94) | (0.54 – 0.92) |

Bias is calculated from the Bland&Altman plot (lower and upper limits of agreement); r is Pearson’s coefficient of correlation; RMSE is the typical error between two indices; CCC is the Lin’s concordance correlation coefficient (95% confidence interval).

* Denotes that bias is different from 0

# Denotes significant difference between MMSS or CP from RCP ($P<0.05$).
Figure 5.4. $\dot{V}O_2$ (L·min$^{-1}$) and work rate (W) (panel A and B, respectively) at the lactate threshold (LT) and respiratory compensation point (RCP). Dashed line represents the aggregated $\dot{V}O_2$ and/or work rate at LT and the average $\dot{V}O_2$ and/or work rate at the maximal metabolic steady-state (MMSS). Panel C and D show the correlation and bias (with limits of agreement), respectively, between the work rate at RCP during the 5 W·min$^{-1}$ ramp-protocol (RCP$_5$) and MMSS.
Discussion

This study demonstrated that the dissociation between constant-\(\dot{V}O_2\) and ramp-\(\dot{V}O_2\) at a given work rate is progressively reduced (or abolished) with slower ramp-protocols. The implications are threefold: i) the \(\dot{V}O_2\) data in response to ramp-exercise need to be left-shifted by less watts (or seconds) when performing slow compared to fast ramp-protocols; ii) a moderate step-transition performed prior to the ramp-test ensures appropriate left-shifting of the \(\dot{V}O_2\) response, regardless of the ramp-slope such that the work rate at LT is constant; iii) the discrepancy between the work rates at ramp-identified RCP and constant-load-identified MMSS disappears if MRT-corrected slow ramp-protocols are used.

\(\dot{V}O_2\) in response to ramp-exercise using different slopes

Compared to the 15 W·min\(^{-1}\) ramp protocol, the overall \(\dot{V}O_2\) vs work rate response reflected a “upward” and “downward” curvilinear increases during the slowest (i.e., 5 and 10 W·min\(^{-1}\) ramps) and the fastest (i.e., 25 and 30 W·min\(^{-1}\) ramps) ramp-protocols, respectively (see Figure 5.1). In line with previous investigations (Scheuermann et al., 2002; Wilcox et al., 2016; Keir et al., 2016a), total G was greatest with the slowest ramp-protocol (i.e., 5 W·min\(^{-1}\)) and progressively decreased as ramp-slope increased. Non-linear dynamics of \(\dot{V}O_2\) (Barstow & Molé, 1991; Spencer et al., 2011; Keir et al., 2016c) were most evident during the 5 W·min\(^{-1}\) and 10 W·min\(^{-1}\) ramp-protocols (see Figure 5.1), where G increased from \(\sim 10\) (\(G_{\text{MOD}}\)) to \(\sim 15\) ml·min\(^{-1}\)·W\(^{-1}\) (\(G_{\text{HVY}}\)). Compared to \(G_{\text{MOD}}\) and \(G_{\text{HVY}}\), \(G_{\text{SVR}}\) was greater with the slowest ramp-protocols (i.e., 5 and 10 W·min\(^{-1}\) ramps), not different (\(\sim 10\) ml·min\(^{-1}\)·W\(^{-1}\)) with the 15 W·min\(^{-1}\) ramp-protocols, and smaller (\(\sim 7\) ml·min\(^{-1}\)·W\(^{-1}\)) during the 25 and 30 W·min\(^{-1}\) ramp-protocols.

With slower ramp-protocols, the magnitude of the dissociation between constant-\(\dot{V}O_2\) and ramp-\(\dot{V}O_2\) at a given work rate (i.e. 100 W) was reduced, such that the MRT\(_{SS}\) became progressively
smaller; thus, a progressively smaller number of watts was required to left-shift the \( \dot{V}O_2 \) response. Furthermore, the work rate at LT was not different and varied minimally amongst ramp slopes lending further support to the use of a “step-ramp-protocol” for accurate and valid quantification of MRT. As discussed elsewhere (Iannetta et al., 2019), this approach is unaffected by variations in \( \dot{V}O_2 \) baseline, \( G_{MOD} \), and breath-by-breath noise. More susceptible to these factors, however, is the “back extrapolation” approach in which an “artificial” prolongation of the MRT can be seen with slower ramp-protocols. Indeed, \( MRT_{LIN} \) was greater on average with slower (5, 10, and 15 W·min\(^{-1}\)) compared to faster ramp-protocols likely because \( G_{MOD} \) increases (Table 1) (Hughson & Inman, 1986; Iannetta et al., 2019) and breath-by-breath noise at baseline reduces certainty of detecting a rise in \( \dot{V}O_2 \) following ramp-onset (Scheuermann et al., 2002; Boone et al., 2008a; Keir et al., 2014). For these reasons, estimations of the work rate at LT based on \( MRT_{LIN} \)-corrected ramp-data were not uniform (smaller with slower ramps) but were identical when a \( MRT_{SS} \) correction was applied (see Figure 5.3B).

Transitioning from the heavy to the severe domain

The \( \dot{V}O_2 \) at RCP was constant regardless of the ramp-protocol adopted and was not different from the \( \dot{V}O_2 \) at MMSS (Figure 5.3C and D), corroborating the observation that the RCP is an invariant parameter of the ramp-exercise (Scheuermann & Kowalchuk, 1998). Although the characteristics of the hyperventilatory compensatory response can be modified by the ramp-slope of choice, the overall dynamics of the gas-exchange and ventilatory patterns are typically unaltered (Scheuermann & Kowalchuk, 1998), with the RCP occurring at very similar \( \dot{V}O_2 \) regardless of the ramp-protocol (Scheuermann & Kowalchuk, 1998; Leo et al., 2017). This is because the range of intensities (or metabolic rates), at which oxidative processes can account for most of the energy required by the task is constant regardless of the “forcing function” (ramp-slope). During exercise
sustained above this “critical metabolic rate” the need to buffer the accumulation of [H+] originating from an accelerated reliance on substrate-level phosphorylation initiates a potent compensatory hyperventilation. That the ČO₂ at RCP is similar to the ČO₂ at MMSS is consistent with previous studies (Keir et al., 2015; Iannetta et al., 2018a, 2018b). For example, it was demonstrated that the ČO₂ associated with these markers changed concordantly in well-trained cyclists over the course of a competitive season (Inglis et al., 2019).

In addition to this, also the work rate at RCP during slow ramp-protocols (after *left-shifting*) was similar and highly concordant with the work rate at MMSS (Figure 5.4B). This close agreement shows that the variability amongst these indices is no longer present when the ČO₂ dynamics during ramp-exercise are appropriately considered and reinforces the idea that the ČO₂ at RCP represents the metabolic rate associated with the transition from heavy to severe-intensity exercise during the ramp (Keir et al., 2015; Iannetta et al., 2017a). These findings are in contrast to Leo et al. (Leo et al., 2017) who concluded that the RCP should not be used to separate the heavy from the severe domain based on their observation of a high variability between the work rates at RCP and CP. While we agree with their conclusion that in most instances the work rate at the RCP should not be used to demarcate the heavy-to-severe boundary, it is important to consider that this study found a near perfect agreement between the work rates at MMSS and RCP with the MRT₆₅-corrected 5 W·min⁻¹ ramp-protocol (CCC > 0.99). It is reasoned that these robust findings stem from: *i*) an accurate estimation of the MRT, and *ii*) an accurate prediction and, subsequently, confirmation of metabolic stability at the work rate estimated by the CP model. For example, the concordance between RCP₅ and MMSS is nearly perfect when RCP₅ is corrected by MRT₆₅, but more variable when using RCP₅ corrected by MRT₆₅, and the work rate at the estimated CP accepted without physiological confirmation (see Table 5.2). Thus, these data provide strong
evidence that the RCP may be used as a surrogate of the heavy-severe boundary providing that slow ramp-protocols are performed (e.g., 5 W·min⁻¹) and the VO₂ dynamics are appropriately considered.

_Mechanistic underpinnings of the different VO₂ dynamics during ramp-exercise_

It has been proposed that within human skeletal muscles there exists a continuum of muscle fibre pools that respond with first-order VO₂ kinetics (Brittain et al., 2001) but are recruited in a hierarchical fashion (Henneman & Mendell, 2011). Each successive pool responds to changes in metabolic demand with slower VO₂ kinetics and lower efficiency to synthesize energy from O₂ and the whole body VO₂ response to any change in work rate reflects the sum of these units (Whipp et al., 2002; DiMenna et al., 2010; Keir et al., 2016a). Our data could be interpreted to support this hypothesis in two ways. First, non-linear VO₂ vs time and work rate relationships were observed for the slowest and fastest ramps (Figure 5.1). When the ramp slope is low (e.g., 5 W·min⁻¹ ramp), the less oxidatively efficient muscle fibre pools are provided more time to dynamically adjust to the change in work rate and contribute to an observable increase in VO₂ gain at the mouth and upward curvature in the VO₂ vs time or work rate relationships. The reverse occurs with faster ramp-protocols. A rapid increase in work rate reduces the contribution of higher-order muscle fiber pools to whole-body VO₂ such that the VO₂ response exhibits a downward curvature as ramp exercise progresses. Second, the MRT (s) increases progressively from the lowest to greatest ramp slope (Figure 5.2A). Compared to slower ramps, with faster ramps it would be expected that a greater proportion of higher-order muscle fibres that are kinetically slower would be recruited at ramp onset and elongate the time before VO₂ increases linearly with ramp work rate.

_Implications of findings and recommendations for exercise prescription_
Important implications for the prescription of exercise intensity from ramp-test data are as follows:

1. Neglecting to or inaccurately left-shifting ramp-\(\dot{V}O_2\) data will carry the risk to artificially increase the work rate associated with LT and incorrectly establish the boundary between moderate and heavy domains; adoption of the “step-ramp” method to quantify the MRT (i.e., MRT\(_{SS}\)) (Iannetta et al., 2019) appears to be a simple strategy to ensure the correct identification of the work rate at LT.

2. After correctly accounting for the MRT of \(\dot{V}O_2\), a dissociation between constant-\(\dot{V}O_2\) and ramp-\(\dot{V}O_2\) at a given work rate remains above LT unless 5 W·min\(^{-1}\) ramp is used. If the goal of the ramp-exercise is accuracy in the exercise prescription, this protocol could simultaneously determine \(\dot{V}O_2_{max}\) (although during very slow ramp-protocols this might not always be the case (Buchfuhrer et al., 1983)), the \(\dot{V}O_2\) and work rates at LT and MMSS (via RCP detection), and establish the \(\dot{V}O_2\)-to-work rate relationship across the moderate and heavy-intensity domains within a single visit.

**Conclusions**

The dynamics of \(\dot{V}O_2\) kinetics of ramp-exercise are affected by ramp-slope. This study provides mechanistic and practical explanations for the issues related to the interpretation of ramp-derived data. With an accurate quantification of MRT and confirmation of maximal metabolic steady-state, firstly the work rate at LT is constant regardless of the ramp-slope selected, and secondly the variability between RCP and MMSS greatly reduced. Furthermore, the long-standing problem of identifying from ramp exercise a work rate that will elicit a target \(\dot{V}O_2\) within the heavy-intensity domain can be solved by performing a 5 W·min\(^{-1}\) ramp-protocol and identifying RCP. Exercise prescriptions derived from ramp-incremental data should carefully consider the specific characteristic of the dynamics of \(\dot{V}O_2\) in response to the ramp-protocol of choice.
CHAPTER VI

A critical evaluation of current methods for exercise prescription in women and men

Introduction

Exercise intensity is the cornerstone of exercise physiology providing a guiding framework by which we perform aerobic testing, interpret physiological responses, prescribe exercise and induce desired adaptations to training (MacInnis & Gibala, 2017). However, although it is widely accepted that exercise training is a cost effective primary intervention to maintain health and to attenuate or reverse a variety of chronic conditions (Martin et al., 2009), the optimal exercise intensity prescription needed to produce a desired effect remains elusive. The contemporary gold standard approach for assignment of exercise intensity involves normalization based on percentages of maximal O\textsubscript{2} uptake (\(\dot{V}\text{O}_{2\text{max}}\)) as identified with cardiopulmonary incremental exercise testing. For practical reasons, alternative methods commonly used involve the identification of other maximal physiological outcomes, such as peak work rate (WR\textsubscript{peak}), and maximal heart rate (HR\textsubscript{max}) that can be linked to a given percent \(\dot{V}\text{O}_{2\text{max}}\). These methods assume that: i) exercise intensity exists upon a continuum; and ii) the characteristics of the metabolic responses and the tolerable duration of exercise amongst individuals exercising at a common percentage of maximum are identical. However, the ability of the aerobic system to maintain a given work rate cannot be ascertained from an incremental-derived maximal measure (Poole & Jones, 2012; Keir et al., 2018a). Rather, it is the dynamic responsiveness of \(\dot{V}\text{O}_{2}\) (or its kinetics) and the achievement (or not) of \(\dot{V}\text{O}_{2}\) steady-state that predict exercise tolerance and unmask the underlying physiological and metabolic stimulus or “intensity” (Poole & Jones, 2012).

In fact, the \(\dot{V}\text{O}_{2}\) kinetics response to step-increases in exercise work rate changes in relation distinct metabolic boundaries: the lactate threshold (LT) and the closely related critical power (CP) and
maximal lactate steady-state (MLSS) (Keir et al., 2015), which demarcate three “domains” of exercise intensity (Poole & Jones, 2012). Within the moderate-intensity domain, (i.e., below the lactate threshold), \( \dot{V}O_2 \) rapidly attains steady-state within 2 to 3 minutes, with an efficiency (\( \Delta \dot{V}O_2 / \Delta \text{work rate} \)) that is highly predictable (Keir et al., 2016c). Exercise within this domain can be sustained for a very long period (~4 h) with no, or modest, accumulation of metabolites and fatigue (Black et al., 2017). Within the heavy-intensity domain, (i.e., between LT and CP/MLSS) the emergence of the \( \dot{V}O_2 \) slow component, which reflects a reduction in efficiency (i.e., greater \( \Delta \dot{V}O_2 / \Delta \text{work rate} \)), delays the attainment of \( \dot{V}O_2 \) steady-state by as much as 15 min (Barstow & Molé, 1991). Here, the greater metabolic perturbations (e.g., elevation of blood [lactate], muscle [inorganic phosphate], H\(^+\)) although stabilized over time, are associated with greater accumulation of fatigue and reduction of exercise tolerance relative to moderate-intensity exercise (Baron et al., 2008; Cannon et al., 2011; Black et al., 2017; Iannetta et al., 2018b). Within the severe-intensity domain (i.e., above CP and MLSS), the rate of ATP resynthesis continues to rise over time (Cannon et al., 2014), time to exhaustion decreases hyperbolically as intensity increases, and end-exercise typically coincides with high levels of metabolites, fatigue accumulation, and attainment (or near attainment) of \( \dot{V}O_{2\text{max}} \) (Keir et al., 2016b; Black et al., 2017; Schäfer et al., 2019).

Between individuals, LT and the CP/MLSS can occur at different percentages of \( \dot{V}O_{2\text{max}} \), thus the ranges of \%\( \dot{V}O_{2\text{max}} \), \%\( WR_{\text{peak}} \), or \%\( HR_{\text{max}} \) that define each intensity domain can vary from person to person (Fontana et al., 2015). Therefore, selection of constant-work rate exercise based on a fixed \%\( \dot{V}O_{2\text{max}} \), \%\( WR_{\text{peak}} \), or \%\( HR_{\text{max}} \) may not guarantee an accurate control of the exercise intensity (DiMenna & Jones, 2009; Scharhag-Rosenberger et al., 2010; Lansley E. et al., 2011; Keir et al., 2018a). Nevertheless, this approach remains as the most commonly used in both clinical and research trials (Bouchard et al., 1999; Burgomaster et al., 2007; Montero & Lundby, 2017;
Roy et al., 2018) and is recommended by current exercise prescription guidelines (Linda S Pescatello; American College of Sports Medicine., 2014). Surprisingly, how each exercise intensity domain is distributed in terms of %\(\dot{VO}_{2\text{max}}\), %WR\(_{\text{peak}}\), and %HR\(_{\text{max}}\) within a large group of individuals, and how sex-related differences may affect these distributions remains unknown.

Thus, using a dataset of one hundred individuals in whom both LT and MLSS were directly measured and verified, the purpose of this study was to quantify the distribution of each intensity domain at discrete fixed %\(\dot{VO}_{2\text{max}}\), %WR\(_{\text{peak}}\), or %HR\(_{\text{max}}\) intensities. It was hypothesized that, given the individual variability in the relative position of LT and MLSS, no fixed %\(\dot{VO}_{2\text{max}}\), %WR\(_{\text{peak}}\), or %HR\(_{\text{max}}\) could guarantee a uniform domain-specific allocation of individuals, with this having important implications for exercise intensity prescriptions.

**Methods**

*Participants*

One-hundred healthy adults (46 women and 54 men) participated in this study. Their physical characteristics are displayed in Table 1. Data from 40 participants have been presented in previous work from our group (Iannetta et al., 2018a, 2018b). The level of fitness of the participants spanned from untrained to well-trained, according to current classification guidelines (De Pauw et al., 2013). Within ten days, all participants completed the following exercise tests on a cycle ergometer: i) a cardiopulmonary ramp-incremental exercise test to exhaustion; ii) two to four 30-min constant-work rate rides for determination of MLSS. This study was approved by the local ethics review board in compliance with the latest version of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

*Data collection*
All testing was performed on an electromagnetic-braked cycle ergometer (Velotron, RacerMate, Seattle, WA). A metabolic cart (Quark, CPET, Cosmed, Rome, Italy) was used to measure ventilatory and gas exchange variables on a breath-by-breath basis. The system was comprised of a low-dead-space turbine for the assessment of inspired and expired volumes and gas analyzers for the assessment of fractional concentrations of respired O₂ and CO₂ which were calibrated according to the manufacture’s recommendation. Blood lactate concentration ([La]ₚ) from a finger pin-prick was assessed with a portable analyzer (Lactate Scout, SensLab GmbH, Leipzig, Germany). Heart rate (HR) was continuously recorded during each testing session using radiotelemetry (Garmin, Olathe, KA). Participants performed the tests on separate days, with a minimum of 48h and a maximum of 72h interval between sessions. All testing sessions were performed at the same time of the day (±30 min). Participants self-selected their preferred cadence (in a range between 75 and 95 rpm) during their first laboratory visit and was maintained the same for subsequent visits. The ramp-tests were stopped when participants could no longer maintain the self-selected cadence by 10 rpm for more than 10 s, or at volitional exhaustion despite strong verbal encouragement. In all conditions, participants were blinded to the work rate and elapsed time but received visual feedback on their cadence.

*Cardiopulmonary ramp-incremental exercise test.* The cardiopulmonary ramp-incremental exercise test was performed to the limit of volitional exhaustion to determine \( \dot{V}O_{2\text{max}} \), LT, \( WR_{\text{peak}} \), and \( HR_{\text{max}} \). A 4-min period of cycling at 50 W preceded the ramp-incremental portion of the test, which consisted of 25 W·min⁻¹ (1 W every 2.4 s) and 30 W·min⁻¹ (1 W every 2 s) increments for women and men, respectively.

*MLSS determination.* Participants performed two to four, 30-min constant-work rate tests for the determination of the work rate at MLSS. MLSS was defined as the highest work rate at which the
difference in $[\text{La}^-]_b$ is less than (or equal to) 1 mmol·L$^{-1}$ between the 10th and 30th min (Beneke, 2003b). Each 30-min constant-work rate test was preceded by a 4-min baseline of 50-80 W cycling, after which the work rate was instantaneously increased to a predetermined value. $[\text{La}^-]_b$ was measured during baseline and every 5-min after the work rate was increased. The resistance for the first 30-min constant-work rate trial was established from a mathematical model recently developed in our laboratory (Iannetta et al., 2018a). Depending on $[\text{La}^-]_b$ responses, the work rate for the subsequent constant-work rate trial was either decreased or increased by 10 W until the highest work rate with stable $[\text{La}^-]_b$ was established (Bellotti et al., 2013).

Data analyses

Ventilatory and gas exchange variables. The raw ventilatory and gas exchange data for each participant were examined by two expert physiologists to identify the $\dot{V}_O2$ corresponding to LT. In the circumstances of a disagreement of more than 100 ml·min$^{-1}$, the experimenters re-evaluated together the profiles until an agreement was reached. Briefly, LT corresponded to the point at which $\dot{V}_CO2$ began to increase out of proportion in relation to $\dot{V}_O2$, coincidental with a systematic rise in the $\dot{V}_E$-to-$\dot{V}_O2$ relation and end-tidal PO$_2$, whereas the ventilatory equivalent of $\dot{V}_CO2$ ($\dot{V}_E/\dot{V}_CO2$) and end-tidal PCO$_2$ were stable (Beaver et al., 1986). For each incremental and constant-work rate trial, the breath-by-breath data were edited and aberrant data lying three SD from the local mean were deleted. Thereafter, they were interpolated on a second-by-second basis. $\dot{V}O2_{max}$ corresponded to the highest $\dot{V}_O2$ during the ramp-test value computed from 30 s rolling-average. To account for the ramp-$\dot{V}_O2$ mean response time (MRT), the “back extrapolation” approach was used (Boone & Bourgois, 2012). Briefly, a piecewise equation that included two linear segments was use:

$$f = \text{if } (t < \text{MRT use } g(t), \text{else } h(t)); g(t) = i_1 + s_1t; i_2 = i_1 + s_1t; h(t) = i_2 + s_2t - \text{MRT}$$
where \( f \) is the piecewise function, \( t \) is time and \( g \) and \( h \) are \( \dot{V}O_2 \), MRT is the time corresponding to the intersection of the two regression lines (MRT), \( i_1 \) and \( i_2 \) are the intercepts of the first and second linear function, respectively, and \( s_1 \) and \( s_2 \) are the slopes. The \( s_1 \) parameter was fixed at “zero”. The two linear segments were fitted from the last 3 min of the pre-ramp baseline (\( t = -180 \text{ s} \)) to the previously established LT (Iannetta et al., 2019). The work rate at LT was identified after left-shifting the ramp-\( \dot{V}O_2 \) data. The \( \dot{V}O_2 \) associated to MLSS was calculated as the average of the last 5 min of the 30-min constant work rate exercise.

**Heart rate responses.** The HR values associated with LT were derived by averaging twenty data points (bpm) around the corresponding \( \dot{V}O_2 \). The HR response during MLSS trial was calculated as the average of the last 20 min of the constant-work rate exercise and, to evaluate the magnitude of the HR “drift” during this trial, 2-min averages around the 10th and 30th min were calculated.

**Constructing the intensity domain schema.** The exercise intensity domains (i.e., moderate, heavy, severe) were identified for each individual by determining LT and MLSS on the basis of absolute and relative \( \dot{V}O_2 \), work rate, and HR. Thereafter, based on the relative position of LT and MLSS, the number of individuals falling within each domain was determined at 5% intervals from 35% to 95% of \( \dot{V}O_{2\text{max}}, \text{WR}_{\text{peak}}, \text{and HR}_{\text{max}} \). These intensities were chosen because they encompass the range of intensities suggested by current exercise prescription guidelines (Linda S Pescatello; American College of Sports Medicine., 2014) and are those generally selected in research and clinical settings. All data processing and editing was performed by using customized functions of a commercially available computer software (Origin, OriginLab Corp., Northampton, MA).

**Statistical analyses**
Data are presented as means ± SD. The Shapiro-Wilk test was performed to assess the normality of the distribution of each dependent variable. Comparisons between men and women in terms of maximal and submaximal indices of cardiorespiratory fitness were made using a series of independent-sample t-test. Pearson’s correlation coefficients were calculated to assess the association between \( \dot{V}O_{2\text{max}} \) and the indices of exercise intensity (i.e., LT and MLSS) expressed in relative terms. The relationship between categorical variables (i.e. the number of individuals distributed in certain domain at a specific fixed percentage) was tested using contingency tables and Pearson’s chi-square (\( \chi^2 \)) tests. Independence of data and expected frequencies assumptions were verified prior to the analysis. To account for any low expected frequency (i.e., \( n = < 5 \)), Fisher’s exact statistics was used. A significant chi-square test was broken down with standardized residuals analysis. Standardized residuals were interpreted as z-scores (using \( z = 1.96 \) as a cut-off value to establish significance at the 0.05 level). Briefly, if within a certain domain (i.e., *moderate*, *heavy* or *severe*) a specific percentage of \( \dot{V}O_{2\text{max}} \), \( WR_{\text{peak}} \), or \( HR_{\text{max}} \) displays a standardized residual higher than 1.96, that signifies that within that domain there is a significant number of individuals with respect to a random allocation. For example, if one prescribes exercise at 75% \( \dot{V}O_{2\text{max}} \) (Figure 3), what is the likelihood that a certain number of individuals will fall in a given domain? Additionally, is the number of individuals falling in a given domain different between women and men? All statistical analyses were performed using R (Version 14, Texas, USA) and \( \alpha = 0.05 \); statistical significance was accepted when \( p < \alpha \). Effect sizes [Cohen’s \( d \), ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (0.80 and greater)] (Cumming, 2014) are also reported as objective and standardized measures of magnitude of effects and as alternative metrics of meaningfulness (Winter *et al.*, 2014).
Results

The physical characteristics of the participants and their incremental exercise results are shown in Table 6.1. Compared to women, men had a greater absolute ($t = 10.4$, df = 98, $p < 0.05$, $d = 2.1$) and relative $\dot{V}O_{2\text{max}}$ ($t = 5.2$, df = 98, $p < 0.05$, $d = 1.0$) and $WR_{\text{peak}}$ (absolute: $t = 10.2$, df = 98, $p < 0.05$, $d = 2.0$ and relative: $t = 4.8$, df = 98, $p < 0.05$, $d = 0.9$) but similar $HR_{\text{max}}$ ($t = 0.2$, df = 98, $p = 0.85$, $d = 0.1$).

| Table 6.1. Participants’ physical characteristics and physiological performance variables measured during the cardiopulmonary incremental exercise test |
|-------------------------------------------------|---------------|-------------|-------------|
|                                  | All (n = 100) | Women (n = 46) | Men (n = 54) |
| Age (years)                     | 28 ± 7        | 26 ± 5       | 30 ± 8 $^a$  |
| Height (cm)                     | 174 ± 8       | 169 ± 8      | 177 ± 7 $^a$ |
| Weight (kg)                     | 72 ± 10       | 67 ± 9       | 76 ± 9 $^a$  |
| $\dot{V}O_{2\text{max}}$ (L·min$^{-1}$) | 3.62 ± 0.72   | 3.06 ± 0.41  | 4.10 ± 0.56 $^a$ |
| $\dot{V}O_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$) | 50.7 ± 8.6    | 46.4 ± 6.1   | 54.4 ± 8.8 $^a$ |
| $WR_{\text{peak}}$ (W)           | 343 ± 66      | 292 ± 37     | 387 ± 53 $^a$ |
| $WR_{\text{peak}}$ (W·kg$^{-1}$)   | 4.8 ± 0.8     | 4.4 ± 0.6    | 5.1 ± 0.8 $^a$ |
| $HR_{\text{max}}$ (bpm)           | 184 ± 9       | 184 ± 9      | 183 ± 9     |

Data are presented as mean ± SD. $\dot{V}O_{2\text{max}}$: maximal oxygen uptake. $WR_{\text{peak}}$: highest work rate achieved at the end of the cardiopulmonary incremental test. $HR_{\text{max}}$: highest heart-rate value recorded during the cardiopulmonary incremental test.

$^a$ Denotes significance between men and women.
The frequency distributions of absolute and relative $\dot{V}O_{2\text{max}}$ for men and women are displayed in Figure 6.1. Percent of $\dot{V}O_{2\text{max}}$ ($r=-0.191; P>0.05$) and the $\%WR_{\text{peak}}$ ($r=0.101; P>0.05$) at LT were not correlated with $\dot{V}O_{2\text{max}}$ (mL·Kg$^{-1}$·min$^{-1}$). There was a moderate correlation between $\dot{V}O_{2\text{max}}$ (mL·Kg$^{-1}$·min$^{-1}$) and $\%WR_{\text{peak}}$ ($r=0.419; P<0.05$) but not with $\%V_{\text{O}2\text{max}}$ ($r=0.135; P>0.05$) at MLSS.

**Figure 6.1.** Distribution of women and men on the basis of absolute and relative $\dot{V}O_{2\text{max}}$. 
Table 6.2 displays the average and range of the \( \dot{V}O_2 \), work rate, and HR at LT and MLSS in both absolute and relative values. Compared to men, the \%\( \dot{V}O_2 \)max and \%HRmax at LT and MLSS were greater in women (\( P<0.05 \)).

<table>
<thead>
<tr>
<th></th>
<th>All (n = 100)</th>
<th>Women (n = 46)</th>
<th>Men (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( L\cdot min^{-1} )</td>
<td>2.17 ± 0.43</td>
<td>1.90 ± 0.23</td>
<td>2.40 ± 0.43a</td>
</tr>
<tr>
<td></td>
<td>(1.50 – 2.50)</td>
<td>(1.60 – 3.30)</td>
<td></td>
</tr>
<tr>
<td>% ( \dot{V}O_2 )max</td>
<td>60.3 ± 6.2</td>
<td>62.5 ± 5.7</td>
<td>58.4 ± 6.0a</td>
</tr>
<tr>
<td></td>
<td>(49.4 – 73.7)</td>
<td>(45.1 – 72.5)</td>
<td></td>
</tr>
<tr>
<td>( W )</td>
<td>146 ± 38</td>
<td>123 ± 25</td>
<td>166 ± 37a</td>
</tr>
<tr>
<td></td>
<td>(62 – 175)</td>
<td>(105 – 250)</td>
<td></td>
</tr>
<tr>
<td>% ( WR_{peak} )</td>
<td>42.4 ± 6.6</td>
<td>42.0 ± 6.6</td>
<td>42.7 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>(23.4 – 55.3)</td>
<td>(29.3 – 57.0)</td>
<td></td>
</tr>
<tr>
<td>( bpm )</td>
<td>137 ± 13</td>
<td>140 ± 14</td>
<td>135 ± 11</td>
</tr>
<tr>
<td></td>
<td>(105 – 170)</td>
<td>(114 – 158)</td>
<td></td>
</tr>
<tr>
<td>% ( HR_{max} )</td>
<td>74.9 ± 5.7</td>
<td>76.1 ± 6.3</td>
<td>73.9 ± 5.1a</td>
</tr>
<tr>
<td></td>
<td>(59.9 – 89.9)</td>
<td>(63.6 – 82.0)</td>
<td></td>
</tr>
<tr>
<td><strong>MLSS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( L\cdot min^{-1} )</td>
<td>2.99 ± 0.64</td>
<td>2.56 ± 0.41</td>
<td>3.37 ± 0.56a</td>
</tr>
<tr>
<td></td>
<td>(1.70 – 3.60)</td>
<td>(2.27 – 4.20)</td>
<td></td>
</tr>
<tr>
<td>% ( \dot{V}O_2 )max</td>
<td>82.6 ± 5.4</td>
<td>83.4 ± 5.2</td>
<td>81.8 ± 5.4a</td>
</tr>
<tr>
<td></td>
<td>(72.6 – 93.8)</td>
<td>(68.5 – 95.8)</td>
<td></td>
</tr>
<tr>
<td>( W )</td>
<td>204 ± 49</td>
<td>174 ± 33</td>
<td>230 ± 46a</td>
</tr>
<tr>
<td></td>
<td>(104 – 248)</td>
<td>(135 – 310)</td>
<td></td>
</tr>
<tr>
<td>% ( WR_{peak} )</td>
<td>59.2 ± 5.1</td>
<td>59.3 ± 5.2</td>
<td>59.0 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>(47.9 – 70.5)</td>
<td>(43.6 – 67.9)</td>
<td></td>
</tr>
<tr>
<td>( bpm )</td>
<td>163 ± 11</td>
<td>167 ± 11</td>
<td>160 ± 10a</td>
</tr>
<tr>
<td></td>
<td>(130 – 184.5)</td>
<td>(139 – 189)</td>
<td></td>
</tr>
<tr>
<td>% ( HR_{max} )</td>
<td>89.0 ± 4.5</td>
<td>90.7 ± 3.9</td>
<td>87.5 ± 4.6a</td>
</tr>
<tr>
<td></td>
<td>(77.4 – 95.7)</td>
<td>(74.9 – 96.9)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD (range). LT: lactate threshold. MLSS: maximum lactate steady-state.

a Denotes significance between women and men.
The HR response during the constant-work rate trial at MLSS increased similarly in men and women progressing between the 10\textsuperscript{th} and 30\textsuperscript{th} minute from the 160±11 bpm to 167±12 bpm ($t$s > 20.0, df = 99, $p < 0.05$, $d$s > 2.0).

The frequency distribution of individuals within each domain at the discrete percentages of $\dot{\text{V}}\text{O}_{2\text{max}}$, WR\text{peak} and HR\text{max} are depicted in Figure 6.2 (panels A, B, C, D, E, and F). These data indicate that within the range of intensities typically used in research and clinical setting, no fixed-percentage of maximum could guarantee a uniform intensity domain distribution amongst the individuals.

Figure 6.3 displays chi-square test analyses and standardized residuals calculation for female and male. There was a significant association between sexes and intensity domains at different discrete percentages of $\dot{\text{V}}\text{O}_{2\text{max}}$, WR\text{peak} and HR\text{max} (all $\chi^2(24) > 1529$, $p<0.001$), meaning that individuals are classified differently based on their gender. Within the same sex, standardized residuals > ±1.96 in Figure 3 allow to verify at which fixed-percentage there exist a significant number of individuals within a given domain in respect to a random allocation.
Figure 6.2. Distribution of individuals in the moderate (M), heavy (H) and severe (S) domains at discrete $\%\text{VO}_{2\text{max}}$, $\%\text{WR}_{\text{peak}}$, and $\%\text{HR}_{\text{max}}$. Women: left panels. Men: right panels.
### Figure 6.3

Classification plot displaying the results of the contingency tables, Pearson’s chi-square ($\chi^2$) tests and standardized residuals analysis. In women and men, at discrete $\%\bar{VO}_2_{\text{max}}$, $\%WR_{\text{peak}}$, and $\%HR_{\text{max}}$, the observed and expected individual’s frequencies in different exercise intensity domains (moderate (M), heavy (H) and severe (S)) were compared. Vertical lines indicate the ranges of intensities ((very-) light (v-L), moderate (M), vigorous (V), and (near)-maximal (n-M)) on the basis on $\%\bar{VO}_2_{\text{max}}$ and $\%HR_{\text{max}}$ identified by the ACSM’s guidelines (Garber et al., 2011). A standardized residual >1.96 (in light grey) indicates a significantly larger observed number of individuals in a certain domain compared to a random allocation at a given percentage of $\bar{VO}_2_{\text{max}}$, $WR_{\text{peak}}$, or $HR_{\text{max}}$. For clarity, only significant classifications are displayed. In addition, the size of the “bubble” represents the cell’s contribution to the overall $\chi^2$ statistics which can be interpreted as a measure of positive “attraction” between relative intensity ($x$) and domain ($y$).
Discussion

In a large dataset including 100 men and women, the present study examined whether the current gold-standard exercise prescription approach, based on fixed-percentages of maximal exercise, could accurately stratify individuals within the exercise intensity domains scheme. Findings demonstrated that, given between-subjects variability in the ranges of \( %\dot{V}O_{2\text{max}} \), \( %WR_{\text{peak}} \), and \( %HR_{\text{max}} \) defining each exercise intensity domain, the fixed-percentage approach cannot guarantee an accurate and homogeneous domain-specific exercise prescription. This carries critical implications for the interpretation of the results of acute and long-term exercise interventions and for the efficacy and applicability of current aerobic exercise prescription frameworks.

Limitations of the fixed-percentage approach to assign exercise intensity in a given domain

After the LT and MLSS were established from the ramp-incremental and constant-work rate trials, the range of \( \dot{V}O_{2} \), work rate and HR associated with moderate, heavy, and severe-intensity domains were identified for each individual. From these ranges, the number of participants exercising within each of these domains could be determined at any specific \( %\dot{V}O_{2\text{max}} \), \( %WR_{\text{peak}} \) and \( %HR_{\text{max}} \). Irrespective of the variable of choice, no single fixed-percentage (within the range of intensities typically used for exercise prescription) could guarantee a uniform domain-specific distribution during constant-work rate exercise. From Figure 6.2, it can be observed that within \( %\dot{V}O_{2\text{max}} \), \( %WR_{\text{peak}} \) and \( %HR_{\text{max}} \) ranges, individuals were distributed between two or across all three domains. This variability occurs due to the large ranges in the relative position of LT and MLSS amongst individuals (Table 6.2). Interestingly, the relative position of LT and MLSS was independent of fitness level (i.e., \( \dot{V}O_{2\text{max}} \)), highlighting how the relative position of these landmarks – and related domains – are highly unpredictable without accurate testing.

“Critical” fixed-percentages and sex-related differences in the domain distribution
This study used the chi-square analysis to verify at which fixed-percentages the frequency (number of individuals) in a given domain was significant in relation to the frequency expected from a random allocation. In Figure 6.3, it is possible to observe significant “overlaps” across domains within the same sex and the differences in distributions at discrete fixed-percentages between sexes. For example, “critical” fixed-percentages at which significant chances of domain-overlap (i.e., moderate-heavy and heavy-severe) occurred were: 55-65% and 75-85% of $\dot{V}O_{2max}$, 35-55% and 60-65% of WR$_{peak}$, and 70% and 85-90% of HR$_{max}$. Regarding sex-differences, greater chances of moderate-domain at 60 and 70% of $\dot{V}O_{2max}$, and of heavy-domain at 75, 80, and 85% of $\dot{V}O_{2max}$ were observed in women compared to men. On the basis of %HR$_{max}$, greater chances of heavy-domain at 70% of HR$_{max}$ and of severe-domain at 85 and 90% of HR$_{max}$ were observed in men compared to women. These sex-related differences reflect the higher %$\dot{V}O_{2max}$ and %HR$_{max}$ at which LT and MLSS were observed in women compared to men (Table 6.2). Although understanding the mechanistic components underpinning these differences is beyond the scope of the present study, some established sex-related differences in muscle fiber composition (Staron et al., 2000), oxidative capacity (Montero et al., 2018), metabolism (Horton et al., 1998), and cardiac function (Howden et al., 2015) may play a role.

Further limitations of the fixed-percent approach: the disconnection between $\dot{V}O_{2}$ and work rate, and HR drift

Concerning exercise prescriptions based on %$\dot{V}O_{2max}$, the present study evaluated the distribution of domains at “true” %$\dot{V}O_{2max}$ (a scenario that would occur when the resistance during constant work rate exercise is “manually” adjusted to elicit the desired %$\dot{V}O_{2max}$ (Scharhag-Rosenberger et al., 2010)). Particularly in research settings, however, it is a common practice to assign a work rate linearly interpolated from the $\dot{V}O_{2}$-to-work rate relationship previously assessed during an
incremental exercise test to a specific %\(\dot{V}O_2\max\). While it could be anticipated that both approaches produce similar domain variability, an additional downfall of using an incremental-derived %\(WR_{\text{peak}}\) is that this practice will yield an unpredictable %\(\dot{V}O_2\) during constant-work rate exercise (most likely greater than desired). The reasons for this have been thoroughly discussed in previous reviews (DiMenna & Jones, 2009; Keir et al., 2018a), and relate fundamentally to the fact that the \(\dot{V}O_2\) control system increasingly lags the changes in work rate during incremental exercise and that steady-state \(\dot{V}O_2\) and work rate are not linearly related across all the intensity domains (DiMenna & Jones, 2009; Keir et al., 2018a). Considering these elements, exercise intensity prescription using a %\(WR_{\text{peak}}\) – even when associated to a ramp-incremental derived %\(\dot{V}O_2\max\) – should be avoided.

For exercise prescription based on fixed %\(HR_{\max}\) it is important to consider that, although a distribution of domains is presented in Figure 2, confidence in establishing exercise intensity boundaries based on HR is low. Indeed, the failure of HR to attain a steady-state response at any intensity during constant-work rate exercise (e.g., the cardiac “drift” while exercising at MLSS was equivalent to ~4% in the present study) hinders the precise association of a univocal %\(HR_{\max}\) to any exercise intensity. Additionally, in experimental settings, to ensure that HR remains at a fixed percentage of \(HR_{\max}\), the work rate would need to be progressively reduced with time, which might cause unintended transition from one domain to another and reduce the metabolic stimulus (i.e., lower \(\dot{V}O_2\)) (Zuccarelli et al., 2018).

Implications for exercise prescription guidelines

The goal of current exercise prescription guidelines from the various health agencies, such as the American Heart Association and the American College of Sport Medicine, is to promote physical activity at the population level through the adoption of “individualized exercise prescription”
(Haskell et al., 2007; Garber et al., 2011; Pescatello, 2014). In doing so, for instance, specific ranges of intensity [i.e., (very-) light, moderate, vigorous, (near-) maximal] based predominately on discrete %\(\dot{V}O_{2\text{max}}\) and %\(HR_{\text{max}}\) have been established (Garber et al., 2011). This framework, however, cannot account for inter-individual variations in LT and MLSS, thus will not guarantee an adequate control of the exercise intensity (Figure 6.3). For example, within the 64-90 %\(\dot{V}O_{2\text{max}}\) and 77-95 %\(HR_{\text{max}}\) ranges corresponding to vigorous exercise (Garber et al., 2011), individuals would be spread across the three intensity domains of exercise (i.e., moderate-heavy-severe). Therefore, although it is recognized that exercise training can result in a wide range of health-related benefits even when using fixed-percentage approaches (Duncan et al., 2005), a re-evaluation of these guidelines might be needed in order to tailor the exercise intensity prescription (also accounting for potential sex-related differences) and optimize the health-related benefits of exercise.

*Physiological implications of “domain misclassifications”*

Different investigations have demonstrated that metabolic efficiency, metabolite accumulation, fatigue etiology, and ultimately exercise tolerance are all linked to the characteristics of the metabolic responses that are ultimately dependant on the intensity domain in which one exercises (Cannon et al., 2011; Black et al., 2017; Davies et al., 2017; Iannetta et al., 2018b; Schäfer et al., 2019). Thus, the exercise intensity has a prominent role in shaping the characteristics of the exercise-induced responses and adaptations (MacInnis & Gibala, 2017). Unfortunately, despite the recognized limitations of the fixed-percentage approach (Katch et al., 1978; Meyer et al., 1999; DiMenna & Jones, 2009; Scharhag-Rosenberger et al., 2010), most studies, including those evaluating training responsiveness, continue to adopt this approach for exercise intensity prescription. The most relevant consequence of applying the fixed-percentage approach is that the
characteristics of the \( \dot{V}O_2 \) response at any percent-derived value will be unpredictable and thus the metabolic stress is poorly controlled. In this context, the issue of responders and non-responders to exercise training has become a popular topic of debate (Mann et al., 2014; Joyner & Lundby, 2018). While the high inter-individual variability in training responsiveness has been attributed to genetic factors (Bouchard et al., 1999), data from the present study suggest that variance in physiological stress profiles could play a bigger role than previously acknowledged. Thus, should we expect homogenous responsiveness to training from a heterogeneous exercise intensity prescription? Interestingly, a recent study showed that the incidence of non-responders is abolished when exercise training is prescribed based on physiological “thresholds” (Weatherwax et al., 2019).

*Practical application of the exercise-intensity Domain schema*

Although the work rate at LT can be accurately derived from gas-exchange and ventilatory profiles from a single ramp-incremental exercise test (provided that the ramp-\( \dot{V}O_2 \) data are *left-shifted* to account for the MRT (Iannetta et al., 2019)), the accurate determination of the heavy-to-severe boundary of exercise intensity requires additional laboratory visits (generally three to five). Alternative strategies to limit the burden of additional testing have been proposed including ramp-incremental test-based equations or analysis of blood lactate profiles during sub-maximal exercise – for MLSS estimation (Fontana et al., 2016; Iannetta et al., 2018a) – and all-out or ramp-sprint tests – for CP estimation (Vanhatalo et al., 2007; Constantini et al., 2014; Murgatroyd et al., 2014). However, the viability of any of these approaches is dependent on the population being tested and the degree of accuracy required. In this context, it must be considered that any approach – including the gold standards – present some level of error (Mattioni Maturana et al., 2018; Jones et al., 2019). Therefore, being aware of the advantages and the limitations of each approach and knowing the
physiological implications of exercising in the heavy-domain versus in the severe-domain can help identifying the best strategy to determine in any context and with accuracy the heavy-to-severe boundary of exercise intensity.

Conclusions

Exercise prescription based on fixed-percentages of maximum provides an inaccurate means for controlling exercise intensity. Given that accurate characterization of the training stimulus is critical to obtain the desired metabolic stimulus and subsequent adaptations to exercise training, a model that considers the exercise intensity domains for exercise prescription is recommended. Application of this approach would optimize health-related outcomes of participants and better characterize the molecular and system level adaptations related to acute and chronic exercise training. Current exercise prescription guidelines that propose percent-based ranges of maximum cannot guarantee an appropriate individualization of the intensity prescription, and this should be carefully considered when presenting exercise and physical activity guidelines.
CHAPTER VII

Overall summary, discussion, and future directions

In Chapter III, the aim was to compare the validity and reproducibility of current approaches to characterize the $\dot{V}O_2$ MRT at ramp-onset that is used to left-shift the ramp-$\dot{V}O_2$ data with a novel method based on a moderate-bout of exercise performed prior to the ramp. Our analyses demonstrated within the same group of participants that: i) current methods based on non-linear and linear regression models used to quantify $\tau'$ and MRT$\text{LIN}$ show high between-day variability; ii) even though two ramp-repeats are averaged together, these methods still elicit values that are unlikely to be accurate; iii) consistent with previous studies (Hughson & Inman, 1986; Boone et al., 2008a), $\tau'$ was affected by between-days variability in baseline $\dot{V}O_2$, whereas MRT was affected by variations in $G$; 4) the novel method (MRT$SS$) showed small between-day and narrow inter-subjects variability. These findings most likely stem from the fact that this method is unaffected by variations in pre-ramp baseline $\dot{V}O_2$ and $G$.

One of the main focuses of this thesis was to evaluate the metabolic- and performance-related consequences when exercising slightly above MLSS (Chapter IV) and to investigate the reasons underpinning the different work rate between the RCP and CP/MLSS (although these markers have been repeatedly reported to occur at the same $\dot{V}O_2$) (Chapter V).

Metabolic and fatigue profiles in response to exercise $>CP$ and $<CP$ have been well characterized (Poole et al., 1988; Vanhatalo et al., 2016). However, CP typically occurs at a slightly higher work rate compared to MLSS (Pringle & Jones, 2002; Dekerle et al., 2003; Keir et al., 2015; Mattioni Maturana et al., 2016). Thus, although described to represent the same physiological event, it was not known whether a small increase above MLSS would produce
disproportional metabolic responses and performance reductions. In line with our hypothesis we found progressive increases in [La\[b\]], ventilation, and RPE during exercising slightly above MLSS which was followed by a disproportional reduction in time-to-exhaustion performance and the attainment of lower peak $\dot{V}O_2$ values (compared to ramp-exercise). Contrary to our hypothesis, however, the overall $\dot{V}O_2$ did not increase significantly from min 10 to min 30 ($\Delta= \sim50$ ml·min\(^{-1}\)) during the 10 W above MLSS trial.

Our interpretation of these findings was that, although $\dot{V}O_2$ did not project to maximal values when exercising for 30 min above MLSS, a disproportional fatigue accumulation was evident in the reduced time-to-exhaustion. Therefore, the detection of an accumulation of lactate in the blood, indicative of accelerated glycolytic activity, may be more sensitive than $\dot{V}O_2$ measurements when evaluating slight changes in work rates at intensities surrounding MLSS. In other words, $\dot{V}O_2$ may not necessarily project to $\dot{V}O_2max$ when the increase in work rate above MLSS is small. In this context, it could be argued whether in this study the work rate at MLSS represented the metabolic rate separating the heavy and the severe domain, above which, by definition, $\dot{V}O_2$ should attain $\dot{V}O_2max$ (Poole et al., 1988). In this perspective, it is important to consider the measurement error associated with the identification of MLSS (~10 W). Indeed, it could be possible that the “true” MLSS could have occurred at a work rate slightly higher than that accepted in this study. It is possible that if a smaller delta work rate between the MLSS determination trials had been used to determine MLSS (i.e., 5 W), then it is likely that a more pronounced projection of $\dot{V}O_2$ towards maximal values could have occurred when exercising above the “true” MLSS. However, this speculation remains to be experimentally tested.

The mechanisms responsible for the reduction in time-to-exhaustion following exercising above MLSS are presently unknown. We proposed that when exercising at intensities slightly
above MLSS, glycogen stores were depleted to a greater extent to support the accelerated glycolytic flux, with this mechanism impairing maximal exercise capacity (Gollnick et al., 1974). Indeed, it has been suggested that low levels of glycogen may not only limit the rate of ATP resynthesis via glycolysis but also reduce ATP utilization by acting as modulator of sarcoplasmic reticulum Ca\(^{2+}\) release (Ørtenblad et al., 2011). This interpretation, however, must be taken with caution, as it was recently observed that the depletion of glycogen stores following two-hour cycling in the heavy domain, although remarkable, was not correlated with reductions in CP estimates (Clark et al., 2019). Therefore, although a greater depletion of glycogen stores might be expected following exercise above MLSS, this might not be the sole mechanism reducing time-to-exhaustion performance. We also proposed that the observed greater perception of effort (RPE) following exercise above MLSS (compared to at MLSS) might have facilitated a more rapid achievement of maximum “tolerable” RPE, which resulted in subsequent task disengagement (Millet, 2011; Pageaux & Lepers, 2016). This is in line with the preposition that task disengagement may occur upon the achievement of a “sensory tolerance limit” (Hureau et al., 2018).

In Chapter V, the reasons for the “dissociation” between the ramp-derived RCP and CP/MLSS were investigated. We found that the work rate at the RCP during the 5 W·min\(^{-1}\) ramp and CP/MLSS were the same. These findings stem from the careful consideration of the dynamics of \(\dot{\text{V}}\text{O}_2\) during ramp-exercise. First, we found that the novel method for left-shifting the ramp-\(\dot{\text{V}}\text{O}_2\) better reflects the \(\dot{\text{V}}\text{O}_2\) parameters at ramp-onset. In this context, it was observed that as G increased with slower ramp-slopes, MRT\(_{SS}\) decreased, demonstrating a smaller dissociation between ramp-\(\dot{\text{V}}\text{O}_2\) and constant-\(\dot{\text{V}}\text{O}_2\) at a given work rate. These findings are opposite to what previous methods using linear and nonlinear regression modelling had reported (i.e., greater MRT...
Second, the slow ramp-slope during the 5 W·min\(^{-1}\) ramp allowed enough time for the \(\dot{V}O_2\) slow-component to develop, which abolished the “dissociation” between \(\dot{V}O_2\) and work rate during the ramp-exercise (at least up to CP/MLSS). Therefore, the correct quantification of the MRT combined with the use of a very slow ramp-slope, resulted in the RCP and CP/MLSS sharing similar work rates. Therefore, the conclusion is that the RCP is a consistent parameter of the ramp-exercise in terms of \(\dot{V}O_2\) and, although variable in terms of work rate, if a slow ramp-slope is used it can be used to separate the heavy and the severe domains.

In explaining the different dynamics of \(\dot{V}O_2\) during the different ramp-slopes performed, we hypothesized that these dynamics are determined by the biochemical and oxidative properties of the pool of muscle fibers being recruited. The underlying assumption is that the human skeletal muscle is comprised by different fiber pools that are recruited in hierarchical order (Henneman & Mendell, 2011), with each pool characterized by progressively greater inefficiency and slower kinetics but all responding with a linear-(first order) dynamic (Brittain et al., 2001). Thus, when the work rate is increased slowly, these fiber pools have “enough” time to adjust to the change in work rate and contribute to the observed \(\dot{V}O_2\), however when the work rate is increased faster they progressively lag the change in work rate and contribute less to the increase in \(\dot{V}O_2\) for a given change in work rate. Based on this hypothesis, it can be observed that the features of the \(\dot{V}O_2\) slow-component are always evident during ramp-exercise such that: \(i\) the progressively increased inefficiency (increase in G) is evident during slow ramp-protocols, whereas \(ii\) the increasingly slower kinetic is evident during fast-ramp protocols. Support to this suggestion of hierarchical recruitment of muscle fiber pools influencing the \(\dot{V}O_2\) dynamics during ramp-exercise is substantiated by the observation that \(i\) each fiber pool exhibits similar oxidative capacity (Nemeth
et al., 1981), ii) there exists considerable differences in oxidative capacity and efficiency between slow- and fast-twitch muscle fibers (Saltin et al., 1977; Reggiani et al., 1997; He et al., 2000; Crow, 2004), and iii) [PCr] is greater in type II compared to type I fibers (Casey et al., 1996; Sahlin et al., 1997). This is in line with the notion that inhibition of creatine kinase speeds up the $\dot{V}O_2$ kinetics (Grassi et al., 2011). Furthermore, in vivo studies have showed that $\dot{V}O_2$ kinetics in response to the changes in work rate of the same magnitude are slower in the upper reach compared to lower reach of the moderate-intensity domain (Hughson & Morrissey, 1982; Brittain et al., 2001; MacPhee et al., 2005; DiMenna et al., 2010; Bowen et al., 2011; Spencer et al., 2011; Keir et al., 2016c, 2016a) and that expression of type I fibers is an independent determinant of fast $\dot{V}O_2$ kinetics responses (Barstow et al., 1996; Pringle et al., 2003). Therefore, it is reasoned that the $\dot{V}O_2$ slow component is characterized by amplitude- and time-based changes that occur when the exercise intensity is progressively increased regardless of the characteristics of the forcing-function (ramp- versus constant-work rate exercise).

However, alternative explanations for this phenomenon are possible. For example, it has been suggested that an increased metabolic rate may alter the intracellular energy state causing a negative feedback for mitochondrial ATP production and delivery and slowing the $\dot{V}O_2$ kinetics (Wüst et al., 2014). In our context, this mechanism would explain the progressive increase in the time required for the $\dot{V}O_2$ to adjust to the change in work rate and contribute to the dissociation between the $\dot{V}O_2$ and work rate with faster ramp-protocols. A different hypothesis relates to the fact that the $\dot{V}O_2$ slow component is representative of a shift in the metabolic system utilized (O’Connell et al., 2017). Indeed, it has been proposed that during step-transitions performed at higher work rates, there is a simultaneous reduction and increase of anaerobic and oxidative contributions, respectively, with time to the ATP turnover (in the presence of unchanged $O_2$ cost
of the exercise within the working muscles) (O’Connell et al., 2017). This hypothesis may explain
the increased G with slower ramp-protocols, although during the ramp-exercise the anaerobic
contribution to the energy turnover should increase rather than decrease.

The aim of Chapter VI was to evaluate the level of concordance between the commonly
used percentage-based approaches for exercise prescription and the intensity domain schema.
Although several studies had pointed out the inadequacy of prescribing exercise using fixed
percentage of maximum parameters and variables measured during incremental-tests, this was the
first investigation quantifying the distribution of individuals at fixed-percentages of maximum
values in a large dataset. As expected, no fixed percentage could guarantee that all individuals
would fall within one domain of intensity (except for those at the extreme of the intensity
spectrum). The variability was exacerbated at intensities surrounding LT and MLSS, which
paradoxically are the ones commonly used in research settings (e.g., 70–80% \( \dot{V}O_2 \text{max} \); 60% of peak
work rate).

**Important findings and future directions**

From the present thesis, there are a number of implications that merit to be highlighted and
given some perspective:

- The methodological approach that can guarantee an accurate identification of LT is the one
  based on the performance of a prior moderate bout of exercise before the ramp-test. These
  findings advocate the use of this approach in future studies when an accurate *left-shifting*
  of the ramp-\( \dot{V}O_2 \) data is required – specifically in those circumstances when the work rate
  at the LT needs to be identified, and more in general when the ramp-\( \dot{V}O_2 \) is linearly
  interpolated to retrieve the corresponding work rate during ramp-exercise in the moderate-
  intensity domain.
• When the $\dot{V}O_2$ dynamics are appropriately considered during ramp-exercise the RCP corresponds to CP/MLSS not only in terms of $\dot{V}O_2$ but also work rate. Future studies will need to substantiate this evidence, perhaps by modulating both the ramp-slope and the inspired fraction of O$_2$. If the concordance will be maintained, then a mechanistic link between these markers could be proposed.

• CP and MLSS in Chapter V were found to occur at the same work rate. This is in contrast with the majority of previous studies (Pringle & Jones, 2002; Dekerle et al., 2003; Mattioni Maturana et al., 2016) except one (Keir et al., 2015). Although it is difficult to explain with certainty the reasons why in some studies CP and MLSS were found to be different, it must be recognized that both methods are subjected to errors in their determination. If one considers that some approaches for CP estimation have been demonstrated to over predict the sustainable work rate (Hill, 1993; Mattioni Maturana et al., 2018; Muniz-Pumares et al., 2019) and that the current criterion for MLSS determination may underestimate this work rate (Jones et al., 2019), it is very likely that if these errors are accounted for, then CP and MLSS would coincide in most instances. In this perspective, it is important to highlight that it is critical to perform a constant-load trial at the estimated CP. This procedure is necessary to ascertain whether the CP estimate is accurate and result in steady-state metabolic responses ($\dot{V}O_2$ and [La$_b$]). This is of paramount importance especially when CP is used to normalize the training stimulus in research settings (such as when examining training responsiveness). On the other hand, it must be recognized that the criterion for MLSS as is may not be accurate enough. As pointed out, the attainment of steady-state in [La$_b$] can be possible also after the 10$^{th}$ min (Jones et al., 2019). Hence, the current criterion for MLSS may result too “conservative”. Therefore, future studies
comparing CP and MLSS, while adopting approaches that minimize the error associated with the estimate of CP, should also re-evaluate the strategies to identify the highest work rate associated with stable [La\(^-\)]\(_b\). In this context, given that [La\(^-\)]\(_b\) is proportional to the rate of lactate formation and oxidation, and an accumulation of lactate in the blood is evidence of continuing contribution of anaerobic metabolism to the energy turnover (Brooks, 2018) (which is what elicits the progressive intracellular disturbances), stability of [La\(^-\)]\(_b\) is a requisite of metabolic steady-state.

- Finally, using fixed-percentages of maximum values for exercise prescription is a practice no longer justifiable, particularly in research settings. Although there is no direct evidence to date highlighting improved training adaptations when the exercise intensity is prescribed based on the domain schema, considering the distinct metabolic and fatigue responses within each domain (Black et al., 2017), it is possible that our understanding of the mechanisms responsible for short- and long-term training adaptations would be facilitated if the exercise intensity is prescribed with knowledge of the relative position of LT and CP/MLSS. Therefore, the fact that exercise intensity is not a linear function of \(\dot{V}O_{2\text{max}}\) should no longer be neglected.
REFERENCES


Haskell WL, Lee I-M, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW,


Iannetta D, Okushima D, Inglis EC, Kondo N, Murias JM & Koga S (2018c). Blood flow occlusion-related O\textsubscript{2} extraction “reserve” is present in different muscles of the quadriceps


Appl Physiol 294, 585–593.


Okushima D, Poole DC, Barstow TJ, Rossiter HB, Kondo N, Bowen TS, Amano T & Koga S (2016). Greater VO$_{2}$peak is correlated with greater skeletal muscle deoxygenation amplitude and hemoglobin concentration within individual muscles during ramp-incremental cycle exercise. *Physiol Rep* **4**, 1–12.


Owles WH (1930). Alterations in the lactic acid content of the blood as a result of light exercise, and associated changes in the CO$_2$ -combining power of the blood and in the alveolar CO$_2$ pressure. *J Physiol* **69**, 214–237.


C172–C178.


Torres-Peralta R, Losa-Reyna J, González-Izal M, Perez-Suarez I, Calle-Herrero J, Izquierdo M


APPENDIX I: Permissions to reproduce any published material

Chapter III: Prior permission from *Medicine and Science in Sports and Exercise* is not required for this purpose.

Chapter IV: Permission from *Scandinavian Journal of Medicine & Science in Sports* is attached below: