

**A critical appraisal of methods to determine
the Maximal Metabolic Steady State in cycling**

**This thesis is presented for the Degree of
Doctor of Philosophy at the University of Kent**

by

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Declaration

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent or any other University or Institution of learning.

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General Abstract

This thesis aimed to examine the validity of different performance markers, namely the maximal lactate steady state (MLSS), functional threshold power (FTP) and critical power (CP), in representing the maximal metabolic steady state or the upper boundary of the heavy intensity domain from a physiological perspective.

Study one (Chapter 3) reported that six out of thirteen participants' blood lactate change between the 15th and 30th minute was below 1 mM ($BLC_{\Delta 1530} < 1\text{mM}$) when exercising 15 W above the MLSS ($MLSS_{+15W}$) determined using the conventional protocol ($BLC_{\Delta 1030} < 1\text{mM}$). It demonstrated that the conventional MLSS determination protocol failed to account for the delayed steady state in blood lactate as the intensity increased. Additionally, the overall $\dot{V}O_2$ response ($\dot{V}O_2$ kinetics and the amplitude of $\dot{V}O_2$ slow component; $\dot{V}O_{2sc}$) corresponding to MLSS and $MLSS_{+15W}$ matched the characteristics of the heavy intensity domain.

Study two (Chapter 4) examined the $\dot{V}O_2$ and blood lactate response when exercising at and 15 W above the FTP (FTP_{+15W}). The overall $\dot{V}O_2$ response ($\dot{V}O_2$ kinetics and the amplitude of $\dot{V}O_{2sc}$) corresponding to FTP and FTP_{+15W} matched the characteristics of the heavy intensity domain. Conversely, the blood lactate appeared to stabilise when exercising at FTP but not during FTP_{+15W} . Thus, there is an apparent dissociation between the blood lactate and $\dot{V}O_2$ kinetics, and the intensity corresponding to FTP does not represent the upper boundary of the heavy intensity domain.

In study 3 (Chapter 5), it was demonstrated that $\dot{V}O_2$ only stabilised when exercised at the intensity corresponding to the CP but not CP_{+15W} . In addition to failing to stabilise, the highest $\dot{V}O_2$ achieved during all CP_{+15W} trials were not significantly different from the $\dot{V}O_{2peak}$. Moreover, the amplitude of $\dot{V}O_{2sc}$ corresponding to all CP_{+15W} trials was significantly higher than exercising at CP. The validity of the 2-parameter CP model in calculating the time to task failure (TTF) at CP_{+15W} was also examined. The actual TTF at three CP_{+15W} trials was significantly shorter than the predicted TTF. However, the actual TTF was not significantly different from the predicted TTF corresponding to CP_{+15W} after one week of HIT training. Therefore, the CP is a valid representation of the upper boundary

of the heavy intensity domain, but its validity in calculating the constant severe intensity exercise performance remains unclear.

In conclusion, the present thesis provides scientific evidence that blood lactate cannot accurately reflect the $\dot{V}O_2$ kinetics. Although there are some limitations of each marker, CP is the only marker examined in the present thesis that can be considered a valid representation of the MMSS or the upper boundary of the heavy intensity domain.

Covid-19 impact on research statement

The ethics application for the first study was approved on 12th March 2020 (see Appendix I). However, following the UK government guidance on 23rd March 2020, the University of Kent laboratory facilities were closed, and face-to-face contact with individuals outside your household was not permitted. The start date of the first study was postponed to January 2021 (see Appendix II). However, the first testing was further delayed to 2nd July 2021 due to multiple lockdowns, Covid restrictions, delay in equipment arrival and resubmitting an ethics application at a different institution (see Appendix III). Given that the time for data collection was severely shortened due to Covid, the number of studies included in the present thesis was less than originally anticipated.

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Abbreviations

Amp	Amplitude
ANOVA	Analysis of variance
BIF	Best individual fit
BLC	Blood lactate concentration
BLC _{FTP}	Blood lactate kinetics corresponding to FTP
BLC _{FTP+15W}	Blood lactate kinetics corresponding to FTP _{+15W}
BLC _{MLSS1}	Blood lactate kinetics corresponding to MLSS ₁
BLC _{MLSS2}	Blood lactate kinetics corresponding to MLSS ₂
BLC _{MLSSav}	The mean of blood lactate data corresponding to MLSS ₁ and MLSS ₂
BLC _{MLSS+15W}	Blood lactate kinetics corresponding to MLSS _{+15W}
BLC _{Δ1030}	The change of blood lactate concentration between the 10 th and 30 th minute
BLC _{Δ1530}	The change of blood lactate concentration between the 15 th and 30 th minute
BLC _{Δ10end}	The change of blood lactate concentration between the 10 th minute and task failure
CV	Coefficient of variation
CP	Critical power
CP _{+15W}	15 watts above the critical power and the test sequence
FTP	Functional threshold power
FTP ₂₀	The FTP determined by 95% of the mean power output during a 20 minute time trial
FTP ₆₀	The FTP determined by the mean power output during a 60 minute time trial
FTP _{+15W}	15 watts above the functional threshold power
GET	Gas exchange threshold
HIT	High-intensity aerobic training
ICC	Intraclass correlation coefficients
MAP	Maximal aerobic power
MLSS	Maximal lactate steady state
MLSS _{+15W}	15 Watts above maximal lactate steady state
MLSS ₁	First testing at the intensity corresponds to MLSS
MLSS ₂	Retest at the intensity corresponds to MLSS

MMSS	Maximal metabolic steady state
RPM	The number of revolutions per minute
TT	Time trial
TTF	Time to task failure
TD	Time delay
Tau	Time constant
$\dot{V}CO_2$	Carbon dioxide production
\dot{V}_E	Ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2base}$	The baseline $\dot{V}O_2$ measured during warm-up
$\dot{V}O_{2peak}$	Highest rate of oxygen consumption
$\dot{V}O_{2max}$	Highest rate of oxygen consumption
$\dot{V}O_{2sc}$	$\dot{V}O_2$ slow component
W	Watts
W'	The finite amount of work can be done above CP

Chapter 1

Introduction

1.1 Background

The maximal oxygen uptake ($\dot{V}O_{2max}$) represents the highest rate of oxygen consumption during exercise. It has long been considered the key determinant factor of success in endurance sports. Although other physiological responses (e.g., $\dot{V}O_2$ kinetics) can better reflect the responses to exercise and are more trainable, it remains one of the priorities for practitioners involved in endurance sports to improve or maintain the $\dot{V}O_{2max}$ when it reaches the maximal capacity as previous studies reported that insufficient or refrained from training would result in $\dot{V}O_{2max}$ reduction (Coyle et al., 1986; Houmard et al., 1992). Therefore, it is essential for coaches and athletes to understand the physiological responses to endurance exercise below, at and above the $\dot{V}O_{2max}$ to optimise race or pacing strategy and avoid premature fatigue. In terms of training, the most time-efficient training mode to improve $\dot{V}O_{2max}$ in cycling is low-volume, high-intensity interval training (HIT) (Helgerud et al., 2007; Matsuo et al., 2014; Turnes et al., 2016). A potential explanation for HIIT being more effective than constant intensity training is because of the significant amount of time spent at or close to $\dot{V}O_{2max}$ (90% to 95% of $\dot{V}O_{2max}$), as there are usually multiple all-out effort intervals (Bossi et al., 2020). The nature of HIT highly demanding from a physiological perspective as it is a means to achieving a volume of work at these intensities that could not be done in a single bout of exercise. Therefore, HIT sessions typically consist of multiple sets of all-out efforts interspersed with recovery periods equal to or longer than the work intervals (Dolci et al., 2020). However, it has been recommended that endurance athletes prioritise lower intensity training over high intensity training (Burnley et al., 2022), given that their competitions can last many hours (Lucia et al., 2001). Moreover, it has been recommended that endurance athletes should prioritise Zone 1 and Zone 2 over Zone 3 in their training (Burnley et al., 2022), whereas Zone 1 refers to exercise intensity below lactate threshold, LT), Zone 2 is above LT but below critical power (CP), and Zone 3 is above the CP. Thus, HIIT should not take up a significant portion of an endurance athlete's overall training structure and is not practical to perform frequently due to the high physical strain caused. It is more important to prescribe the minimal power output that would trigger the appropriate physiological adaptation to improve or maintain the $\dot{V}O_{2max}$ within an overall long duration and zone 1 and 2 intensity-dominated training sessions.

Generally, practitioners in cycling use seven intensity zones to prescribe training intensity as % of a specific threshold maker in Allen and Coggan's publication (2010), it is done using

Functional threshold power (FTP) (Table 1.1) or using other threshold markers, e.g., heart rate (HR). To practitioners, it is “common sense” that exercising above 90% of the maximal HR or 106% of FTP can improve $\dot{V}O_{2\max}$ (Allen & Coggan, 2010). However, neither one can be a valid approach to ensure the appropriate stimulus for $\dot{V}O_{2\max}$ improvement. For example, it has been reported that HR has a large day-to-day variability and does not correlate to the time spent above 90 % of $\dot{V}O_{2\max}$ (Achten & Jeukendrup, 2003; Bossi et al., 2020). On the other hand, the rationale for using FTP to inform training is that it is a valid alternative to other performance markers representing the exercise intensity corresponding to the maximal metabolic steady state (MMSS). The MMSS is the threshold which separates two intensities (heavy and severe intensity domains) with significantly different physiological responses, including $\dot{V}O_2$, lactate and muscle pH (Jones et al., 2019). Unfortunately, the gold standard for the MMSS determination remains controversial. Therefore, it remains unknown whether the FTP is the most appropriate and accurate performance marker for prescribing training to improve or maintain the $\dot{V}O_{2\max}$.

Table 1.1 Training intensity zones using a 7-zone system

Zone	Training aim	% FTP power	% Max heart rate
1	Active recovery	< 55	50 – 60
2	Endurance	55 – 75	60 – 70
3	Tempo	76 – 90	70 – 80
4	Threshold	91 – 105	80 – 90
5	$\dot{V}O_{2\max}$	106 – 120	90 – 100
6	Anaerobic	121 – 150	n/a
7	Neuromuscular power	> 150	n/a

Adapted from Allen & Coggan, 2010. P. 48

1.2 The maximal metabolic steady state and the issues related to its determination

In sports and exercise science, there is a 3-intensity domain system categorised by distinguishingly different $\dot{V}O_2$ responses, and they are moderate, heavy, and severe intensity domains. While the threshold between moderate and heavy intensity domain has been well accepted as gas exchange threshold (GET) or lactate threshold (LT). The threshold separating the heavy and severe intensity domain has been considered the maximal

sustainable oxidative metabolic rate or the MMSS (Jones et al., 2019). It represents the highest intensity at which the $\dot{V}O_2$ can remain steady, whereas when exercising above it, the $\dot{V}O_2$ will project towards $\dot{V}O_{2max}$, and the task failure will occur significantly sooner (Burnley & Jones, 2007; 2018). The MMSS can be determined by identifying the intensity corresponding to critical power (CP) or maximal lactate steady state (MLSS). Given that the determination protocol of CP and MLSS requires multiple trials, thus, Allen & Coggan (2006) proposed FTP which only requires a 20 minute maximal effort time trial for determination and suggested it is a valid alternative to both CP and MLSS. An accurate determination of the MMSS is vital to practitioners and scientific research. Hence, in the past three years, the topic of which performance marker can appropriately represent this threshold has received extensive attention in the sports science community (Altuna & Hopker, 2022; Burnley, 2022; 2023; Black et al., 2022; Broxterman et al., 2022; Dotan, 2022a; 2022b; 2022c; Gorostiaga et al., 2022a; 2022b; 2022c; Nixon et al., 2021) but the answer remained debated. Although previous studies reported that PCr, Pi, lactate, K^+ & $\dot{V}O_2$ were significantly different (Jones et al., 2008; Vanhatalo et al., 2016; Black et al., 2017) when exercising above CP compared to below CP, the validity of CP is questioned because CP is approximately 7 to 11% higher than the MLSS (Galan-Rioja et al., 2020). Given that it is proposed that continuous blood lactate accumulation reflects the instability in $\dot{V}O_2$ response (Svedahl & MacIntosh, 2003), it is considered the lactate steady state to reflect the oxygen steady state; thus, the MLSS is the gold standard of MMSS. With this presumption, it is often concluded that the CP overestimated the MMSS (Dotan, 2022a). Moreover, the accuracy of the determination protocol of CP is questioned because it is suggested to lack a standardisation (Dotan, 2022a). However, those who supported MLSS being the gold standard of representing the MMSS failed to acknowledge that the MLSS also shares the same fundamental methodological limitation as CP, which is a lack of standardised determination protocol. In addition, it has been suggested that the conventional determination protocol for MLSS potentially ignores delayed blood lactate steady state (Jones et al., 2019), which might underestimate the intensity corresponding to the “true MLSS”. On the other hand, the intensity corresponding to the FTP was proposed as a valid alternative to CP and MLSS by Allen & Coggan (2006) when they first introduced the concept. Surprisingly, despite FTP being the most popular performance marker in cycling, it has received very little scientific examination. Previous research on the validity between FTP and other performance markers tends to be limited to examining the correlation and

approximating the time to task failure instead of the physiological response of FTP. For example, whether the blood lactate difference between the 10th and 30th minute would be below 1 mM when exercising at FTP or whether the $\dot{V}O_2$ response can stabilise when exercising above the FTP. Therefore, some of the fundamental aspects of the FTP remained unaddressed.

The research interest in determining the gold standard for representing the MMSS has increased since the work of Jones et al. (2019) (Altuna & Hopker, 2022; Burnley, 2022; 2023; Black et al., 2022; Broxterman et al., 2022; Dotan, 2022a; 2022b; 2022c; Gorostiaga et al., 2021; 2022a; 2022b; 2022c; Nixon et al., 2021) but no decisive conclusion has been made regarding each performance marker's appropriateness to represent the threshold. Previous studies also tried to establish a new determination protocol for MMSS (Iannetta et al., 2020) and minimise the difference between the estimation of CP and MLSS by modifying the determination protocol of MLSS to using the blood lactate change between 15 to 30 or 20 to 30 minutes instead of the conventional 10 to 30 minutes blood lactate difference (Iannetta et al., 2021). Since the 3 intensity domain system is based on the different $\dot{V}O_2$ responses, the research focus would be more appropriate to highlight the difference in physiological responses between the different markers explicitly when attempting to identify the gold standard of the upper boundary of the heavy intensity domain. Specifically, it is essential to examine and consider the $\dot{V}O_2$ response of CP, MLSS and FTP along with their respective limitation mentioned above independently before a sound and scientific conclusion can be made.

1.3 Summary

The main goal of the present thesis is to determine which of MLSS, FTP and CP above is a valid representation of the MMSS, separating the heavy and severe exercise intensity domains. In addition, the present thesis also examined different unaddressed issues regarding each performance marker (ie. whether the conventional MLSS determination protocol ignored the delayed lactate steady state; the $\dot{V}O_2$ response of FTP and the validity of CP on predicting severe intensity exercise).

Chapter 2

Literature Review

2.1 Literature search strategy

A literature search was conducted using Google Scholar and PubMed. The keywords used for the search were: maximal lactate steady state, oxygen kinetics, oxygen uptake, lactate, critical power, $\dot{V}O_2$ slow component, cycling, functional threshold power, anaerobic threshold, $\dot{V}O_{2max}$, high intensity interval training, and maximal metabolic steady state. Some studies were also identified from research reference lists of other publications. The inclusion criteria were that the paper or book must be published in English and research papers must be approved by Ethics committees if including human participants.

2.2 Exercise intensity domains and the physiological response within each domain

2.2.1 Physiological responses to exercise

Exercise physiologists have long recognised the difference in the physiological response to different exercise intensities (e.g. blood lactate increases as high intensity exercise continues, Hill et al., 1924). In the 1930's, Owles identified a certain exercise intensity above which there was an observable increase in blood lactate, ventilation, and carbon dioxide excretion. Wasserman and McIlroy (1964) later suggested the term “anaerobic threshold” (AT) represents the intensity identified by Owles (1930) and proposed a non-invasive method of determination by analysing the ventilatory and gas exchange profiles. This research group subsequently identified two ventilatory thresholds which can be determined within a single ramp incremental test (Wasserman et al., 1973; Beaver et al., 1986; Solberg et al., 2005; See Figure 2.1). The first ventilatory threshold (VT_1) is also known as the gas exchange threshold (GET) and can be determined by identifying the lactate threshold (LT) (Jones et al., 2011). It corresponds to the lowest intensity at which the $\dot{V}_E/\dot{V}O_2$ exhibited a systematic increase without a concomitant rise in $\dot{V}_E/\dot{V}CO_2$ (Solberg et al., 2005; Lillo-Bevia et al., 2022). When exercising above the VT_1 , there will be a non-linear increase in $\dot{V}CO_2$ due to the blood lactate rising above the resting levels, causing bicarbonate buffering of H^+ (Wasserman et al., 1973). The second ventilatory threshold (VT_2) is also known as the respiratory compensation point (RCP). It is the second breakpoint in ventilation response and can be identified from the $\dot{V}_E/\dot{V}CO_2$ relationship (Beaver et al., 1986; Bergstrom et al., 2013), and represents the highest intensity to exercise without causing metabolic acidosis and hyperventilation (Broxterman et al., 2018; Keir et al., 2018). Exercise physiologists have since developed

three exercise intensity domains separated by these two thresholds based on their distinctively different ventilatory and oxygen uptake ($\dot{V}O_2$) responses (Whipp & Wasserman, 1972; Burnley & Jones, 2007). Knowing these thresholds and intensity domains provides invaluable information for athletes and coaches to design training intensities that could accurately target the desired physiological response to improve fitness.

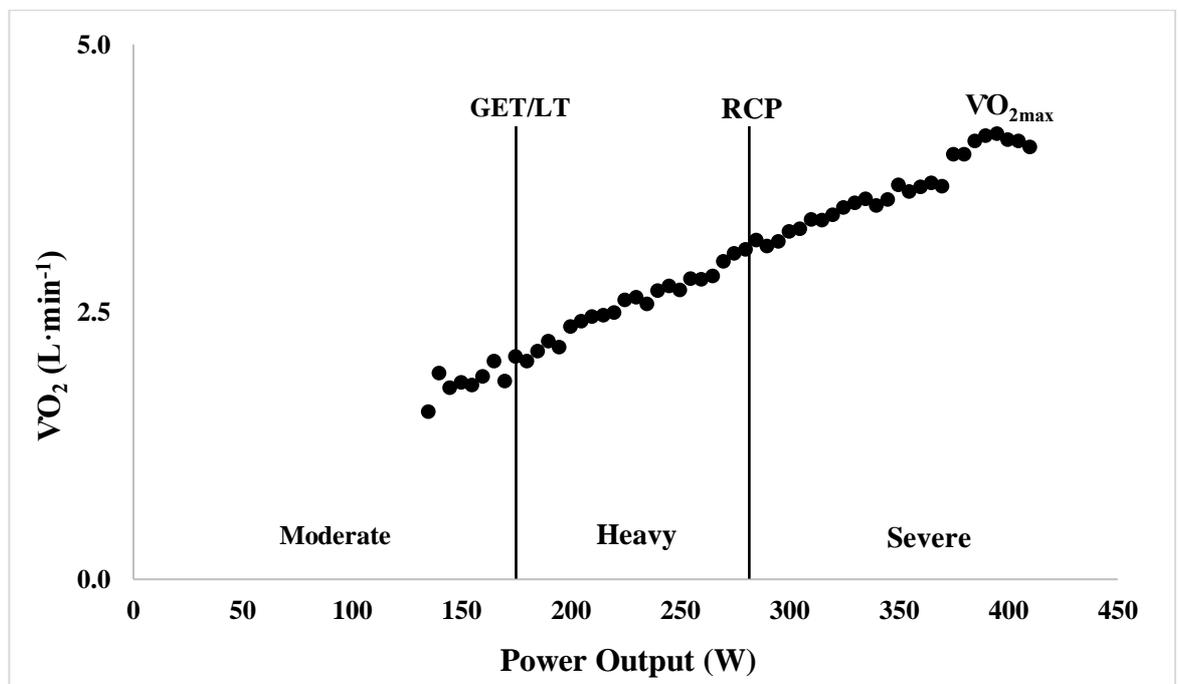


Figure 2.1 Illustration of the approximate location of the key intensity thresholds on the incremental ramp test

2.2.2 Oxygen uptake kinetics corresponding to different exercise intensity domains

The $\dot{V}O_2$ kinetics response is defined as the pattern of $\dot{V}O_2$ responses to a set of exercise challenges (Burnley & Jones, 2007). Exercising at different intensities will invoke different $\dot{V}O_2$ responses, and based on their dynamic behaviour, three exercise intensity domains have been developed – moderate, heavy, and severe (Poole et al., 1988; Gaesser & Poole, 1996; Hill et al., 2002; Wilkerson et al., 2004). The distinct features of $\dot{V}O_2$ responses to each intensity domain are depicted in Figure 2.2.

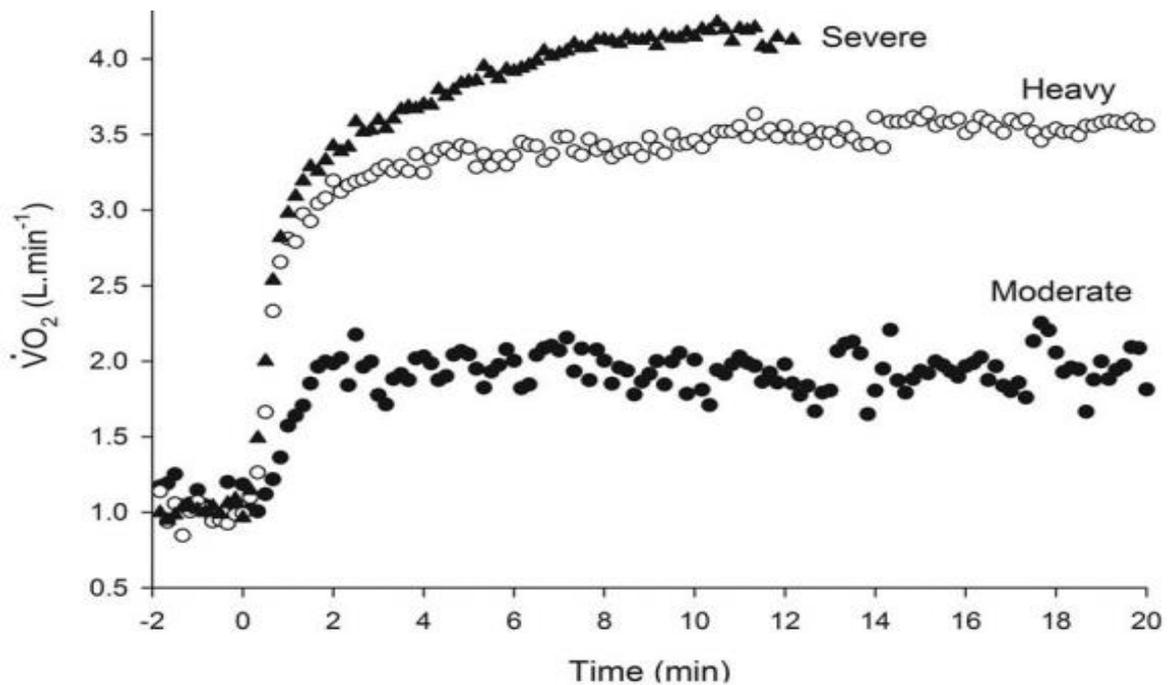


Figure 2.2. The $\dot{V}O_2$ response at different intensity zones (Burnley & Jones, 2018)

Moderate intensity applies to any exercise below the LT or VT_1 (Whipp & Wasserman, 1972; Burnley & Jones, 2007). As shown by Figure 2.2, in the moderate intensity domain, the $\dot{V}O_2$ rises monoexponentially during the cardio-dynamic phase (Phase I) and achieves a steady state (Phase III) within two to three minutes from the onset of the exercise (Linnarsson, 1974; Whipp & Wasserman, 1972). The heavy intensity domain refers to the work rate that exceeds LT or VT_1 but remains below the maximal metabolic steady state (MMSS). Here, the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) is evident after the primary phase (Phase II), which is approximately 3 to 6 minutes after the exercise start (Burnley & Jones, 2007). The $\dot{V}O_{2sc}$ may require 10 minutes to reach a steady state, stabilising at an elevated $\dot{V}O_2$ (Roston et al., 1987; Poole et al., 1988; Jones & Carter, 2000). Finally, the severe intensity domain refers to any exercise intensity above the MMSS. The $\dot{V}O_{2sc}$ is unable to stabilise, and the $\dot{V}O_2$ will continue to rise until the individuals reach their $\dot{V}O_{2max}$ if the exercise continues for long enough and eventually terminate the exercise (Poole et al., 1988; Burnley & Jones, 2007; Jones et al., 2011). In addition to the $\dot{V}O_2$, previous studies also provided other physiological evidence regarding the existence of the maximal metabolic steady state. Poole et al. (1988) reported that blood lactate achieved a delayed steady state at approximately 18 to 22 minutes during a 24 minutes constant intensity exercise at Critical Power (CP), but not when exercising 15 W above it. In addition, Jones et al. (2008) demonstrated that muscle metabolic responses are significantly different when exercising below and above the CP. The

phosphorylcreatine (PCr), pH and inorganic phosphate concentrations (Pi) could stabilise rapidly and remained close to baseline values when exercising below the CP. However, PCr and pH continued to fall while the Pi rose precipitously towards the end of the exercise when exercising above CP. A similar result shows that 90% of the magnitude of the $\dot{V}O_{2sc}$ is mirrored in the PCr response (Rossiter et al., 2002). The difference in the $\dot{V}O_2$ kinetics between the heavy and severe intensity domains will be the primary focus of this thesis and will be used to identify different intensity domains.

2.2.3 Mechanism of $\dot{V}O_{2sc}$ and its effect on exercise performance

It has been shown that at least 85% of the $\dot{V}O_{2sc}$ arises from the contracting muscles (Poole et al., 1991; Rossiter et al., 2002), whereas the remaining portion corresponds to the increased O₂ cost of ventilatory and cardiac work. The exact underpinning of the $\dot{V}O_{2sc}$ remains debatable in the literature as several mechanisms are suggested to explain the cause of $\dot{V}O_{2sc}$, including the rise in muscle temperature, reduced muscle pH caused by lactate accumulation and change in muscle fiber recruitment (from fatigued type I muscle fiber to poorly efficient type II muscle fiber) (Cannon et al., 2011; Jones et al., 2011). However, research evidence demonstrated that increased muscle temperature (Ferguson et al., 2006; Krstrup et al., 2004) and acidity (Gaesser et al., 1994; Poole et al., 1994) are less critical contributors to the development of $\dot{V}O_{2sc}$. Koga et al. (1997) reported no significant difference in $\dot{V}O_{2sc}$ when exercising muscle temperature increased by 2 to 3 degrees. Similarly, it was demonstrated that neither increased blood lactate concentration nor reduced pH significantly affects the amplitude of the $\dot{V}O_{2sc}$ during heavy intensity exercise (Gaesser et al., 1994). Conversely, there is a clear association between type II muscle fibre and $\dot{V}O_{2sc}$. Barstow et al. (1996) and Pringle et al. (2003) reported that individuals with a higher % of type II muscle fibre have a larger $\dot{V}O_{2sc}$. Additionally, the amplitude of $\dot{V}O_{2sc}$ is higher when exercising at high cadence (100 to 135 rpm) compared to low cadence because more type II muscle fibre is recruited when exercising with higher rpm (Beelen & Sargeant., 1993; He et al., 2000; Pringle et al., 2003). Other studies also reported a similar conclusion showing that both type II muscle fibers recruitment and the development of $\dot{V}O_{2sc}$ only occur during exercise intensity above the heavy intensity domain but not moderate-intensity exercise (Krstrup et al., 2004; 2008). For example, Krstrup et al. (2004) examined the muscle biopsies during both moderate (50% of $\dot{V}O_{2max}$) and heavy (80% of $\dot{V}O_{2max}$) intensity exercises. It was reported that glycogen content in type I and II muscle fibre reduced

significantly during heavy intensity, where the $\dot{V}O_{2sc}$ developed, whereas during moderate intensity only the glycogen in type I muscle fibre decreased. Given that the appearance of $\dot{V}O_{2sc}$ is mainly due to the continuous recruitment of type II muscle fibers and its phosphate to oxygen ratio is 18% higher than type I fibre, more oxygen is required to produce the same level of ATP for muscle contraction activity during at and above heavy intensity exercise (Billat et al., 2001; Poole & Jones, 2013; Grassi et al., 2015). Therefore, the $\dot{V}O_{2sc}$ signifies an additional oxygen cost, leading to a faster depletion rate of the body's limited energy stores (predominately the glycogen reserve) and results in a shorter exercise tolerance duration than if it was not present (Burnley & Jones, 2007). Overall, the $\dot{V}O_{2sc}$ is associated with the development of negative physiological consequences such as metabolic instability (e.g., muscle pH, ADP, Pi, PCr) and reduced fiber recruitment contribution (Rossiter et al., 2002; Jones et al., 2008; Dimenna et al., 2010; Colosio et al., 2020; 2021). The reduced muscle efficiency is the primary factor causing a significant difference in time to task failure (Jones et al., 2011; Murgatroyd et al., 2011; Colosio et al., 2020; 2021). Thus, whether the $\dot{V}O_2$ and $\dot{V}O_{2sc}$ can stabilise during exercise is an important indicator of fatigue development because of its fundamental linkage to other physiological variables that directly determine the time to task failure and endurance performance. Indeed, previous research has demonstrated a significant improvement in exercise tolerance when the magnitude of $\dot{V}O_{2sc}$ is reduced (Poole & Jones, 2013). Therefore, it is crucial to accurately determine the exercise intensity corresponding to the threshold that will invoke a significant difference in the $\dot{V}O_2$ and $\dot{V}O_{2sc}$ response. Based on the current literature, that threshold is represented by the MMSS as it is the highest exercise intensity that corresponds to the maximal sustainable oxidative metabolic rate, i.e., the stabilisation of $\dot{V}O_2$ and $\dot{V}O_{2sc}$ (Jones et al., 2019; Poole et al., 2021; Nixon et al., 2021).

2.3 Performance markers representing the MMSS and their limitations

2.3.1 Overview

Identifying the work rates associated with an individual's exercise intensity domains is an interest in the academic world and has important implications for applied sports scientists or clinicians working with athletic and clinical populations. However, identifying the appropriate performance marker to represent the threshold between the heavy and severe intensity domain or the MMSS remains controversial in the current literature. Some

scientists suggest the MLSS should be the “gold standard” (Beneke & von Duvillard, 1996; Billat et al., 2003; Faude et al., 2009; Garcia-Tabar & Gorostiaga, 2019; 2021; Dotan, 2022a), whereas others support the use of CP (Burnley & Jones, 2016; Jones et al., 2019; Nixon et al., 2021; Poole et al., 2021). In addition, another group propose that the two concepts are, in fact, interchangeable along with a newly developed and popular performance marker – the FTP (Allen & Coggan, 2006; 2010; Borszcz et al., 2019; Lillo-Beviá et al., 2022). However, there are some logical and scientific flaws in the arguments supporting MLSS or FTP to represent the MMSS or suggesting that the MLSS, FTP, and CP are interchangeable.

2.3.2 *Maximal Lactate Steady State*

The body's demand for energy increases as the duration of the exercise continues. Given that the ATP stored in the human body is extremely limited, the primary source of energy production shifts to the glycolytic pathway, (using muscle glycogen or blood glucose to produce ATP), producing ATP and pyruvate (Carins, 2006). While the ATP is used by working muscle immediately, the pyruvate undergoes oxidative phosphorylation in the mitochondria and produces ATP. However, the mitochondria would be unable to oxidise all the pyruvate during intense or submaximal-intensity exercise. As a result, pyruvate is converted to lactate by the enzyme lactate dehydrogenase (LDH). This reaction generates oxidised nicotinamide adenine dinucleotide (NAD⁺) from NADH, which is required for the continuation of glycolysis (Hall et al., 2016).

Lactate has several fates within and outside the muscle cell which all are facilitated via the monocarboxylate transport proteins. Within the cell, lactate can be oxidised by the mitochondria to produce ATP. This process is known as lactate oxidation or the Cori cycle. The lactate can also enter the bloodstream or be taken up and used as fuel by other tissues, such as the muscle, liver, kidney, heart, and brain, where it can be oxidised or converted back to glucose via gluconeogenesis (Brooks, 2009; Van Hall, 2010). Given that the total lactate produced is distributed to different parts of the human body, it is suggested that measuring blood lactate does not reflect the lactate kinetics in skeletal muscle (Brooks et al., 1999; Jones et al., 2019)

During moderate-intensity exercise, the net muscle lactate production depends on the balance between the lactate production rate and the lactate clearance rate. When the blood

lactate is in a steady state, the lactate production rate equals the removal rate, which is known as an equilibrium in turnover. However, as exercise intensity increases to heavy or severe, the rate of lactate production increases, and the clearance rate fails to keep up, resulting in an increase in blood lactate concentration. Various mechanisms are suggested to contribute to the increased blood lactate including mitochondrial capacity, the imbalance between lactate production and removal rate, and inadequate oxygen delivery (Hall et al., 2016).

The MLSS is defined as the highest exercise intensity at which the lactate concentration in the blood can be maintained without continual increase during constant intensity exercise (Beneke, 1995; Beneke et al., 2000). It also represents the intensity at which equilibrium is achieved in the lactate turnover rate (Heck et al., 1985). The evidence that a lactate steady state can be reached during prolonged exercise at a constant moderate and heavy intensity was first documented by Wasserman et al. (1967). Similar results were also observed by Scheen et al. (1981), as the lactate kinetics of 66 participants all showed a clear steady-state during moderate intensity (48%, 52% and 57% of $\dot{V}O_{2max}$) and a delayed steady-state at heavy intensity (63% of $\dot{V}O_{2max}$) exercise. However, the original aim of both studies was neither to examine nor to determine the lactate steady state. Wasserman et al. (1967) explored the interaction of physiological mechanisms during different exercise intensities, while Scheen et al. (1981) aimed to analyse the VT_1 during exercise at constant intensity. Snyder et al. (1980) were the first to specifically attempt to determine the MLSS as they believed it is an optimal training intensity for athletes. The criteria for the blood lactate steady state proposed by Snyder et al. (1980) was a change of less than 0.05 mM/min for blood lactate concentration (BLC), which alternatively is understood as no more than 1 mM during the final 20 minutes of a constant intensity exercise ($BLC_{\Delta 1030} < 1\text{mM}$; Heck et al., 1985). The $BLC_{\Delta 1030} < 1\text{mM}$ protocol represents the conventional MLSS protocol from this point on. In 1992, Aunola and Rusko questioned whether the testing duration of 20 to 25 minutes is too short to confirm the existence of true lactate steady state and whether the tolerance limit of 1 mM is too great to account for intraindividual differences. Hence, they suggested that the testing duration should be longer than 25 minutes and the tolerance limit should be set as 0.5 mM to ensure accuracy. Although the testing duration of 30 minutes or longer and a tougher tolerance limit such as 0.2 mM have been used in previous studies (Haverly et al., 1988; Beneke, 1995; Beneke & von Duvillard, 1996).

The MLSS concept has been considered a valid parameter of endurance sports performance, and the method of determining MLSS has been universally accepted. Previous studies have examined the reasons for the termination of exercise at the MLSS intensity (Baron et al., 2008), the physiological response when exercising at and above MLSS (Baron et al., 2003; 2008; Iannetta et al., 2018; Brauer & Smekal, 2020), the MLSS application in different sports (Beneke, 1996; Fontana et al., 2009), the reliability of the testing protocol (Beneke et al., 2000; Beneke, 2003a; 2003b; Hauser et al., 2013; Faude et al., 2016) and its validity concerning other performance markers such as CP and FTP (Pringle & Jones, 2002; Kier et al., 2015; Maturana et al., 2016; Lillo-Beviá et al., 2018; 2022; Borszcz et al., 2019; Nixon et al., 2021). Although the intensity corresponding to MLSS was reported to be highly correlated to 8 km running ($r = 0.92$), 5 km running ($r = 0.87$) and 40 km cycling TT performance ($r = 0.84$) (Jones & Doust, 1998; Haverty et al., 1988; Harnish et al., 2001), Niemeier et al. (2022) reported that the power output corresponding to MLSS is not an independent predictor of the supra MLSS time trial performance and questioned its validity to determine endurance performance. In addition, previous studies reported that individuals could exercise for 30 minutes at an intensity well above the MLSS (Iannetta et al., 2018; Hill et al., 2021). Most importantly, a few fundamental issues regarding the conventional MLSS remain unaddressed by the previous research.

2.3.3 Limitations to Maximal Lactate Steady State

The MLSS was once used as a method to validate VT_1 (Stegman & Kindermann, 1982; Heck et al., 1985; Aunola & Rusko, 1992), Pallarés et al. (2016) demonstrated that the $\dot{V}O_2$ and intensity corresponding to MLSS lies between the VT_1 and the respiratory compensation threshold (VT_2), which was found to agree with other studies (Dekerle et al., 2003; Denadai et al., 2004; Grossl et al., 2012; Kier et al., 2015). In addition, determining the work rate at MLSS is time-consuming, invasive, and labour-intensive for both researchers and subjects. It requires individuals to give multiple blood lactate samples and exercise for 30 minutes at a submaximal constant intensity of three to five times on separate days in a laboratory. Although numerous studies have attempted to simplify the conventional protocol to a single visit, the results remain controversial (Kilding & Jones, 2005; Laplaud et al., 2006; Lillo-Bevia et al., 2018). However, there remain other unanswered questions in regard to the adequacy of the MLSS to represent the MMSS. Specifically, whether the $\dot{V}O_2$ can stabilise when exercising above the MLSS determined using the conventional protocol and whether

the conventional method ignored the delayed lactate steady state. These problems would directly affect the fundamentals of the MLSS and its validity in representing the MMSS (Jones et al., 2019; Jamnick et al., 2020). For example, the conventional MLSS determination protocol would underestimate the “true MLSS” if it fails to account for a delayed lactate steady state. Additionally, if the $\dot{V}O_2$ can stabilise when exercising above the MLSS determined by the conventional protocol, it would mean the MLSS does not represent the threshold between the heavy and severe intensity domain.

From the exercise physiology perspective, lactate is a by-product the body produces during exercise and is responsible for providing energy to the working tissue during exercise (Clarke & Skiba, 2013; Poole et al., 2021). In addition, contrary to common sense in the sports world, lactate is neither the cause of muscular fatigue nor confined to anaerobic conditions (Hall et al., 2016; Poole et al., 2021), and it does not reflect the metabolic status of the contracting muscle (Jorfeldt et al., 1978; Tesch et al., 1982; Bergman et al., 1999; Vanhatalo et al., 2016). Therefore, measuring BLC or the MLSS might have limited use for informing and evaluating endurance sports performance. Smith & Jones (2001) previously reported no significant difference between the intensity of critical velocity (CV), MLSS and Lactate Turn Point and recommended that the MLSS be the preferred method for determining the upper boundary of the heavy exercise domain. However, for a concept to demarcate the upper limit of the heavy intensity domain, it is essential to consider its $\dot{V}O_2$ kinetics when exercise is performed at and above the specific intensity (Burnley & Jones, 2007; Bergstrom et al., 2017; Jones et al., 2019) because the $\dot{V}O_2$ kinetics is the foundation of characterising different exercise intensity domains (Poole et al., 1988; Gaesser & Poole, 1996; Hill et al., 2002; Wilkerson et al., 2004). It must be stressed that blood lactate is not the most important determinant in endurance sports performance, let alone the sole indicator (Clarke & Skiba, 2013). It might be considered premature if some try to encourage the perception of MLSS as the “gold standard” of representing the threshold between heavy and severe intensity without considering the $\dot{V}O_2$ kinetics. Although several studies did not use the conventional method of MLSS, they consistently reported that the $\dot{V}O_2$ stabilised when exercise was carried out at and above the MLSS intensity (Keir et al., 2015; Matirana et al., 2016; Iannetta et al., 2018; Bräuer & Smekal, 2020; Hill et al., 2021). The steady state in $\dot{V}O_2$ during exercise is the typical $\dot{V}O_2$ response corresponding to heavy intensity (Burnley & Jones, 2007) (see Figure 2.2). These results challenged the legitimacy of using MLSS as

the upper boundary of the heavy intensity domain, as the $\dot{V}O_2$ does not reflect the characteristics of the severe intensity domain when exercising above the MLSS.

Apart from the $\dot{V}O_2$ results suggesting that the MLSS is not a valid representation of MMSS, the criterion of BLC for the conventional MLSS determination should also be examined before considering whether it is appropriate to represent the MMSS. The criterion of the BLC during the MLSS test was initially set as < 0.05 mM/min when Synder et al. (1980) first proposed measuring MLSS. Subsequently, Heck et al. (1985) reintroduced the criterion to be < 1 mM during the last 20 minutes of a 30 minute constant intensity exercise. Although Heck et al. (1985) did not provide any rationale for the very specific criterion of 1 mM, it appears to be based on 0.05×20 minutes = 1 mM. The validity of using the BLC difference of less than 1 mM between two arbitrary pre-set time points (10th and 30th minute) to represent blood lactate steady state has been questioned (Jones et al., 2019). It has been reported that the rate of change in the BLC tends to be more significant in the first 10 minutes than in the final 5 to 10 minutes during the conventional 30 minutes MLSS test (Sheen et al., 1981; Jones & Doust, 1998; Beneke et al., 2003). Similar findings were repeatedly highlighted by Beneke et al. (2009; 2011), which show that 92% of the final steady-state of BLC is attained only after the 10th minute (approximately between the 15th and 20th minute). Therefore, such a blood lactate kinetics profile should be considered the achievement of a delayed steady state in BLC. The conventional protocol might therefore ignore the existence of a delayed steady state (Jones et al., 2019). In addition, Dotan (2022a) has suggested that the BLC requires more time to stabilise as the intensity increases. There is, therefore, a possibility that the exercise intensity considered as above the conventional MLSS is, in fact, an underestimation of the true MLSS because the BLC stabilised or declined towards the end of the 30 minutes constant intensity test (Van Schuylenbergh et al., 2004). Taken together, the conventional MLSS determination protocol merits a more rigorous examination, as it is poorly justified and has a high risk of underestimating the actual MLSS exercise intensity.

2.3.4 Functional Threshold Power

The equipment, time and training required to conduct and interpret MLSS tests hinder its practical application. Thus, researchers and practitioners have tried to develop an alternative performance-related indicator to assess the MMSS that is more assessable and has minimal

equipment and time demands. One proposal put forward by Allen and Coggan (2006) was a concept called the Functional Threshold Power (FTP). The FTP is defined as the highest mean power output that an individual can sustain for 60 minutes (Allen & Coggan, 2010). Although the rationale and research behind the FTP are unclear, the first study investigating FTP was conducted by Nimmericher et al. (2011), five years after the concept was introduced by Allen & Coggan (2006). FTP has become a popular method within the cycling community to assess the upper limit of sustainable exercise in cycling for two reasons: firstly, the only equipment required is a bicycle and a power meter, which are both commonly owned by cyclists; secondly, the 60-minute mean power output has been reported to be the strongest indicator of 40-kilometre time-trial (TT) performance for cyclists during the competitive season (Coyle et al., 1991). The power output corresponding to FTP is also commonly used to inform cyclists' performance levels and design training intensity to target specific physiological outcomes (Allen & Coggan, 2010) (see Table 2.1). However, the FTP determined by performing a 60-minute TT (FTP₆₀) appears to be highly stressful and physically demanding for athletes. It is especially difficult to complete when assessing performance on open roads. The protocol was modified to a 20-minute test as Allen and Coggan (2010) found that 95% of the mean power output during a 20-minute TT performance was equivalent to the mean power output achieved during a 60-minute TT performance. Subsequently, the testing method for FTP determination (see Table 2.2) comprises a 45-minute specific warm-up followed by a 20-minute TT, given that the FTP is suggested to be able to estimate the highest power output for maintaining 60 minutes, which theoretically makes it the ideal indicator for the "hour record" event in cycling. However, the power output corresponding to FTP₂₀ tends to be significantly higher than the average power during a 60 minute TT (Maclinnis et al., 2019). It has also repeatedly been reported that the FTP₂₀ exercise intensity cannot be sustained for 60 minutes regardless of the individual's experience, performance level, and aerobic fitness level (Borszcz et al., 2018; Sitko et al., 2022). According to Sitko et al. (2022), the time to task failure at FTP across four different performance level categories (recreationally trained, trained, well trained and professional cyclists) was all shorter than 60 minutes (35, 42, 47 and 51 minutes, respectively). Therefore, the validity of using the intensity estimated from FTP₂₀ to approximate the FTP₆₀ remains controversial. Nevertheless, previous studies have suggested that the FTP concept is interchangeable with MLSS (Borszcz et al., 2019) and CP (Allen & Coggan, 2010).

Table 2.1 Functional threshold power based training levels

Zone	Description	% of FTP	RPE	% HR at FTP
1	Active Recovery	< 55	< 2	<68
2	Endurance	56-75	2-3	69-83
3	Tempo	76-90	3-4	84-94
4	Lactate Threshold	91-105	4-5	95-105
5	$\dot{V}O_{2max}$	106-120	6-7	>106
6	Anaerobic Capacity	121-150	>7	N/A
7	Neuromuscular Power	N/A	Maximal	N/A

Adapted from Allen & Coggan, 2010. P. 48

Table 2.2 The original testing procedure for FTP

	Time	Description	% FTP
Warm Up	20 min	Endurance pace	65
	3 x 1 min (separated by 1 minute rest)	Fast pedalling, 100 rpm	N/A
	5 min	Easy riding	65
Main Set	5 min	All-out effort	Max
	10 min	Easy riding	65
Test	20 min	Time trial	100

Adapted from Allen & Coggan, 2010. P. 47

2.3.5 Limitations to Functional Threshold Power

The FTP is proposed to represent the exercise intensity that can exercise for 60 minutes. However, as mentioned in the preceding paragraphs, the exercise tolerance at the intensity corresponding to FTP_{20} is often shorter than 60 minutes (Borszcz et al., 2018; Sitko et al., 2022). In other words, FTP has deviated from what it is proposed to represent. Conversely, using the FTP to prescribe training intensity is a common practice for cyclists. However, studies have yet to examine the appropriateness of this method. For example, whether exercising at 106% of FTP (Table 2.1) would achieve the necessary physiological response and enough stress to the respiratory system to improve the $\dot{V}O_{2max}$ remained unaddressed.

The validity of the FTP concept as a marker of the MMSS and its relationship to other performance markers is debated within the current scientific literature. For example, Borszcz et al. (2019) suggested that the FTP can be used for MLSS estimation, as their results demonstrated a strong correlation ($r = 0.91$) with high limits of agreement (LoA) of $1.4 \pm 9.2\%$. In contrast, a study by Inglis et al. (2019) suggested that the FTP_{95%} significantly overestimated the MLSS by 17W, and the agreement between the exercise intensity of MLSS and FTP only improved when the percentage of FTP was adjusted from 95% to 88%. The apparent conflict in findings between the two studies is likely due to Borszcz et al. (2019) used a 45-minute warm-up which involved a high intensity 5 minutes time trial, potentially causing a higher degree of fatigue and energy depletion prior to the 20 minutes TT, whereas Inglis et al. (2019) only prescribed eight-minute baseline cycling at 80W. However, Barranco-Gill et al. (2020) reported that the FTP is independent of the intensity of the warm-up. Interestingly, no study has examined the lactate kinetics when exercising at FTP to determine its validity as an alternative to MLSS by fulfilling the requirements of the conventional MLSS determination criteria.

One of the significant values of FTP proposed by Allen and Coggan (2006) is that it is a valid alternative method to determine the critical power (CP). The possible linkage between FTP and CP could be based on the research from Coyle et al. (1991) and Smith et al. (1999). In the work by Coyle et al. (1991), it was suggested that the 1-hour laboratory performance test was highly correlated with the actual road racing 40 km TT performance ($r = -0.88$, $p < 0.001$). Whereas Smith et al. (1999) reported that the power output corresponding to CP is highly related to the 40 km TT performances. When combining both results, it is tempting to consider the 1-hour-based FTP as an alternative to CP or the MMSS. However, an indirect comparison of the TT performance and correlation only provided a superficial and weak linkage between the FTP and CP. Thus, a more rigorous scientific examination is required before making any meaningful conclusion between the FTP and CP. Specifically when it is trying to develop a new performance marker to represent the MMSS, which has significant value to sports scientists and practitioners.

Since FTP is a relatively new concept, little research has been conducted on this topic. Less than 30 FTP-related research papers have been published, with only around 20 papers directly examined the relationship between FTP and other “traditional” performance markers.

The current literature examined the validity of FTP representing the MMSS by simply investigating the correlation and limits of agreements between the FTP and other performance markers representing the MMSS, such as MLSS and CP. As mentioned previously, the determination of its validity to be considered an indicator for MMSS should be based on the physiological response, $\dot{V}O_2$ kinetics specifically, when exercising at and above the FTP. For FTP to be the threshold between the heavy and severe intensity domain, it is vital to examine whether exercise around the FTP can trigger the unique $\dot{V}O_2$ kinetics characteristics presented in Figure 2.2 as that is the foundation of the MMSS and the three intensity domains system. Without the availability of such information, the FTP should not be further considered as a replacement for MLSS or CP to represent the MMSS from a physiological standpoint.

2.3.6 Power duration relationship and the concept of Critical Power

The relationship between exercise intensity and duration was first proposed by Hill (1925) in his work examining speed–time/power–duration curves from world record performance to investigate the function of aerobic and anaerobic energy sources during physical performance. In 1965, Monod and Scherrer also observed this hyperbolic relationship between power and duration (PD) from an isolated muscle group. More recently, the concept has been extended to whole body exercise, including cycling (Smith et al., 1999; Pringle & Jones, 2002), running (Kolbe et al., 1995; Florence & Weir, 1997), swimming (Dekerle et al., 2006) and rowing (Shimoda & Kawakami, 2005). The hyperbolic form of the PD relationship can be described using the following equation (Morton, 2006):

$$\text{Hyperbolic Model: } T_{\text{lim}} = W' / (P - CP) \quad [\text{Equation 1}]$$

Where P is power and T_{lim} is the duration for which the power lasted. When applied in sports such as running, the W'/W and P would be expressed as D'/D (Distance) and S (Speed), respectively. CP is critical power, and it was originally defined as “the maximal work rate that can be maintained for a very long time without fatigue”. However, the duration that can be sustained during exercise at CP has been quantified to be approximately 30 minutes in recent research (Poole et al., 2016). The CP or its analogous Critical Speed (CS) represents the upper limit for exercise sustained by aerobic metabolism, and the W' is the finite amount

of work that can be performed above the CP (Jones et al., 2011; Poole et al., 2021). Equation 1 can also formulate into two other equations:

$$\text{Linear Total Work Model: } W = (\text{CP} \times T_{\text{lim}}) + W' \quad [\text{Equation 2}]$$

$$\text{Linear 1/Time Model: } P = (W' \times 1/T_{\text{lim}}) + \text{CP} \quad [\text{Equation 3}]$$

These simple mathematical equations describing the PD relationship and the information of CP and W' can provide valuable information to practitioners. For example, designing different racing strategies to maximise their metabolic advantages or act accordingly to opponents' metabolic strength (Fukuba & Whipp, 1999). The best performance over any severe intensity exercise can also be accurately calculated (Jones & Whipp, 2002; Jones et al., 2010; Vanhatalo et al., 2011; Jones & Vanhatalo, 2017) and achieved through an optimised pacing strategy (Jones et al., 2010). Although CP and W' are equally important in the concept of CP, the present thesis will focus on the CP and its validity as the representation of the MMSS.

Even though CP is determined from the mathematical relationship between performance and time to exhaustion, several previous research papers have demonstrated its ability to estimate the MMSS or the upper boundary of the heavy intensity domain based on the $\dot{V}O_2$ kinetics (De Lucas et al., 2013; Vanatalo et al., 2016; Black et al., 2017; Nixon et al., 2021). Poole et al. (1988) reported that the blood lactate and $\dot{V}O_2$ reached a delayed steady state during exercise at the intensity corresponding to the CP, but continued to rise and reached $\dot{V}O_{2\text{max}}$ when exercising above the CP. A similar result was reported by Nixon et al. (2021) that the $\dot{V}O_2$ stabilised when exercising at a speed of approximately 0.5 km/h below the CS but failed to stabilise and reached $\dot{V}O_{2\text{max}}$ when the exercise intensity was 2.4% above the CS. Similar results were also found in cycling as De Lucas et al (2013) demonstrated that contrary to the appearance of a $\dot{V}O_2$ steady state when exercising at CP, $\dot{V}O_{2\text{max}}$ was attained when exercising 5% above the CP. Indeed, previous research has also shown that working at and above CP will trigger distinct physiological responses in multiple physiological indexes such as PCr, pH and Pi (Jones et al., 2008). Studies that examined the muscle metabolic responses at and above CP also reported similar findings. Jones et al. (2008) demonstrated that muscle metabolic responses significantly differ when exercising 10% below and 10% above the CP.

The PCr, pH and Pi stabilised rapidly and remained close to baseline when exercising below the CP. However, PCr and pH continued to fall while the Pi rose precipitously towards the end of the exercise when exercising above the CP. Vanhatalo et al. (2016) demonstrated that the muscle PCr, lactate and PCr remained stable between 12 minutes and the end test during the test < CP trial but now during > CP trial. Additionally, the $\dot{V}O_2$ data is consistent with previous studies showing a steady state during < CP trial but the $\dot{V}O_2$ did not stabilise and rise towards $\dot{V}O_{2max}$ during > CP trial. Collectively, CP separates two intensity domains which have different physiological responses. Most importantly, the $\dot{V}O_2$ response to exercising at and above CP matches the heavy and severe intensity domain characteristics, respectively (Poole et al., 1988; Gaesser & Poole, 1996; Hill et al., 2002; Wilkerson et al., 2004).

2.3.7 Considerations associated with the Critical Power and its conventional determination method

Although there were studies that challenged the validity of CP representing the MMSS (Keir et al., 2015; Maturana et al., 2016), there are some flaws behind the rationale of their conclusion. In the work of Keir et al. (2015), the mean difference between the intensity corresponding to CP and MLSS was 2 ± 12 W, and the $\dot{V}O_2$ for each intensity was 3.29 ± 0.48 and 3.27 ± 0.44 L/min⁻¹, respectively. Thus, it was concluded that both CP and MLSS are physiological equivalent and valid representations of the MMSS. However, the $\dot{V}O_2$ clearly stabilised when exercising at and 10 W above the conventional MLSS, with the end test $\dot{V}O_2$ both corresponding to 79% of $\dot{V}O_{2max}$. It indicated that the conventional MLSS does not separate the heavy and severe intensity domains. On the other hand, Maturana et al. (2016) concluded that the CP overestimated the MMSS because the intensity corresponding to CP was higher than MLSS (253 ± 44 W vs 233 ± 41 W). They used the rationale to support the MLSS as the reference for MMSS because both blood lactate and $\dot{V}O_2$ can be stabilised at MLSS. However, as mentioned in the MLSS section, the conventional MLSS potentially ignored the delayed steady state in blood lactate, which underestimated the true MLSS. Additionally, a clear $\dot{V}O_2$ steady state was evidenced both at and 10 W above MLSS. The discrepancy between the $\dot{V}O_2$ response corresponding to MLSS_{+10W} and the characteristics of the severe domain was reported repeatedly (Keir et al., 2015; Maturana et al., 2016). Therefore, it is reasonable and logical to conclude that CP and MLSS are not interchangeable

and the difference between the two should not be the bases to challenge the validity of CP representing the MMSS.

Despite multiple research studies supporting the use of CP as the gold standard for determining the MMSS (De Lucas et al., 2013; Jones et al., 2019; Nixon et al., 2021), some concerns persist regarding the accuracy of CP estimation (Muniz-Pumares et al., 2019). The conventional CP determination protocol requires 3 to 5 different points (power outputs for given durations) on the PD relationship in the severe exercise intensity domain. All determination trials must be between 2 and 15 minutes (Muniz-Pumares et al., 2019). Any intensity that lasts less than 2 minutes or more than 15 minutes would risk the intensity being outside the severe domain or unable to attain $\dot{V}O_{2max}$, which would affect the CP estimation (Hill et al., 2002; Vanhatalo et al., 2016; Burnley, 2022). Indeed, the CP reported by Pallarés et al. (2020) was 2% lower than MLSS instead of the commonly suggested 7 to 11% higher than MLSS (Jones et al., 2019; Saif et al., 2022) because the longest trial used was approximately 80 minutes. Additionally, there should be at least five minutes between the shortest and the longest trial (Poole et al., 1988). The estimated CP would be significantly inflated if the trials were exclusively short (e.g., 68 to 193s) (Bishop et al., 1998). Besides following the duration requirement, the need for the attainment of $\dot{V}O_{2max}$ is also vital for a valid determination of CP. It can ensure that the prescribed intensity for the determination trial is severe intensity (Burnley & Jones, 2007). Multiple research studies support the notion that the W' and $\dot{V}O_{2SC}$ are positively and mechanistically related (Murgatroyd et al., 2011; Vanhatalo et al., 2007; 2011; 2016; Goulding et al., 2021). The $\dot{V}O_{2max}$ attainment represents a complete W' depletion because of the maximum $\dot{V}O_{2SC}$ amplitude developed (Burnley, 2023). Thus, reaching the $\dot{V}O_{2max}$ can ensure the participants performed to the limit of tolerance at different determination trials and the estimation of the CP and W' .

From a practical perspective, one of the reasons that render the determination of the CP cumbersome is that the recovery period between each maximal effort test is usually between 24 to 48 hours, which is similar to the determination procedure of MLSS. Theoretically, the minimal number for the determination trial can be two. However, this approach would require at least two familiarisation trials before the actual determination trials for a valid result. Furthermore, the calculation for CP would be limited to only linear equations (Simpson & Kordi, 2017). The limitation of only using the linear equation would be unable

to determine the model with “best individual fit” (BIF) to minimise the combined error for the CP and W’ to less than 5% and 10%, respectively. Therefore, using at least three constant severe intensity trials is recommended. Besides using the BIF approach to improve the accuracy of CP and W’, a previous study has also reported that Equation 1 is the favourable choice to assess the MMSS (Altuna & Hopker, 2021). There are two approaches to determining the CP: the conventional method in which the CP is calculated based on the performance at a fixed time or fixed power/speed (Black et al., 2015), or a 3 minute all-out test during which the participants must exercise against a fixed resistance maximally for 3 minutes (Burnley et al., 2006b; Vanhatalo et al., 2007). The present thesis will focus on discussing the conventional approach. Multiple research studies support the use of CP as the gold standard for determining the MMSS (De Lucas et al., 2013; Jones et al., 2019; Nixon et al., 2021).

Great care must be taken in all aspects including the number, duration and intensity of the determination trials and the modelling of CP. Although theoretically the CP can be calculated using 2 determination trials, the optimal and conventional number of determinations trials is 3 to 5 (Pringle & Jones, 2002; Vanhatalo et al., 2007; Black et al., 2015). conventional CP determination protocol requires 3 to 5 different points (power outputs for given durations) on the PD relationship in the severe exercise intensity domain. The main principle of the duration for the TT approach is that all determination trials must be between 2 and 15 minutes (Muniz-Pumares et al., 2019). Any intensity that lasts less than 2 minutes or more than 15 minutes would risk the intensity being outside the severe domain and affecting the CP estimation (Burnley, 2023). Indeed, the CP reported by Pallarés et al. (2020) was 2% lower than MLSS instead of the commonly observed 7 to 11% higher than MLSS (Jones et al., 2019; Saif et al., 2022) because the longest determination trial for CP was approximately 80 minutes in their study. On the other hand, there should be at least five minutes between the shortest and the longest determination trial (Poole et al., 1988). The estimated CP would be significantly inflated if the trials were exclusively short (e.g., 68 to 193s) (Bishop et al., 1998). Additionally, using the same number of trials but different duration could result in a different CS estimation. Triska et al. (2018) examined the difference in the CS determined between two fixed time protocols; protocol 1 (12, 7 and 3 minutes) and protocol 2 (10, 5 and 2 minutes), which were both within the recommendation range. The CS for the same group of participants was significantly different between

protocols (4.17 vs. 4.29 m/s⁻¹) and the longer combined durations (protocol 1) consistently resulted in a lower CS, which is in line with the assumption of Gorostiaga et al. (2022a). It is suggested the CS was affected because participants with lower CS (< 4 m/s⁻¹) did not fully deplete their D' during the 12 minutes run. On the other hand, it was suggested that the physiological or psychological residual fatigue from the 12 minutes trial affected the 7 minutes trial performance and thereby the D' estimation.

The exercise intensity corresponding to CP estimated using the TT has been demonstrated to overestimate the CP determined using the constant work rate approach (Black et al., 2015). This discrepancy may be because participants adopted a fast start pacing strategy during the time trials but not the constant work rate trials. Bailey et al. (2011) showed that 3-min time trials with fast-start pacing yielded greater total work, mean power, and end-spurt power than time trials with even pacing ($p < 0.05$), the same strategy used in Black et al.'s (2015) constant work rate trials. A fast start may alter $\dot{V}O_2$ kinetics, reducing metabolic stress early in the trial and enabling higher power outputs and $\dot{V}O_{2max}$ utilisation, especially in trials lasting under 3 min. Specifically, the fast start could allow individuals to maximise the finite work capacity (W') available for very short, high-intensity trials. In contrast, the even pacing of the constant work rate trials may have caused participants to finish with spare W' capacity, leading to a lower CP estimate. The CP can be determined using this fixed work rate method to minimise the effect of different pacing and duration. Generally, the work rates assigned to the constant work rate tests were based on the individual's ramp incremental test performance. The assigned intensity with monitoring the $\dot{V}O_2$ response during determination trials ensures the prescribed work rate is severe intensity and the W' is fully depleted upon task failure. However, there is no standardised intensity for the fixed work rate approach (Vanhatalo et al., 2007; Black et al., 2015). Therefore, a consistent and standardised protocol (i.e., fixed work rate at the same % of power output) is recommended to minimise any noise on the CP estimation (Black et al., 2015; Maturana et al., 2018).

From a practical perspective, one of the reasons that render the determination of the CP cumbersome is that the recovery period between each maximal effort test is usually between 24 to 48 hours, which is similar to the determination procedure of MLSS. Theoretically, the minimal number for the determination trial can be two. However, this approach would require at least two familiarisation trials before the actual determination trials for a valid

estimation of CP and W'. Furthermore, the calculation for CP using only 2 trials would be limited to only linear equations (Simpson & Kordi, 2017). The limitation of only using the linear equation would be unable to determine the model with "best individual fit" (BIF) to minimise the combined error for the CP and W' to less than 5% and 10%, respectively. Therefore, using at least three constant severe intensity trials is recommended and minimising the recovery time between each determination trial appears to be the favoured approach.

Multiple studies investigated different recovery times between trials with the least effect on the estimation of CP and W'. Karsten et al. (2017) previously explored the validity of reducing the recovery period between trials from 24 hours to 3 hours or 30 minutes. Compared to the 24 hour recovery period, the average prediction error of CP of 3 hours and 30 minutes was 2.5% and 3.7%, respectively. However, a large prediction error was reported for W'; 25.6% (3 hours) and 32.9% (30 minutes). Therefore, the study concluded that both recovery time protocols are only valid for determining CP but not W'. Karsten et al. (2017) examined the 60 minutes recovery and showcased similar results, concluding that it could be a valid alternative testing protocol for estimating the CP. However, consistent with the findings from Karsten et al. (2017), the results cannot transfer to W'. On the contrary, Burnley et al. (2011) reported that prior severe exercise has no significant effect on exercise tolerance, CP and the W' after severe intensity exercise despite increased blood lactate and primed $\dot{V}O_2$ kinetics. The 60 minute recovery between each determination trial was also supported by MUNIX-Pumares et al. (2019) as the most suitable approach for CP estimation to shorten the total duration of the multiple testing trials method. Although the PCr, pH and W' can typically be restored within 25 minutes (Baker et al., 1993; Ferguson et al., 2010; Skiba et al., 2012), the rationale for 60 minutes recovery period is that it minimises the priming effects in $\dot{V}O_2$ kinetics from the previous determination trials (Burnley et al., 2006a; Karsten et al., 2017).

2.3.8 Limitations of Critical Power

Even though the BIF approach can address the difference between the CP and W' determined by different equations, the determination method still arguably lacks a standardised protocol (Dotan, 2022a). As mentioned, performing 3 to 5 fixed time or power tests which last 2 to 15 minutes to determine the CP and W' is suggested. However, using a different duration

would result in a different CS estimation (Triska et al., 2018). The difference between the CS was determined using protocol 1 (12, 7 and 3 minutes) and protocol 2 (10, 5 and 2 minutes), both within the recommendation range. The CS for the same group of participants significantly differed between protocols (4.17 v.s. 4.29 m/s⁻¹). It was concluded that the longer combined durations consistently resulted in lower CS, which is in line with the assumption of Gorostiaga et al. (2022a). The fixed time/duration testing protocol also reported a significantly higher CP estimation in cycling compared to the fixed intensity protocol (Black et al., 2015). This discrepancy in the CP estimation between the two protocols may be because participants adopted a fast start pacing strategy during the fixed time trials but not the constant work rate trials. Bailey et al. (2011) showed that 3-min time trials with fast-start pacing yielded greater total work, mean power, and end-spurt power than time trials with even pacing ($p < 0.05$). Similarly, it is suggested that because a fast start would lead to rapid $\dot{V}O_2$ kinetics and reduced O₂ deficit, therefore it dictates a higher CP (Goulding et al., 2021; Goulding & Marwood., 2023). For constant work rate protocol, the assigned intensities are generally based on the individual's ramp incremental test performance. However, there is no guarantee that all individuals can sustain the minimally required 2 minutes at the highest work rate determination trial (100% of the intensity at $\dot{V}O_{2max}$). Given that both approaches have their respective limitations as mentioned above, it is recommended to use a consistent protocol (i.e., fixed work rate with the same % of power output) to avoid the overestimation of the CP and standardise the determination protocol (Black et al., 2015; Maturana et al., 2018).

Recent work by Gorostiaga et al. (2021; 2022a) challenged the CP model and suggested that it is merely a statistical artifact and challenged its validity for demarcating the heavy from severe intensity exercise domains. They found that a hyperbolic model could not adequately fit the relationship between speed and time across different track running performances. As a result, CP tended to closely correspond to 95-99% of the intensity sustained for the longest trial duration. However, Gorostiaga et al. (2021) ignored each intensity domain's unique physiological characteristics. Their study provided no physiological evidence (e.g., $\dot{V}O_2$ or lactate responses) demonstrating a similar physiological response when exercising below and above CP. The result of De Lucas et al. (2013) reported that the $\dot{V}O_2$ response corresponding to exercising at and 5% above the CP challenged the validity of CP representing the MMSS. The $\dot{V}O_2$ kinetics illustrated in their work showed a non-steady

state both at and above the CP. However, the $\dot{V}O_{2sc}$ was lower in the CP_{+5%} trial compared to the CP trial (247 vs 222 ml/min), which is contrary to the typical $\dot{V}O_{2sc}$ profile of heavy and severe intensity exercise (Pringle et al., 2003). It is highly possible that the CP was overestimated as they only used the linear equation instead of BIF or Equation 1 suggested by Altuna & Hopker (2021). Therefore, there is a high chance that both intensities examined belonged to the severe domain. The current limitation of the CP concept is mainly on the mathematical aspect. From a physiological perspective, although previous studies provided a relatively comprehensive overview of the different physiological responses (Vanatalo et al., 2016; Black et al., 2017; Nixon et al., 2021), the mixed result was reported due to different determination protocols and analysis methods.

2.4 Summary

The work rate corresponding with the MMSS provides important insight into endurance sports and fatigue-related mechanisms, which are unique compared to other performance markers. However, this threshold has received far less attention than other physiological indicators, such as $\dot{V}O_{2max}$ and LT. The primary reason for this lack of popularity is the confused definition and disagreement amongst researchers concerning the suggested procedures of its estimation.

The previous sections highlighted how it might not be appropriate to consider MLSS, FTP and CP as conceptually similar or interchangeable. While the definition and the specific $\dot{V}O_2$ kinetics characteristics of heavy and severe domains are clearly stated, some researchers appear to have disregarded them. Instead, they focused on the lactate response and the correlation between the threshold markers. Based on the current literature, only research associated with the CP sought to investigate the $\dot{V}O_2$ kinetic response as an indicator of the MMSS. Such research is lacking concerning MLSS and FTP. Moreover, the conventional determination protocol for MLSS could be considered arbitrary and poorly justified. Specifically, the $BLC_{\Delta 1030} < 1\text{mM}$ protocol could miss a delayed steady state in lactate, leading to an underestimation of the intensity corresponding to the true MLSS. Similarly, the argument of using FTP to estimate MMSS is also subject to review because previous studies have only been examined based on correlational data and lack evidence on either

$\dot{V}O_2$ or lactate kinetics. It is essential to examine MLSS, FTP and CP using the same standard to evaluate their validity in representing the exercise intensity associated with the MMSS.

2.5 Thesis Aims and hypotheses

The present study aims to provide scientific evidence to determine which performance marker (MLSS, FTP and CP) should represent the MMSS. In addition, this thesis will also examine the limitation associated with MLSS, FTP and CP.

Chapter 3 – There were three aims in this experimental chapter, 1). Determine the validity of the conventional MLSS for representing the MMSS by examining the $\dot{V}O_2$ kinetics; 2). Examine the possibility that the conventional MLSS determination protocol fails to account for delayed steady state by examining the blood lactate response and finally; 3). The reliability of the blood lactate response when exercising at the intensity corresponding to the conventional MLSS.

Chapter 4 – This experimental chapter examined the physiological difference between exercising at and 15 W above the FTP.

Chapter 5 – The main aims of this experimental chapter were 1). to examine the physiological difference between exercising at and 15 W above the CP (CP_{+15W}) and 2). The validity of the concept of CP on informing the time to task failure (TTF) at a severe constant intensity exercise. The study also investigated the effectiveness of using 1 week of HIT training in reducing the difference between the actual and predicted TTF for CP_{+15W} .

Chapter 3

The Maximal Lactate Steady State is not a valid exercise intensity threshold

3.1 Abstract

Purpose: The maximal lactate steady state (MLSS) has been proposed as the threshold between the heavy and severe intensity domains. However, this is yet to be fully supported by research evidence.

Methods: The present study examined the blood lactate and $\dot{V}O_2$ kinetics corresponding to at and 15 W above (MLSS_{+15W}) and the test-retest reliability of MLSS. Thirteen cyclists completed three to five 30 minute constant intensity exercise trials to determine the work rate corresponding to MLSS and the associated physiological responses when exercising at MLSS and MLSS_{+15W}. Oxygen consumption was recorded continuously, with blood lactate measured at baseline and every 5 minutes.

Results: BLC showed a low coefficient of variation (0.3%) and strong intraclass correlation coefficients ($r = 0.96$) between the test and retest. The end test BLC was significantly higher when exercising at MLSS_{+15W} compared to MLSS ($p < 0.001$). The percentage of $\dot{V}O_{2peak}$ was higher during MLSS_{+15W} ($78 \pm 7\%$) vs at MLSS ($72 \pm 9\%$). The $\dot{V}O_2$ stabilised at both intensities, and the magnitude of the slow component was not significantly different between the two intensities ($355 \pm 155 \text{ mL}\cdot\text{min}^{-1}$ vs $382 \pm 183 \text{ mL}\cdot\text{min}^{-1}$, $p = 0.453$).

Conclusion: Although the BLC response between test and retest is reliable, two trials at the MLSS are recommended to confirm the result. Given that the conventional determination protocol for MLSS cannot account for delayed blood lactate steady state, the $\dot{V}O_2$ response corresponding to MLSS and MLSS_{+15W} were consistent with the characteristics of the heavy intensity domain. Therefore, MLSS is not a valid threshold for separating heavy and severe intensity domains.

3.2 Introduction

The maximal lactate steady state (MLSS) is the highest intensity at which the blood lactate concentration (BLC) can remain stable during a 30 minute constant intensity exercise (Heck et al., 1985; Beneke, 1995; Beneke et al., 2000; Smith & Jones, 2001). It was first established by Snyder et al. (1980), and the steady state of BLC was defined as a change of < 0.05 mM/min but did not specify the duration over which this should be determined during a 30 minute constant intensity trial. The determination protocol was later modified to require the BLC change between the 10th and 30th minute ($BLC_{\Delta 1030}$) to be less than 1 mM during a 30 minute constant intensity exercise (Heck et al., 1985; Mader & Heck, 1986). This protocol is represented as the conventional protocol from this point on. Multiple studies have examined the MLSS by investigating the physiological response when exercising at and above it (Baron et al. 2003, 2008; Iannetta et al. 2018; Bräuer & Smekal, 2020), its reliability (Beneke et al., 2000; Beneke, 2003a; 2003b; Hauser et al. 2013; Faude et al. 2016; Pallarés et al. 2016) and validity (Pringle & Jones 2002; Denadai et al. 2004; Grossl et al. 2012; Kier et al. 2015; Maturana et al. 2018; Lillo-Beviá et al. 2018, 2022; Borszcz et al. 2019; Nixon et al. 2021), as well its application in different sports (Beneke & von Duvillard, 1996; Fontana et al. 2009). However, there remain some fundamental issues of the MLSS unaddressed.

One of the issues about the conventional protocol is the validity of the criterion used for identifying lactate steady state. It has been reported that ~92% of the final steady state in BLC during a constant load trial at MLSS is attained approximately between the 15th and 20th minute (Beneke et al., 2009; 2011). Additionally, Dotan (2022), who suggested the MLSS is the preferable performance marker representing the upper boundary of the heavy intensity domain, also acknowledged that blood lactate would require more time to stabilise, if possible, as the intensity increases. The conventional protocol assumes the blood lactate would stabilise at the 10th minute regardless of the testing intensity, which contradicts the findings that the blood lactate concentration tends to stabilise over the last 10 to 15 minutes of the exercise (Beneke, 2003a; Beneke et al., 2011). Hence, the 10th to 30th minutes might ignore the delayed steady state in blood lactate, resulting in identifying as an intensity above the MLSS, even though the BLC stabilises or declines towards the end of the test (Jones et al., 2019). The reliability of the conventional protocol is another aspect that has been

overlooked. A previous study reported that the end test BLC corresponding to MLSS (coefficient of variation (CV): 16.6%) has higher day-to-day variability than the power output at MLSS (CV: 3%) (Hauser et al., 2013). However, the reliability of the conventional protocol should be assessed by the overall BLC kinetics rather than just the end test BLC. If the $BLC_{\Delta 1030}$ exceeds 1 mM during the re-test, the conventional protocol should be deemed unreliable, regardless of a strong CV or intraclass correlation coefficient (ICC).

Another controversial aspect of MLSS is its validity in representing the threshold separating the heavy and severe intensity domain or the maximal metabolic steady state (MMSS) (Jones et al., 2019). Unfortunately, no compelling conclusion has been made potentially because previous research focused on investigating the correlation between MLSS and other performance markers that represent MMSS (Dekerle et al., 2003; Maturana et al., 2016), rather than specifically examine $\dot{V}O_2$ response when exercising at and above the MLSS. The MLSS has been considered the gold standard for representing the MMSS (Faude et al., 2009; Dotan, 2022) because the MMSS represents the highest intensity at which the oxygen uptake ($\dot{V}O_2$) can remain stable and blood lactate accumulation is assumed to be caused by insufficient oxygen (O_2) and results in unstable $\dot{V}O_2$ kinetics (Billat et al., 2003; Svedahl & MacIntosh, 2003; Faude et al., 2009; Dotan, 2022a). However, lactate is not confined to anaerobic conditions and is produced continuously under fully aerobic conditions (Rogatzki et al., 2015; Brooks et al., 2021). The foundation of distinguishing different exercise intensity domains should rely on the characteristics of the $\dot{V}O_2$ kinetics (Burnley & Jones, 2007). When exercising within the heavy intensity domain, the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) is evident and stabilised at an elevated level. Conversely, $\dot{V}O_2$ will be unable to stabilise, and $\dot{V}O_{2sc}$ will project upwards until the $\dot{V}O_{2max}$ is reached if the exercise continues for long enough during severe intensity exercise (Burnley & Jones, 2007). Previous studies that examined the $\dot{V}O_2$ response when exercising above the MLSS consistently reported it stabilised and did not reach $\dot{V}O_{2max}$ when exercising above the MLSS (Brauer & Smekal, 2020; Hill et al., 2021; Nixon et al., 2021). However, two studies did not use the conventional protocol for MLSS determination; Bräuer & Smekal (2020) defined the blood lactate steady state as no more than 0.5 mM between the 10th and 30th minute, whereas Hill et al. (2021) collected blood samples after 6 to 8 and 27 to 29 minutes. While Nixon et al. (2021) used the conventional protocol, the study examined the $\dot{V}O_2$ of runners, which has significantly different characteristics of $\dot{V}O_2$ kinetics compared to cycling (Hill et al., 2003). Therefore,

these studies are not appropriate for determining the validity of the MLSS determined using the conventional protocol as the threshold separating heavy and severe domains in cycling.

The purpose of this study was to 1) examine the test-retest reliability of the BLC kinetics when exercising at MLSS; 2) to determine any significant difference between the change in BLC during the 10th to 30th and the 15th to 30th minute when exercising at and above MLSS; 3) to examine the difference in $\dot{V}O_2$ kinetics when exercising at and above MLSS.

3.3 Methods

3.3.1 Participants

Thirteen cyclists (male $n = 12$, female $n = 1$, age 23 ± 4 years, height 172.8 ± 5.0 cm, mass 60.4 ± 7.4 kg, $\dot{V}O_{2peak}$ 3.6 ± 0.7 L·min⁻¹ or 57.0 ± 10.0 mL·kg⁻¹·min⁻¹) volunteered to participate in this study. Participants' levels were classified into four categories based on their relative and absolute $\dot{V}O_{2peak}$ (De Pauw et al., 2013; see Table 3.1). The inclusion criteria were at least three years of cycling experience with at least two hours of cycling exercise per week. Before providing written informed consent, participants were fully informed about the nature of the study, all associated risks, and their right to withdraw at any time. The study was ethically approved by the Human Research Ethics Committee at the Education University of Hong Kong (E2020-2021-0063) in line with the requirements of the declaration of Helsinki.

Table 3.1 Participant characteristics

Participant	Height (cm)	Mass (kg)	GET (W)	Relative $\dot{V}O_{2peak}$ (mL·kg⁻¹·min⁻¹)	Absolute $\dot{V}O_{2peak}$ (L·min⁻¹)	Performance Level (Relative/Absolute)
1	177	75	175	70	5.24	WT / WT
2	170	62	195	70	4.32	WT / WT
3	168	55	135	69	3.81	WT / RT
4	177	56	155	54	3.05	RT / UT
5	175	67	140	56	3.71	T / RT
6	167	64	105	41	2.63	UT / UT
7	180	80	115	43	3.41	UT / UT
8	173	55	170	67	3.70	WT / RT
9*	168	58	167	53	3.04	RT / UT
10	175	61	155	53	3.22	RT / UT
11	176	68	175	50	3.40	RT / UT
12	180	65	185	63	4.12	T / RT
13	174	53	125	50	2.67	RT / UT

UT = Untrained; RT = Recreationally Trained; T = Trained; WT = Well Trained; GET = Gas Exchange Threshold, *Female participant, W = Watts

3.3.2 Study Design

The study comprised four to six laboratory visits in total. Visits were separated by 24 to 72 hours. In visit 1, participants undertook a ramp incremental exercise test to volitional exhaustion. During visits 2 to 5, participants completed multiple 30 minutes constant intensity trials (one per visit). The final visit was exercised at the work rate corresponding to MLSS. Participants were requested to avoid all training and additional exercise throughout the testing period. In addition, they were required to arrive at the laboratory fully hydrated and without consuming food and caffeine for three hours before each test and to maintain the same diet 24 hours prior to each test. All tests were performed on the same cycle ergometer (Lode Excalibur Sport, The Netherlands) adjusted for participant comfort. The pedal frequency was set at the participants' preferred rate between 80 and 90 rpm, determined by the participant prior to any testing by pedalling on a cycle ergometer without resistance; the selected rpm was held constant throughout all tests (± 2 rpm). Pulmonary gas

exchange was measured on a breath-by-breath basis using Cortex Metalyzer 3B (Cortex, Leipzig, Germany) during all trials.

3.3.3 Incremental ramp test

The incremental ramp test commenced with a warm up at 50 W for four minutes, the work rate was increased by 30 W·min⁻¹ for male participants and 25 W·min⁻¹ for the female participant until volitional exhaustion or failure to maintain the preferred rpm for more than 10 rpm for more than 5 consecutive seconds despite strong verbal encouragement (Inglis et al., 2019). The gas exchange threshold (GET) was identified using the v-slope method, defined as the $\dot{V}O_2$ value corresponding to the intersection of two linear regression lines derived separately from the data points below and above the breakpoint in the carbon dioxide production rate ($\dot{V}CO_2$)-versus $\dot{V}O_2$ relationship (Solberg et al., 2005). The power output observed as GET was corrected by subtracting 20 W and 17 W for male and female participants, respectively (Davison et al., 2022). $\dot{V}O_{2peak}$ was defined as the highest 30 s average during the incremental ramp test (Nixon et al., 2021).

3.3.4 Constant work rate tests to determine maximal lactate steady state

Participants completed a five minute warm up at 80 W, following which the work rate was instantaneously increased to the corresponding intensity. The intensity for the first determination trial (at the estimated MLSS or + 15 W) was randomly assigned using a website (<https://www.random.org/lists/>) (Inglis et al., 2019). The work rate corresponding to the MLSS was estimated using the following formula (Saif et al., 2022):

$$\text{MLSS (W)} = (\text{GET} \times 1.3) \times 0.89$$

Where GET is the power output corresponding to the gas exchange threshold.

All trials lasted 30 minutes or until volitional exhaustion, whichever occurred first. Fingertip capillary blood samples were collected before the test, every fifth minute of exercise and at the end test in duplicate (Biosen C-Line, EKF Diagnostics, GmbH, Barleben, Germany). The average of the two blood lactate samples was used to represent the BLC at each time point. The MLSS was defined as the highest work rate in W, during which the $\text{BLC}_{\Delta 1030}$ has to be less than 1 mM (Beneke et al., 1996; Jones et al., 1998). If the $\text{BLC}_{\Delta 1030}$ at the first

determination trial was ≥ 1 mM, the subsequent test was completed at a 15 W lower work rate. If the $BLC_{\Delta 1030}$ was < 1 mM at the first determination trial, the work rate in the subsequent test was increased by 15 W (Burnley et al., 2006b). A change in work rate of ± 15 W was selected due to previous research using lower values, e.g. 10 W (Maturana et al., 2016; Iannetta et al., 2021) and 5% of $\dot{V}O_{2max}$ (Dekerle et al., 2003), were suggested to be too low to provide conclusive changes in BLC and $\dot{V}O_2$ response (Jones et al., 2019). Participants proceeded to the next stage (test-retest trial) when performed one trial at the MLSS and one trial at the 15 W above the MLSS ($MLSS_{+15W}$). From this point on, the trial corresponding to MLSS determined during visits 2 to 5 is represented as $MLSS_1$. The BLC response corresponding to $MLSS_1$ and $MLSS_{+15W}$ are represented as BLC_{MLSS_1} and $BLC_{MLSS_{+15W}}$, respectively.

3.3.5 Test-retest reliability of maximal lactate steady state

The final laboratory visit aimed to investigate the test-retest reliability of the BLC kinetics when exercising at the intensity corresponding to the MLSS. Following the determination of $MLSS_1$, participants were asked to cycle at a constant work rate identical to $MLSS_1$ for 30 minutes in the final visit, and this trial is termed $MLSS_2$. Fingertip capillary blood samples were collected before the test and every fifth minute of exercise and in duplicate (Biosen C-Line, EKF Diagnostics, GmbH, Barleben, Germany). The average of the two blood lactate samples was used to represent the BLC at each time point. The BLC response corresponding to the retest is represented as BLC_{MLSS_2} .

3.3.6 Data Analysis

Breath-by-breath $\dot{V}O_2$ data from the ramp test and constant intensity trials were averaged in 10 s intervals to determine GET and the $\dot{V}O_2$ response. The $\dot{V}O_2$ data was edited to eliminate the effects of coughs or swallows on the measurement. Only those data points beyond the three standard deviations of the mean value were excluded (Burnley et al., 2006a). For $\dot{V}O_{2sc}$ calculation, the first 20 s of the $\dot{V}O_2$ data following the onset of exercise were removed to eliminate the phase I component from the analysis. The first two minutes of the $\dot{V}O_2$ data (20 to 120 s) were then analysed using the monoexponential model (Rossiter et al., 2001; Burnley et al., 2005; 2006a):

$$\dot{V}O_2(t) = \dot{V}O_{2base} + amp * (1 - e^{-(t-TD)/tau})$$

Where $\dot{V}O_2(t) = \dot{V}O_2$ at time, $\dot{V}O_{2\text{base}}$ = the baseline $\dot{V}O_2$ measured in the four minutes before the transition in work rate, amp = amplitude, TD = time delay, and tau = time constant of the primary (phase II) response. The amplitude of the $\dot{V}O_{2\text{sc}}$ was determined by the highest $\dot{V}O_2$ value achieved during the 30 minutes of constant intensity exercise and subtracting the “absolute” primary amplitude ($\dot{V}O_{2\text{base}} + \text{amp}$) (Burnley et al., 2005; 2006a). The monoexponential model was chosen because a more complex model will significantly degrade the confidence intervals and create a lot of parameter interdependence (Burnley et al., 2005). The $\dot{V}O_2$ measured during MLSS and MLSS_{+15W} trials were divided into baseline, 5th, 10th, 15th, 20th, 25th, and 30th minutes. The $\dot{V}O_2$ corresponding to the desired time points was determined by the average $\dot{V}O_2$ over the prior 60 s. The $\dot{V}O_2$ corresponding to each intensity was determined by averaging the $\dot{V}O_2$ of the last 10 minutes. The mean of the BLC_{MLSS1} and BLC_{MLSS2} is represented as BLC_{MLSSav}.

3.3.7 Statistical Analysis

Two-way ANOVA repeated measures was used to assess the difference in $\dot{V}O_2$ and blood lactate response between two intensities/trials (MLSS vs MLSS_{+15W}/BLC_{MLSS1} vs BLC_{MLSS2}/BLC_{MLSSav} vs BLC_{MLSS+15W}) and seven time points (baseline, 5th, 10th, 15th, 20th, 25th and 30th minute). The significant interaction and main effects were determined using LSD post hoc tests. When sphericity was violated, the F value was adjusted using Greenhouse-Geisser. The amplitude of the $\dot{V}O_{2\text{sc}}$, BLC_{Δ1030} and the BLC change between 15 and 30 minutes (BLC_{Δ1530}) corresponding to MLSS and MLSS_{+15W} were analysed using paired sample t-test. Analyses were performed using IBM SPSS statistics 26.0 (Chicago, IL, USA). The reliability of the BLC_{MLSS1} and BLC_{MLSS2} was analysed using the CV and ICC in Microsoft Excel (Excel, Microsoft, Redmond, Washington). Data are reported as mean ± SD unless otherwise stated.

3.4 Results

3.4.1 Test-retest reliability of the BLC response at MLSS

The mean power output corresponding to the MLSS was 170 ± 27 W. There was good reliability with a CV of 0.3% and a strong ICC (r = 0.96) between BLC_{MLSS1} and BLC_{MLSS2}. There was no significant interaction effect for the test and timepoint (F = 0.261; p = 0.953) comparing BLC_{MLSS1} and BLC_{MLSS2} and no main effect of the test (F = 0.207; p = 0.658).

However, there was a significant main effect for timepoint ($F = 37.620$; $p < 0.001$). Post hoc tests showed no significant difference in all time points between the two trials. The BLC at baseline and the 5th minute significantly differed from the rest of the time points in both trials (see Figure. 3.1). The BLC_{MLSS1} was 4.2 ± 1.4 , 4.3 ± 1.1 and 4.6 ± 1.3 mM at the 10th, 20th and 30th minute, respectively) and the BLC_{MLSS2} also stabilised (4.1 ± 1.2 , 4.3 ± 1.3 and 4.5 ± 1.3 mM). However, three participants' $BLC_{\Delta 1030}$ was > 1 mM (1.4 ± 0.2 mM) during the MLSS₂ trial, but only one participant's $BLC_{\Delta 1530}$ remained above 1 mM (1.3 mM).

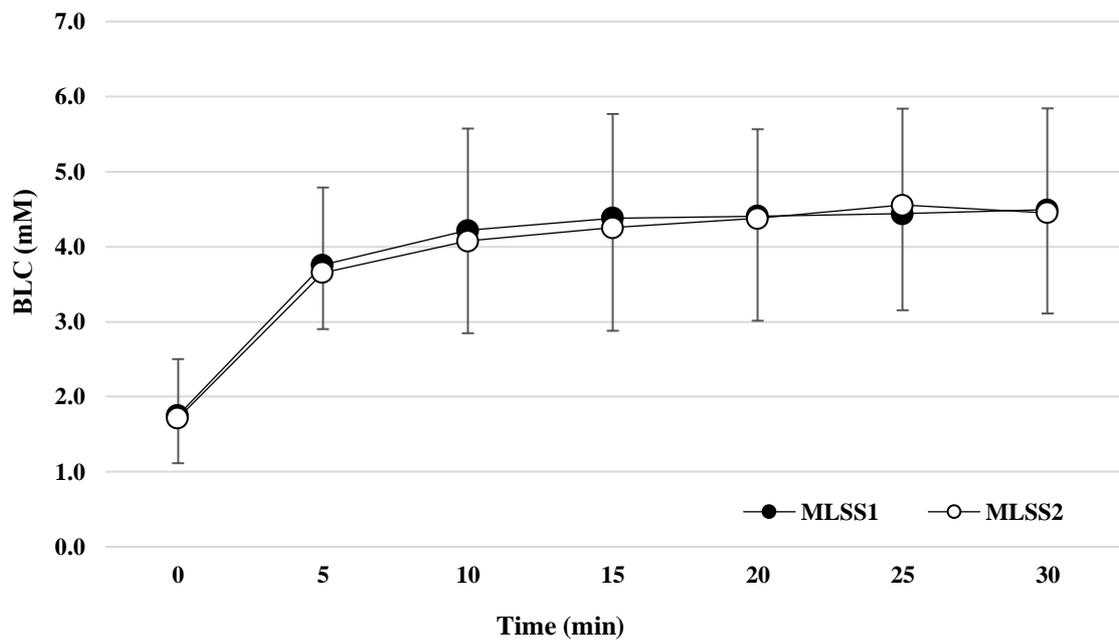


Figure 3.1. Lactate kinetics during MLSS₁ and MLSS₂ in 5 minute intervals.

3.4.2 Blood lactate kinetics at MLSS_{av} vs MLSS_{+15W}

A significant interaction effect was evident between intensity and timepoint ($F = 24.333$, $p < 0.001$). There was also a significant main effect of intensity ($F = 33.558$, $p < 0.001$) and timepoint ($F = 49.610$, $p < 0.001$). For BLC_{MLSSav} , the BLC at baseline and 5th minute were significantly different from every other time point and the BLC at the 10th minute and 30th minute was significantly different. However, there was no significant difference between the BLC in the 15th and 30th minute. Post hoc analysis revealed that the BLC was significantly different between the two intensities at all time points, except for baseline. The BLC_{MLSSav} was stable (4.1 ± 1.3 , 4.4 ± 1.2 and 4.5 ± 1.3 mM at the 10th, 20th and 30th minute, respectively). Conversely, $BLC_{MLSS+15W}$ increased throughout the test (5.4 ± 1.9 , 6.6 ± 2.5 and 7.2 ± 2.1 mM at the 10th, 20th and 30th minute, respectively, see Figure. 3.2)

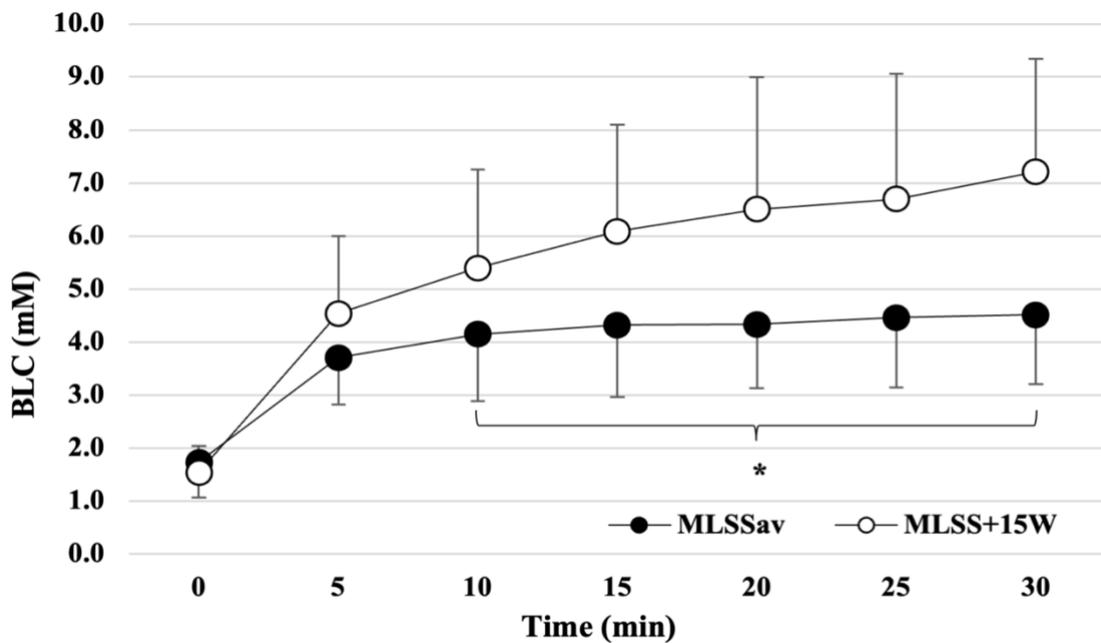


Figure. 3.2. Lactate kinetics during $MLSS_{av}$ and $MLSS_{+15W}$ in 5 minute intervals. * significant difference ($p > 0.05$).

3.4.3 The change in BLC between the 10-30 minute and 15-30 minute

When examining the $BLC_{\Delta 1530}$ for $BLC_{MLSS+15W}$, the change was below 1 mM in six out of thirteen participants. It reduced from 1.5 ± 0.3 mM ($BLC_{\Delta 1030}$) to 0.7 ± 0.3 mM ($BLC_{\Delta 1530}$) for the six participants. The mean BLC difference for the remaining seven participants was 2.1 ± 0.5 mM ($BLC_{\Delta 1030}$) and reduced to 1.5 ± 0.3 mM ($BLC_{\Delta 1530}$) for $BLC_{MLSS+15W}$.

3.4.4 $\dot{V}O_2$ kinetics and $\dot{V}O_2$ slow component at and above MLSS

The $\dot{V}O_2$ corresponding to the trial at MLSS and $MLSS_{+15W}$ were equivalent to $72 \pm 9\%$ (2.54 ± 0.39 L \cdot min $^{-1}$) and $78 \pm 7\%$ (2.73 ± 0.37 L \cdot min $^{-1}$) of $\dot{V}O_{2peak}$, respectively. There was a significant interaction effect between time and test ($F = 5.250$, $p < 0.001$). A significant main effect was evident for time ($F = 123.803$, $p < 0.001$) and intensity ($F = 26.973$, $p < 0.001$). The post hoc test revealed that the $\dot{V}O_2$ at all time points was significantly different between the two intensities, except for the baseline. The $\dot{V}O_2$ did not change significantly between the 10th minute and 30th minute during both intensities (see Figure. 3.3). Additionally, there was no significant difference in the amplitude of the $\dot{V}O_{2sc}$ between MLSS and $MLSS_{+15W}$ (355 ± 154 vs 382 ± 183 ml \cdot min $^{-1}$, respectively; $p = 0.453$).

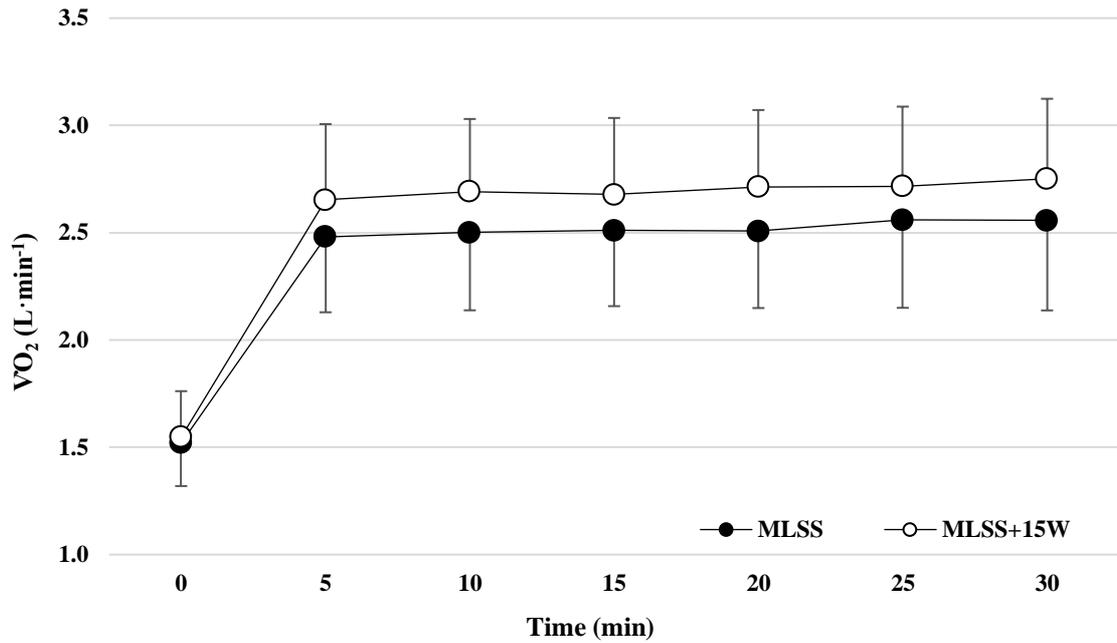


Figure. 3.3 $\dot{V}O_2$ kinetics during $MLSS_{av}$ and $MLSS_{+15W}$ in 5 minute intervals.

3.5 Discussion

3.5.1 Overview

The main findings of the present study are 1) six out of thirteen participants' $BLC_{\Delta 1530}$ was less than 1 mM during the trial at $MLSS_{+15W}$; 2) Although the CV and ICC suggested high reliability between BLC_{MLSS1} and BLC_{MLSS2} , three participants' $BLC_{\Delta 1030}$ exceeded 1 mM in BLC_{MLSS2} ; 3) The amplitude of the $\dot{V}O_{2sc}$ corresponding to MLSS and $MLSS_{+15W}$ was not significantly different, and a $\dot{V}O_2$ steady state was evident at both intensities. The $BLC_{MLSS+15W}$ indicated that the conventional MLSS protocol ($BLC_{\Delta 1030} < 1$ mM) failed to account for the delayed steady state in BLC as the intensity increases, which results in underestimating the intensity corresponding to the true MLSS. Additionally, the BLC_{MLSS2} suggested that it should take at least two trials at the same intensity to confirm the work rate corresponding to MLSS when using the conventional protocol. Based on the $\dot{V}O_2$ kinetics, the trial corresponding to $MLSS_{+15W}$ was not within the severe intensity domain as the $\dot{V}O_2$ stabilised and did not project towards the $\dot{V}O_{2peak}$ before end test. Therefore, both the intensity corresponding to MLSS and $MLSS_{+15W}$ examined in the present research should be considered to be within the same exercise intensity domain (i.e. heavy intensity), suggesting that the MLSS determined using the conventional protocol should not be regarded as the threshold to separate the heavy and severe intensity domains in cycling.

3.5.2 Reliability of MLSS

There was no significant difference ($p > 0.05$) between the BLC_{MLSS1} and BLC_{MLSS2} at all time points, and good levels of reliability (CV%: 0.3; ICC: $r = 0.96$) were found. It could be interpreted as the overall blood lactate kinetics during the MLSS having good reliability with low day-to-day variability, contrary to the conclusion made by Hauser et al., 2013. The high mean constant load trials (11.8 ± 2.0 per subject) and the lengthy period (six weeks) to establish the reliability (Hauser et al., 2013) may explain the lack of reliability in the MLSS end test BLC. The high mean total test number was likely to cause a training effect on the low baseline fitness level participants ($\dot{V}O_{2max}$: 55.5 ± 6.6 ml/min/kg; recreationally trained) and, therefore, also affected the reliability result (Jones et al., 2019). As mentioned previously, it is essential to consider whether $BLC_{\Delta 1030}$ met the 1 mM criterion upon re-test when determining the reliability (Hopkins, 2000). In the present study, three participants' $BLC_{\Delta 1030}$ exceeded the 1 mM at MLSS₂. In other words, the three participants' MLSS was at least 15 W lower only a few days after the first determination, which contradicts Hauser et al.'s (2013) conclusion that the work rate corresponding to MLSS shows a low day to day variability. However, when analysing the $BLC_{\Delta 1530}$ during the MLSS₂ trial, only one participant's BLC difference remained above 1 mM. Such results suggested that $BLC_{\Delta 1530} < 1$ mM could be a reasonable approach to improve the reliability of the MLSS determination protocol. It is recommended to perform at least two trials at the intensity corresponding to MLSS to confirm the BLC kinetics if using the conventional MLSS determination protocol.

3.5.3 Blood lactate kinetics at and above the MLSS

The rationale behind the $BLC_{\Delta 1030} < 1$ mM criterion has been questioned recently (Iannetta et al., 2021; Nixon et al., 2021). Thus, Nixon et al. (2021) proposed a new criterion, $BLC_{\Delta 1020} < 2$ mM. Applying this higher criterion for BLC change and a new time window to the current data led to the work rate corresponding to MLSS_{+15W} being classified as MLSS for all participants. Similarly, the result from the present study indicated that the criterion of $BLC_{\Delta 1530} < 1$ mM significantly changed the blood lactate difference and MLSS determination results. When using $BLC_{\Delta 1530} < 1$ mM, six out of thirteen participants' $BLC_{MLSS+15W}$ can be determined as stabilised ($BLC_{\Delta 1530} = 0.7 \pm 0.3$ mM) during the intensity originally classified as MLSS_{+15W} by the conventional protocol ($BLC_{\Delta 1030} < 1$ mM). Although Heck et al. (1985) and Mader and Heck (1986) did not specifically justify the criterion, the rationale of the conventional $BLC_{\Delta 1030} < 1$ mM protocol appears to be 0.05

mM x 20 minutes. Based on that, the $BLC_{\Delta 1530}$ (0.7 ± 0.3 mM) of the six participants' $BLC_{MLSS+15W}$ in the present study would also satisfy the MLSS criterion suggested by Snyder et al. (1980) (0.05 mM x 15 minutes = 0.75 mM). Additionally, the BLC_{MLSSav} changed significantly between the 10th and 30th minute ($p < 0.05$) (see Figure 3.2) despite $BLC_{\Delta 1030}$ being less than 1 mM, but there was no significant difference between the BLC at the 15th and 30th minute. It highlighted the limitation of using the conventional $BLC_{\Delta 1030} < 1$ mM criterion to inform the differences in blood lactate change. Additionally, the mean end test BLC corresponding to the $MLSS_{+15W}$ for the six participants was 6.5 mM and it has been suggested that MLSS of approximately 7 mM may take 15 to 20 minutes for blood lactate to stabilise (Beneke et al., 2011). It is, therefore, reasonable to conclude that it took 15 minutes for the BLC to adjust to the corresponding intensity for the six participants. Using the conventional $BLC_{\Delta 1030} < 1$ mM protocol may therefore fail to account for the apparent delayed blood lactate response as the intensity increases (i.e. the $MLSS_{+15W}$ in the present study). If the MLSS is determined by the conventional protocol and used to inform training intensity or program, it could result in a 15 to 30 W underestimation of the “true MLSS”. For example, the work rate corresponding to the MLSS for a participant in the present study was 196 W, determined using the $BLC_{\Delta 1030} < 1$ mM (i.e. when exercising at 211 W, $BLC_{\Delta 1030} = 1.3$). However, this higher exercise intensity of 211 W ($MLSS_{+15W}$) could also be accepted as MLSS when using the $BLC_{\Delta 1530} < 1$ or < 0.75 mM criterion as the $BLC_{\Delta 1530}$ was 0.6 mM. Interestingly, $BLC_{\Delta 1530} < 1$ mM for MLSS determination was also recommended by Iannetta et al. (2021) because they reported that the work rate corresponding to MLSS determined using the $BLC_{\Delta 1530} < 1$ mM criterion demonstrated the highest degree of accuracy in representing the MMSS. Thus, the $BLC_{\Delta 1530} < 1$ mM could be a better criterion as it accounts for the delayed lactate steady state and represents the MMSS with a higher accuracy. On the other hand, these results also question the validity of the conventional criterion because the blood lactate concentration required more than 10 minutes to stabilise with increasing intensity. In short, different protocols for determining the MLSS result in a wide variety of equivalent power outputs and affect the lactate steady state interpretation. This may be problematic when trying to prescribe specific training and racing intensities for athletes from the MLSS. Most importantly, the results demonstrated that the conventional $BLC_{\Delta 1030} < 1$ mM could not account for the delayed lactate steady state as the intensity increases.

3.5.4 Validity of MLSS to represent the boundary between heavy and severe domains

For MLSS to be accepted as the “gold standard” to separate the heavy and severe domains, the $\dot{V}O_2$ response should present different characteristics when exercising above and below it. Specifically, the $\dot{V}O_2$ response should demonstrate a steady state when exercising up to the MLSS. On the contrary, there should be no steady state when exercising above MLSS because the $\dot{V}O_2$ should project towards the $\dot{V}O_{2max}$, and a greater $\dot{V}O_{2sc}$ should also be evident (Pringle et al., 2003; Burnley & Jones, 2007). The $\dot{V}O_2$ response in the present study was consistent with previous studies, which all demonstrated that the $\dot{V}O_2$ did not increase towards $\dot{V}O_{2peak}$ during exercise at and above the MLSS, even though previous studies did not use the conventional protocol and used a smaller incremental rate (Brauer & Smekal., 2020: no more than 0.5 mM between 10th and 30th minute and ± 10 W; Hill et al., 2021: blood lactate difference between 6 to 8 min and 27 to 29 min) and Nixon et al. (2021) examined the MLSS in running. However, the results of the present study are in contrast to the work of Hill et al. (2021), who examined the $\dot{V}O_2$ response below (-2 ± 1 W), slightly above (4 ± 1 W) and well above (19 ± 8 W) the MLSS. Interestingly, the $\dot{V}O_2$ at the $MLSS_{+15W}$ ($78 \pm 7\% \dot{V}O_{2peak}$) in the present study was considerably lower than Hill et al.’s (2021) $\dot{V}O_2$ when exercising ‘well above’ the MLSS ($94 \pm 4\% \dot{V}O_{2peak}$), but similar to the $\dot{V}O_2$ when exercising ‘slightly above’ MLSS ($79 \pm 5\% \dot{V}O_{2peak}$). The differences in participant cohorts must be acknowledged between the current study and Hill et al. (2021). Specifically, the participants in the present study are cyclists with a mean $\dot{V}O_{2peak}$ of 57.0 ± 10 mL·kg⁻¹·min⁻¹, whereas those in the study of Hill et al. (2021) were healthy individuals with a mean $\dot{V}O_{2peak}$ of 42.2 ± 6.4 mL·kg⁻¹·min⁻¹. It is well known that capillary density and mitochondrial density increase with training, which enhance the oxygen delivery capacity. Studies have also shown the ability to increase the density of monocarboxylate transporter, which would be expected to improve lactate uptake and utilisation and be reflected in lower blood lactate levels. (Dubouchaud et al., 2000). Additionally, trained respiratory muscles could use more lactate as fuel for their own activity. (Spengler et al., 1999), which leads to less blood lactate accumulation. Therefore, the differences between the results of the present study and Hill et al. (2021) might suggest that the MLSS could represent the threshold between heavy and severe intensity domains in physically active and healthy individuals but not in cyclists.

To consider the MLSS as the upper boundary of the heavy intensity domain, the amplitude of $\dot{V}O_{2sc}$ when exercising at MLSS should be smaller than $MLSS_{+15W}$ (Pringle et al., 2003). Indeed, Pringle et al. (2003) and Colosio et al. (2020) demonstrated that the $\dot{V}O_{2sc}$ during exercise in the severe domain is significantly larger than when exercising at a heavy intensity. However, the results of the present study reported no significant difference in the $\dot{V}O_{2sc}$ when exercising at MLSS and $MLSS_{+15W}$. Such results are similar to Carter et al.'s (2000) work which reported a similar amplitude of $\dot{V}O_{2sc}$ during two heavy intensity exercises. In addition to the difference in $\dot{V}O_{2sc}$, the attainment of a $\dot{V}O_2$ steady state is another determinant factor for classifying different intensity domains (Burnley & Jones, 2007). Maturana et al. (2016) concluded that the MLSS is a suitable performance marker representing the maximal physiological steady state because of the lactate response. However, the $\dot{V}O_2$ stabilised and did not increase towards $\dot{V}O_{2max}$ at the end of the 30 minutes constant intensity exercise corresponding to 10 W above MLSS (Maturana et al., 2016), which is similar to the present study. Given that the pulmonary $\dot{V}O_2$ kinetics is closely associated with muscle $\dot{V}O_2$ and PCr (Rossiter et al., 2002; Poole & Jones, 2012; Jones et al., 2011), it is reasonable to conclude that the muscle metabolism also remained stable when exercising at $MLSS_{+15W}$. On the contrary, the disassociation between blood lactate and pulmonary $\dot{V}O_2$ shown in the present study and previous studies (Sheen et al., 1981; Poole et al., 1988; Maturana et al., 2016; Nixon et al., 2021) therefore demonstrated that blood lactate cannot reflect the muscle $\dot{V}O_2$ and PCr kinetics. Additionally, it is well established that measuring blood lactate cannot accurately inform the muscle lactate kinetics (Stainsby & Brooks, 1990; Brooks et al., 1999). These results challenged the validity of using blood lactate to inform the MMSS as the blood lactate itself is not a sensitive or appropriate marker to reflect the overall metabolic status.

3.6 Conclusion

The results of the present study suggested that an additional test should be performed to confirm the blood lactate kinetics when using the $BLC_{\Delta 1030} < 1$ mM protocol. The present study also demonstrated that the conventional protocol could not account for a delayed steady state in blood lactate and therefore underestimated the highest intensity at which the blood lactate can stabilise in six out of thirteen participants. Future research should examine the validity of $BLC_{\Delta 1530} < 1$ mM in informing the blood lactate steady state. In addition, it

was demonstrated that the $\dot{V}O_2$ stabilised when exercising at MLSS and MLSS_{+15W}, which indicated that the MLSS determined using the conventional protocol does not represent the boundary between heavy and severe exercise intensity domains.

Chapter 4

**Functional threshold power is not a valid marker
of the maximal metabolic steady state**

4.1 Abstract

Purpose: Functional Threshold Power (FTP) has been considered a valid alternative to other performance markers that represent the upper boundary of the heavy intensity domain. However, such a claim has not been empirically examined from a physiological perspective.

Method: This study examined the blood lactate and $\dot{V}O_2$ response when exercising at and 15 W above the FTP (FTP_{+15W}) to identify whether it can discriminate between heavy and severe exercise domains. Thirteen cyclists completed an incremental test to determine $\dot{V}O_{2peak}$, a 20-minute time trial to determine FTP, and two constant work rate tests to examine the physiological response when exercising at FTP and FTP_{+15W}. The $\dot{V}O_2$ was recorded continuously throughout FTP and FTP_{+15W}, with blood lactate measured before the test, every 10 minute and at task failure. Data were subsequently analysed using two-way ANOVA.

Results: The time to task failure at FTP and FTP_{+15W} were 33.7 ± 7.6 and 22.0 ± 5.7 minutes ($p < 0.001$), respectively. The $\dot{V}O_{2peak}$ was not attained when exercising at FTP_{+15W} ($\dot{V}O_{2peak}$: 3.61 ± 0.81 vs FTP_{+15W} 3.33 ± 0.68 L·min⁻¹, $p < 0.001$). The $\dot{V}O_2$ slow component (399 ± 177 mL·min⁻¹ vs 409 ± 185 mL·min⁻¹) was not significantly different between the two intensities ($p > 0.05$). However, the end test blood lactate corresponding to FTP and FTP_{+15W} was significantly different (6.7 ± 2.1 mM vs 9.2 ± 2.9 mM; $p < 0.05$).

Conclusion: The $\dot{V}O_2$ response corresponding to FTP and FTP_{+15W} suggests that FTP should not be considered a threshold marker between heavy and severe intensity.

4.2 Introduction

The last chapter concludes that the work rate corresponding to the MLSS is not a valid representation of the threshold between the heavy and severe intensity domains. Therefore, this experimental chapter aims to examine the validity of another marker to represent the threshold. Over recent years, field-based testing methods for assessing athletic endurance performance potential have become more popular. This has been facilitated by the progress in micro-technologies such as the cycle computer and power meter, which are now essential components for most cyclists. One commonly used field-based test for assessing cycling performance potential is the Functional Threshold Power (FTP) test (Allen & Coggan, 2006; 2010). The FTP is the highest power output a cyclist can maintain for one hour (Allen & Coggan, 2010). Determining the power output corresponding to FTP originally required cyclists to perform a maximal effort trial over one hour (FTP₆₀), but it was deemed impractical to conduct this test regularly. Thus, the determination protocol was modified to require an individual to perform a 20 minute maximal effort time trial (TT) and 95% of the mean power output is subsequently calculated for the intensity corresponding to FTP₆₀ (FTP₂₀) (Morgan et al., 2019; Inglis et al., 2019). Indeed, a strong correlation has been shown between the work rate corresponding to FTP₂₀ and FTP₆₀ ($r=0.88$; Borszcz et al., 2018). However, it has been questioned whether the power output from FTP₂₀ and FTP₆₀ can be considered interchangeable on an individual basis, despite no statistically significant differences between the two on a group basis (Borszcz et al., 2018).

The FTP₂₀ has also been proposed as a surrogate of some well-known performance markers representing the maximal metabolic steady state (MMSS). For example, critical power (CP) (Jones et al., 2019) and maximal lactate steady state (MLSS) (Dotan, 2022a) because exercise sustained at intensities slightly greater than FTP₂₀ ($> 106\%$ of FTP₂₀) is suggested to result in $\dot{V}O_{2max}$ attainment (Allen & Coggan, 2010). However, the validity between FTP₂₀ and other performance markers lacks physiological examination. It is interesting that Allen & Coggan. (2006) did not provide a rationale for suggesting that the FTP₂₀ is a valid alternative to CP and MLSS, as the first paper that mentioned FTP was by Nimmericher et al. (2011), five years after the FTP was first introduced. The potential linkage between FTP₆₀ and CP might be based on the research from Coyle et al. (1991) and Smith et al. (1999). It was suggested that the 1-hour laboratory performance test was highly correlated with the

actual road racing 40 km TT performance ($r = -0.88$, $p < 0.001$) (Coyle et al., 1991). Whereas Smith et al. (1999) reported, the power output corresponding to CP is highly related to the 40 km TT performances. When combining both results, it is tempting to consider the FTP₆₀ as an alternative to CP or the MMSS. However, an indirect comparison of the TT performance and correlation only provided a superficial and weak linkage between the FTP₆₀ and CP. Thus, a more rigorous scientific examination is required before making any meaningful conclusion between the FTP and MMSS.

The MMSS has been considered to represent an exercise intensity that can be sustained without a progressive loss of homeostasis and demarcates the heavy and severe exercise domains (Jones et al., 2019). The threshold between the heavy and severe intensity domains represents the upper boundary of whether the $\dot{V}O_2$ can remain in a steady state and the ability of the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) to stabilise. In the heavy domain, the $\dot{V}O_{2sc}$ can stabilise, whereas when exercising within the severe intensity domain, the $\dot{V}O_2$ has been shown to project upwards, rising to $\dot{V}O_{2max}$ without a discernible steady state (Poole et al., 1988; Hill et al., 2002; De Lucas et al., 2013; Jones et al., 2019). Given that the amplitude of the $\dot{V}O_{2sc}$ is closely related to muscle fatigue development and exercise tolerance, the TTF for severe intensity exercise would be shorter than heavy intensity exercise (Burnley & Jones, 2007; Colosio et al., 2020). The determination of MLSS and CP are the two common approaches to identifying the work rate corresponding to the MMSS (Billat et al., 2003; Faude et al., 2009; Poole et al., 2021). However, Chapter 3 provided physiological evidence demonstrating that MLSS is not a valid representation of the MMSS. Both methods require individuals to undertake at least three to four short submaximal effort trials to determine the intensity corresponding to the threshold, which is a time consuming and labour-intensive process. Thus, a single 20-minute maximal effort TT for FTP₆₀ estimation (Morgan et al., 2019; Inglis et al., 2019) could efficiently determine the work rate corresponding to MMSS. However, previous studies have reported a low level of agreement between the measured power outputs associated with CP and FTP₂₀ and should not be used interchangeably (Karsten, 2018; Morgan et al., 2019; Karsten et al., 2021; McGrath et al., 2021). Similarly, Inglis et al. (2019) reported that the power output corresponding to FTP₂₀ was significantly higher than the MLSS; therefore, not a valid marker of the threshold between heavy and severe intensity domains. In short, previous research suggests that FTP₂₀ should not be used interchangeably with CP and MLSS or as a marker of the MMSS. Nonetheless, the use of

FTP₂₀ to inform training and design training programs by coaches and athletes continues to grow (Allen & Coggan, 2010; Borszcz et al., 2018). A possible reason for this is that the little previous research that has been conducted investigating FTP₂₀ and those investigated the topic has tended to focus on the statistical perspective (correlation and limits of agreement) with other well-known performance markers rather than the physiological basis of FTP₂₀ itself (Borszcz et al., 2018; Inglis et al., 2019; Karsten et al., 2021; McGrath et al., 2021). Therefore, to determine the validity of FTP₂₀ representing the MMSS, there is a need to examine the physiological responses to exercise at and above the FTP₂₀ and identify whether they correspond to the heavy and severe intensity domain, respectively.

The present study aimed to investigate the validity of FTP₂₀ being the threshold separating the heavy and severe intensity domains by examining the physiological response when exercising at and 15 W above FTP₂₀ (FTP_{+15W}). Specifically, the study compared the $\dot{V}O_{2sc}$ response between exercising at and above FTP₂₀ due to its ability to discriminate between heavy and severe exercise intensity domains (Burnley & Jones, 2007). The FTP₂₀ will refer as FTP from this point on. The null hypothesis of the present study was that there would be no significant difference in $\dot{V}O_{2sc}$, % $\dot{V}O_{2peak}$ and blood lactate when exercising at FTP and FTP_{+15W}.

4.3 Methods

4.3.1 Participants

Thirteen cyclists (male n = 11; Age = 23.5 ± 3.9 years; $\dot{V}O_{2peak}$ = 60.0 ± 4.7 mL·kg⁻¹·min⁻¹; female n = 2; Age = 26 ± 9.8 years; $\dot{V}O_{2peak}$ = 48.0 ± 4.0 mL·kg⁻¹·min⁻¹) were recruited for this study. Participants were classified into four categories based on their relative and absolute $\dot{V}O_{2peak}$ (De Pauw et al., 2013; see Table 4.1). The inclusion criteria were 1) at least three years of cycling experience, 2) a minimum of four hours of training per week, and 3) previous experience with the FTP determination test. Participants were fully informed about the nature of the study, all associated risks, and their right to withdraw at any time before providing written consent. The study was ethically approved by the Human Research Ethics Committee at the (E2021-2022-0003) in line with the requirements of the declaration of Helsinki.

Table 4.1 Participant characteristics

Participant	Height (cm)	Mass (kg)	Relative $\dot{V}O_{2peak}$ (mL·kg⁻¹·min⁻¹)	Absolute $\dot{V}O_{2peak}$ (L·min⁻¹)	Performance Level (Relative/Absolute)
1	177	70	64	4.49	T / T
2	177	56	60	3.36	T / RT
3	180	65	61	3.95	T / RT
4	173	63	65	4.07	WT / RT
5	176	68	57	3.89	T / RT
6	170	62	64	3.97	T / RT
7*	164	53	51	2.68	RT / UT
8	174	63	55	3.44	T / RT
9	170	58	62	3.59	T / RT
10*	162	42	45	1.89	UT / UT
11	171	53	50	2.66	R / UT
12	180	63	64	4.04	T / RT
13	177	83	58	4.84	T / T

UT = Untrained; RT = Recreationally Trained; T = Trained; WT = Well Trained; *Female participant

4.3.2 Study Design

The study comprised four laboratory visits separated by 24 to 48 hours. During the first visit, participants were required to undertake a ramp incremental exercise test to determine their maximal oxygen uptake ($\dot{V}O_{2peak}$). Visit 2 was conducted to determine the participant's FTP, which was subsequently used in visits 3 and 4, the main experimental trials. The order of the 3rd and 4th visits was randomised and required the participant to cycle for 40 minutes or to task failure, whichever occurred first, either at an exercise intensity equivalent to their FTP or FTP_{+15W}.

Participants were asked not to engage in strenuous exercise 24 hours before testing and were required to avoid adding new training to their habitual routine during the testing period. They were required to maintain the same diet 24 hours before each test and arrive at the laboratory hydrated without consuming food and caffeine in the preceding three hours. The incremental

ramp test and constant intensity tests at FTP and FTP_{+15W} were performed on a laboratory cycle ergometer (Lode Excalibur Sport, The Netherlands). The ergometer was calibrated according to the manufacturer's recommendations and adjusted for participant comfort before every use. The FTP determination trial was performed on the participants' bike attached to a stationary bike trainer (Wahoo KICKER v.5; Wahoo Fitness, Atlanta, GA), which was previously shown to possess a high level of accuracy and reliability (Hoon et al., 2016). The pedal frequency was set at the participants' preferred rate between 80 and 90 rpm and held constant throughout the ramp incremental and constant intensity tests (± 2 rpm). Pulmonary gas exchange was measured on a breath-by-breath basis using Cortex Metalyzer 3B (Cortex, Leipzig, Germany).

4.3.3 Incremental ramp test

The incremental ramp test commenced with a warm-up at 50 W for four minutes. The work rate was increased by 30 W.min⁻¹ for male participants and 25 W.min⁻¹ for female participants until volitional exhaustion. Breath-by-breath $\dot{V}O_2$ data were subsequently averaged in 30 s bins to determine the $\dot{V}O_{2peak}$ (Nixon et al., 2021).

4.3.4 Determination of the FTP

The FTP test started with five minutes of baseline pedalling at 100 W using the preferred cadence, followed by a 20-minute maximal, self-paced TT. The aim of the TT was for the participant to achieve the highest mean power output possible across the 20 minutes with no verbal encouragement from the researcher. Participants were allowed to see the time and cadence to support appropriate pacing (Morgan et al., 2019; Inglis et al., 2019). Indoor cycling training software (Zwift, v1.0.85684, Zwift Inc, US) was used to record PO from all FTP determination trials.

4.3.5 Constant intensity trials equivalent to FTP and FTP_{+15W}

These visits aimed to determine the participant's $\dot{V}O_2$ and blood lactate responses when exercising at the intensity corresponding to FTP and FTP_{+15W}. A change in work rate of ± 15 W was selected due to previous research that examined similar threshold markers such as CP and MLSS using incremental rates such as 10 W (Maturana et al., 2016; Iannetta et al., 2021) and 5% of $\dot{V}O_{2max}$ (Dekerle et al., 2003), were suggested to be too low to provide conclusive changes in BLC and $\dot{V}O_2$ response (Jones et al., 2019). Tests began with a five-

minute warm-up at 100 W using the participant's preferred cadence. Participants were then required to cycle at a constant intensity, either equivalent to their FTP or FTP_{+15W}, for 40 minutes or until task failure, whichever occurred first. The intensity for the first trial (FTP or FTP_{+15W}) was randomly assigned using a website (<https://www.random.org/lists/>). Task failure was defined as the point at which the participant could no longer maintain a cadence of at least 50 rpm for more than 5 consecutive seconds despite strong verbal encouragement (Murgatroyd et al., 2011). Blood lactate samples were collected in duplicate from their fingertip before the test (baseline), every 10th minutes throughout the test session, and upon task failure (Biosen C-Line, EKF Diagnostics, GmbH, Barleben, Germany).

4.3.6 Data Analysis

Breath-by-breath $\dot{V}O_2$ data from the constant intensity trials were averaged in 10 seconds intervals to determine the $\dot{V}O_2$ response. The $\dot{V}O_2$ data was edited to eliminate the effects of coughs or swallows on the measurement. Only those data points beyond the three standard deviations of the mean value were excluded (Burnley et al., 2006a). The first 20 s of the $\dot{V}O_2$ data following the onset of exercise were removed to eliminate the phase I component from the analysis. The first 2 minutes of the $\dot{V}O_2$ data (20 to 120 s) were then analysed using the monoexponential model (Rossiter et al., 2001; Burnley et al., 2005; 2006a):

$$\dot{V}O_2(t) = \dot{V}O_{2\text{base}} + \text{amp} * (1 - e^{-(t-TD)/\text{tau}})$$

Where $\dot{V}O_2(t)$ = $\dot{V}O_2$ at time, $\dot{V}O_{2\text{base}}$ = the baseline $\dot{V}O_2$ measured in the four minutes before the transition in work rate, amp = amplitude, TD = time delay, and tau = time constant of the primary (phase II) response. The amplitude of the $\dot{V}O_{2\text{sc}}$ was determined by the highest $\dot{V}O_2$ value achieved during the constant intensity trial and subtracting the "absolute" primary amplitude ($\dot{V}O_{2\text{base}} + \text{amp}$) (Burnley et al., 2005; 2006a). The monoexponential model was chosen because a more complex model will significantly degrade the confidence intervals and create extra parameter interdependence (Burnley et al., 2005). Given that the time to task failure (TTF) at FTP and FTP_{+15W} varied between participants, the $\dot{V}O_2$ data were analysed using the individual isotime method and expressed in relative time (baseline, 25, 50, 75 and 100%) where the baseline is indicated as 0 in the figure, to avoid any data loss (Nicolò et al., 2019). The $\dot{V}O_2$ corresponding to the desired time points was determined by

the average $\dot{V}O_2$ over the prior 60 s. The mean of the last two segments (75% and 100%) was considered the $\dot{V}O_2$ corresponding to each trial.

Two sets of blood lactate samples were collected before the test, at every 10th minute and task failure. The mean of the two blood lactate samples was used for subsequent analysis. The blood lactate kinetic response was interpolated with a linear function using Microsoft Excel (Excel, Microsoft, Redmond, Washington) and represented as BLC_{FTP} and $BLC_{FTP+15W}$. The estimated blood lactate concentration (BLC) corresponding to 25, 50, 75 and 100% of the test duration were used to represent the blood lactate kinetics corresponding to FTP (BLC_{FTP}) and FTP_{+15W} ($BLC_{FTP+15W}$) (Nicolo et al., 2019). The actual difference in BLC between the 10th minute and end test ($BLC_{\Delta 10end}$) and the actual end test value corresponding to FTP and FTP_{+15W} trials were also calculated and subsequently used for statistical analysis purposes.

4.3.7 Statistical Analysis

The $\dot{V}O_2$ data were analysed using two-way ANOVA with repeated measures across two tests (FTP vs FTP_{+15W}) and five time points (Baseline, 25%, 50%, 75% and 100% of the total test duration). The end test $\dot{V}O_2$, the $BLC_{\Delta 10end}$ and the end test BLC corresponding to FTP and FTP_{+15W} were analysed using paired t-tests. The estimated blood lactate kinetics data interpolated with a linear function was analysed using two-way ANOVA with repeated measures across two tests (BLC_{FTP} vs $BLC_{FTP+15W}$) and five time points (Baseline, 25%, 50%, 75% and 100% of the total test duration). When sphericity was violated, the F value was adjusted using Greenhouse-Geisser. The significant interaction and main effects were determined using LSD post hoc tests. Analyses were performed using IBM SPSS statistics 26.0 (Chicago, IL, USA). Data are reported as mean \pm SD unless otherwise stated.

4.4 Results

4.4.1 General results

The mean cycling power output was 222 ± 51 W and 237 ± 51 W at FTP and FTP_{+15W}, respectively. Only seven out of thirteen participants were able to sustain exercise at FTP for 40 minutes. The mean TTF at FTP and FTP_{+15W} was 33.7 ± 7.6 and 22.0 ± 5.7 min ($p < 0.05$), respectively. There was a small but significant difference between the end test $\dot{V}O_2$

(calculated using the average of 75% and 100% of the total duration) corresponding to FTP and FTP_{+15W} (2.97 ± 0.66 vs 3.13 ± 0.67 L·min⁻¹, respectively; $p < 0.05$). The highest $\dot{V}O_2$ achieved during both intensities in 10 s average was significantly lower than the $\dot{V}O_{2peak}$ measured during the incremental ramp test ($\dot{V}O_{2peak}$: 3.61 ± 0.81 vs FTP: 3.21 ± 0.69 , $p < 0.001$ and FTP_{+15W} 3.33 ± 0.68 L·min⁻¹, $p < 0.001$).

4.4.2 The oxygen kinetics at FTP and FTP_{+15W}

The $\dot{V}O_2$ kinetics analysed using the individual isotime method demonstrated a significant interaction effect between test and time for $\dot{V}O_2$ response ($F = 2.827$, $p < 0.05$), the main effect of the test ($F = 19.015$, $p < 0.001$) and time ($F = 85.535$, $p < 0.001$). The $\dot{V}O_2$ was significantly different at all time points between the two intensities, except for baseline. Post hoc analysis showed a significant difference in $\dot{V}O_2$ between the baseline and the rest of the time points when exercising at FTP and FTP_{+15W}. The $\dot{V}O_2$ did not change significantly between 25% of the total duration and the end of the exercise during both FTP and FTP_{+15W} (see Figure. 4.1). There was no significant difference in the magnitude of the $\dot{V}O_{2sc}$ when exercising at FTP and FTP_{+15W} (399 ± 177 mL·min⁻¹ vs 409 ± 185 mL·min⁻¹, $p > 0.05$).

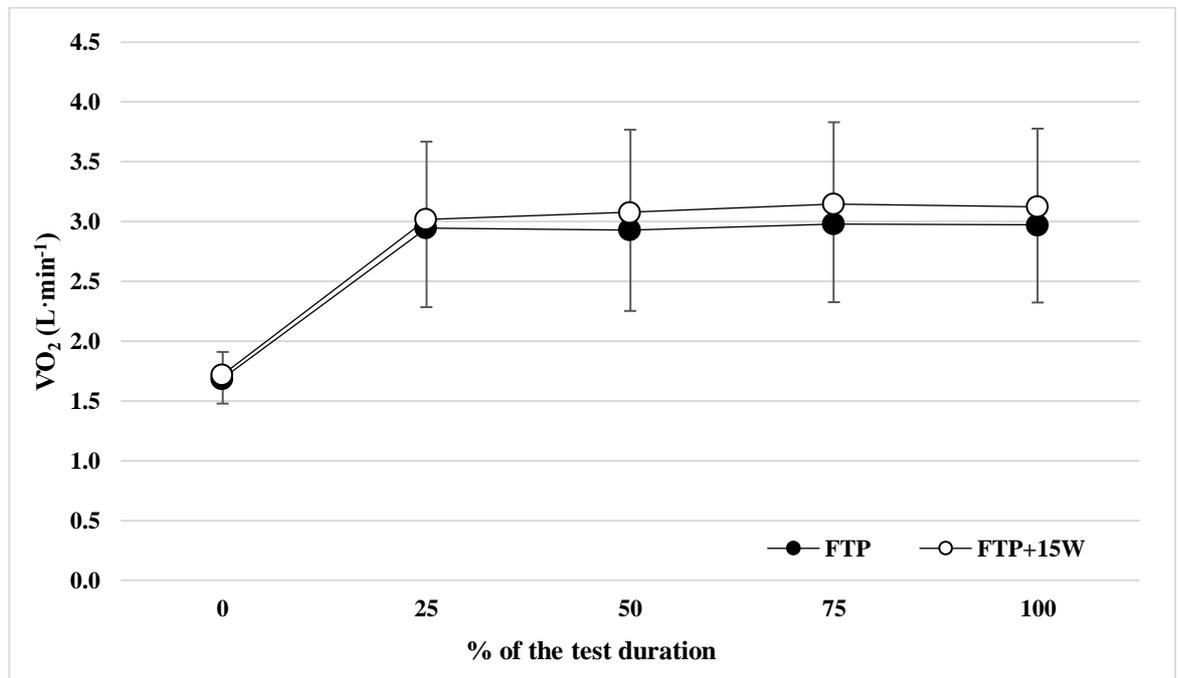


Figure. 4.1 The $\dot{V}O_2$ response as a percentage of trial duration when exercising at the intensities corresponding to FTP and FTP_{+15W}.

4.4.3 The blood lactate response at FTP and FTP_{+15W}

For the blood lactate kinetics estimated using linear regression, there was a significant interaction between test and time ($F = 12.871$, $p < 0.001$), a significant main effect of time ($F = 88.110$, $p < 0.001$) and for test ($F = 3.12$, $p = 0.09$). Post hoc analysis showed a significant difference between all timepoints for BLC_{FTP} and $BLC_{FTP+15W}$. There was also a significant difference in the BLC from 50 % of the test duration to the end test between BLC_{FTP} and $BLC_{FTP+15W}$ (see Figure. 4.2), except for the baseline. The estimated BLC_{FTP} and $BLC_{FTP+15W}$ at each time point were 1.6 ± 0.6 , 5.6 ± 2 , 6.0 ± 2.0 , 6.4 ± 2.0 , 6.8 ± 2.1 mM and 1.5 ± 0.5 , 6.2 ± 2.0 , 7.3 ± 2.2 , 8.3 ± 2.5 and 9.3 ± 3.0 mM, respectively. The blood lactate difference between 50% and 100% of the test duration was 0.8 ± 0.7 and 2.0 ± 1.4 mM for FTP and FTP_{+15W}, respectively. The actual end test BLC (FTP: 6.7 ± 2.1 vs FTP_{+15W}: 9.2 ± 2.9 mM, $p < 0.05$) and the $BLC_{\Delta 10\text{end}}$ (FTP: 1.1 ± 0.9 vs FTP_{+15W}: 2.8 ± 2.3 mM, $p < 0.05$) were significantly different between two intensities. Nine participants were able to sustain 30 minutes when exercising at the intensity corresponding to FTP and the $BLC_{\Delta 1030}$ was 1.1 ± 0.2 mM.

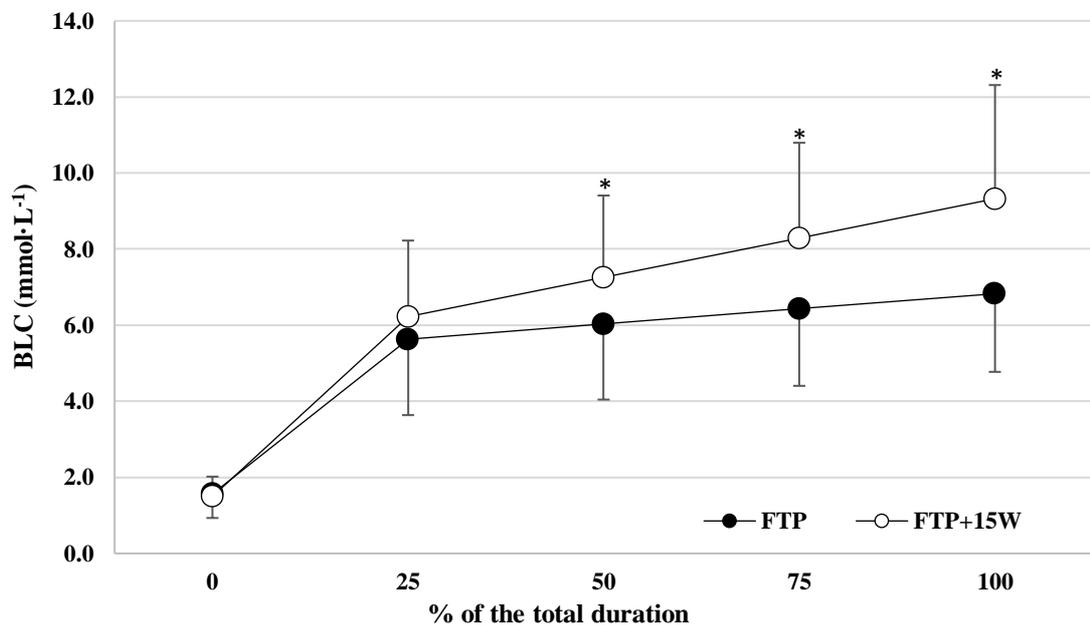


Figure. 4.2 The blood lactate response as a percentage of trial duration when exercising at intensities corresponding to FTP and FTP_{+15W}. *significantly different from FTP ($p < 0.05$)

4.5 Discussion

4.5.1 Overview

The present study examined the physiological response when exercising at and above FTP to determine whether it validly represents the threshold between the heavy and severe intensity domains. The key findings were that i) $\dot{V}O_{2peak}$ was not reached at both intensities; ii) $\dot{V}O_2$ stabilised at both intensities; iii) there was no significant difference in the amplitude of the $\dot{V}O_{2sc}$ between two intensities; iv) the actual end test BLC and the $BLC_{\Delta 10end}$ were both significantly higher during FTP_{+15W} ; v) six out of thirteen participants reached task failure before 40 minutes when exercising at FTP. Therefore, although the present study did not set out to examine the validity between FTP and hour performance, the results demonstrated that the FTP determined using 95% of a 20-minute TT performance is not a valid estimation of maximal hourly performance. The present study also demonstrated that FTP and FTP_{+15W} are within the heavy intensity domain and should not be used to represent the physiological threshold between the heavy and severe intensity domains.

4.5.2 The validity of FTP

The FTP estimated by 20 minutes TT is suggested to represent the maximal power output one can sustain for an hour (Allen & Coggan, 2010, p.47). Although the original goal of the present study was not to examine the TTF of FTP, the results were in line with previous studies as all demonstrated that the TTF is shorter than 60 minutes when exercising at the intensity corresponding to the FTP determined by either the original protocol (Borszcz et al., 2018; Sitko et al., 2022), 20 minutes time trial (the present study) or 60 minutes time trial protocol (Borszcz et al., 2018). In the present study, only seven out of thirteen participants were able to complete 40 minutes at FTP. The mean TTF for the six participants who reached TTF before 40 minutes was 26 ± 4 minutes demonstrating that FTP is not a valid estimation of the one-hour maximal performance. Contrary to previous research (McGrath et al., 2019) reported that 89% of the participants sustained 60 minutes when exercising at an intensity equivalent to FTP. A possible explanation is that their participants were allowed to undergo 60 minutes of exercise without wearing a face mask from 11 to 49 minutes and drink a carbohydrate beverage, which would help to prolong their TTF and improve performance. Although they recruited highly trained subjects therefore potentially with a higher pain/fatigue tolerance ($\dot{V}O_{2max}$: Male 66.3 ± 5.5 mL·kg·min⁻¹; Female 59.3 ± 6.9 mL·kg·min⁻¹

¹), whereas the participants in the present study have a lower mean $\dot{V}O_{2\text{peak}}$ (Male 60.0 ± 4.7 ; Female 48.0 ± 4.0 mL·kg·min⁻¹). According to Sitko et al. (2022), even professional cyclists with a $\dot{V}O_{2\text{max}}$ of 74.3 ± 3.9 mL·kg·min⁻¹ and more than 15 years of cycling experience were unable to sustain 60 minutes at the intensity corresponding to the FTP determined by the original protocol (mean TTF: 51 minutes, ranged from 44 to 59 minutes). It is important to note that even the FTP determined using Allen & Coggan's (2006) original determination protocol (50 minutes of specific warm up plus 20 minutes TT) cannot be sustained for 60 minutes (Sitko et al., 2022). Therefore, the problem is that none of the FTP determination protocols proposed by Allen & Coggan (2006; 2010; 2012) and previous studies can accurately determine the intensity that can be sustained for 60 minutes. Sitko et al. (2022) suggested that the difference in TTF between recreationally trained and elite could be because of the better pain tolerance and pacing strategies which allow the elite to achieve a longer TTF. However, the reason why the FTP₂₀ is a poor predictor of FTP was not examined or discussed before. The potential explanation could be the variability in pacing and its effect on energy depletion and physiological response. For example, if the participants chose to conserve energy until the later part of the TT and progressively increase the power output towards the end it is highly possible that the W' was not fully depleted upon the TT finished. Therefore, the estimated FTP might not reflect the highest power output that can be sustained for 20 minutes because the best performance cannot be achieved (Fukuba & Whipp, 1999). On the contrary, if the cyclist decides to have a strong start or a less experienced cyclist does not have a good concept regarding the appropriate power output allocation throughout the TT, it is possible the W' is fully depleted in the first 5 to 10 minutes, therefore, affecting the TT performance. Therefore, the FTP should not be considered a valid representation of what it originally proposed, even when accounting for variables such as cyclists' performance level, experience, and aerobic fitness level (i.e., $\dot{V}O_{2\text{max}}$). Although the fatigue development in the heavy intensity domain is complex (Burnley & Jones, 2018), future studies could explore why cyclists reach task failure before the 60 minutes mark when exercising at FTP.

4.5.3 Oxygen kinetics when exercising at FTP and FTP_{+15W}

The threshold between the heavy and severe intensity domain separates whether the $\dot{V}O_2$ can remain stable or not and rise towards the $\dot{V}O_{2\text{max}}$ (Hill et al., 2002). Therefore, in our view, the question of whether FTP represents the upper boundary of the heavy intensity domain is best assessed by the physiological characteristics, specifically the $\dot{V}O_2$ response.

In the present study, the $\dot{V}O_2$ was not significantly different between 25% of the total duration and the end test when exercising at FTP and FTP_{+15W}, indicating a clear $\dot{V}O_2$ steady state during both intensities. Contrary to the results reported by Nixon et al. (2021), in which the $\dot{V}O_2$ changed significantly between the 5th minute and at task failure when exercising slightly above critical speed (CS), the representation of the threshold between the heavy and severe domain in running (Jones et al., 2019). Consistent with our hypothesis, the amplitude of $\dot{V}O_{2sc}$ was not significantly different between the two intensities, indicating that FTP and FTP_{+15W} are the same intensity. It has been previously demonstrated that the $\dot{V}O_{2sc}$ is significantly lower during the heavy intensity domain compared to the severe intensity domain (Pringle et al., 2003) because the $\dot{V}O_{2sc}$ cannot be stabilised and rise towards $\dot{V}O_{2max}$ in the severe domain (Burnley & Jones, 2007). Another result from the present study that demonstrates FTP should not be considered as the threshold between heavy and severe is that the percentage of $\dot{V}O_{2peak}$ corresponding to FTP and FTP_{+15W} was $83 \pm 4\%$ and $87 \pm 3\%$, respectively. Although the increment rate was fixed to 15 W in the present study, it was equivalent to a 9% to 12% increase for 3 participants. The percentage of $\dot{V}O_{2peak}$ corresponding to the FTP_{+15W} remained below 90% for the 3 participants, whereas when exercising within the severe intensity domain until task failure, the $\dot{V}O_2$ should rise inexorably towards $\dot{V}O_{2max}$ (Poole et al., 1988; De Lucas et al., 2013; Nixon et al., 2021). Therefore, based on both the overall $\dot{V}O_2$ kinetics and the inability to attain $\dot{V}O_{2max}$, the power output corresponding to FTP_{+15W} does not represent the severe intensity domain. The intensity corresponding to FTP and FTP_{+15W} may be within the heavy intensity domain as the $\dot{V}O_2$ stabilised during both intensities (Hill et al., 2002; Burnley & Jones, 2007). Additionally, 106% of FTP is the minimal intensity that could be expected to increase $\dot{V}O_{2max}$ according to Allen and Coggan (2010). However, the present study demonstrated that the suggested intensity has a high chance of overestimating the $\dot{V}O_2$ response as the end test $\dot{V}O_2$ was below 90% of the $\dot{V}O_{2max}$ when exercising above that recommended intensity. Such $\dot{V}O_2$ response is not enough to promote the physiological adaptations to enhance $\dot{V}O_{2max}$ (Bacon et al., 2013). Therefore, FTP may not be appropriate to use as a reference for designing training programs because it overestimates the $\dot{V}O_2$ response which would result in over or underestimating the training intensity.

4.5.4 Lactate kinetics of FTP and FTP_{+15W}

Allen & Coggan (2012) suggested that the FTP could be used interchangeably with the MLSS. Three studies have examined whether FTP and MLSS can be used to represent each other from a statistical perspective. Borszcz et al. (2019) concluded that the FTP is a valid alternative for estimating MLSS because of the high correlation ($r = 0.91$) and the limit of agreement was $1.4 \pm 9.2\%$ between the two. The other two studies suggested that a 88% (Inglis et al., 2019) or 91% (Lillo-Bevia et al., 2022) correction factor, instead of 95%, should be used to provide a more accurate estimation of MLSS. However, the most appropriate approach to determine whether FTP is a valid alternative to MLSS should directly examine the blood lactate response when exercising at the FTP. Results of the current study demonstrated that the difference in the actual BLC from the 10th to 30th minute for those who sustained the FTP intensity for at least 30 minutes was 1.1 ± 0.2 mM ($n = 9$), which exceeds the suggested conventional criterion for the determination of the conventional MLSS (change in BLC < 1.0 mM between the 10th and 30th minute). Therefore, using the conventional determination criterion for MLSS suggested that the power output corresponding to FTP is not a valid surrogate of MLSS as the BLC_{FTP} was not in a steady state. However, a paper recently published by Nixon et al. (2021) proposed that the criterion for BLC should be 2 mM between 10 to 20 minutes instead of the conventional criterion because the MLSS determined using this protocol eliminated the difference with Critical Speed (CS). When adopting this modified approach, the FTP examined in the present study fulfilled the criterion for being accepted as MLSS for all thirteen participants as they all sustained 20 minutes when exercising at FTP and the blood lactate difference between 10 and 20 minutes was below 2 mM (BLC _{Δ 1020} = 0.8 ± 0.6 mM). Similarly, Iannetta et al. (2021) also proposed a modified MLSS criterion of using data from the time window of 20 to 30 minutes instead of the conventional method because it has a higher agreement with MMSS. In the present study, the actual BLC difference between 20 to 30 minutes was 0.3 ± 0.6 mM for those who cycled for at least 30 minutes when exercising at FTP ($n = 9$), which also meets the modified criterion for MLSS proposed by (Iannetta et al., 2021). Therefore, the ability of FTP to provide an approximation of the MLSS appears to be influenced by the criterion used to determine the MLSS. On the other hand, the blood lactate data estimated using the linear function showed a clear dissociation between blood lactate and $\dot{V}O_2$ kinetics. It demonstrated that relying solely on blood lactate to inform the systemic homeostasis and the threshold between heavy and severe intensity domains is inappropriate as it does not

reflect the $\dot{V}O_2$ kinetics. As such, there is a need for scientific validation of the criterion for determining MLSS and blood lactate steady state, and it is premature to conclude whether FTP can be used to represent MLSS.

4.6 Conclusion

The present study demonstrated that FTP should not be considered a marker of the threshold separating the heavy and severe domains for cyclists. Therefore, not a valid representation of the MMSS. The conclusion from the present study is based on the $\dot{V}O_2$ response when exercising at FTP and FTP_{+15W}. There was no significant difference in the $\dot{V}O_{2sc}$, and a clear $\dot{V}O_2$ steady state was shown when exercising at both intensities. Most importantly, the $\dot{V}O_2$ did not project towards $\dot{V}O_{2max}$ when exercising at FTP and FTP_{+15W}. Future studies should examine the value of FTP, other than a valid 20-minute TT indicator, and whether it can be used as an alternative to conventional and any modified MLSS. Therefore, the next Chapter of the present thesis will examine the concept of Critical Power.

Chapter 5

The validity of Critical Power as the threshold for the maximal metabolic steady state in cycling

5.1 Abstract

Purpose: Critical Power (CP) represents the upper boundary of the heavy intensity domain, and its validity in performance prediction has been repeatedly challenged. The present study aims to provide physiological data to examine the topic.

Method: This study examined the $\dot{V}O_2$ response, blood lactate, and time to task failure (TTF) when exercising at and 15 W above the CP (CP_{+15W}). Thirteen cyclists completed an incremental test to determine $\dot{V}O_{2peak}$ and gas exchange threshold, four constant severe intensity exercises to determine CP, and four constant intensity exercises to determine the TTF and physiological response at and above CP. Participants were then assigned one week of HIT training and completed one constant intensity exercise at CP_{+15W} . The $\dot{V}O_2$ was recorded continuously throughout CP and CP_{+15W} , with blood lactate measured at TTF. The $\dot{V}O_2$ data was expressed in relative time (baseline, 25%, 50%, 75% and 100%) and subsequently analysed using two-way ANOVA.

Results: The end test $\dot{V}O_2$ and the magnitude of $\dot{V}O_2$ slow component were both significantly lower at CP compared to all CP_{+15W} trials ($p < 0.05$). The $\dot{V}O_2$ stabilised at CP but increased significantly between 25% of the total duration and the end test during all CP_{+15W} trials ($p < 0.05$). The TTF for CP_{+15W} trials before training was significantly shorter than the predicted TTF but not after training.

Conclusion: The $\dot{V}O_2$ response to exercising at CP and CP_{+15W} suggests that CP should be considered the threshold between heavy and severe intensity. However, the validity of severe intensity exercise performance prediction remains unclear.

5.2 Introduction

In the previous chapters, the results demonstrated that contrary to what previous research suggested, exercising 15 W above the Maximal Lactate Steady State (MLSS) and Functional Threshold Power (FTP) does not trigger the typical physiological response corresponding to the severe intensity domain. Therefore, this experimental chapter aimed to examine the validity of Critical Power (CP) in representing the threshold. The concept of CP has been considered the gold standard for representing the threshold between the heavy and severe intensity domain or the Maximal Metabolic Steady State (MMSS) (Smith & Jones, 2001; Pringle et al., 2002; Jones et al., 2008; Maturana et al., 2016; Vanhatalo et al., 2016; Karsten, 2017; Jones et al., 2019; Karsten et al., 2021; McGrath et al., 2021; Nixon et al., 2021). However, the validity of the CP has been repeatedly challenged from different perspectives (Pallarés et al., 2020; Dotan, 2022a; Gorostiaga et al., 2021; 2022a). For example, the MLSS has been suggested to represent the MMSS (Dotan, 2022a) and CP is, on average, 7% to 11% higher than the power associated with the MLSS (Galán-Rioja et al., 2020). Therefore, the intensity of CP is often considered to overestimate the MMSS (Dotan, 2022a). However, given that the $\dot{V}O_2$ kinetics response is distinct to each intensity domain (Burnley & Jones, 2007), the question of whether the CP should be considered as a measure of the threshold is better addressed by examining the $\dot{V}O_2$ response when exercising both at, and above CP. Briefly, the $\dot{V}O_2$ response to constant intensity exercise in the heavy intensity domain demonstrates a steady state response, albeit at an elevated level due to the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) (Burnley & Jones, 2007; 2018). Constant intensity exercise in the severe domain causes $\dot{V}O_2$ to project upwards until reaching the $\dot{V}O_{2max}$ if the exercise continues for long enough without any evidence of a steady state-like response (Burnley & Jones, 2007; 2018). Surprisingly, the $\dot{V}O_2$ response to exercise at and above CP has received limited attention compared to other aspects, such as comparisons with other performance markers (Dekerle et al., 2003; Karsten et al., 2018; 2021) and its application in designing training and informing pacing strategies (Morton & Billat, 2004; Vanhatalo et al., 2011; Jones & Vanhatalo, 2017; Ashtiani et al., 2019; Kirby et al., 2021). Previous research reported no significant difference between the $\dot{V}O_{2sc}$ when exercising at and 5% above the CP (247 vs 222 ml·min⁻¹) (De Lucas et al., 2013). Based on the $\dot{V}O_{2sc}$ results, both intensities in their study should be considered within the same intensity domain, which leads to the conclusion that the CP is not a valid representation of the threshold between the heavy and severe

intensity domains. However, it is important to note that the amplitude of $\dot{V}O_{2sc}$ in their work was the difference between two rigid time points (i.e. the $\dot{V}O_2$ corresponding to the point of exhaustion and the 3rd minute of the exercise). This determination method has been shown to significantly underestimate the true amplitude of the $\dot{V}O_{2sc}$ calculated using the nonlinear regression model (Santana et al., 2007).

The 2 parameter model defines the relationship between the intensity (power output or speed) and time to task failure from a mathematical perspective, therefore it can be used to calculate the TTF at any intensity above the CP or within the severe intensity domain (Vanhatalo et al., 2011). It makes the concept of CP so unique because no other performance marker can offer similar information. Previous studies have examined the validity of using the 2 parameter model to predict severe intensity exercise performance in different sports. Hill et al. (2003) reported that the 2 parameter model can provide close estimation of the TTF of a 2000 m trial in rowing. The model can also provide a prediction of performance with high accuracy in running. Jones & Vanhatalo (2017) reported that the mean marathon finish speed of 12 elite marathon runners was correspond to $96 \pm 2\%$ of the CS. The 2 parameter model has also been proposed as a valid tool for estimating running performance and designing appropriate pacing strategies accordingly (Jones & Whipp, 2002; Jones et al., 2010; Pettitt, 2016; Ashitiani, 2021). Similarly, Kirby et al. (2021) reported that the achievement of the best performance time calculated using the 2 parameter model depends on whether the pacing strategy can allow the athlete to utilise the D optimally'. Nevertheless, the result demonstrated that the group average predicted time of 5000 m and 10000 m races were close to the actual race time (5000 m 13 min 09 s vs 13 min 36 s; 10000m 27 min 00 s vs 27 min 04 s).

In cycling, a previous study (Chidnok et al., 2012) reported that the two parameter CP model could provide a valid estimation of the intensity that would result in a time to task failure (TTF) of 6 min. Morgan et al., (2019) reported that the predicted TTF was significantly correlated with the actual 16.1 km TT performance (27.5 ± 3.3 min vs 26.7 ± 2.2 min, $r = 0.88$, $p < 0.01$). Although the mean power output during the 16.1 km TT was above the CP (TT: 296 ± 38 W vs CP: 275 ± 42 W) and did not drop below the CP throughout the TT. The variation of cadence and power output allowed during the TT may assist participants with fatigue resistance and $\dot{V}O_2$ priming, thus enhancing performance (Black et al., 2015).

Besides using the model to calculate the best performance over a specific distance, studies also used the model to calculate the highest intensity that would trigger a specific TTF, and the results were consistently reliable (Vanhatalo et al. 2011: 180 s vs 177 ± 29 s; Chidnok et al., 2012; 360 s vs 384 ± 48 s). Conversely, Pallarés et al. (2020) recently reported that the model could not accurately predict TTF during constant intensity cycling. However, the determination trials' duration potentially affected the CP estimation. A total of 4 trials were used to determine the CP and W' , with the shortest and longest trials being $00:29 \pm 00:06$ min:ss (mean TTF at Wingate test) and $76:05 \pm 13:53$ min:ss (mean TTF at MLSS), respectively. Both trials were outside the suggested testing duration of 2 to 15 minutes for CP and not within the severe intensity domain (Jones et al., 2019). Using a very long determination trial may lead to the CP being heavily influenced by the lowest intensity resulting in an inaccurate estimation of the CP (Gorostiaga et al., 2021). Consequently, the CP reported by Pallarés et al. (2020) was 2% below the MLSS, contrary to previous research suggesting CP should be approximately 7 to 11% higher than MLSS (Jones et al., 2019; Saif et al., 2022). In addition to the testing methods, factors such as daily variation in performance (Bommasamudram et al., 2022) and fitness status (Bailey et al., 2009; Clark et al., 2013) could also affect the TTF at a constant severe intensity exercise. If the predicted TTF overestimated the actual TTF of a constant severe intensity exercise, it would be interesting to consider whether training could improve performance such that the actual TTF matched the predicted TTF. Indeed, different studies have reported that taper training in a high-intensity aerobic training (HIT) structure could sufficiently improve well-trained cyclists' endurance performance (Clark et al., 2013; Rønnestad & Vikmoen, 2019) and specificity of the performance at an intensity closely related to the CP (Rønnestad et al., 2021). Therefore one week of HIT shock microcycle from the work of Rønnestad et al. (2021) was used in the present study as they demonstrated the training program induced improvement in endurance performance-related indicators (e.g. power output at blood lactate concentration (BLC) at 4 mM).

The present study aimed 1) to determine the validity of the CP representing the upper boundary of the heavy intensity domain from a physiological perspective and 2) to examine the accuracy of the 2 parameter formula in predicting constant severe intensity exercise performance. We hypothesised that 1) the end test $\dot{V}O_2$, lactate, and the amplitude of $\dot{V}O_{2sc}$ would be significantly higher when exercising at and 15 W above the CP (CP_{+15W}) than at

CP; 2) there will be a significant difference between the actual (before training intervention) and calculated TTF; and 3) the actual TTF at CP_{+15W} would not be significantly different from the predicted TTF after 1 week of HIT training.

5.3 Methods

5.3.1 Participants

Thirteen cyclists (male n = 10, female n = 3, age 23 ± 5 years, height 170.3 ± 6.9 cm, mass 61.9 ± 8.6 kg, $\dot{V}O_{2peak}$ 3.7 ± 0.7 L·min⁻¹ or 60.0 ± 7.9 mL·kg⁻¹·min⁻¹) volunteered to participate in this study. Participants were classified into four categories based on their relative and absolute $\dot{V}O_{2peak}$ (De Pauw et al., 2013; see Table 5.1). The inclusion criteria were at least three years of cycling experience and four hours of training per week. Before providing written informed consent, participants were fully informed about the nature of the study, all associated risks, and their right to withdraw at any time. The study was ethically approved by the Human Research Ethics Committee at the Education University of Hong Kong (2021-2022-0186) in line with the requirements of the declaration of Helsinki.

Table 5.1 Participant characteristics

Participant	Height (cm)	Mass (kg)	GET (W)	Relative $\dot{V}O_{2peak}$ (mL·kg⁻¹·min⁻¹)	Absolute $\dot{V}O_{2peak}$ (L·min⁻¹)	Performance Level (Relative/Absolute)
1	177	70	160	58	4.07	T / RT
2	177	83	210	56	4.66	T / T
3	170	65	185	59	3.83	T / RT
4	180	65	155	59	3.86	T / RT
5	173	63	155	60	3.78	T / RT
6*	163	50	125	44	2.21	UT / UT
7*	158	57	150	57	3.23	T / UT
8	170	62	185	67	4.14	WT / RT
9	168	54	135	66	3.58	WT / RT
10*	164	53	123	48	2.54	T / UT
11	180	63	195	67	4.24	WT / T
12	165	54	185	74	3.93	PT / RT
13	170	66	180	60	3.95	RT / RT

UT = Untrained; RT = Recreationally Trained; T = Trained; WT = Well Trained; PT = Professionally trained, GET = Gas Exchange Threshold; *Female participant

5.3.2 Study Design

Participants completed one incremental ramp test to determine $\dot{V}O_{2peak}$ and gas exchange threshold (GET), four constant intensity trials to determine CP and W', and four constant intensity trials to examine the physiological responses to exercise at CP and CP_{+15W} within a total of four laboratory visits. Following this initial testing period, participants completed a one week training microcycle which included five supervised HIT sessions (see the below details), prior to returning to the laboratory for a final constant intensity test. All laboratory visits were separated for at least 24 to 72 hours. Participants were prohibited from strenuous exercise 24 hours before all tests. They were required to be adequately hydrated without consuming food and caffeine for three hours before each test. They were asked to maintain the same diet for 24 hours prior to each test. All tests were performed on the same cycle ergometer (Lode Excalibur Sport, The Netherlands), handlebar and seat were adjusted for participant comfort.

5.3.3 Visit 1 - Ramp incremental test and one constant intensity test

The aim of this visit was to determine the participant's $\dot{V}O_{2peak}$ and GET using an incremental test to volitional exhaustion and the TTF at one of the following intensities: 60% Δ , 70% Δ , 80% Δ and 100% of $\dot{V}O_{2peak}$ (Vanhatalo et al., 2007; Hunter et al., 2021). The incremental test required participants to cycle at 50 W for 4 minutes, followed by increments of 30 W.min⁻¹ for male participants and 25 W.min⁻¹ for female participants until volitional exhaustion or failure to maintain the targeted rpm for more than 10 rpm for more than 5 consecutive seconds despite strong verbal encouragement (Inglis et al., 2019). $\dot{V}O_{2peak}$ was defined as the highest 30 s average $\dot{V}O_2$ during the test (Nixon et al., 2021). Pulmonary gas exchange was measured on a breath-by-breath basis using Cortex Metalyzer 3B (Cortex, Leipzig, Germany) throughout the test. After 60 minutes of seated rest, participants performed a constant work rate test at an intensity between 60% Δ , 70% Δ , 80% Δ and 100% of $\dot{V}O_{2peak}$, where Δ refers to the power output difference between the GET and $\dot{V}O_{2peak}$ (Burnley et al., 2006a; Vanhatalo et al., 2007; Hunter et al., 2021). The intensity was randomly assigned using a website (<https://www.random.org/lists/>). After 4 minutes of warm-up at 100 W, the work rate instantaneously increased to the intensity assigned to that session. The TTF was recorded in the closest second and determined by the point where the participant stopped pedalling or the pedal rate fell more than 10 rpm below the chosen cadence for more than 5 seconds despite strong verbal encouragement. This constant intensity trial's TTF and power output were used in calculating CP and W'.

5.3.4 Visit 2 – Three constant intensity tests

Three severe intensity trials were performed in random order during this visit to enable the estimation of CP and W'. 60 minutes of seated rest separated each effort (Burnley et al., 2006a; Karsten et al., 2017; Hunter et al., 2021). Each test started with 4 minutes of warm-up at 100 W, following which the intensity instantaneously increased to the target resistance. Participants were blinded from time and power output throughout all constant load tests. Strong encouragement was provided by the researcher throughout the test. The TTF was recorded to the closest second and determined by the point at which the participant stopped pedalling, or the pedal rate fell more than 10 rpm below the chosen cadence for more than 5 seconds despite strong verbal encouragement. The TTF and power output of these constant intensity trials were used in calculating CP and W'.

5.3.5 Visits 3 and 4 – Two constant intensity tests per visit

There were four constant intensity trials during visits 3 and 4 (two per visit) in which the participants were required to cycle to task failure. One trial was conducted at CP and three trials at CP_{+15W} to enable the calculation of reliability of time to exhaustion and physiological responses during exercise above the CP. After a 4 minutes warm-up at 100W, the work rate instantaneously increased to the prescribed intensity. Participants were instructed to pedal at their preferred cadence until task failure, which was the point when subjects could no longer maintain a cadence of 50 rpm for more than 5 consecutive seconds despite strong verbal encouragement (Murgatroyd et al., 2011). $\dot{V}O_2$ data was measured on a breath-by-breath basis using Cortex Metalyzer 3B (Cortex, Leipzig, Germany) throughout the test. After 60 minutes of seated rest, participants performed another constant intensity test to task failure. The trial order was randomly generated using a website (<https://www.random.org/lists/>). Two blood lactate samples were collected from the participant's fingertip at baseline prior to the test, every 5 minutes during all trials and within 1 minute of task failure (Biosen C-Line, EKF Diagnostics, GmbH, Barleben, Germany).

5.3.6 Training

After the 4th visit, participants performed a one-week HIT microcycle, which required one HIT session on days 1 to 3, a recovery day on day 4, a HIT session on day 5, a recovery on day 6, and a final HIT session on day 7. All HIT sessions were performed with the participants' bikes mounted on an electromagnetically braked trainer (Wahoo KICKER v.5; Wahoo Fitness, Atlanta, GA). Participants were allowed to train at home using their bike trainer or in the laboratory using a stationary bike trainer provided by the researcher (Wahoo KICKER v.5; Wahoo Fitness, Atlanta, GA). The one week of HIT shock microcycle from the work of Rønnestad et al. (2021) was used in the present study. There were 5 series of 12 work intervals lasting 30 second, separated by 15 second recovery periods. Each series was followed by a 150 second recovery period. During the HIT work intervals, the participants were instructed to achieve an RPE after each series equal to 17 to 19 on Borg's 6 to 20 scale, which they had previously been familiarised with during the incremental test in visit 1 and subsequently used during the constant intensity trials. During the recovery periods, participants were instructed to recover at around 50% of the power output during the work intervals. Power output during all work intervals was recorded using indoor cycling training

software (Zwift, v1.0.85684, Zwift Inc, US) and exported into Excel format for further analysis.

5.3.7 Visit 5 – One constant intensity test at CP_{+15W} ($CP_{+15W\ post}$)

A final test was conducted no later than 72 hours after the last training session. Participants completed a constant intensity test at the power output corresponding to CP_{+15W} (determined in Visit 2) until task failure using the abovementioned procedures for visits 3 and 4. The trial is represented as $CP_{+15W\ post}$ from this point on.

5.3.8 Data Analysis

Breath-by-breath $\dot{V}O_2$ data from the ramp test and constant intensity trials were averaged in 10 seconds intervals to determine GET and the $\dot{V}O_2$ response. The $\dot{V}O_2$ data were edited to eliminate the effects of coughs or swallows on the measurement. Only those data points beyond the three standard deviations of the mean value were excluded (Burnley et al., 2006a). The GET was identified using the v-slope method, which is defined as the $\dot{V}O_2$ value corresponding to the intersection of two linear regression lines derived separately from the data points below and above the breakpoint in the carbon dioxide production rate ($\dot{V}CO_2$)-versus $\dot{V}O_2$ relationship (Solberg et al., 2005; Bergstrom et al., 2013). The power output observed as GET was corrected by subtracting 20 W and 17 W for male and female participants, respectively (Davison et al., 2022). The power output and TTF during the CP determination trials were used to determine CP and W' using IBM SPSS statistics 26.0 (Chicago, IL, USA). The 2 parameter hyperbolic model (see below) was used as previous research concluded it is the favourable choice to assess MMSS (Altuna & Hopker, 2021)

$$T_{lim} = W'/(P-CP)$$

The first 20 s of the $\dot{V}O_2$ data following the onset of exercise were removed to eliminate the phase I component from the analysis. The first 2 minutes of the $\dot{V}O_2$ data (20 to 120 s) were then analysed using the monoexponential model (Rossiter et al., 2001; Burnley et al., 2005; 2006a):

$$\dot{V}O_2(t) = \dot{V}O_{2\text{base}} + \text{amp} * (1 - e^{-(t-TD/\text{tau})})$$

Where $\dot{V}O_2(t) = \dot{V}O_2$ at time, $\dot{V}O_{2\text{base}}$ = the baseline $\dot{V}O_2$ measured in the four minutes before the transition in work rate, amp = amplitude, TD = time delay, and tau = time constant of the primary (phase II) response. The amplitude of the $\dot{V}O_{2\text{sc}}$ was determined by the highest $\dot{V}O_2$ value achieved during the constant intensity trial and subtracting the “absolute” primary amplitude ($\dot{V}O_{2\text{base}} + \text{amp}$). The monoexponential model was chosen because a more complex model will significantly degrade the confidence intervals and create extra parameter interdependence (Burnley et al., 2005). Given that the time to task failure (TTF) at CP and CP_{+15W} varied between participants and trials, the $\dot{V}O_2$ data were analysed using the individual isotime method and expressed in relative time (baseline, 25, 50, 75 and 100%) where the baseline is indicated as 0 in the figure, to avoid any data loss (Nicolò et al., 2019). The $\dot{V}O_2$ corresponding to the desired time points was determined by the average $\dot{V}O_2$ over the prior 60 s. The mean of the last 2 segments (75% and 100%) was considered the $\dot{V}O_2$ corresponding to each trial.

Two sets of blood lactate samples were collected before the test, at every 5th minute and at task failure. The mean of the two blood lactate samples was used for subsequent analysis. The blood lactate kinetics response was interpolated with a linear function using Microsoft Excel (Excel, Microsoft, Redmond, Washington). Values corresponding to 25%, 50%, 75% and 100% of the test duration were used to represent the response during the CP and CP_{+15W} test (Nicolò et al., 2019).

5.3.9 Statistical Analysis

The blood lactate and $\dot{V}O_2$ data were analysed using two-way ANOVA with repeated measures to analyse the 5 trials (CP, CP_{+15W1,2&3} and CP_{+15Wpost}) x 5 time points (baseline, 25%, 50%, 75% and 100% of the total test duration). The end test $\dot{V}O_2$, highest $\dot{V}O_2$, TTF (s) and the amplitude of $\dot{V}O_{2\text{sc}}$ corresponding to exercise at CP, CP_{+15W1,2&3} and CP_{+15Wpost} were analysed using one-way repeated measure ANOVA. When sphericity was violated, the F value was adjusted using Greenhouse-Geisser. The significant interaction and main effects were determined using LSD post hoc tests. The predicted TTF at CP_{+15W} was calculated in Microsoft Excel (Excel, Microsoft, Redmond, Washington) using participants' CP and W' and the 2 parameter hyperbolic formula. Analyses were performed using SPSS. Data are reported as mean \pm SD unless otherwise stated.

5.4 Results

5.4.1 CP determination trials

The details of all CP determination trials were presented in Table 5.2 The resultant mean CP and W' were 248 ± 43 W (95% CI: 225 W, 272 W) and 13236 ± 3172 J (95% CI: 11512 J, 14960 J), respectively. The difference between CP and CP_{+15W} was $6 \pm 1\%$ (ranging from 5% to 10%; CP: ranged from 154 W to 301 W, and CP_{+15W} : ranged from 169 W to 316 W). The CP and W' from 1/time and linear model are Mean CP = 249 W (95% CI: 225 W, 272 W). Mean $W' = 13236$ J (95% CI: 16383 J, 11732 J) and CP = 249 W (95% CI: 226 W, 272 W). Mean $W' = 13336$ J (95% CI: 11670 J, 15001 J), respectively.

Table. 5.2 The mean power output (in Watts) and time to task failure (s) for CP determination trials

	60% Δ	70% Δ	80% Δ	100 % of $\dot{V}O_{2peak}$
Power (W)	277 ± 43	296 ± 46	315 ± 39	327 ± 108
Time to task failure (s)	477 ± 111	277 ± 63	202 ± 38	124 ± 43

5.4.2 Training

All participants completed all assigned training sessions. Mean and SD in power output (in Watts), relative power output (% $\dot{V}O_{2peak}$), time spent above $\dot{V}O_{2peak}$ (s) and time spent above CP (s) during “on” intervals in sessions 1 to 5 are presented in Table 5.3.

Table. 5.3 Mean power output (in Watts), Relative power output (% $\dot{V}O_{2peak}$), Time spent above $\dot{V}O_{2peak}$ (s) and Time spent above CP (s) during training sessions 1 to 5.

	Power (W)	%$\dot{V}O_{2peak}$	Time spent above $\dot{V}O_{2peak}$ (s)	Time spent above CP (s)
Session 1	265 ± 50	75 ± 10	176 ± 111	1276 ± 376
Session 2	279 ± 58	79 ± 9	185 ± 136	1373 ± 282
Session 3	270 ± 60	77 ± 12	242 ± 233	1329 ± 323
Session 4	279 ± 61	79 ± 10	254 ± 296	1417 ± 305
Session 5	284 ± 64	81 ± 10	209 ± 253	1454 ± 264
Mean	275	78	213	1370
SD	8	2	34	70

5.4.3 Time to task failure

There was a significant difference in the TTF across 5 constant intensity trials and the predicted TTF estimated using the CP and W' from pre-training ($F = 10.943$, $p < 0.001$). There was no significant difference between the 3 CP_{+15W} trials before training ($p > 0.05$) with the ICC of 0.77 (95% confidence limits 0.49-0.92) and CV of $14.2 \pm 9.9\%$. Both $CP_{+15Wpost}$ and the predicted TTF were significantly longer than all the CP_{+15W} trials before training ($p < 0.05$). Following training, there was no significant difference between the $CP_{+15Wpost}$ and the predicted TTF estimated using the pre-training CP and W' ($p > 0.05$; Table 5.4).

Table. 5.4 Time to task failure (s) at CP, $CP_{+15W1,2,3\&post}$, and the predicted TTF

	At CP	CP_{+15W1}	CP_{+15W2}	CP_{+15W3}	$CP_{+15Wpost}$	Predicted
Mean (s)	1021	628 ^a	678 ^a	664	878 ^{b,c,d}	882 ^{b,c,d}
SD	418	190	163	214	260	211

^asignificant different from at CP ($p < 0.05$); ^bsignificant different from at CP_{+15W1} ($p < 0.05$); ^csignificant different from at CP_{+15W2} ($p < 0.05$); ^dsignificant different from at CP_{+15W3} ($p < 0.05$)

5.4.4 Blood lactate response and end test blood lactate

There was a significant interaction effect between time and intensity for the estimated blood lactate response ($F = 6.190$, $p < 0.001$), for the main effect of time ($F = 254.904$, $p < 0.001$) and intensity ($F = 5.327$, $p < 0.001$). The estimated blood lactate at all time points was

significantly different between each time point during all trials (at and above CP, before and after training; see Figure. 5.1). The end test BLC at CP_{+15W1} was the lowest of the three pre-training CP_{+15W} trials (9.9 ± 0.8 mM vs. 11.9 ± 0.5 mM and 11.5 ± 0.5 mM, for CP_{+15W} trial 1, 2 and 3, respectively). There was no significant difference between the end test BLC at CP and CP_{+15W1} trial (9.5 ± 0.7 mM vs. 9.9 ± 0.8 mM). Although not significantly different, the end test BLC at the CP_{+15Wpost} trial (12.3 ± 2.0 mM) was on average 4% and 7% higher than the second and third CP_{+15W} trials, respectively.

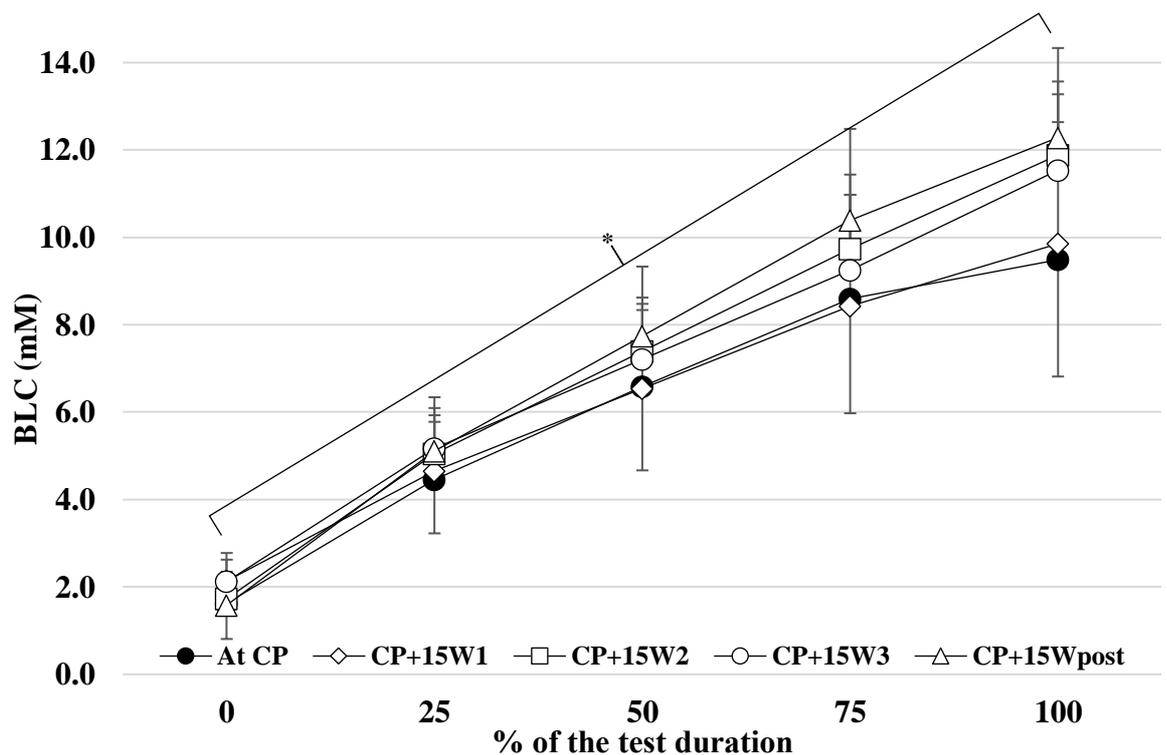


Figure. 5.1 The blood lactate response as a percentage of trial duration when exercising at the intensities corresponding to CP, CP_{+15W1,2,3} and CP_{+15Wpost} *significant difference ($p < 0.05$)

5.4.5 $\dot{V}O_2$ responses corresponding to CP, CP_{+15W} and CP_{+15Wpost}

There was a significant interaction effect between time and intensity for the $\dot{V}O_2$ response ($F = 6.459$, $p < 0.001$), for the main effect of time ($F = 186.970$, $p < 0.001$) and intensity ($F = 11.351$, $p < 0.001$). Post hoc analysis showed that the $\dot{V}O_2$ corresponding to 50%, 75% and 100% at CP was significantly lower than all CP_{+15W} trials. However, there was no significant difference between all CP_{+15W} trials during that time window. During exercise at CP, there was no significant difference between the $\dot{V}O_2$ at 25% and 100% of the total

duration. On the contrary, the $\dot{V}O_2$ changed significantly between 25% and 100% of the total duration during all CP_{+15W} trials before and after training (see Figure. 5.2).

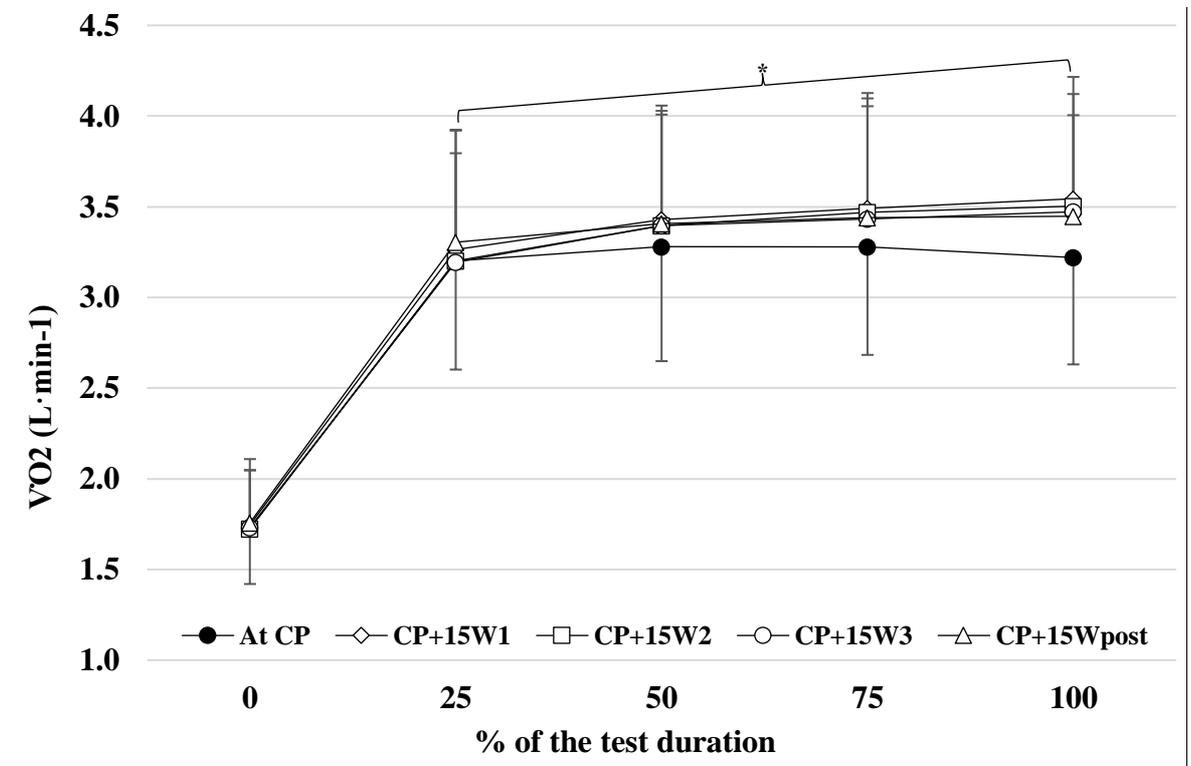


Figure. 5.2 The $\dot{V}O_2$ response as a percentage of trial duration when exercising at the intensities corresponding to CP, CP_{+15W1,2,3} and CP_{+15Wpost}. *significant difference ($p < 0.05$)

There was a significant difference in the amplitude of $\dot{V}O_{2sc}$ between trials ($F = 2.961$, $p = 0.029$). Post hoc analysis revealed that the $\dot{V}O_{2sc}$ corresponding to CP was significantly lower than all CP_{+15W} trials (Table 5.5). A significant difference was also found between the highest $\dot{V}O_2$ across 6 tests (5 constant intensity trials and the ramp test) ($F = 17.192$, $p < 0.001$). The post hoc test shows that the highest $\dot{V}O_2$ achieved during the trial at CP was significantly lower than in all CP_{+15W} trials and $\dot{V}O_{2peak}$. No significant difference was found between the highest $\dot{V}O_2$ achieved in all CP_{+15W} trials and the $\dot{V}O_{2peak}$ from the ramp test.

Table. 5.5 The $\dot{V}O_2$, the percentage of $\dot{V}O_{2peak}$ and the $\dot{V}O_{2sc}$ correspond to each constant intensity test

	$\dot{V}O_2$ (L·min ⁻¹)	% of $\dot{V}O_{2peak}$	$\dot{V}O_{2sc}$ (ml·min ⁻¹)
At CP	3.25 ± 0.59	88 ± 3%	274 ± 95
CP_{+15W1}	3.52 ± 0.65 ^a	95 ± 3%	427 ± 202 ^a
CP_{+15W2}	3.49 ± 0.59 ^a	95 ± 3%	422 ± 178 ^a
CP_{+15W3}	3.45 ± 0.59 ^a	94 ± 4%	411 ± 137 ^a
CP_{+15Wpost}	3.44 ± 0.58 ^a	94 ± 4%	393 ± 160 ^a

^aSignificant different from exercise at CP (p < 0.05)

5.5 Discussion

5.5.1 Overview

The main aim of this study was to examine the validity of CP representing the upper boundary of the heavy intensity domain. The results demonstrated 1) the $\dot{V}O_2$ response stabilised when exercising at CP, whereas exercising 15W above CP leads to the $\dot{V}O_2$ increasing towards the $\dot{V}O_{2peak}$ and without apparent stabilisation (see Figure 5.2); and 2) the amplitude of $\dot{V}O_{2sc}$ was consistently larger during all CP_{+15W} trials compared to the test at CP. The second aim of this study was to evaluate the accuracy of the 2 parameters hyperbolic model for predicting constant severe intensity exercise performance. Results demonstrated that the TTF for the 3 CP_{+15W} trials before training was significantly shorter than the predicted TTF (76 ± 3%). After the 1-week microcycle training sessions, the TTF at CP_{+15Wpost} had increased and was not significantly different from the TTF predicted pre-training, without significant changes in both the $\dot{V}O_2$ and blood lactate response.

5.5.2 The validity of CP being the threshold between heavy and severe intensity domain

Since the proposal that CP is the gold standard measurement for determining the threshold separating the heavy and severe intensity domains (Jones et al., 2019), there has been debate in the literature as to whether this is the case (Altuna & Hopker, 2022; Burnley, 2022; 2023; Black et al., 2022; Broxterman et al., 2022; Dotan, 2022a; 2022b; Gorostiaga et al., 2022a; 2022b; 2022c; Nixon et al., 2021). The results of the present study demonstrated that the $\dot{V}O_2$ was able to stabilise (see Figure 5.2) while the blood lactate concentration increased continuously when exercising at CP (see Figure 5.1). These results demonstrated the

inadequacy of using blood lactate kinetics-based performance markers such as MLSS to inform the MMSS. Similar to the current study's findings, De Lucas et al. (2013) reported that $\dot{V}O_{2peak}$ was reached when exercising at 5% above CP (98% of $\dot{V}O_{2peak}$). However, based on the statistically significant difference between the $\dot{V}O_{2peak}$ and the $\dot{V}O_2$ corresponding to CP, the authors concluded that the $\dot{V}O_{2peak}$ was not elicited during the constant intensity test at CP (94% of $\dot{V}O_{2peak}$). However, Katch et al. (1982) suggested that $\dot{V}O_{2peak}$ can be expected to have a measurement error of $\pm 5.6\%$ between trials. Thus, it is possible that the $\dot{V}O_{2peak}$ was achieved when exercising at CP even though it did not ultimately reach the same magnitude as the 5% above CP trial in their study. Interestingly, De Lucas et al. (2013) also report a non-steady-state in the $\dot{V}O_2$ response during exercise at CP. Combining both the result of the highest $\dot{V}O_2$ and the $\dot{V}O_2$ response, the CP in their study should be classified as severe intensity. Data from the present study demonstrate that the highest and the end test $\dot{V}O_2$ achieved during the test at CP was significantly lower than the $\dot{V}O_{2peak}$ (Table 5.5), with a clear stabilisation in the $\dot{V}O_2$ response (see Figure 5.2). Similar to the results from Nixon et al. (2021), the highest $\dot{V}O_2$ achieved during all CP_{+15W} trials in the current study was not significantly different from the $\dot{V}O_{2peak}$ recorded during the ramp test ($p > 0.05$). Moreover, the mean end test $\dot{V}O_2$ corresponding to all CP_{+15W} trials was $95 \pm 1\%$, which met the suggested verification of $\dot{V}O_{2peak}$ attainment during constant intensity exercise (Jones et al., 2019). The $\dot{V}O_2$ also increased significantly in all CP_{+15W} trials from 25% of the total duration until the point of task failure, which is evidence of non-steady state response (see Figure 5.2). Both $\dot{V}O_{2peak}$ achievement and non-steady state $\dot{V}O_2$ responses when exercised at CP_{+15W} matched the characteristics of the severe intensity domain (Jones et al., 2019).

The change in amplitude of $\dot{V}O_{2sc}$ represents a loss of the steady state in $\dot{V}O_2$ during severe intensity exercise (Burnley & Jones, 2007). It can therefore be used as an indicator of whether exercise is within the heavy or severe intensity domain, i.e. below or above the MMSS. As reported by Pringle et al. (2003), the $\dot{V}O_{2sc}$ when exercising within the heavy intensity domain should be smaller than in severe intensity ($p < 0.05$). Interestingly, De Lucas et al. (2013) reported no significant difference and a smaller $\dot{V}O_{2sc}$ when exercising 5% above CP ($222 \pm 106 \text{ ml}\cdot\text{min}^{-1}$) compared to trials at CP ($247 \pm 82 \text{ ml}\cdot\text{min}^{-1}$). Such results are in contrast to the amplitude of $\dot{V}O_{2sc}$ reported in both the present study (Table 5.5) and that of Pringle et al. (2003) and do not appear to reflect the typical oxygen uptake kinetics in the two different intensity domains, i.e. heavy and severe. (Burnley & Jones, 2007;

Pringle et al., 2003). A possible reason for these apparent differences could be De Lucas et al. (2013) calculated the $\dot{V}O_{2sc}$ as the difference in $\dot{V}O_2$ between the 3rd minute and task failure, which has been shown to significantly underestimate the $\dot{V}O_{2sc}$ calculated using a nonlinear regression model (Santana et al., 2007). Based on the overall $\dot{V}O_2$ kinetics during both at and 5% above the CP, it is also possible they overestimated the power corresponding to the CP. This apparent over-representation of the CP could be explained by differences in the methodological approach to estimating CP between studies. De Lucas et al. (2013) used the linear 1/time model to determine the CP and W', whereas the present study used a 2 parameter hyperbolic model. According to Altuna and Hopker (2021), the linear inverse-time model produces a significantly higher CP than the hyperbolic model, leading to an overestimation of the boundary between the heavy and severe domains. Therefore, the potential overestimation of CP is likely to have also led to the non-steady state in $\dot{V}O_2$, $\dot{V}O_{2peak}$ reached, and account for the lack of difference in the $\dot{V}O_{2sc}$ reported by De Lucas et al. (2013). Although the blood lactate was unable to stabilise at both CP and CP_{+15w} trials (Figure 5.1), the overall $\dot{V}O_2$ results indicated that exercising 15 W above the CP estimated from 2 parameter hyperbolic model is within the severe intensity domain, with exercise at CP should be categorised as the heavy intensity domain. As such, the current study's findings support that the CP determined by the 2 parameter hyperbolic model provides a valid determination of the MMSS.

5.5.3 The validity of using the 2 parameter hyperbolic model to predict performance

Pallarés et al. (2020) previously examined the difference between the predicted and measured TTF at different exercise intensities. The authors reported that the CP model overestimated the actual TTF by 86% and 6% at Wingate and MLSS intensities, respectively. However, it is essential to note that the shortest (00:29 ± 00:06 min:ss) and longest (76:05 ± 13:53 min:ss) were outside the suggested testing duration for CP determination trials, minimal 2 minutes and maximal 15 minutes (Jones et al., 2019). Additionally, the CP model is only suitable for informing the exercise performance of severe intensity exercise. (Vanhatalo et al., 2011) and does not apply to extreme (Wingate) and heavy (MLSS) (Leo et al., 2022; Jones et al., 2019). Therefore, the results of Pallarés et al. (2020) might provide a misleading conclusion regarding the validity of using the 2 parameter model to predict TTF. Whereas the present study used a conventional fixed work rate determination method to ensure the duration of all determination trials were within the suggested 2 to 15 minutes

range, and only severe intensity work rate were prescribed. Surprisingly, the actual TTF of the 3 CP_{+15W} trials performed before training were all significantly different from the predicted TTF (Table 5.3) and the mean actual TTF was only $76 \pm 3\%$ of the predicted TTF. There was a moderate reliability level in the measure of TTF from the 3 pre training CP_{+15W} trials (ICC = 0.77; CV: 14.2%). Interestingly, after one week of HIT training, the TTF at CP_{+15W}post was not significantly different from the predicted TTF estimated using the CP and W' from pre training ($p > 0.05$). Unfortunately, given that the present study did not perform an additional ramp test and CP determination trials after the training, it remains unknown whether the performance improvement was because of an enhanced CP and W' after training or whether the validity of prediction relies on whether the individuals' performance is optimised. Additionally, because there was no standardised script or method for verbal encouragement to participants, the inconsistencies in how verbal encouragement was provided could have impacted the TTF during all CP determination trials and thereby the CP and W' estimation.

The power corresponding to CP can be improved by performing 4 to 8 weeks of continuous or interval endurance training (Gaesser & Wilson; 1998; Jenkins & Quigley, 1992; Vanhatalo et al., 2008). Previous research has also reported that individuals with a higher proportion of type I muscle fibres tend to have a higher CP and a negative correlation between CP and the proportion of type II muscle fibers (Goulding et al., 2021; Goulding & Marwood, 2023; Mitchell et al., 2018). Considering the duration (5 hours in total within one week) and the nature (repeated all out sprint) of the training prescribed in the present study, it is highly unlikely that the conversion from type II to type I muscle fibers occurred (Kohn et al., 2011), and CP improved. Conversely, endurance training would cause a reduction in both $\dot{V}O_{2sc}$ (Cleuziou et al., 2005; Demarle et al., 2001) and W' (Gaesser & Wilson, 1988; Jenkins & Quigley, 1992). Prior glycogen depletion and high intensity exercise have also been reported to decrease the amplitude of $\dot{V}O_{2sc}$ and W' (Bailey et al., 2009; Burnley et al., 2000; Ferguson et al., 2007; 2010; Carter et al., 2004; Miura et al., 2000). In addition, Murgatroyd et al. (2011) reported a strong and positive relationship between $\dot{V}O_{2sc}$ and W' and suggested that the depletion of W' when exercising above the CP is reflected in the development of the $\dot{V}O_{2sc}$, which consistently supported the notion that the amplitude of $\dot{V}O_{2sc}$ and W' are mechanistically related (Goulding et al., 2021). On the other hand, the results from previous studies regarding the training effect of HIIT on W' is mixed. Jenkins

& Quigley (1993) reported a significant increase in W' after 8 weeks of HIIT training while Thomas et al. (2020) reported the contrary after 6 weeks of training intervention. In the present study, the mean difference in $\dot{V}O_{2sc}$ between CP_{+15W1} and CP_{+15W3} was $16 \text{ m}\cdot\text{min}^{-1}$, which is similar to the difference between CP_{+15W3} and $CP_{+15Wpost}$, $18 \text{ m}\cdot\text{min}^{-1}$. Additionally, the amplitude of $\dot{V}O_{2sc}$ also was not significantly different between all CP_{+15W} trials (pre and post). It is important to note that the highest $\dot{V}O_2$ during all CP_{+15W} trials (pre and post training) was not significantly different to the $\dot{V}O_{2peak}$, the W' and the amplitude of $\dot{V}O_{2sc}$ should therefore fully depleted and maximised, respectively, at task failure during all CP_{+15W} trials (Murgatroyd et al., 2011). Therefore, the training intervention does not appear to alter the W' according to the amplitude of the $\dot{V}O_{2sc}$. Collectively, physiological data does not indicate that CP and W' improved after the 1 week training intervention prescribed in the present study and the performance improvement is possible because of other reasons which will be discussed in the following paragraph.

The end test $\dot{V}O_2$ and the amplitude of $\dot{V}O_{2sc}$ were, on average reduced by $0.04 \pm 0.03 \text{ L}\cdot\text{min}^{-1}$ and $26 \pm 13 \text{ ml}\cdot\text{min}^{-1}$, respectively, after the training block, suggestive of improved cycling efficiency. Indeed, previous research has supported the suggestion of an improvement in TTF during exercise in the severe domain being explained by a reduction in the amplitude of $\dot{V}O_{2sc}$ (Bailey et al., 2009). A reduction in $\dot{V}O_{2sc}$ is suggested to increase TTF by reducing the demand on PCr stores and glycogen reserve (Krustrup et al., 2008), lowering the level of muscular fatigue, delaying the accumulation of fatiguing metabolite accumulation during severe intensity exercise (Burnley & Jones, 2007). Conversely, the end test blood lactate at $CP_{+15Wpost}$ increased by 4% and 7% compared to CP_{+15W2} and CP_{+15W3} , respectively, providing another possible explanation for the TTF improvement after the training microcycle. First, lactate has been suggested as an essential energy substrate that the body can utilise during endurance exercise (Cairns, 2006; Hall et al., 2016). Hence, the greater capacity to generate lactate would mean more energy can be utilised during exercise. Second, blood lactate has been suggested as an exercise stress indicator, producing pain sensation according to the exercise intensity and terminating exercise to avoid any significant damage to muscle and other organs (Philp et al., 2005). In the present study, the higher accumulated blood lactate and prolonged TTF at $CP_{+15Wpost}$ indicated the participants could tolerate a higher level of pain and metabolite accumulation for an extended period, which potentially allowed the participants to fully expend the W' during the $CP_{+15Wpost}$ trial

(Salam et al., 2018) and therefore the TTF after training matched the model prediction. However, it must be stressed that the contributions of $\dot{V}O_2$ and blood lactate underpinning the enhancement in TTF at $CP_{+15W_{post}}$ is unclear as there was no significant difference between before and after training for both physiological parameters.

Even though the recovery period between each determination trial was 1 hour, not the conventional 24 hours, the use of a 1 hour recovery period between each determination trial in the present study was recommended and used by previous studies (Karsten et al., 2018; Muniz-Pumares et al., 2019; Hunter et al., 2021). Karsten et al. (2017) previously reported that the recovery period could be as low as 30 minutes, but it can only provide a valid estimation of CP but not W' . The potential explanations were that the 30 minutes recovery does not allow full reconstitution of W' and the priming effect of $\dot{V}O_2$ kinetics from the previous determination trial. Different studies reported that the time required for W' reconstitution following exhaustion is approximately 25 minutes (Ferguson et al., 2010; Skiba et al., 2012), therefore, the latter explanation is likely to be a more contributing factor. Indeed, previous severe intensity exercise has a priming effect on the $\dot{V}O_2$ kinetics by reducing the $\dot{V}O_{2SC}$ and increasing the $\dot{V}O_{2peak}$ (Burnley et al., 2011) which both could prolong the TTF during determination trials. It has been shown that the priming effect would last for at least 45 minutes and the overall $\dot{V}O_2$ response was not significantly different from the control data after 60 minutes (Burnley et al., 2006a). Such result is consistent with Karsten et al. (2018) findings, which reported a 60 minutes recovery period between estimation trials as a valid determination protocol. Therefore, a recovery period of 60 minutes is recommended by Muniz-Pumares et al. (2019) as it allows sufficient time to restore W' and avoid the priming effect on $\dot{V}O_2$ kinetics.

5.6 Conclusion

Collectively, the $\dot{V}O_2$ response indicated that the CP determined from 2 parameter hyperbolic model is a valid representation of the threshold separating the heavy and severe intensity domains. Based on this finding, practitioners are recommended to use CP to prescribe training as it accurately represents the highest intensity at which the $\dot{V}O_{2peak}$ is not attained, and the $\dot{V}O_2$ can be stabilised. Conversely, the TTF for the three CP_{+15W} trials before the one week of HIT training was significantly shorter than the predicted TTF but not

after the training. Therefore, the validity of using the 2 parameter hyperbolic model to predict constant severe intensity performance requires further examination.

Chapter 6

General discussion

6.1 Overview

The topic of which performance marker represents the MMSS is a topic of intense debate in the sports science community (Altuna & Hopker, 2022; Burnley, 2022; 2023; Black et al., 2022; Broxterman et al., 2022; Dotan, 2022a; 2022b; 2022c; Gorostiaga et al., 2021; 2022a; 2022b; 2022c; Nixon et al., 2021). Different arguments have been raised to challenge the validity of the markers examined in the present thesis. For MLSS, the use of blood lactate has been suggested as an invalid marker to represent the oxygen kinetics and the conventional determination protocol for MLSS might ignore the delayed blood lactate steady state (Jones et al., 2019). For FTP, the research regarding the physiological response when exercising at and above the marker is substantially lacking. The concept of CP has been extensively studied but also challenged by previous studies. Overall, the criticisms of CP are that 1) it contradicts its own definition, 2) it lacks a standardised determination protocol 3) it overestimates the MLSS and 4) statistical artifact without clear physiological meanings (Millet et al., 2022; Rodrigo-Carranza et al., 2022; Dotan, 2022a; 2023). However, it is important to recognise that these limitations might be more suitable to describe MLSS and FTP.

6.2 Main research findings

Study 1 (Chapter 3):

- a) The $\dot{V}O_2$ was able to stabilise when exercising 15 W ($9 \pm 2\%$) above the conventional MLSS.
- b) The amplitude of $\dot{V}O_{2sc}$ and end test $\dot{V}O_2$ were not significantly different between exercising at and 15W above the conventional MLSS.
- c) The conventional MLSS protocol could not account for the delayed steady state in lactate when exercising 15 W above the MLSS.

Study 2 (Chapter 4):

- a) $\dot{V}O_2$ stabilised when exercising at and 15 W ($7 \pm 2\%$) above FTP.
- b) The amplitude of $\dot{V}O_{2sc}$ and end test $\dot{V}O_2$ were not significantly different between exercising at and 15 W above the FTP.

- c) The blood lactate kinetics corresponding to FTP trials fulfilled the modified criterion of MLSS proposed by Nixon et al. (2021).
- d) Not all participants could sustain 40 minutes of exercise at the power output corresponding to FTP.

Study 3 (Chapter 5):

- a) The highest $\dot{V}O_2$ during all CP_{+15W} trials was not significantly different from the $\dot{V}O_{2peak}$ from the incremental ramp test.
- b) The amplitude of $\dot{V}O_{2sc}$ and end test $\dot{V}O_2$ were significantly lower during exercise at CP than in CP_{+15W} trials.
- c) The $\dot{V}O_2$ was able to stabilise during exercise at CP but did not stabilise during all CP_{+15W} trials.
- d) The TTF was significantly shorter than predicted before training but not after training.

The novelty of the present thesis:

- a) MLSS (conventional protocol ignored the delayed steady state in lactate) and FTP (reached task failure before 40 minutes) have deviated from their original definition.
- b) The $BLC_{\Delta 1530} < 1mM$ could be a valid criterion for MLSS determination which accounts for the delayed steady state in blood lactate.
- c) The CP determined using 2 parameter hyperbolic model is a valid representation of the upper boundary of the heavy intensity domain, not MLSS and FTP.
- d) The validity of the 2 parameter hyperbolic model in predicting severe intensity performance remains unclear.
- e) One week of HIT training is potentially an effective method to improve the work rate corresponding to CP.
- f) Blood lactate kinetics does not reflect the $\dot{V}O_2$ kinetics.
- g) MLSS, FTP and CP should not be used interchangeably from a physiological perspective.

6.2.1 *The physiological response corresponding to MLSS, FTP and CP*

The blood lactate has been considered a reliable bio-maker to inform the oxidative metabolic status (Keir et al., 2018). Hence, it became the rationale of MLSS being the gold standard that other performance markers such as CP and FTP must compare to in terms of validating as the representation of MMSS. Previous research has suggested that MLSS is equivalent to the MMSS because it accurately determines the highest intensity at which the blood lactate production rate equals the clearance rate (Caen et al., 2021; Iannetta et al., 2021; Dotan, 2022a). However, a dissociation between the lactate in blood and working muscle has been documented (Stanisby & Brooks, 1990; Galdden, 2004; Brooks, 2018), and the blood lactate may not accurately reflect the metabolic status of respiring cells (Marwood et al., 2019). The blood lactate kinetics was proposed as an index of $\dot{V}O_2$ kinetics based on the notion that increased blood lactate concentration is due to limited oxygen available to working muscle (Hill & Lupton, 1923), and therefore deemed to be suitable in determining the boundary between heavy and severe intensity domains. This narrative has now been overruled as the lactate formation is not because of a lack of available oxygen delivery to mitochondria (Gladden, 2004; Svedahl & MacIntosh, 2003; Rogatzki et al., 2015) nor a fatigue-inducing metabolite during exercise (Poole et al., 2021).

The present thesis provided direct comparisons of the $\dot{V}O_2$ and blood lactate kinetics when exercising at and 15 W above the MLSS, FTP and CP. In Chapter 3, the blood lactate concentration was unable to stabilise based on the conventional MLSS requirement ($BLC_{\Delta 1030} < 1\text{mM}$) when exercising at $MLSS_{+15W}$, but the $\dot{V}O_2$ was not significantly different between the same time points ($p > 0.05$). Furthermore, when exercising at FTP, FTP_{+15W} and CP, the blood lactate significantly differed between 25% of the total duration and the end test (Figure 4.2 & 5.1). On the contrary, the corresponding $\dot{V}O_2$ at the same time points demonstrated an ability to stabilise (Figure 4.1 & 5.2). The $\dot{V}O_2$ and blood lactate kinetics from Chapter 4 and 5 demonstrated a dissociation between the $\dot{V}O_2$ and the blood lactate steady state according to both the definition of MLSS ($BLC_{\Delta 1030} < 1\text{mM}$) and the statistical difference. These results are consistent with Scheen et al. (1981) and Poole et al's (1988) findings demonstrating that blood lactate does not reflect whole body oxidative metabolic rate because the blood lactate was consistently unable to achieve a steady state (by the conventional MLSS determination standard or statistical difference) while the $\dot{V}O_2$ stabilised. Therefore, it is inappropriate to consider the representation of MMSS dependent

on the blood lactate response because it is not a valid surrogate of $\dot{V}O_2$ kinetics and it does not reflect the metabolic status of working muscle.

The MMSS is the threshold separating the heavy and severe intensity domain, and the difference in the overall $\dot{V}O_2$ kinetic is the foundation of categorising the two intensity domains. In short, the $\dot{V}O_2$ is able to stabilise without $\dot{V}O_{2max}$ attainment during heavy intensity exercise and the contrary in the severe intensity domain (Burnley & Jones, 2007). Furthermore, the amplitude of $\dot{V}O_{2sc}$ corresponding to the heavy intensity domain is smaller than the severe intensity domain because the $\dot{V}O_{2sc}$ is able to stabilise during heavy intensity but not severe intensity exercise (Pringle et al., 2003; Burnley & Jones, 2007). Therefore, the present thesis examined the $\dot{V}O_2$ kinetics response at work rates corresponding to MLSS, FTP and CP as well as 15 W above each to determine the validity of each threshold marker in representing the MMSS. As presented in Chapters 3 and 4, the $\dot{V}O_2$ was able to stabilise when exercising at the intensity corresponding to MLSS, MLSS_{+15W}, FTP and FTP_{+15W}. The $\dot{V}O_2$ corresponding to each intensity was $72 \pm 9\%$, $78 \pm 7\%$, $83 \pm 4\%$ and $87 \pm 3\%$ of $\dot{V}O_{2peak}$, respectively. Moreover, the $\dot{V}O_{2sc}$ was not significantly different when exercising at and 15 W above the MLSS and FTP, respectively. Based on these results and the definition of the threshold-based exercise intensity domain system, both MLSS and FTP cannot be considered as the upper boundary of the heavy intensity domain and, therefore representative of the MMSS. As shown in Figure 5.1, a clear steady state in $\dot{V}O_2$ exists when exercising at CP. On the contrary, the $\dot{V}O_2$ changed significantly during all CP_{+15W} trials, both before and after one week of HIT training. The amplitude of $\dot{V}O_{2sc}$ during all the CP_{+15W} trials was consistently higher than exercise at CP ($p < 0.05$), which is in line with the findings of a previous study indicating that the amplitude of $\dot{V}O_{2sc}$ is significantly different between heavy and severe intensity exercise (Pringle et al., 2003). Furthermore, in agreement with results from Nixon et al. (2021), the highest $\dot{V}O_2$ during all the CP_{+15W} trials was not significantly different from the participant's $\dot{V}O_{2peak}$ ($p > 0.05$), but this is not the case when exercise was conducted at CP intensity. In other words, the $\dot{V}O_2$ response at and 15 W above CP matched the heavy and severe intensity domains' characteristics, respectively. Since the threshold-based intensity domains are developed based on the distinct $\dot{V}O_2$ response (Burnley & Jones, 2007), which has a determining effect on performance, the physiological response should be the only consideration when determining the threshold. Therefore, from a physiological perspective, MLSS, FTP and CP should not be considered interchangeable.

6.2.2 *Contradiction to the concept's original definition*

Despite extensive research demonstrating that CP represents the highest intensity that can remain in a metabolic steady state, the validity of CP representing the MMSS has been continuously challenged. One of the most mentioned limitations of CP is that it contradicts the concept's original definition made by Monod and Scherrer in 1965, which is “being sustainable for a long time without fatigue” (Dotan, 2022a; 2022b; 2022c). Thus, it has been suggested that the concept of CP should be considered a flawed phenomenon and a mathematical artefact (Dotan, 2022a; 2022b; 2022c; Gorostiaga et al., 2022a). However, The description of “being sustainable for a long time without fatigue” is merely a flawed interpretation, not a definition, and that does not detract from the utility of CP (Burnley, 2022; Marwood & Goulding, 2022). On the contrary, suitable for describing MLSS and FTP as they both indeed deviated from what they originally proposed to measure and represent (i.e the MLSS is not the highest intensity the blood lactate can stabilise and the cycling at FTP cannot be sustained for 60 minutes).

The MLSS is defined as the highest intensity at which blood lactate is maintained in a steady state (Snyder et al., 1980) and believed that the MLSS is the optimal training intensity to improve endurance exercise performance. The original criterion for MLSS determination was a change in blood lactate of less than 0.05 mM every minute (Snyder et al., 1980), which evolved into a change of less than 1 mM between the 10th and 30th minute. The choice of this very specific time frame has been repeatedly questioned as being arbitrary (Jones et al., 2019; Marwood et al., 2019). Indeed, Mader and Heck (1986) did not provide any justification or rationale behind this very specific time frame. Additionally, the choice of the 10th minute might be too early and therefore not a valid starting time to examine blood lactate kinetics. It has been reported that the blood lactate steady state is attained approximately between 15 to 20 minutes (Poole et al., 1988; Beneke, 2003a; Beneke et al., 2009; 2011), which is similar to the results from Chapter 3. The mean blood lactate at the 10th minute was significantly lower than at the 30 minute during MLSS suggesting the BLC was not yet reaching a steady state at the 10th minute. Whereas the BLC at the 15th and 30th minute corresponding to MLSS was not significantly different (Figure 3.2), indicating that the blood lactate began to stabilise. Other than the starting time point of the 10th minute being too early, the conventional requirement of $BLC_{\Delta 1030} < 1$ mM also merits further examination. Dotan (2022a) pointed out that the time required for blood lactate to stabilise, if possible, increases

with the intensity. Hence, it is possible that the blood lactate failed to meet the conventional MLSS criterion ($BLC_{\Delta 1030} < 1 \text{ mM}$) but was able to reach a delayed steady state at a later time point (e.g. $BLC_{\Delta 1530} < 1 \text{ mM}$). Suppose the rationale for the conventional 1 mM criterion is based on Snyder et al.'s suggestion; 0.05 mM difference every minute during the last 20 minutes of a 30 minutes constant intensity exercise ($0.05 \text{ mM} \times 20 \text{ minutes} = 1 \text{ mM}$). In that case, an intensity at which the blood lactate change can remain less than 0.75 mM during the last 15 minutes of a 30 minute constant intensity trial would also meet the criterion of steady state proposed by Snyder et al. (1980) and should be considered as the MLSS. Using data from Chapter 3 of the current thesis, MLSS for one participant can be identified as 196 W because $BLC_{\Delta 1030} = 1.3 \text{ mM}$ when exercising at the next higher work rate of 211 W ($MLSS_{+15W}$). However, the $BLC_{\Delta 1530}$ was 0.6 mM calculated from the same 30 minute $MLSS_{+15W}$ constant intensity trial. Based on the $BLC_{\Delta 1530} < 1$ or $< 0.75 \text{ mM}$ criterion, the 211W which was originally classified as $MLSS_{+15W}$ could also be considered as MLSS. Such results showed that the conventional $BLC_{\Delta 1030} < 1 \text{ mM}$ criterion for MLSS determination failed to account for the delayed steady state in lactate as the intensity increases. In other words, the conventional MLSS does not equal the highest intensity at which the blood lactate can remain in a steady state, which deviated from the original definition as the blood lactate could stabilise at an intensity higher than MLSS based on either 1 mM or the original criterion proposed by Snyder et al. (1980). Therefore, the conventional MLSS should be considered a flawed performance marker. Alternatively, the $BLC_{\Delta 1530}$ should be considered as a more appropriate period to judge MLSS as it has been demonstrated to be able to account for the delayed blood lactate steady state.

The intensity corresponding to FTP is the power output that can be sustained for 60 minutes (Allen & Coggan, 2006; 2010). However, results from both Chapter 4 and previous research (Borszcz et al., 2018; Inglis et al., 2019; Karsten et al., 2021; Sitko et al., 2022) have shown that it is difficult and often impossible for individuals to sustain the power output corresponding to the FTP for 60 minutes, regardless of fitness, experience level and which determination protocol used. Sitko et al. (2002) used the determination protocol proposed by Allen & Coggan (2006; 2010) and the TTF at FTP was 35, 42, 47 and 51 minutes for recreationally trained ($\dot{V}O_{2\text{peak}}$: $46.9 \pm 5 \text{ ml/min/kg}$), trained ($59.5 \pm 3.1 \text{ ml/min/kg}$), well trained ($66.4 \pm 1.3 \text{ ml/min/kg}$) and professional level ($74.3 \pm 3.9 \text{ ml/min/kg}$) cyclists, respectively. Although Chapter 4 used another determination protocol which has also been

used by previous study for FTP estimation, a similar TTF of 33.7 minutes for participants with a mean $\dot{V}O_{2peak}$ of 58.2 ± 6.3 ml/min/kg was reported. On the other hand, the FTP has also been suggested as an alternative to MLSS (Borszcz et al., 2018). However, the blood lactate kinetics results demonstrated that the BLC change between the 10th and 30th was above the 1 mM tolerance limit, which makes the FTP not a valid alternative to MLSS. Unfortunately, blood lactate samples were not collected in the 5 minute intervals in Chapter 4, so it is impossible to identify whether the blood lactate response at FTP can meet the $BLC_{\Delta 1530} < 1$ mM criteria proposed in Chapter 3. An interesting finding from Chapter 4 is that the FTP could be used to determine MLSS because the blood lactate kinetics for FTP ($BLC_{\Delta 1020} = 0.8 \pm 0.6$ mM) matched the modified criteria for MLSS ($BLC_{\Delta 1020} < 2$ mM) proposed by Nixon et al. (2021). These results also suggested that the ability of FTP to provide an approximation of the MLSS appears to be influenced by the criterion used to determine the MLSS. Therefore, further research is needed to examine the validity between MLSS and FTP, as any modified criteria for the MLSS would require a more robust scientific examination. Overall, similarly to the MLSS, the concept of FTP also does not match its definition.

When Monod and Scherrer (1965) first proposed the CP, it was described but not defined as the intensity of “being sustainable for a long time without fatigue”. The definition of CP is the slope of the work-time relationship during muscular work performed to task failure. While it is true that the exercise intensity at CP cannot be sustained for a very long time, as presented in Chapter 5 (mean TTF at CP: 657s). Burnley (2022) has explained why the durability of exercising at CP should not be justified as the bases of considering CP a flawed phenomenon and a mathematical artefact. Moreover, Black et al. (2022) explained that the short TTF does not affect the validity of the CP representing the upper boundary of the heavy intensity domain because of the extensive and unequivocal physiological evidence presented by previous studies (Chapter 5; Jones et al., 2008; Nixon et al., 2021). It has become a common practice in research and coaching to determine a cyclist’s physiological profile based on whether the power output can be sustained for a fixed time (i.e. 60 minutes). However, neither MLSS nor FTP, which is fixed time based, can accurately reflect the physiological response according to Chapters 3 and 4, and the duration of both tests is arbitrary with no direct association with competitive events. Previous research has also repeatedly demonstrated high variability in durability when exercising at these fixed time-

oriented performance markers (Fontana et al., 2009; Pallarés et al., 2020; Sitko et al., 2022; Chapter 4). To summarise, determining the threshold separating heavy and severe intensity domains should not simply be based on whether the exercise intensity can be sustained for a fixed period (e.g. 30 or 60 minutes) as the TTF corresponding to different intensity domains (CP and CP_{+15W}) or performance marker (FTP) varies between individuals (Chapters 4 and 5; Sitko et al., 2022). Practitioners are therefore recommended to avoid using fixed time based performance markers to estimate physiological response and design training programs accordingly. The result from the present thesis also demonstrated that it is inappropriate to consider CP a flawed concept or inferior to MLSS and FTP simply because the intensity corresponding to CP cannot be sustained for “a very long time” because it is MLSS and FTP that are in fact deviated from their original definition.

6.2.3 The determination protocol of each performance marker

6.2.3.1 Maximal Lactate Steady State

The validity of whether the MLSS determined using the conventional protocol can represent the upper boundary of the heavy intensity domain and reflect the true lactate steady state was examined in Chapter 3. The conventional determination protocol of MLSS depends on whether the blood lactate change between two arbitrary pre-set time points (10 and 30 minutes) can remain below a fixed value of 1 mM. However, the MLSS determination protocol modification remains a popular area of debate in the sports science community (Iannetta et al., 2021; Nixon et al., 2021), which at some level reflects that the sports science community is uncertain about the conventional criterion. Indeed, using the conventional protocol ($BLC_{\Delta 1030} < 1 \text{ mM}$) has been suggested and demonstrated to be unable to account for the possibility of delayed steady-state in lactate (Jones et al., 2019; Chapter 3). In Chapter 3, six out of thirteen participants' blood lactate difference between the 15th and 30th minute was able to meet the 1 mM criterion when exercising at MLSS_{+15W}. It is therefore suggested that $BLC_{\Delta 1530} < 1 \text{ mM}$ could be a better criterion than $BLC_{\Delta 1030} < 1 \text{ mM}$ for MLSS determination. Similar to Chapter 3, the $BLC_{\Delta 1530} < 1 \text{ mM}$ criterion was also recommended by Iannetta et al. (2021) because the MLSS determined using this criterion was found to be the best approximation to the power output corresponding to CP.

The MLSS lacks a standardised protocol for the choice of the first MLSS determination trial as well as the stage incrementation between determination trials. Identifying the MLSS requires multiple constant intensity trials and a fixed increase rate between each trial. Additionally, given the high day-to-day variability in blood lactate (Hauser et al., 2013), the difference in starting intensity could result in a very different MLSS intensity even for the same individual. Therefore, the closer the first test is to the MLSS, the fewer tests are required, which helps minimise the training effect and reduce the labour-intensive nature of the MLSS determination protocol (Jones et al., 2019). Various intensities have been used as the first MLSS determination test in previous studies, including $75\% \pm 5\%$ of $\dot{V}O_{2\max}$ (Dekerle et al., 2003); 80% of $\dot{V}O_{2\max} \pm 10$ W (Hauser et al., 2013); $FTP \pm 5\%$ (Borszcz et al., 2018); $CP \pm 10$ W (Maturana et al., 2016); self-developed formula ± 10 W (Iannetta et al., 2018) and the power corresponding to lactate minimum ± 5 W (Fontana et al., 2009). Whereas the intensity corresponding to the first MLSS determination trial in Chapter 3 was calculated based on Saif et al.'s findings (2022) and the number of estimation trials was two to three for most participants, which could be an option to solve the labour intensive problem. On the other hand, the lack of a standardised fixed increase rate between trials would also affect the estimation of the MLSS. It has been suggested that a small difference in power output should be used to improve the precision of the MLSS estimation (Jones et al., 2019). A difference of 15 W between trials was used in Chapter 3, as Jones et al. (2019) suggested. Although the choice of ± 15 W can ensure the exercise examined is above the MLSS, it could be challenged as too large, thereby providing a poor resolution of the MLSS estimation. Therefore, it is important for future studies to develop a standardised starting intensity and a universally agreed incremental rate for MLSS determination. Additionally, the conventional $BLC_{\Delta 1030} < 1$ mM criterion has been demonstrated to be unable to account for the complexity of the blood lactate kinetics. Thus, the underestimation of the true MLSS was at least 15 to 30 W based on the incremental rate used in Chapter 3.

6.2.3.2 Functional Threshold Power

In terms of FTP, although Chapter 4 focused on the physiological response instead of directly examining the limitation of the FTP determination protocol, the fact that the FTP lacks a standardised protocol has been repeatedly highlighted by previous research (Carmichael & Rutberg, 2012; Borszcs et al., 2018; Barranco-Gil et al., 2020; Tramontin et al., 2022). The determination protocol has been modified several times since it was first

introduced by Allen and Coggan (2006; 2010). It was suggested that the FTP could be estimated by 90% of an 8-minute TT (Carmichael & Rutberg, 2012), which was used by Gavin et al. (2012) and Sanders et al. (2020). However, a previous study concluded that the FTP determined by 60 and 20 minute tests should not be used interchangeably (Borszcz et al., 2018; MacInnis et al., 2019). This conclusion is in line with Chapter 4 because six out of thirteen participants reached task failure before 40 minutes when exercising at the constant intensity corresponding to the FTP. Whereas for the 8-minute TT, Carmichael & Rutberg (2012) claimed the method is developed based on their own experience rather than any scientific evidence, and both Gavin et al. (2012) and Sanders et al. (2020) did not examine the validity between the FTP₈ and FTP₂₀ or FTP₆₀. Therefore, the problem with the FTP determination protocol is that it lacks a standardised protocol as there are at least three protocols that can be used for FTP estimation and the FTP determined between different protocols cannot be used interchangeably.

6.2.3.3 Critical Power

Multiple methods and equations (i.e. fixed time/power trials and equations 1 to 3) can be used to determine the exercise intensity corresponding to CP (Leo et al., 2022; Saif et al., 2022). Even though the present thesis did not examine all determination protocols and equations, extensive research has been done to determine the validity of different models and determination protocols (Altuna & Hopker, 2021; Bartram et al., 2017; Burnley et al., 2006a; Vanhatalo et al., 2007; Wright et al., 2018). Based on Chapter 5, the CP determined using fixed power trials and the 2 parameter hyperbolic equation can provide a reliable estimation of the threshold separating the heavy and severe intensity domain, and a valid solution to address the lack of standardised determination protocol. Therefore, future studies related to the upper boundary of the heavy intensity domain could use the determination protocol in Chapter 5 for accurate threshold estimation. Nevertheless, previous studies also raised concerns regarding the other aspects of the CP determination trials.

In a recent paper, Gorostiaga et al. (2021) suggested that CP determination protocol is unnecessary and that CP is a mathematical artifact because CS corresponds to 95 % to 99% of the average speed of the longest determination trial. However, the exercise intensity at CP in Chapter 5 corresponds to $89 \pm 3\%$ of the lowest intensity determination trial, ranging from 80% to 93%. Therefore, it is clear that the conclusion made by Gorostiaga et al. (2021) might

not have accounted for individual differences and do not apply to cyclists because the CP is well below 95 to 99% of the lowest intensity determination trial. Another aspect of the CP determination protocol that has been repeatedly challenged is the choice of duration for the determination trials (Gorostiaga et al., 2021; Dotan, 2022a). The suggested duration for CP determination trials is between 2 to 15 minutes, with a minimum of 5 minutes difference between the shortest and longest trial (Vanhatalo et al., 2011; Jones et al., 2019). The purpose of the suggested 2 to 15 minute duration is to ensure that severe exercise is performed and that the energetic store is fully depleted at task failure (Burnley, 2023). It provides **valid** guidelines to follow if athletes and coaches cannot perform an incremental ramp test to determine the correct intensity for fixed power determination trials based on the information of power output corresponding to the GET, as used in Chapter 5. However, it must be acknowledged that the choice of different duration trials would significantly affect the CP estimation despite using the same number of trials (Triska et al., 2018). Moreover, fixed time determination trials have been suggested to inflate the estimation of CP (Black et al., 2015). Thus, the fixed power/intensity approach should be preferred over the fixed time approach as the standardised protocol.

The validity of the CP determination protocol has also been questioned because trials longer than 15 minutes are rarely used in the determination trials (Dotan, 2022a; 2022b). Dotan (2022c) suggested the reason for not using longer trials is “to avoid confronting longer trials that would result in MLSS-like CP”. Indeed, it has been reported that using long determination trials (e.g. 76 minutes) resulted in the CP being 2% lower than the intensity corresponding to the MLSS (Pallarés et al., 2020). However, the aim of establishing CP is to identify the highest intensity at which the $\dot{V}O_2$ can stabilise without increasing towards $\dot{V}O_{2max}$. Therefore, longer determination trials would not only influence the result to “MLSS-like CP”, but also lose the essence of CP. Results from Chapter 3 of this thesis demonstrate that MLSS is not the upper boundary of the heavy intensity domain because $\dot{V}O_2$ stabilised, and $\dot{V}O_{2peak}$ was not reached when exercising 15 W above it. On the contrary, the $\dot{V}O_2$ failed to stabilise, and $\dot{V}O_{2peak}$ was achieved when exercising 15 W the above CP, as demonstrated in Chapter 5. Therefore, using determination trials longer than 15 minutes would risk performing a determination trial at the heavy intensity domain (Burnley, 2023), and the result of the “MLSS-like CP” would underestimate the MMSS.

6.2.4 Practical implications

Given that the $\dot{V}O_{2max}$ is one of the important factors that determine endurance sports performance (Joyner & Coyle, 2008), it is essential that practitioners can accurately categorise different intensity zones that target specific physiologic responses. In this case, the correct intensity zone leads to $\dot{V}O_{2max}$ attainment, hence, beneficial for $\dot{V}O_{2max}$ improvement. Practitioners in cycling tend to use FTP to inform the power output corresponding to different training zones. It is commonly assumed that $\dot{V}O_{2max}$ will be achieved when exercising at or above 106% of the FTP (Table 1.1). Similar to MLSS, it is considered a valid representation of MMSS by some researchers therefore $\dot{V}O_{2max}$ is thought to be reached when exercising above it. However, the training session would be without specificity if the prescribed intensity cannot trigger the required physiological stress (e.g. $\dot{V}O_{2max}$ attainment). In Chapters 3 and 4, it is demonstrated that the end test $\dot{V}O_2$ corresponding to $9 \pm 2\%$ and $7 \pm 2\%$ above the MLSS and FTP, respectively, was well below the $\dot{V}O_{2peak}$ (MLSS_{+15W}: $78 \pm 7\%$ of $\dot{V}O_{2peak}$ and FTP_{+15W}: $87 \pm 3\%$ of $\dot{V}O_{2peak}$). Whereas the $\dot{V}O_{2peak}$ was achieved when exercising 6% above the CP ($95 \pm 1\%$ of $\dot{V}O_{2peak}$). Based on these findings, it is suggested that practitioners should abandon the use of FTP and MLSS and adapt CP for correct training perception to ensure the desired training adaptation is achieved for $\dot{V}O_{2max}$ improvement.

One of the significant values of CP that MLSS and FTP cannot offer is that the concept of CP theoretically can be used to calculate the TTF over any severe intensity exercise. In Chapter 5, the mean TTF for CP_{+15W} trials before the one week HIT was $76 \pm 15\%$ of the predicted performance. However, after 5 hours of HIT training, the TTF at CP_{+15Wpost} was not significantly different from the predicted time calculated using pretraining data, and the end test lactate was higher than in all the pre-training CP_{+15W} trials. Such results could be owing to the fact that CP or W' or both increased after the training. Although the CP and W' were not re-determined after the training intervention, it is unlikely that CP and W' increased based on the nature of the prescribed training program and the $\dot{V}O_2$ data. It is well established that individuals with a relatively higher proportion of type I muscle fibres will have a higher CP because the enhanced capillarisation allows the muscle to maintain higher oxygenated blood perfusion and interstitial PO₂ values during rest and exercise (Goulding & Marwood, 2023). Contrary to the HIT training prescribed in Chapter 5 which consists of repeated 30 s all out cycling, endurance training predominantly recruits type I muscle fibre causing

hypertrophy specific to type I muscle fibre (Jones & Carter, 2000). Moreover, regular endurance training could cause a transition from type II to type I muscle fibre which results in an increased percentage of type I muscle fibre (Sale et al., 1990; Luden et al., 2012; Wilson et al., 2012) and endurance training has been reported as an effective method to increase CP accordingly (Gaesser & Wilson, 1988; Jenkins & Quigley, 1992). It is unlikely that the 1 week/5 hours of HIT improved the CP in Chapter 5. In terms of the W' , given that the W' is correlated with the amplitude of $\dot{V}O_{2sc}$ (Goulding et al., 2021) and the $\dot{V}O_{2sc}$ corresponding to CP_{+15W3} and $CP_{+15Wpost}$ was not significantly different. Therefore, it suggested that the W' also does not appear to be increased after the training intervention in Chapter 5. The performance improvement could be a result of other factors.

At the post-training retest, the TTF at $CP_{+15Wpost}$ was not significantly different from the predicted time estimate pre-training, and the end test lactate was higher than in all CP_{+15W} trials before training. Such results indicate a possibility that before the training, participants' fitness status was not optimised to allow them to expend their W fully' (Salam et al., 2018). After training, it was demonstrated that the 2-parameter hyperbolic CP model could closely estimate the best severe intensity exercise performance. It is possible that the CP can accurately predict the best performance that an individual can physiologically achieve. Still, an appropriate training program must be assigned to maximise performance gain first. In that case, the predicted time of constant severe intensity exercise can indicate the fitness state and gives coaches an indicator of when to retest and reassign appropriate intensity to ensure continued fitness development. Alternatively, the results of Chapter 5 could be that the CP model overestimated the TTF at constant severe intensity exercise. It is also possible that the CP improved after the training as the end test $\dot{V}O_2$ slightly reduced. Thus, the TTF at the same power output was prolonged after training. The results from Chapter 5 cannot conclude which of the abovementioned possibilities is the case. Further research is required to examine the validity of the concept of CP in predicting constant severe intensity exercise.

6.3 Conclusion

The primary objective of the present thesis was to determine whether MLSS, FTP or CP is a valid representation of the threshold between the heavy and severe intensity domains, i.e. the MMSS. Instead of addressing this question from a statistical perspective or whether

cyclists can sustain for a fixed amount of time when exercising at and above each exercise intensity, the physiological response corresponds to, at, and 15 W above the MLSS, FTP and CP was examined. Although MLSS and FTP are popular and considered valid performance markers in both research and practical settings, the results in Chapters 3 and 4 demonstrated that the physiological response (i.e. $\dot{V}O_2$ stabilised and $\dot{V}O_{2peak}$ not reached) when exercising at and above MLSS and FTP matched the characteristics of the heavy intensity domain. Furthermore, chapters 3 and 4 demonstrated an apparent dissociation between the $\dot{V}O_2$ and lactate kinetics, indicating that relying solely on blood lactate is inappropriate to identify the upper boundary of the heavy intensity domain. Contrary to exercising above the MLSS and FTP, the physiological response when exercising above the CP matched the expected characteristics of severe intensity exercise (i.e. the $\dot{V}O_2$ failed to stabilise and $\dot{V}O_{2peak}$ was achieved). Therefore, the exercise intensity corresponding to CP should be the one to represent the threshold between the heavy and severe intensity domain. Further work is required to examine the issues of MLSS, FTP and CP highlighted in the present thesis i.e. how to account for the delayed steady state in blood lactate; the usefulness of FTP; and the ability to use CP and W' to predict severe intensity performance.

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Appendices

ETHICS REVIEW CHECKLIST FOR RESEARCH WITH HUMAN PARTICIPANTS – FACULTY OF SCIENCES



A checklist should be completed for every research project in order to identify whether a full application for ethics approval needs to be submitted. The principal investigator or, where the principal investigator is a student, the supervisor, is responsible for exercising appropriate professional judgement in this review.

This checklist must be completed before potential participants are approached to take part in any research. All forms must be signed by the School’s Research Ethics Advisory Group representative.

Section I: Project details	
Project title:	The reliability of maximal lactate steady state
Planned start date: 01/02/2020	Planned end date: 30/06/2020
Funder:	The applicant

Section II: Applicant details			
Applicant name:		Wong Ting Qun	
School/Department:		School of Sport and Exercise Science	
Email:	Tw398@kent.ac.uk	Telephone number:	07307741364
Contact address:			
Undergraduate <input type="checkbox"/>	Taught Postgraduate <input type="checkbox"/>	Research Postgraduate <input type="checkbox"/>	Staff <input type="checkbox"/>

Section III: Declaration and signatures			
Please note that it is your responsibility to follow, and to ensure that, all researchers involved with your project follow accepted ethical practice and appropriate professional ethical guidelines in the conduct of your study. You must take all reasonable steps to protect the dignity, rights, safety and well-being of participants. This includes providing participants with appropriate information sheets, ensuring informed consent and ensuring confidentiality in the storage and use of data.			

Applicant signature		Date	21/01/2020
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Supervisor name	Mark Burnley	Date	6/1/2020
Supervisor signature		Date	6/1/2020

School REAG rep signature (required for both staff and students)		Date	12/03/20
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If the question “Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) or other biological samples from participants, or the use of existing samples?” in Section IV(A) is answered ‘yes’ if the human tissue samples taken are tested as soon as possible (within hours or days) and immediately disposed of, or rendered acellular you should reference the SSES Ethical Clearance for Standard Laboratory & Field Procedures document (<https://www.kent.ac.uk/stms/research-ethics/sses-ethical-clearance-2015.docx>) section 5 on research with human samples that sets out the methods by which the samples are rendered acellular or immediately disposed of in the full ethics application. This will confirm that the study goes not fall under the Human Tissues Act.

1. Complete full application form together with supporting documentation
2. Send to the N.Khan-360@kent.ac.uk for review by SSES (REAG)

If any other question in Section IV(A) is answered ‘yes’ or human tissue samples taken are not tested as soon as possible (within hours or days) and immediately disposed of, or rendered acellular tissue:

1. Contact Nicole Palmer (University Research Ethics & Governance Officer) for advice

SSES REQUEST FOR AMENDMENT TO RESEARCH ETHICS

School of Sport & Exercise Sciences (SSES)
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent, ME4 4AG

Original Ethics Reference No.: Prop
40_2019_20

Date of request: 15th December 2020

Name of person making request:

Wong Ting Qun

Details of proposed amendment(s):

Amend the planned start and end date:

Start date amend from 01/02/2020 to End of Jan 2021

End date amend from 30/06/2020 to June 2021

Please attach copies of any new or revised documentation used in the research study. If documentation has been revised please highlight changes.

Reason for amendment(s):

Delayed in testing due to Covid-19.

Amendment category:



A. Minor amendments – signed off by supervisor & submitted to SSES REAG

Examples - typographical errors, grammatical mistakes or formatting issues. Use of inappropriate templates for participant information sheets and consent forms. Use of personal contact details on participant information sheets.



B. Minor amendments/alterations – require SSES REAG Chair approval

Examples – weak procedures for ensuring confidentiality and data management. Improvement needed to participant information. Inequitable recruitment procedures. Overstatement of benefits. Researcher safety arrangements need more consideration.



C. A total redraft of the proposal – requires resubmission for full review

Examples – study design unsuitable to produce valid and reliable data and will be a waste of participants' time. Evidence of inducement or coercion. Risks to participants outweigh benefits to an unacceptable level. Researcher safety seriously at risk.

Approval granted: YES /

Reason if approval not granted:

Signed – ~~Cat A Student Supervisor / Cat B & C SSES REAG Chair~~ K. Hambley

Print name: KAREN HAMBLEY Date of decision: 16/12/20

SSES REAG AMENDMENTS APPLICATION OCTOBER 2019

31 May 2021

Mr WONG Ting Qun
Visiting Research Student Scheme
Department of Health and Physical Education

Dear Mr Wong,

Ethical Review <Ref. no. E2020-2021-0063>

The Human Research Ethics Committee (HREC) has noted and reviewed the Ethical Approval obtained from the University of Kent of your research proposal "*The Reliability of Maximal Lactate Steady State*". It is not necessary for your proposal to undergo review by the HREC.

Best wishes for your research.

Yours sincerely,



Patsy Chung (Ms)
Secretary

Human Research Ethics Committee

c.c. Professor CHOU Kee Lee, Chairperson, Human Research Ethics Committee

6 October 2021

Mr WONG Ting Qun
Visiting Student Programme
Department of Health and Physical Education

Dear Mr Wong,

Application for Ethical Review <Ref. no. 2021-2022-0003>

I am pleased to inform you that approval has been given by the Human Research Ethics Committee (HREC) for your research project:

Project title: Physiological Response when Exercising at and above Functional Threshold Power

Ethical approval is granted for the project period from 6 October 2021 to 30 November 2021. If a project extension is applied for lasting more than 3 months, HREC should be contacted with information regarding the nature of and the reason for the extension. If any substantial changes have been made to the project, a new HREC application will be required.

Please note that you are responsible for informing the HREC in advance of any proposed substantive changes to the research proposal or procedures which may affect the validity of this ethical approval. You will receive separate notification should a fresh approval be required.

Thank you for your kind attention and we wish you well with your research.

Yours sincerely,



Patsy Chung (Ms)
Secretary

Human Research Ethics Committee

c.c. Professor CHOU Kee Lee, Chairperson, Human Research Ethics Committee

10 February 2022

Mr WONG Ting Qun
Visiting Student Programme
Department of Health and Physical Education

Dear Mr Wong,

Application for Ethical Review <Ref. no. 2021-2022-0186>

I am pleased to inform you that approval has been given by the Human Research Ethics Committee (HREC) for your research project:

Project title: Physiological Response and the Validity of Critical Power

Ethical approval is granted for the project period from 10 February 2022 to 6 May 2022. If a project extension is applied for lasting more than 3 months, HREC should be contacted with information regarding the nature of and the reason for the extension. If any substantial changes have been made to the project, a new HREC application will be required.

Please note that you are responsible for informing the HREC in advance of any proposed substantive changes to the research proposal or procedures which may affect the validity of this ethical approval. You will receive separate notification should a fresh approval be required.

Thank you for your kind attention and we wish you well with your research.

Yours sincerely,



Patsy Chung (Ms)
Secretary

Human Research Ethics Committee

c.c. Professor CHOU Kee Lee, Chairperson, Human Research Ethics Committee