Beyond the second ventilatory threshold: The prevalence and reliability of a second respiratory compensation point and third ventilatory threshold.

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Abbreviation	Definition
%PPO	Percentage of peak power output
%TTE	Percentage time to exhaustion
$\%_V\dot{V}O_{2Peak}$	Percentage peak velocity
AI	Artificial intelligence
ANOVA	Analysis of variance
AT	Anaerobic threshold
ATP	Adenosine triphosphate
CPET	Cardiopulmonary exercise testing
CP	Critical power
CI	Confidence intervals
CV	Coefficient of variation
EPOC	Excess post oxygen consumption
EQ	Breathing equivalent
$EQCO_2$	Breathing equivalent of carbon dioxide
EQO_2	Breathing equivalent of oxygen
ES	Effect size
$ExCO_2$	Excess CO_2 method
f	Breathing frequency
FITT	Frequency. Intensity. Time. Type.
GET1	First gas exchange threshold

GET2	Second gas exchange threshold
H^+	Hydrogen ions
HCO_3	Bicarbonate
ICC	Interclass correlation coefficient
K^+	Potassium
LBPP	Lower body partial pressure
LT1	First lactate threshold
LT2	Second lactate threshold
MDC	Minimal detectable change
Na	Sodium
NAD^+	Nicotinamide adenine dinucleotide
NOF	Norwegian Olympic federation
P:Wt	Power to weight ratio
PAR-Q	Participant activity readiness questionnaire
$PaCO_2$	Partial pressure of carbon dioxide
$PETCO_2$	End-tidal pressure of carbon dioxide
$PETO_2$	End-tidal pressure of oxygen
PO	Power output
PPO	Peak power output
RCP	Respiratory compensation point
RCP1	First respiratory compensation point
RCP2	Second respiratory compensation Point
RER	Respiratory exchange ratio

rpm	Revolutions per minute
SEM	Standard error or the mean
TTE	Time to exhaustion
$\dot{V}CO_2$	Volume of carbon dioxide
V_E	Minute ventilation
VEQ	Ventilatory equivalent
$\dot{V}O_2$	Volume of oxygen
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2Peak}$	Peak oxygen uptake
VT	Ventilatory Threshold
VT1	First Ventilatory Threshold
VT2	Second Ventilatory Threshold
VT3	Third Ventilatory Threshold

1. Thesis overview

1.1. Abstract

Respiratory data obtained from cardiopulmonary exercise testing is commonly used to study ventilatory thresholds (VT). Traditionally, these thresholds correspond to two physiological transition points where the cardiorespiratory and metabolic systems shift in response to increasing exercise. However, the terminology and methodology used to describe submaximal thresholds is contradictory. The anaerobic threshold, second ventilatory threshold (VT2), or respiratory compensation point (RCP) are different terms that describe the second threshold. Despite many papers using this terminology interchangeably, different methods exist to identify them. Recent literature suggests that RCP overestimates VT2 in elite athletes, suggesting a third ventilatory threshold (VT3).

Study one explored whether RCP overestimates VT2 and if a third threshold, beyond the second transition point, can be identified within cyclists. Overall VT2 and RCP1 reflected the same physiological transition point, both occurring around 70% peak power output (PPO). Furhtermore, VT3/RCP2 was identified at ~90% PPO in 72% of participants. Participants presenting VT3/RCP2 were younger (p=0.01) and demonstrated a longer time to exhaustion, power-to-weight ratio and PPO (p=0.01-0.02), and a moderate effect size (ES) for BMI and $\dot{V}O_{2Peak}$(ES=0.5).

Study two reported the interrater and test re-test reliability of all the thresholds during cycling and running. Interrater reliability reported excellent interclass correlation (ICC) (r=0.96-1.00) across all thresholds and sporting modalities. There was moderate-excellent test, re-test reliability between visits across all thresholds and modalities

(*r*=0.71-0.97), and excellent ICC for VT3/RCP2 (*r*=0.91-0.97). Overall VT3/RCP2 was repeatedly identified within 6 of 7 cylists and 2 of 13 runners.

Overall, this study expands on the traditional understanding of VT, demonstrating that a third threshold can be confidently and repeatedly identified within cycle tests. The prevelance of a third threshold is likely to be associated with athletes of a higher trained status. However the prevelance of VT3/RCP2 during treadmill tests is not as clear and requires further exploration.

Keywords: ventilatory threshold, cycling, running, performance, exercise.

1.2. Thesis justification

Cardiopulmonary exercise testing (CPET) is frequently used to determine athletes' aerobic capacity and cardiorespiratory functionality. CPET has become an essential tool in tailoring and prescribing training for athletes in medical and sports settings. CPET involves breath-by-breath analysis of gas and ventilatory responses to increasing exercise intensities.[1] These responses reflect the cardiorespiratory and metabolic systems' transitions as exercise intensity and demand increase. Traditionally, two physiological transition points have been identified during CPET: the first threshold, occurring at lower exercise intensities,[2] representing the transition from the isotonic buffering phase to the isocapnic buffering phase; and the second threshold, occurring at moderate to high or severe exercise intensities, reflecting the transition from the isocapnic buffering phase to the hypercapnic buffering phase.[3] Understanding these physiological transitions is crucial in prescribing training regimens that optimize athletic performance.[4-7] Therefore, there is a need to explore the methodology and terminology surrounding the identification of these thresholds to understand their precision and accuracy.

The use of breath-by-breath gas analysis during CPET provides valuable information for tailoring training programs for athletes. Specifically, the identification of ventilatory thresholds (VT), including the second ventilatory threshold (VT2) and respiratory compensation point (RCP), has been used to inform exercise prescription. However, recent research has suggested that RCP may overestimate VT2[8, 9] and even represent a third ventilatory threshold (VT3) in well-trained and elite endurance athletes.[10] Despite the potential clinical significance of these findings, there has been limited research

exploring the existence and clinical implications of VT3. Furthermore, despite this lack of supporting research, the concept of VT3 has already been introduced by metabolic cart manufacturers.[2] Thus, further investigation into the existence and clinical relevance of VT3 is warranted to better understand the physiological implications of this threshold and evaluate the potential usefulness of this concept for optimizing training protocols for athletes. This research will contribute to our understanding of VTs during exercise and inform the development of exercise prescription guidelines, particularly for elite athletes.

To further justify the need for research in this area, it is important to consider the reliability of threshold detection, particularly in the context of a newly described third VT3. While the existence of VT3 has been speculated in the literature,[10] little research is available to confirm its presence, let alone its reliability as a measure of physiological response to exercise. As such, it is necessary to investigate the test-retest reliability of threshold detection and the inter-rater reliability of threshold detection, particularly for this newly described threshold.

Reliability is a crucial factor in determining the validity and usefulness of any physiological measurement. Without a high level of reliability, it is difficult to make accurate conclusions about the effectiveness of interventions or the progression of athletes over time.[7, 11-13] Therefore, it is imperative that the reliability of VT3 detection is thoroughly investigated to determine its usefulness as a measure of physiological response to exercise. Additionally, it is important to consider the potential impact that unreliable threshold detection could have on training prescriptions for athletes. If threshold detection is unreliable, training programs based on these measurements may

be ineffective or even detrimental to an athlete's performance.[14, 15] Therefore, it is essential to investigate the reliability of threshold detection, particularly for VT3, to ensure that training prescriptions are accurate and effective.

1.3. Thesis aims

This thesis aims to explore the inflexion points observed in ventilatory and respiratory gas parameters, with the specific objective of examining the comparability of the VT2, RCP, and the recently proposed VT3. This aim will be achieved by conducting two studies.

1.3.1. Study 1: Prevalence of a third threshold within healthy individuals

The specific aims of this study were:

1. To compare methods used to identify thresholds at VT1, GET1, VT2, and the first respiratory compensation point (RCP1) during a maximal incremental cycle test.
2. To assess the presence of VT3 and the second respiratory compensation point (RCP2) beyond VT2 and RCP1
3. To investigate the relationship between performance and the prevalence of a third threshold.

1.3.2. Study 2: Reproducibility and reliability of a third threshold within healthy individuals

The specific aims of this study were:

1. Assess the interrater reliability of VT1, GET1, VT2, RCP1, VT3, and RCP2 between researchers for cycle ergometer and treadmill tests.
2. Explore the test re-test reliability of each threshold, in particular, VT3/RCP2
3. Assess the prevalence of VT3/RCP2 in different endurance modalities
4. Evaluate the difference in physiological and performance parameters between participants that do and do not present VT3/RCP2.

1.4. Thesis outline/structure

The first section (thesis overview/current section) and the second section (literature review) encompass the overarching research, methodologies, and understandings of exercise testing, prescription training, and the physiological underpinnings of threshold training. These sections help to direct the general basis and rational of this thesis.

The third section (the first experimental study) was designed to:

a) Compare multiple methods used to identify the first and second threshold

b) Investigate if alternative methods identify a third threshold and

c) Investigate the relationship between performance and the prevalence of a third threshold.

The fourth section (the second experimental study) was designed to:

a) Assess the interrater and test re-test reliability of each threshold

b) Explore the prevalence of a third threshold within running as well as cycling,

c) Explore the influence of performance level of prevalence of VT3/RCP2.

The fifth and final section (summary chapter) of the thesis encapsulates the findings of the two experimental studies, discusses recommendations for future research, and expands the potential application and wider use of the findings.

2. Literature review

2.1. Abstract

Within endurance training, there is an abundance of literature surrounding the diverse training methodologies, how they are currently used within athlete testing to optimise training, and how threshold training compares to other forms of endurance-based training. This literature review explores the two physiological transition points, how they are analysed and identified, and what physiological mechanisms underpin them. Throughout the literature, the terminology and methodology used to identify these thresholds are vast and inconsistent, complicating the research's clarity and comparability. The most commonly referred to thresholds are first and second ventilatory threshold (VT1 and VT2). However, the respiratory compensation point (RCP), associated with the second transition point, has shown to be inconsistent and unreliable when compared to VT2, with others accusing RCP of overestimating the second transition point. This invites the discussion of the reliability of threshold identification methods, and the potential of a third threshold. For consistency with VT1 and VT2, the novel third threshold will be referred to as the third ventilatory threshold (VT3). Studies were selected if they included terminology surrounding ventilatory thresholds, anaerobic or aerobic thresholds, gas exchange thresholds, sub-maximal thresholds, or respiratory or ventilatory compensation point. There is a comprehensive amount of research surrounding treadmill or cycle tests within a variety of participants, from those with metabolic illnesses to elite-level endurance athletes. Respiratory parameters commonly reported within the literature include minute ventilation, the volume of oxygen and carbon dioxide, breathing equivalents, end-tidal partial

pressures, breathing frequency, and tidal volume. There is a large body of research surrounding the physiological responses to incremental exercise and exercise at differing intensities. Whilst there is a strong understanding of the underlying physiology around VT1, there is less certainty that the physiological responses around VT2, RCP, and heavy-severe exercise are unclear and widely debated. Therefore, the understanding and reasoning for the disparities observed between methods used to identify a comparable threshold are largely hypothesised throughout the literature.

Key words: endurance training, threshold training, ventilatory thresholds, respiratory compensation point.

2.2. Introduction

CPET is the gold standard for evaluating pulmonary, cardiovascular, and cellular responses to exercise testing.[1] CPET is utilised within various clinical and medical settings to help predict health outcomes within vulnerable populations. It is also widely used within sports performance research, enabling researchers to evaluate athletes' performance.[16, 17] Gas analysers are incorporated into CPET to collect breath-by-breath data, allowing the evaluation of ventilatory responses to exercise. CPET protocols that involve progressive exercise testing, often to exhaustion, gain an insight into an athlete's endurance performance and exercise capacity.[13, 18] Analysis of breath-by-breath data can be applied to estimate two physiological change points that occur in response to the increased exercise intensity of an incremental exercise test.[2] Throughout the literature, ongoing debates exist about what terms best identify these respiratory break points.[2, 19-21] Wasserman and McIlroy initially conceptualised the anaerobic threshold in 1964, aiming to identify an exercise intensity that provided substantial, safe levels of exercise stress within patients diagnosed with cardiovascular disease.[22] Confusion surrounding terminology began as research built upon the original findings.[21] Further thresholds were identified[23] with the modernisation of laboratory equipment and suggestions of new methods of threshold identification using various parameters, e.g. lactate, gas exchange, ventilatory, heart rate, and glucose.[21] This resulted in the overlapping terminology in the literature today. However, once identified, these thresholds enable training prescriptions bespoke to the individual athlete. Optimising training for athletes and improving aerobic capacity, efficiency and exercise tolerance is essential to augment athletic progression and performance.[4-7]

Determining training zones based on the respiratory phases is a widely used and accepted method to prescribe individualised training for athletes.[24]

The first threshold has been referred to as VT1,[25] GET1,[10] VT,[7] aerobic threshold, or first lactate threshold (LT1). For the second threshold, common terminology within research includes VT2,[25] anaerobic threshold,[18] RCP,[26] second lactate threshold (LT2) and the second gas exchange threshold (GET2).[10] Terms like anaerobic and VT[5] have been used in literature to describe both individual thresholds. The variety and overlapping terms used for these individual parameters make it difficult to distinguish and identify what thresholds are being referred to within literature without detailed analysis of the methods implemented (*Figure 1*).[18, 19, 27]

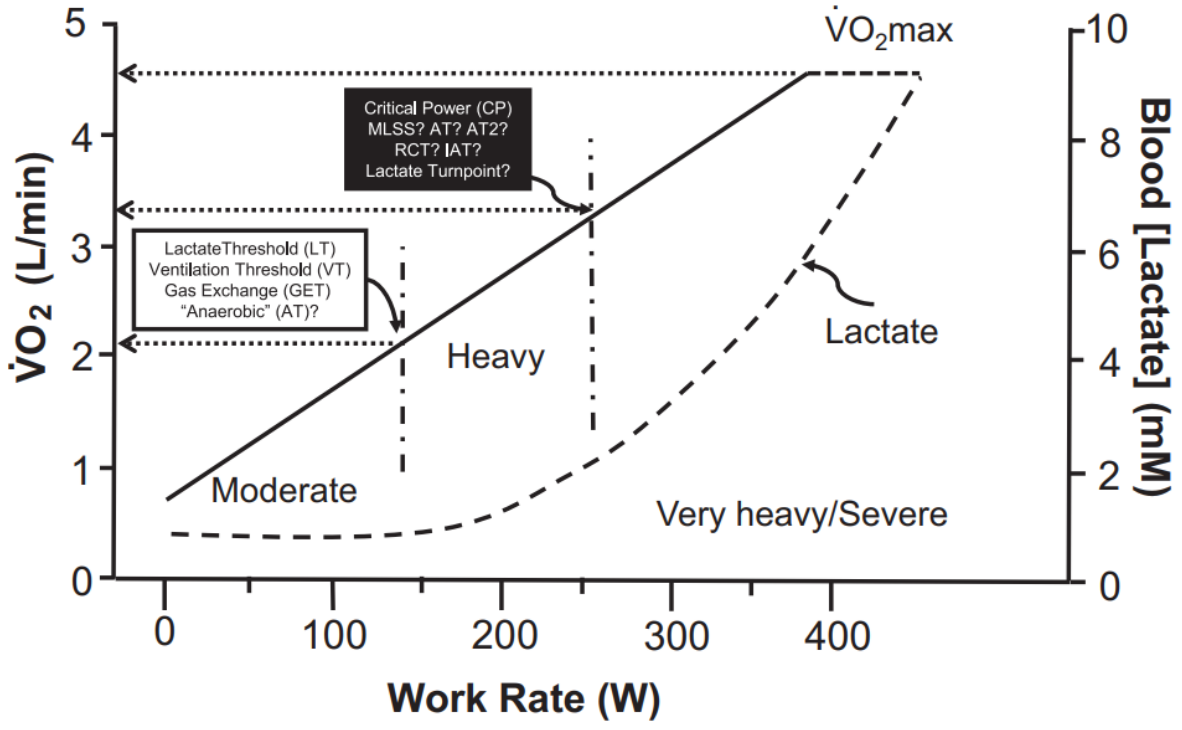

Figure 1. Thresholds used within the literature to define submaximal thresholds and moderate to severe intensity domains. Figure extracted from Poole *et al*, 2021.[19]

However, some clarity can be achieved by specifying the parameters and methods used to identify each threshold. For example, GET1 and GET2 reflect the interactions and utilisation of oxygen and carbon dioxide (O_2 and CO_2, respectively).[19] Whereas ventilatory thresholds (VT) are typically determined by changes in ventilatory rate or V_E. Consistent application of methodology and terminology within the literature would help clarify how thresholds are being interpreted; however, this is not currently the case.[19] The term RCP has been used interchangeably with VT2 [28] but is often defined as the onset of exercise-induced hyperventilation, demonstrated via the $V_E/\dot{V}CO_2$ slope.[8, 28]

The understanding of ventilation beyond VT2 is under much speculation.[19] Exercise-induced hyperventilation was initially associated with the transition from the isocapnic buffering phase into hypercapnia. However, a delay in the onset of hyperventilation (identified via the $V_E/\dot{V}CO_2$ slope) has been noted in some studies.[8] This suggests that the identification of exercise-induced hyperventilation overestimates VT2 and creates an additional exercise intensity domain between heavy and severe exercise intensities. This theory was reinforced by Ozkaya *et al*, demonstrating a later threshold within respiratory parameters, beyond VT2 and before maximal exertion, and can be seen marketed within some CPET software.[9, 10]

Throughout this thesis, the referral of the two thresholds will be the VT1 and VT2, following the three-phase model of incremental exercise presented by Binder *et al*.[18] This terminology avoids misnomers, as seen with the use of anaerobic or aerobic threshold, especially when at no point is energy supply during exercise ever exclusively either aerobic or anaerobic. Moreover, further application of this terminology within

exercise prescription is easily accessible.[18] VT3, as referenced by Ozkaya *et al*,[10] will define the potential third threshold and allow for continuity.

2.3. Threshold testing and training

Individualised training prescription is helpful to a wide range of individuals, from cardiovascular patients to elite-level athletes. The main parameters involved with prescribing exercise follow the FITT concept: frequency, intensity, time and type. The application of VT to training helps to specify exercise intensity domains. Exercise intensity refers to the 'rate of metabolic energy demand during exercise'.[29, 30] The respiratory breakpoints observed during CPET testing reflect the point at which exercise inflicts metabolic disturbance, resulting in physiological responses aiming to regain homeostasis. Training at these intensities is where beneficial adaptations can be optimised.[29, 31] Thresholds can be estimated using heart rate, peak power output (PPO) or the peak uptake of oxygen ($\dot{V}O_{2Peak}$); however, implications of age, fitness and comorbidities influence the exact point a threshold is likely to be determined. For example, using heart rate training, or training at a percentage of your maximal heart rate, has been a traditional approach to training.[32] Using heart rate to estimate thresholds is unreliable compared to lactate and ventilatory thresholds due to the physiological variability between athletes' responses to exercise stress.[15] Therefore, training should be individualised and prescribed according to the point at which exercise intensity elicits metabolic disturbance.[29]

A randomised control trial involving healthy, sedentary males and females compared the impact of threshold-based exercise prescription and heart rate training (using the relative percentage concept) across 12 weeks. Participant resting heart rate reserve was

calculated by the difference between maximal and resting heart rates. The percentage of the participants' resting heart rate reserve was then calculated to prescribe training for one group, whereas a second group was prescribed training according to VT1 and VT2. A 12-week programme was then followed, prescribing training around VT1 (or an average target heart rate of 126 bpm) or 40-45% heart rate reserve (or an average target heart rate of 109 bpm) for the first four weeks. This increased to between VT1 and VT2 (or an average target heart rate of 137-140 bpm) or 50-55% heart rate reserve (or an average target heart rate of 116-117 bpm) for four weeks, and then to VT2 (or an average target heart rate of 153 bpm) or 60-65% heart rate reserve (or an average target heart rate of 127 bpm) for the final four weeks. Following heart rate training, 41.7% of the participants presented significant improvements in $\dot{V}O_{2Peak}$ values, whereas 100% of the group following threshold training demonstrated improvements in $\dot{V}O_{2Peak}$.[33] Overall, this study demonstrated that threshold training was a more consistent and reliable method for sedentary individuals to elicit improvements in $\dot{V}O_{2Peak}$ and endurance performance. However, it is important to note, that the target heart rate and recorded heart rate throughout the 12-week training period were consistently 10-20 bpm higher in the threshold training group. Therefore, the subsequent results could ultimately be a result of the threshold training group working harder.

The metabolic response to exercise differs between athletes; therefore, relying on methods not prescriptive to an individual, such as estimated maximum heart rate values, fails to account for metabolic variability.[34] This applies when training a group of individuals with the same training goals and sport. Research demonstrated that athletes

with similar aerobic capacities had differences in blood lactate concentrations when exercising at comparable percentages of their $\dot{V}O_{2Peak}$. It was hypothesised that the wide variability across participants resulted from the multiple factors contributing to performance and sustainability of constant work rates, including blood lactate, exercise economy, fat oxidation and anaerobic capacity. Given percentages of $\dot{V}O_{2Peak}$ elicited different levels of metabolic strain, labelling the variability in lactate responses at a percentage of $\dot{V}O_{2Peak,}$ as "inhomogeneous".[35] Thus, prescribing training using generalised methods is unlikely to pin point the intensity necessary to optimise the development of beneficial adaptations. Moreover, personalised training at thresholds bespoke to that individual maximises physiological adaptations[25, 36], including aerobic capacity, muscular endurance[14] and cardiovascular efficiency.[25] This consequently improves the athlete's physiological capacity to deal with exercise-induced stress and improves the athlete's performance.[4-7] Using ventilatory thresholds can also aid in preventing overtraining, which can result in injuries and burn out.[14, 15]

Zone training focuses on an exercise intensity continuum. There are different ideologies, with some including up to five training zones, which can be prescribed relative to $\dot{V}O_{2Peak}$, heart rate[24, 37] or lactate.[38, 39] The five-zone intensity scale, published and used by the Norwegian Olympic Federation (NOF) to prescribe and monitor training[37], reflects the relevant intensities and duration associated with each zone (*Table 1)*. However, this method does not account for the relationship between heart rate, blood lactate and individual variation. [37, 40] Further anaerobic zones (zone 6, 7 and 8) are sometimes used to accommodate sprint and strength training and anaerobic capacity.[37]

Table 1. Scale implemented by the Norwegian Olympic federation (NOF) implementing a 5-zone scale to prescribe training for endurance athletes based on cross-country skiers, biathletes and rowers.

Zone (NOF Model)	Heart Rate (% of max)	$\dot{V}O_{2Peak}$ (% of peak)	Lactate ($mmol.L^{-1}$)	Duration spent in zone
1	55-75	45-65	0.8-1.5	1-6 h
2	75-85	66-80	1.5-2.5	1-3 h
3	85-90	81-87	2.5-4	50-90 min
4	90-95	88-93	4-6	30-60 min
5	95-100	94-100	6-10	15-30 min

A three-zone model has also been greatly effective at improving endurance performance. Expanding on the zone training method,[24] the three-zone model overlays the five-zone intensity scale by defining thresholds based on markers anchored to physiological events occurring in response to exercise intensity. The physiological events are identifiable via respiratory data and blood lactate levels specific to an athlete's training.[40, 41] Each zone reflects a different physiological response to the respective intensity, with the ventilatory thresholds highlighting the transition from one phase into another (*figure 2*).

The first zone (zone 1), also referred to as the isotonic buffering phase, reflects a light exercise intensity, where there is an increase in oxidative carbon dioxide production

($\dot{V}CO_2$). VT1 then demarcates the shift from zone 1 into the second zone (zone 2). Zone 2 is also referred to as the isocapnic buffering phase and reflects a moderate to high exercise intensity. During the isocapnic buffering phase, $PETCO_2$ remains stable and exercise-induced lactate accumulation is fully buffered; however, a nonlinear rise in V_E against oxygen production ($\dot{V}O_2$) results in increases in the $PETO_2$.[3] VT2 then demarcates the transition from the isocapnic buffering phase and zone 2 into the third zone (Zone 3). Zone 3, also called the hypercapnic response phase, can be characterised by a rise in $\dot{V}CO_2$ and VE, combined with a fall in end tidal partial pressure of CO_2 potentially occurring because of metabolic acidosis due to buffering capabilities being exceeded. This zone reflects high to severe exercise intensities that cannot be sustained.

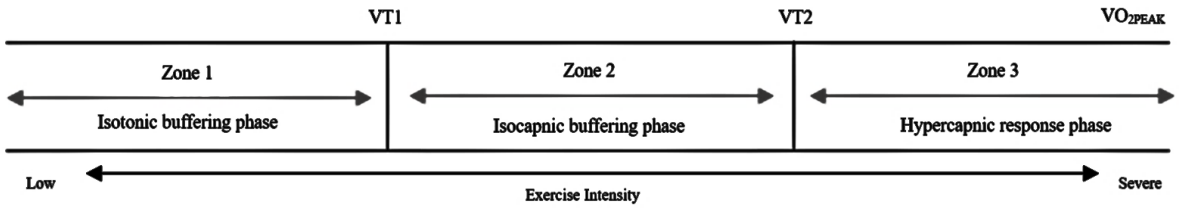

Figure 2. Scale demonstrating where the first and second ventilatory thresholds demarcate the metabolic transitions through the isotonic, isocapnic and hypercapnic buffering phases and the relative to training zones.

Polarised and pyramidal training adopted the three-zone model and proved more effective at promoting performance improvements over the threshold model. Polarised training, introduced by Stephen Seiler, focuses on spending 75-80% of training in zone 1, and 15-20% in zone 3, with only 5-10% within Zone 2.[42] Similarly, pyramidal

training spends 80% in zone 1, spreading the final 20% of training between zone 2 and zone 3. Both techniques focus on majority zone 1 training.[43] On the other hand, the more traditional threshold training intensity distribution model encourages a greater volume of training (35-55%) within Zone 2 and only 45-55% in zone 1.[42, 44] This style of training is far more intense and can be compared to high volumes of training at 'race pace'.[43] A systematic review and meta-analysis compared the effect of polarised training with threshold training within endurance sports and found when comparing time trial performance, polarised training invoked more significant improvements in endurance performance with respect to $\dot{V}O_{2Peak,}$ time to exhaustion (TTE) and exercise economy, reporting a moderate effect size (ES = -0.66; 95% CI:-1.17 to -0.15) in favour of polarised training.[42] A couple of other systematic reviews found similar findings, with one comparing polarised, pyramidal and threshold training[43] and the other focusing on time intensity and distribution of training within endurance sports.[44] Both found that polarised and pyramidal training and training incorporating high volumes of low-intensity and low volumes of high-intensity training (comparable to polarised/pyramidal training) were more effective at improving endurance performance over threshold-based training. Both reviews concluded that training adaptations and overall endurance performance could be optimised when training predominantly involved high volumes of zone 1 training. [43, 44] Despite this, progress and improvement can be seen following training threshold training VT2 or "race pace". It has been highlighted that while some of the best marathon runners in the world follow a threshold training model (favouring more intense zone 2 training),[43] this training style is often reduced closer to the competitive season.[45] Also, aspects of polarised training are used

prior to and in preparation for threshold-based training.[44] Therefore, the sporting season dictated training time, intensity and distribution.

2.4. Physiological adaptations in response to exercise stress

Training-related adaptations occur in response to exercise-induced stress on physiological structures, triggering adaptive responses that enhance an athlete's physiological capacity. The athlete's goals will influence the intesnity and distribution of training, as this determines what physiological adaptations are required. Following training, structural changes within mitochondria, especially within type 1 muscle fibres, can be evident following a single bout of exercise.[46] Such changes are a result of increases of blood flow to the muscle instigating an influx of hormones that promote receptor-mediated responses. This increases metabolic demand driving increases in oxygen consumption, depletion in ATP-PC stores, glycogen stores and lactate accumulation. These responses disrupt the homeostatic balance. The intensity and distribution of the training determine the level of disturbance, which instigates the activation of stress-activated proteins.[47]

The internal environment created as a result of exercise promotes a stress response.[48] During exercise, particularly at higher intensities, exercise stress triggers temperature increases, pH decreases, ischemia, and glucose deprivation.[49] When an athlete is exposed to exercise stress, there is an increased yield of antioxidants and proteins within the skeletal muscle, known as cytoprotective proteins or heat response proteins.[49, 50] The influx of cytoprotective proteins increases tolerance to exercise stressors to aide in maintaining homeostasis. They also facilitate repair and increase 'protection' against related stressors.[48-51] Cytoprotective proteins are also integral to facilitating the cellular

remodelling process, which is fundamental to the physiological adaptations achieved in response to training.[50] Both endurance and resistance training invokes physiological responses, however, the adaptations achieved differ depending on the exercise modality and exercise intensity.[50]Among endurance athletes, type 1 muscle fibres are favourable over type IIa and type IIb, due to their greater capillary density and oxidative capacity, resulting in greater metabolic efficiency. Endurance-based training prescribed at high volumes around zone 1 facilitates the formation of type I muscle fibres over type IIa and IIb, which fatigue faster.[47] Moreover, high-volume, low-intensity training, as seen within pyramidal and polarised training, incurr lower levels of exercise stress, and drive increases in oxidative capacity, and other endurance-related adaptations.[25, 36] Metabolic and morphological adaptations can be observed within the cardiovascular, musculoskeletal, and hematopoietic systems following; tailored, long-term, low-intensity, high-duration training. Structural changes within the cardiovascular system that optimise endurance performance include enlarged left ventricular cavity and wall thickness and increased heart mass. These structural changes increase stroke volume, cardiac output, venous return and reduce overall peripheral resistance, enabling more effective transportation of blood, $\dot{V}O_2$ and VCO_2, to and from respiring muscles. Haematological changes involve increases in red cell mass and plasma volume. The increase in total blood volume and red blood cells increases the oxygen carrying capacity and overall perfusion. Capillary supply to skeletal muscle also improves, reducing the distances for diffusion of substrates and gases, improving the effectiveness and efficiency of oxygen delivery and the removal of carbon dioxide and other metabolic by products.

Zone 1 training invokes structural and functional change within the skeletal muscle. Particular adaptations incurred during exercise stress involve increased mitochondrial density, greater capillary density, specification of muscle fibre types (i.e. increase in type 1 muscle fibres for endurance athletes), greater mitochondrial oxidative enzymes, and greater neural recruitment. These adaptations increase energy and force production alongside greater oxidative capacity, enabling athletes to work at greater exercise intensities and incur less fatigue. Improvements in mitochondrial density and oxidative enzyme activity are highest within the muscles engaged in training, which implies mitochondrial adaptations are local rather than systemic.[47]

Further adaptations seen within endurance athletes involve the reduced rate of muscle glycogen store depletion compared to untrained athletes. Often endurance athletes have decreased carbohydrate use, which is compensated for via increased fat oxidation. This is reflected through lower respiratory exchange ratios (RER) at relative exercise intensities. This improved oxidative substrate utilisation has also been attributed to increased mitochondrial density improving respiratory control and sensitivity.[47] The respiratory system, on the other hand, has little training responses to exercise other than improved endurance and strength of respiratory muscles, much like what can be observed within skeletal muscle. Among recreational and untrained individuals, the capacity of the respiratory system is typically greater than the cardiovascular or muscular system. However, following the optimisation of other systems, the lack of adaptability of the respiratory system means it can be the limiting factor within high-level endurance performance.

The addition of interval training around zone 3 is beneficial to increase the aerobic potential of type IIa muscle fibres and improve fatigue resistance, improving athletes' ability to sustain fast running speeds. However, only small durations of high-intensity bouts are recommended, as it increases the risk of overtraining and injury.[44] Moreover, one study comparing rats undertaking more intense but shorter exercise bouts showed similar mitochondrial improvements per gram of muscle compared to those undertaking more prolonged bouts of submaximal activity. However, increasing exercise intensity increases the recruitment of fast twitch glycolytic fibres. Reducing type 1 muscle fibre recruitment reduces beneficial type 1 fibre adaptations, hindering endurance performance capacity.[47] As such, researchers initially suggested that a combination of high-intensity, resistance-based, and endurance training was not optimal for endurance performance.[52-54] Although, recent literature does not agree with this stance, suggesting high-intensity training has a place within endurance sports (*Section 5.3 [paragraph 2]*).

2.5. Respiratory parameters

Within exercise physiology, $\dot{V}O_2$ and carbon dioxide production $\dot{V}CO_2$ are standard measures used to assess physiologic response to exercise intensity, aerobic capacity and energy expenditure.[12] At rest, an average young male's $\dot{V}O_2$ is around 250ml·min^{-1}. At submaximal intensities, $\dot{V}O_2$ increases linearly and can reach up to 5000 ml·min^{-1} within endurance athletes at high exercise intensities.[55] During progressive exercise tests, $\dot{V}O_2$ and $\dot{V}CO_2$ can be combined or used with other ventilatory parameters to identify ventilatory thresholds and assess substrate utilisation.[12, 56]

Changes and increases in pulmonary ventilation can be attributed to a combination of increased respiratory frequency and tidal volume, which closely matches $\dot{V}O_2$ and $\dot{V}CO_2$.[55] Respiratory frequency (the number of breaths made per minute) and tidal volume (the volume of air passing through the lungs per respiratory cycle) can be multiplied to calculate V_E. V_E demonstrates the volume of air respired per minute. At rest, tidal volume sits at around 400mL per breath for females and 500mL per breath for males.[57] During incremental exercise testing, initially at low intensities, tidal volume increases to meet the increasing oxygen demands whilst respiratory frequency remains stable.[58] This continues until around 50% to 60% of the athlete's maximal capacity, where tidal volume begins to plateau. Respiratory frequency accelerates as the work rate increases to compensate for the growing exercise intensity and oxygen demand.[51, 58] The spike in respiratory frequency results in a visible and disproportionate increase in V_E, especially when compared with $\dot{V}CO_2$.[59, 60]

2.5.1. Ventilatory equivalents of oxygen and carbon dioxide

Ventilatory equivalents (VEQ) provide a further measure of ventilatory performance using the ventilatory equivalents of oxygen (EQO_2) and the ventilatory equivalents of carbon dioxide ($EQCO_2$ or $V_E/\dot{V}CO_2$). EQO_2 and $EQCO_2$ are calculated using a ratio between dead space (the residual volume of air that is not involved within gas exchange) and tidal volume (the volume of air inspired per breath),[2, 58] demonstrating how many litres (L) of breath is needed to produce 1L of $\dot{V}CO_2$ and consume 1L of $\dot{V}O_2$.[58] The EQO_2 and $EQCO_2$ for the average male at rest is around 20-25L and 25-30L respectively. At the beginning of an incremental exercise test, there is an initial rise in EQO_2 and $EQCO_2$ due to a high volume of dead space relative to a low tidal volume

ratio. As the intensity progresses, tidal volume increases, resulting in a decrease in the EQ. Increases in EQO_2 and $EQCO_2$ suggest that gas exchange and ventilatory performance efficiency are impaired (*Figure 3)*.[61] When referring to EQO_2 and $EQCO_2$ as a method to identify VT1 or VT2, the abreviation more commonly used is $V_E/ \dot{V}O_2$ for EQO_2 and $V_E/ \dot{V}CO_2$ for $EQCO_2$. Therefore $V_E/ \dot{V}O_2$ and $V_E/ \dot{V}CO_2$ will be used to describe the respiratory paramaters used to identify VT1 and VT2 (*2.7.1. Ventilatory equivalents method).*

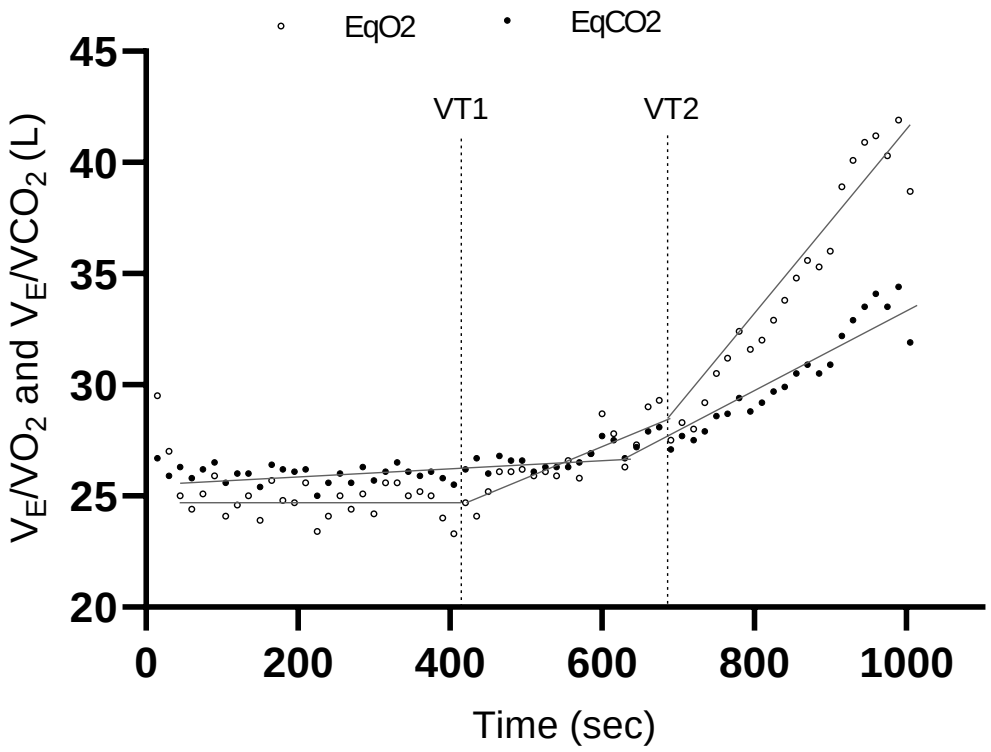

Figure 3. A Theoretical graph demonstrating the identification of the first and second ventilatory threshold (VT1 and VT2, respectively) via the ventilatory equivalents (VEQ) method, tracing the ventilatory equivalent of oxygen ($V_E/\dot{V}O_2$) and the ventilatory equivalent of carbon dioxide ($V_E/\dot{V}CO_2$) against time.

2.5.2. End-tidal partial pressure

$PETO_2$ and $PETCO_2$ can also be used to calculate the VTs independently or in conjunction with the VEQ method.[2] $PETO_2$ and $PETCO_2$ quantify the partial pressure of O_2 and CO_2 at the end of expiration, often mirroring the partial pressure of arterial and alveolar O_2 and CO_2.[61-63] Therefore, $PETO_2$ and $PETCO_2$ are a good reflection of ventilatory and perfusion efficiency. At rest, the average value of $PETO_2$ and $PETCO_2$ is 100-110 mmHg and 36-42mmHg, respectively.[61, 62] The curves of $PETO_2$ and $PETCO_2$ progress in a reversed manner to each other; during the initial stages of an incremental test $PETO_2$ takes a downward slope, whilst $PETCO_2$ rises until VT1.[2, 61] At and beyond VT1, $PETCO_2$ plateaus or begins a downward slope until volitional exhaustion, whereas $PETO_2$ rises. The interaction between $PETCO_2$, $PETO_2$ and time can depict the time points at which VT1 and VT2 occur (*Figure 4)*.[2]

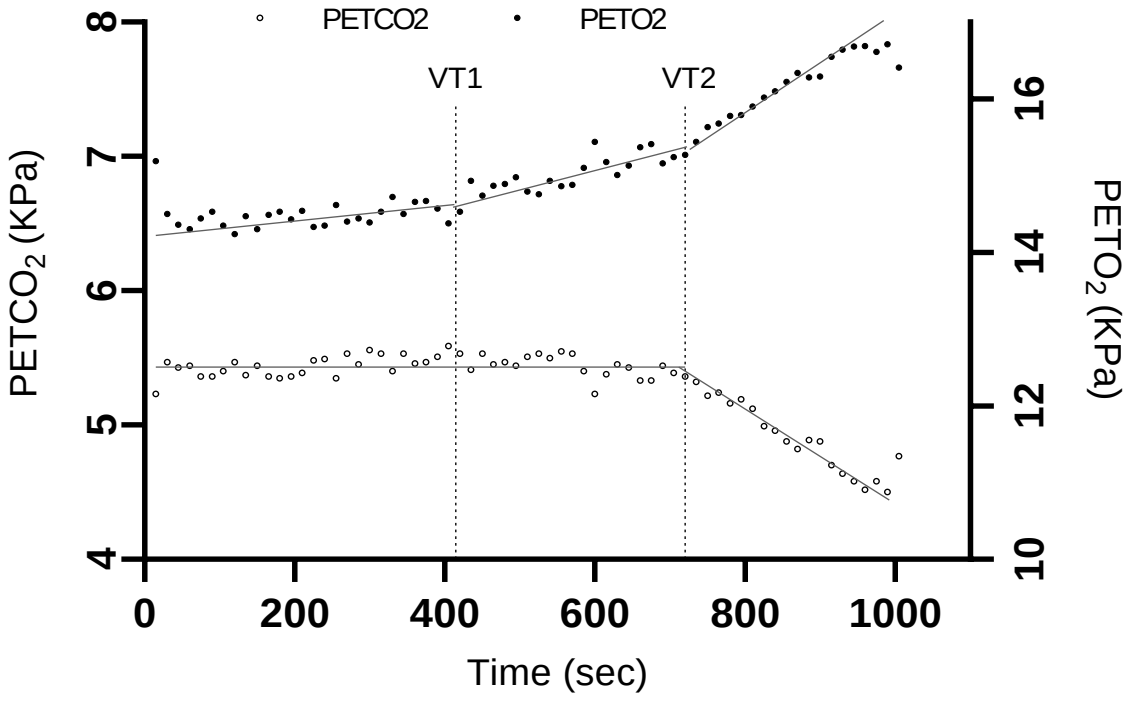

Figure 4. Theoretical graph demonstrating the interaction between the end-tidal partial pressure of oxygen ($PETO_2$) and the end-tidal partial pressure of carbon dioxide ($PETCO_2$), and how it can be used to identify the first ventilatory threshold (VT1) and the second ventilatory threshold (VT2).

2.6. First ventilatory threshold (VT1)

VT1 occurs during low to moderate exercise intensities. [2, 64] Exercise completed around VT1 is fuelled predominantly through aerobic mechanisms, such as lipid oxidation, enabling prolonged time to exercise at such intensities.[64] $\dot{V}CO_2$ and $\dot{V}O_2$ increase linearly to VT1, where the threshold can be identified following an increase in the $\dot{V}O_2$ uptake in response to exercise (the isotonic buffering period).[64, 65] This parameter can be used as a measure of an individual's aerobic capacity. For endurance

athletes, training prescribed at VT1 is beneficial for developing an athlete's oxidative capacity enabling prolonged performance at higher intensities. Further applications of VT1 identification include measuring the effects of endurance training, predicting endurance performance and characterising endurance athletes.[4]

2.7. Methods to identify the first ventilatory threshold

2.7.1. Ventilatory equivalents method (VEQ method)

VEQ encompasses V_E, $\dot{V}O_2$ and $\dot{V}CO_2$, drawing a comparison between $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$. However, it does not reflect the responses of V_E to increasing $\dot{V}O_2$ and $\dot{V}CO_2$ levels but represents the characteristics of ventilatory control during exercise,[66] reflective of EQO_2 and $EQCO_2$ (*2.5.1. Ventilatory equivalents of oxygen and carbon dioxide*)Initially, the ventilatory equivalents method was viewed as one of the most reputable ways of identifying VT1 [4] as it demonstrates the ventilatory drive for a given level of oxygen consumption.[67] VE/ṾCO2 follows a mostly linear pattern between rest and low-moderate exercise intensities, whereas $V_E/\dot{V}O_2$ presents a more positive linear curve. A rise in $V_E/\dot{V}O_2$ without any rise in $V_E/\dot{V}CO_2$ is visible as a result of the metabolic buffering of bicarbonate (HCO_3).[68] However, more recent research suggests that irregular breathing, inappropriate protocols (see *chapter 1.13* Stepwise vs Ramp protocol) and poor ventilatory responses from the participant can impact the reliability of threshold identification via this method.[68] The VEQ method demonstrated in *Figure 3* plots $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ on the y-axis against time or work rate on the x-axis. At the beginning of exercise, $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ will have a flat or declining curve.[7] VT1 can then be identified via the first non-linear rise in $V_E/\dot{V}O_2$ without a concurrent rise in $V_E/\dot{V}CO_2$, where $V_E/\dot{V}CO_2$ remains linear or at a negative slope.[7, 66, 69]

2.7.2. End-tidal partial pressure of oxygen and carbon dioxide

At the start of exercise $PETO_2$ is high, and $PETCO_2$ is low.[68] As exercise progresses during the early stages, $PETO_2$ decreases due to changes in the physiological dead space to tidal volume ratio. However, $PETO_2$ reaches a minimum point, after which it increases. Alongside this, V_E rises out of proportion with $\dot{V}O_2$. $PETCO_2$, on the other hand, initially increases, then as VT1 is achieved, it plateaus and remains constant.[18] This method, demonstrated in *Figure 4*, plots $PETO_2$ and $PETCO_2$ against time, where an inflexion point in $PETO_2$ and a concomitant deflection or plateau in $PETCO_2$ can be visually identified.

2.7.3. Ventilatory equivalent method with end-tidal partial pressure of oxygen

The combination of the VEQ method with $PETO_2$ was first determined by Wasserman *et al*[68] to promote a more accurate estimation of VT1 and is widely adopted throughout the literature.[2, 11, 24, 25, 70-72] Whilst the VEQ method is widely used, noisy data can make it difficult to interpret. Combining $PETO_2$ with the VEQ method helps prevent pseudo VT1 identification.[71, 73]At the beginning of an incremental test, the physiological dead space to tidal volume ratio decreases, which then plateaus as exercise increases. At VT1, the $V_E/\dot{V}O_2$ and $PETO_2$ rise as a result of isocapnic buffering, whereas a rise in $V_E/\dot{V}CO_2$ occurs beyond VT1 (*Figure 5*).[70] Across the two methods, the time point identified might not be identical as each method reflects differing mechanisms with different response times.[68] There are fast, neurogenic factors from the peripheral and central nervous system and slow humoral stimuli such as chemical and physical stimuli from motor receptors.[74, 75] Therefore, ventilatory thresholds do not occur at one specific time

point but instead represent a point of physiological transition across differing exercise intensities.[2]

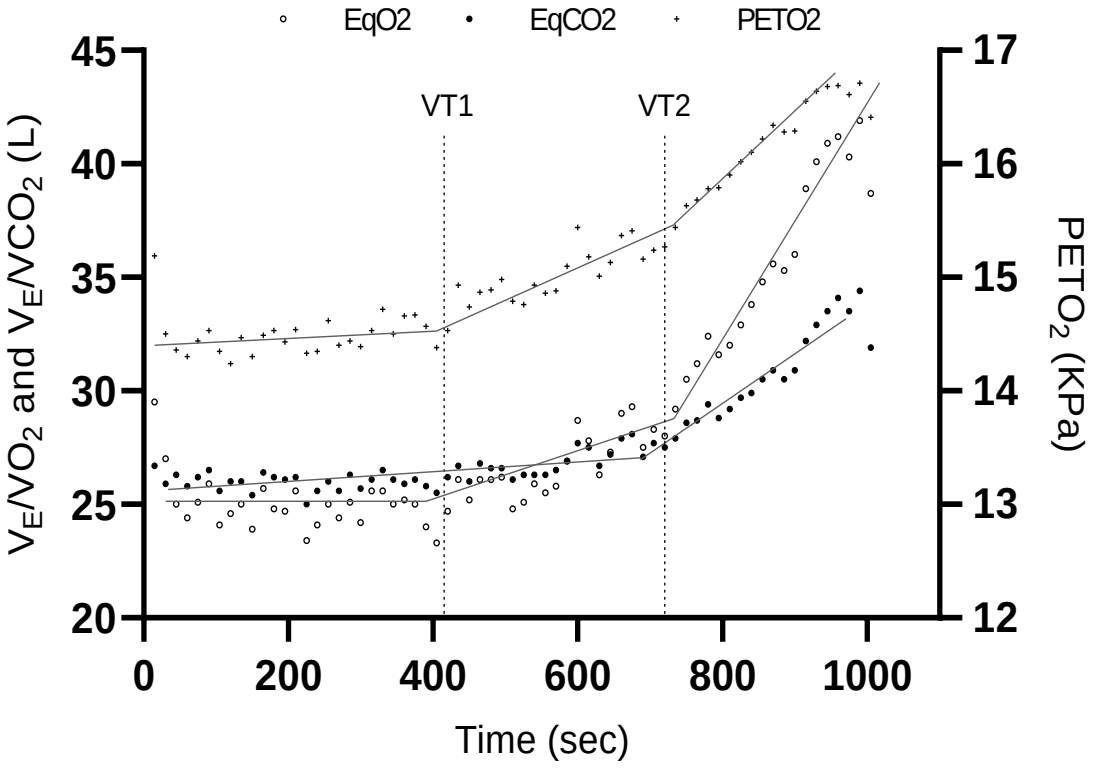

Figure 5. Theoretical graph demonstrating where the identification of the first and second ventilatory threshold (VT1 and VT2, respectively) might be identified via the interaction between the ventilatory equivalents method (VEQ) using the breathing equivalents of oxygen ($V_E/\dot{V}O_2$) and the ventilatory equivalent of carbon dioxide ($V_E/\dot{V}CO_2$) alongside the end-tidal partial pressure of carbon dioxide ($PETO_2$), against time.

2.7.4. V-Slope method

The theory surrounding the interaction between $\dot{V}CO_2$ and $\dot{V}O_2$ was initially identified by William Beaver and Karlman Wasserman, suggesting that VT1, then termed the

'anaerobic threshold', could be identified non-invasively through the analysis of ventilatory parameters and their responses to progressive exercise testing.[76, 77] As exercise intensifies, there is an increase in lactic acid production. Hydrogen ions (H^+) produced by respiring cells are often a result of anaerobic respiration lactic acid dissociation. H^+ is then buffered by HCO_3, which generates an increase in cell CO_2 production. This excess yield of CO_2 accelerates $\dot{V}CO_2$ and V_E whilst $\dot{V}O_2$ remains linear, changing the $\dot{V}CO_2/\dot{V}O_2$ relationship and steepening the plot. [61, 76] Therefore, visual inspection of this graph can identify a point of inflexion where there is a non-linear increase in $\dot{V}CO_2$ against $\dot{V}O_2$.[77] However, visual inspection can be time-consuming and subjective, impacting the reproducibility and accuracy of threshold identification.[78]

V-Slope method, demonstrated in *Figure 6*, was constructed by Beaver *et al* to track the behaviour between $\dot{V}CO_2$ and $\dot{V}O_2$ during progressive exercise testing via computerised regression analysis. Breath-by-breath data was smoothed by a moving average filter, sometimes evading the first few minutes following the start of exercise. The remaining data points were then partitioned into two linear segments, where the point of intersection was taken as the threshold.[77] Typically, VT1 is identified between 40%-60% $\dot{V}O_{2Peak.}$[61] However, this method is often more comparable to blood lactate threshold, however, is agreeable with VT1.[79]

More simplified versions of the V-Slope method have been developed to increase usability and reduce exclusivity regarding equipment.[79] One example includes the use of 30-second averages instead of breath-by-breath analysis developed by Walsh and Davis *et al*.[79, 80] A further adaptation includes the modified V-Slope method, which

identifies VT1 when the rate of $\dot{V}CO_2$ and $\dot{V}O_2$ rises above 1.00, depicted by a 45° triangle/line, drawn parallel to the $\dot{V}CO_2/\dot{V}O_2$ data set.[61, 81] However, whilst the modified method is simple to execute, it can overestimate VT1 compared to the computerised method, as it does not account for the data points above the gas exchange threshold.[79] This is potentially due to the computerised method integrating all the data before and after the threshold, whereas the visual methods disregard data exceeding

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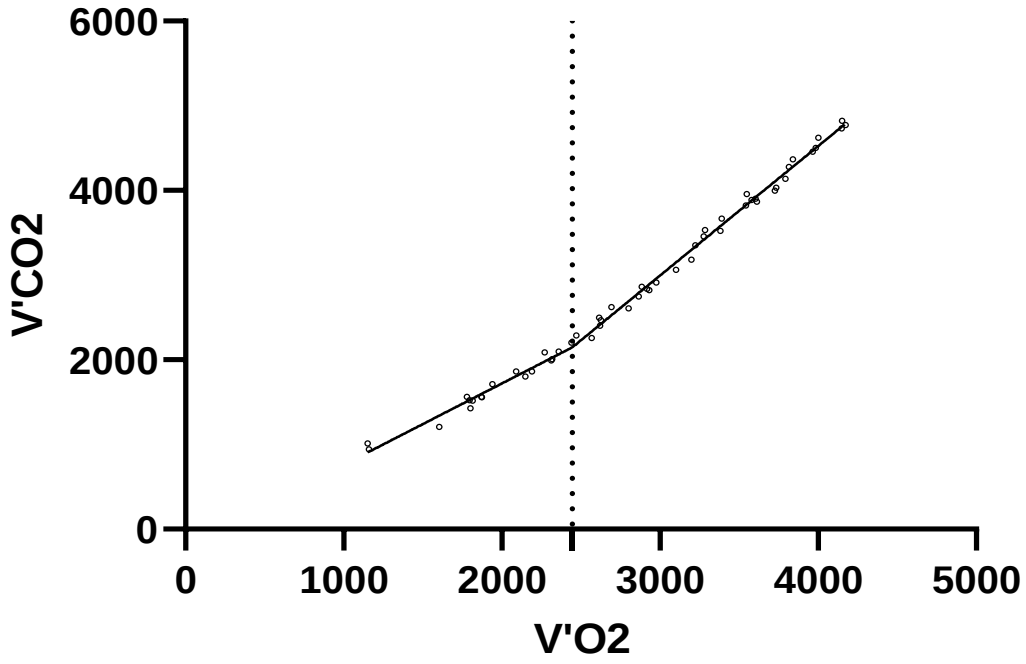

Figure 6. Theoretical graph demonstrating the V-Slope method used to identify the first ventilatory threshold (VT1) via the interaction between $\dot{V}CO_2$ and $\dot{V}O_2$.

2.7.5. Excess CO_2 Method

This method focuses on determining excess CO_2 ($exCO_2$) production resulting from the buffering of lactate, marking VT1 where the first sustained rise in $exCO_2$ against work done can be seen.[82] Firstly, to calculate $exCO_2$, the increment of the respiratory exchange ratio is calculated by the difference between the actual R-value and an estimated R-value reflecting a rested state ($\Delta R = R_{work} - 0.75$). $ExCO_2$ is then determined by the following calculation:

$$ExCO_2 = \Delta R \cdot \dot{V}O_2 = \dot{V}CO_2 - R_{rest}\dot{V}O_2.$$

(Equation 1).

Little application of this method can be seen throughout the literature. However, Gaskill's paper comparing the modified V-Slope method, VEQ method and $ExCO_2$ demonstrated that this method could effectively increase the accuracy of VT1 identification relative to LT when combined with the modified V-Slope method.[7, 82]

Table 2. Summary of 5 methods used to identify the first threshold; breathing equivalent (VEQ) method, end-tidal partial pressure of oxygen and carbon dioxide ($PETO_2$ and $PETCO_2$) method, combined VEQ and $PETO_2$ method, V-slope method, and the excess carbon dioxide ($ExCO_2$) method with founding author.

Name	Method	Author
VEQ method	$V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ are plotted against time/load on the x-axis. $V_E/\dot{V}O_2$ curve rises, resulting in a non-linear inflexion, whilst $V_E/\dot{V}CO_2$ curve remains constant.[28, 83]	Wasserman *et al* 1973
$PETO_2$ and $PETCO_2$ method	$PETO_2$ and $PETCO_2$ plotted against time/load. A non-linear rise in $PETO_2$ increases alongside a plateau or slight decrease in $PETCO_2$.[22]	Wasserman *et al* 1973
Combined VEQ and $PETO_2$ method	$V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ are plotted on the y-axis, with $PETO_2$ plotted on the z-axis against time/load on the x-axis. An increase in $V_E/\dot{V}O_2$ and $PETO_2$ demarcates an inflexion point without a concurrent rise in $V_E/\dot{V}CO_2$.[76]	Wasserman *et al 1984*
V-Slope method	$\dot{V}CO_2$ plotted against $\dot{V}O_2$, with a non-linear increase in $\dot{V}CO_2$ demarcating a point of inflection.[77]	Beaver and Wasserman *et al* 1986
$ExCO_2$ method	The $ExCO_2$ method states the exercise intensity resulting in an increase from steady state to excess production of CO_2 marks a rise, calculated by $((\dot{V}CO_2^2/\dot{V}O_2)-\dot{V}CO_2)$[7]	Nikolai *et al 2003*

Whilst no study comparing all methods could be found, one study compared the V-Slope method, VEQ method, $PETO_2$ method and R/Time method (2.*9.1 R against Time/Work rate*). Overall, the study found the $PETO_2$ method and V-Slope method were most consistent at identifying VT1 (96.9% and 92.9%, respectively). Whereas using the R/Time and VEQ methods independently had a lower detection rate at 83.6% and 78.1%, respectively.[84] Whilst there is a high identification rate among the V-Slope and the $PETO_2$ method, a combination of methods of threshold identification is preferable and has been shown to improve the accuracy and rate of VT identification.[7]

2.8. Second ventilatory threshold (VT2)

VT2 occurs beyond VT1 during moderate to high exercise intensities and is the highest sustainable intensity where energy requirements and is a good measure of an individual's anaerobic capacity.[2] For the resynthesis of ATP to continue via oxidative phosphorylation. One of which involves oxygen, which serves as the final electron acceptor in the respiratory chain, combining with hydrogen to form water. As exercise intensity increases, energy demands require greater volumes of oxygen than can be delivered.[51] The inadequacy of in oxygen delivery, or utilisation creates an imbalance with no oxygen available to accept hydrogen at the final stage of the electron transport chain. This causes hydrogen to accumulate across the electron transport chain and bind to NAD^+ and FAD. However, for glycolysis to continue, NAD^+ needs to be available to oxidize 3-phosphoglyceraldehyde. Subsequently, the non-oxidised hydrogens bind to pyruvate via the enzyme lactate dehydrogenase, forming lactate. This anaerobic glycolysis, results in increased production of $\dot{V}CO_2$, lactate and H^+.[51] Once the production of these metabolic by-products exceeds the buffering capacity, then a state

of metabolic acidosis is reached [6, 19, 83], resulting in muscular fatigue, acidosis and unmaintainable energy production.[64, 65] Prescribed training at VT2 optimises training adaptations which enhance metabolic efficiency, buffering capacity, and skeletal muscle physiology.[5, 6] These adaptations improve physiological tolerance to exercise stress through increasing buffering capacity to reduce the accumulation of lactate and H^+ in the blood and skeletal muscle.[29] Such adaptations improve metabolic efficiency, increasing exercise tolerance. Subsequently, the exercise intensity VT2 occurs at increases as higher exercise intensities can be maintained at a steady state.[7, 15]

2.8.1. Physiological adaptations incurred via training at VT2

As exercise intensity progresses within incremental exercise testing, there is a non-linear increase in ventilation alongside increased activity of buffering systems. For example, the bicarbonate system minimises lactate accumulation and the extent of pH change. H^+ react with HCO-3 to form carbonic acid, dissociating to form H_2O and CO_2. Once buffering capacities are exceeded, pH starts to decrease, which initiates increases in ventilatory stimulation, driving greater expulsion of CO_2. This process is concurrent with the transition from the isocapnic buffering period to hypercapnia, or VT2.[6]

VT2 is often identified within a moderate to high exercise intensity and can reflect an individual's anaerobic capacity.[2, 64] At and below VT2 energy requirement can be supplemented by aerobic respiration. However, beyond VT2, the energy demands exceed oxidative capacities increasing the recruitment of anaerobic metabolic processes like glycolysis. During glycolysis, nicotinamide adenine dinucleotide (NAD^+), a coenzyme, is reduced, forming $NAD+H^+$. For glycolysis to proceed, $NAD+H^+$ needs to be re-oxidised. However, once oxidative capacities have been exceeded, $NAD+H^+$

cannot be re-oxidised aerobically via the mitochondria. Therefore, pyruvate regenerates $NAD+H^+$ without oxygen, forming NAD^+ and lactate. For every anaerobic regeneration of $NAD+H^+$ to NAD^+ and lactate, there is also an H+, impacting the acid-base balance and lactate accumulation.[68] Wasserman *et al's* primary rationale for VT2 (originally termed anaerobic threshold) was to objectively measure exercise-induced stress in individuals with cardiovascular disease without completing a full CPET to exhaustion.[6]

2.9. Methods of identifying second ventilatory threshold

2.9.1. Ventilatory equivalent method (VEQ method)

Utilising the VEQ method to identify VT2 combines the same parameters but tracks their interactions beyond VT1. During moderate to high exercise intensities, the primary aim of the ventilatory system is to expel excess CO_2 accumulation. Rises in CO_2 and declines in pH result in the ventilatory shift reflected within the VEQ Method. $V_E/\dot{V}CO_2$ demonstrates the volume of $\dot{V}CO_2$ (produced by the active tissues) that needs to be eliminated via ventilation and is directly influenced by the partial pressure of carbon dioxide ($PaCO_2$).[67] Comparable to the VEQ VT1 method, $V_E/\dot{V}O_2$ $V_E/\dot{V}CO_2$ are plotted against time or work rate. VT2 can be identified via a secondary, non-linear rise in $V_E/\dot{V}O_2$ with the first concomitant non-linear rise in $V_E/\dot{V}CO_2$ (F*igure 3).*

2.9.2. End-tidal partial pressure of oxygen and carbon dioxide

$PETCO_2$ reflects ventilatory and perfusion efficiency, which often declines following VT2. The onset of exercise-induced hyperventilation results from the decrease in $PETCO_2$; subsequently, there is an increased arterial-venous partial pressure difference.

This increased concentration gradient allows for more efficient removal of CO_2 from the respiring tissues, delaying dramatic reductions in pH and increases in $PETO_2$. At VT2, a decrease in $PETCO_2$ can be identified with an increase in V_E, counterbalancing the reduced arterial pH. Subsequently, a breakpoint can be identified via a steep decline in $PETCO_2$ when plotted against time or load, demonstrated in *Figure 4.*

2.9.3. R against time/work rate

This method of VT2 identification is not always the most accurate; however, it is potentially more accurate when measured within trained athletes with an increased aerobic capacity. R represents RER. RER is the ratio between $\dot{V}O_2$ and $\dot{V}CO_2$. Once the $\dot{V}CO_2$ value exceeds the $\dot{V}O_2$ value, an RER of 1 or greater is reported. During exercise, the buffering of lactic acid increases $\dot{V}CO_2$, subsequently increasing RER.[61] An RER of 1 or higher implies anaerobic glycolysis is the primary pathway being utilised for energy production, which is also reminiscent of VT2.[4, 77, 85] An RER of 1.1 or greater can also be used as a parameter for 'exhaustion'.[67] When an RER of 1 is achieved during a maximal incremental exercise, VT2 can be estimated.[86]

2.9.4. $V_E/\dot{V}CO_2$ Slope

The $V_E/\dot{V}CO_2$ slope method is popularly used to identify RCP. This method depicts the onset of exercise-induced hyperventilation, otherwise referred to as 'hot ventilation' or 'panic breathing' that occurs within the heavy-severe exercise intensity domain (*Section 3.10.1.$V_E/\dot{V}CO_2$ Slope*).[2]

2.9.5. Ventilatory equivalent method with end-tidal partial pressure of carbon dioxide.

Similar to VT1, combining the VEQ method paired with $PETCO_2$ is a common method used to improve accuracy when visually identifying VT2.[24, 25, 71] This method identifies VT2 via a deflection in $PETCO_2$ against a rise in $V_E/\dot{V}CO_2$ and a secondary rise in $V_E/\dot{V}O_2$ against time or work load. Demonstrated in *Figure 5*.

Table 3. Summary of 5 methods used to identify the second threshold; breathing equivalent (VEQ) method, end-tidal partial pressure of oxygen and carbon dioxide ($PETO_2$ and $PETCO_2$) method, respiratory exchange ratio method with work rate/time (R method), minute ventilation and volume of expelled carbon dioxide slope ($V_E/\dot{V}CO_2$ Slope) and the combined VEQ and $PETO_2$ method with founding author.

Name	Method	Author
VEQ method	$V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ are plotted against time/load on the x-axis. Secondary rise in $V_E/\dot{V}O_2$ with a concurrent nonlinear rise in $V_E/\dot{V}CO_2$.[73]	Wasserman *et al 1973*
$PETO_2$ and $PETCO_2$ method	$PETO_2$ and $PETCO_2$ plotted against time/load. Rise in $PETO_2$ alongside a steep decline in $PETCO_2$.[22]	Wasserman *et al* 1973
R method	Achieved an RER of 1 or higher.[22]	Wasserman *et al* 1973
$V_E/\dot{V}CO_2$ Slope	V_E plotted against $\dot{V}CO_2$. A no linear increase in V_E compared to $\dot{V}CO_2$ resulting in a point of inflexion.[77, 87]	Wasserman and Beaver *et al 1981*
Combined VEQ and $PETCO_2$	VEQ method combined with $PETCO_2$, presenting a second deflection in $PETCO_2$ concurrent with second inflection in $V_E/\dot{V}O_2$ and primary $V_E/\dot{V}CO_2$ infection point.[76]	Wasserman *et al 1984*

2.10. Respiratory compensation point and the third ventilatory threshold

A more comprehensive understanding surrounding the physiological responses to incremental exercise beyond VT2 is constantly sought within the literature. Whilst VT2 reflects the upper boundary of the isocapnic buffering phase, continuing into hypercapnia, a further physiological response does occur. The transition into hypercapnia occurs when there is elevated $PaCO_2$ in the blood (greater than 45mmHg).[88] This triggers exercise-induced hyperventilation during high to severe exercise intensities.[89] The onset of exercise-induced hyperventilation drives an increase in breathing frequency, increasing the expulsion of CO_2, subsequently reducing $PaCO_2$.[2, 19, 31, 90] RCP has been defined as exercise-induced hyperventilation[59] or hot ventilation,[2] beyond the isocapnic buffering phase into the hypercapnic phase. The onset of hyperventilation following hypercapnia is then used to identify RCP via the $V_E/\dot{V}CO_2$ slope. In some studies, the occurrence of RCP is delayed compared to VT2 (and other thresholds), leading to accusations of the RCP overestimating other breakpoints and subsequently not being seen as a valid parameter.

2.10.1. $V_E/\dot{V}CO_2$ slope

The V_E and $\dot{V}CO_2$ are closely coupled throughout increasing exercise intensities until V_E rises unproportionally to $\dot{V}CO_2$, reflecting hyperventilation. The definitive understanding surrounding the physiological mechanisms instigating non-linear increases in V_E beyond this breakpoint is unclear. One widely held theory suggests metabolic acidosis is a key instigator, theorising rising $\dot{V}CO_2$ levels, as a result of metabolic acidosis, drives an increase in breathing frequency as tidal volume remains stable.[8] Such a response achieves increased expulsion of $\dot{V}CO_2$ and maintains a normal

pH. This response instigates a non-linear steepening of the $V_E/\dot{V}CO_2$ slope, creating a breakpoint which identifies RCP (*Figure 7)*. However, further investigation into the physiology driving involuntary exercise-induced hyperventilation is necessary as the research is currently unclear and divided.

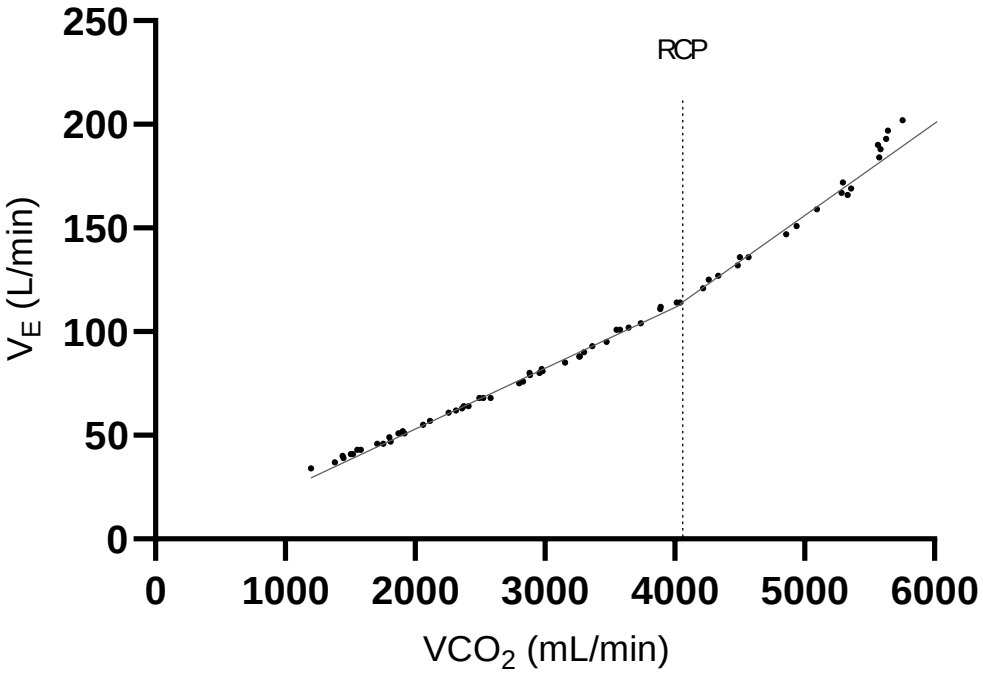

Figure 7. A theoretical graph demonstrating where the identification of the respiratory compensation point (RCP) via the $V_E/\dot{V}CO_2$ Slope, plotting minute ventilation (V_E) against the volume of expelled carbon dioxide ($\dot{V}CO_2$).

When evaluating participants struggling with obesity, occasionally airflow obstruction, chemoreceptor insensitivity and ventilatory responses 'lag' against metabolic responses.[91] In these cases, measures focusing on GET via $\dot{V}O_2$ and $\dot{V}CO_2$ changes

can be preferable to ventilatory responses that rely on V_E.[77] Moreover, the accuracy and reliability of this method have also been questioned among elite-level athletes compared to recreationally active individuals. As recreationally active individuals transition from the upper boundary of the isocapnic buffering phase into hypercapnia, there is a marked fall in $PETCO_2$ (F*igure 3*). This fall is coupled with exercise-induced hyperventilation, identifiable by the $V_E/\dot{V}CO_2$ slope. However, in well-trained athletes, hyperventilation does not coincide with hypercapnia (the drop in $PETCO_2$) and thus has been accused of overestimating this VT2.

It is thought that hyperventilation at RCP results from both neurogenic and metabolic stimuli. The increase in V_E reflects the onset of hyperventilation as it is attributed to an increase in respiratory frequency rather than increases in tidal volume. Increases in *f* are theorised to be principally driven by carotid body stimulation.[92] Chemoreceptor responses are 'slower' than neurogenic responses, which could potentially justify the 'delay' seen from RCP when compared with VT2. However, changes in respiratory parameters during high exercise intensities can also be associated with hormonal, hemodynamic and thermal changes. There is also research to suggest mechanical feedback from vagal pulmonary receptors and chest wall receptors further drives ventilation (*2.13.2 Peripheral influence*).[92]

Ozkaya *et al* has since combined V_E with $PETCO_2$ over time to investigate whether RCP overestimates VT2 or is an independent threshold.[10] The combination of $PETCO_2$ with V_E over time corresponded well with traditional threshold intensities and demonstrated that within recreationally active individuals, the RCP corresponded with VT2 ($p>0.05$). However, among well-trained athletes, RCP clearly overestimated VT2

(p<0.001) despite other threshold identification methods not being statistically different. The overestimation of VT2 was only evident in well-trained athletes and not with recreationally active participants. The transition period between VT2 and RCP and $\dot{V}O_{2Peak}$ has been referred to as the 'grey zone' by Ozkaya *et al* with the third threshold, distinguishable via an independent rise in V_E coined VT3.[10] Overall, Ozkaya *et al* concluded that a VT3 is present in well-trained athletes, following maximal incremental cycle testing. The delayed onset of hyperventilation observed supports previous theories surrounding RCP overestimating VT2 within elite-level athletes.[9] The current hypothesis of VT3 theorises that recreationally trained people have lower endurance capacities and fewer sport-specific physiological adaptations. Thus, they cannot achieve the intensity required to stimulate the hyperventilatory response distinguishing VT3.[93] Well-trained and elite-level athletes, with greater endurance capacities, are more capable of achieving such intensities and reaching a later threshold. It is important to note this study used a ramp protocol. Whilst this method is more frequently used to assess the V_E/CO_2 slope, it is more likely to overestimate threshold parameters, thus encouraging the likelihood of identifying RCP over estimating VT2. The premise of VT3 is also notable within the Vyaire booklet; however there is no supporting research to standby/confirm such claims. There is wide speculation surrounding what physiological mechanisms regulate ventilatory and hyperventilatory responses during exercise beyond VT2 and between moderate to severe exercise.[8, 13, 29, 59, 94]

2.11. Use of artificial intelligence and algorithms to identify VT1 and VT2

Recent progression within threshold identification has been via the incorporation of artificial intelligence (AI) and algorithms used alongside or in place of visual

identification of thresholds. Computerised methods help to remove subjective identification of thresholds.[95] One popular method includes piecewise linear regression lines. Two or three adjoining linear segments are repeatedly fitted into a data set, identifying points via the overlapping and convergences of two adjacent lines. The main concern with this method is the assumption regarding the number of breakpoints. Prior assumptions need to be made for the number of thresholds being identified, as this dictates whether bi-segmental or tri-segmental methods should be permitted. Moreover, with each break point requiring 'abrupt changes' in the gradient, the often short time span of the data can result in inaccurate identification.[96] Bever *et al* stated when assessing VT1 via the V-slope method; the regression lines sometimes identified a bend beyond RCP. Therefore, data points above VT2 should be excluded from the calculation to achieve accurate threshold identification.[77]

A further method, known as polynomial spline smoothing, identifies thresholds via fitting a continuous spline to a data set, whereby an optimally fitted curve is drawn through the data. Any deviations from the line can then identify accelerations or decelerations within the data, which is subsequently marked as your threshold.[97] This can be beneficial as it allows threshold identification regardless of whether the data is continuous or segmental. One paper demonstrated that when identifying VT2, this automated method was more precise than the piece-wise and visual identification methods, as it entirely removes subjective analysis. However, automatic threshold detection can often be hindered due to the high signal-to-noise ratio frequent within CPET data.[98] Alternatively, machine learning algorithms are being used more frequently to process CPET data. This method is a form of AI that instigates neural

networks to reveal complex non-linear relationships between variables. However, the inclusion of too many variables, i.e. age, stature, body mass, gender, heart rate or arterial blood saturation as a function of the exertion, hinders the algorithm's ability to identify meaningful relationships within the data.[98]

2.12. Stepwise vs ramp protocol

The protocol used when completing an incremental exercise test to identify ventilatory thresholds can influence the clarity and accuracy of their identification. Ramp protocols have recently been a popular protocol to use. This protocol allows for continuous and constant increases in workloads for the participant, i.e., speed and gradient increased every 30 seconds, resulting in a test to exhaustion typically lasting around 8–10 minutes in healthy individuals.[67] When the ramp protocol time is kept below 12 minutes[99, 100] the estimation of exercise capacity and validity of $\dot{V}O_{2Peak}$ is greater when compared to stepwise protocols, demonstrating more linear physiological responses[101] within a shorter time frame. Not all physiological responses used to assess exercise intensity are immediate and require slow increments and moments of steady state. Linear increases in exercise intensity, like ramp protocols, do not allow physiological parameters to reach a steady state, as the metabolic demand continuously changes in response to the increasing intensity.[68, 99] Subsequently, a physiological lag between the exercise intensity and the relevant metabolic needs is more likely.[91, 102] The delayed response causes the work rate identified at steady state $\dot{V}O_2$ to be underestimated[103] and other submaximal parameters (RCP) to be overestimated.[104, 105]. Therefore, the prescription of training intensities will exceed an athlete's optimal training intensity, as the PO or %PPO will not align with the physiological threshold.[106, 107] Alternatively, a stepwise

protocol consists of a longer stage duration, i.e. 2-4 minutes, with larger workload increments. Whilst this allows for a point of 'steady-state' to be achieved, the stepped increase takes more adjustment from the participant.[101, 108] Furthermore, this protocol increases the test duration, but this can also be a benefit. Stepwise protocols are preferable when identifying submaximal capacities as the increase in intensity occurs in stages, allowing 2-4 minutes of steady state exercise. This increases the sensitivity of physiological changes to exercise,[107] and prevents a lag between the physiological and metabolic responses and the metabolic demand.[68, 99] Therefore, when looking to identify thresholds, the most effective protocols are 3-minute step durations, with smaller increments, lasting longer than 12 minutes.[99, 109] Though it is important to note that the longer protocol hinders the accuracy of maximal exercise capacity estimations.[108, 110]

2.13. The physiology driving ventilation during intense exercise

During incremental exercise, there is a trigger point at which respiratory parameters adjust to provide for the increased exercise intensity. However, the understanding of what is needed to initiate physiological changes during exercise at and beyond VT2 and the hypercapnic phase is debated. The leading theory surrounding physiological drivers of ventilation during high exercise intensities beyond VT2 is chemoreceptors responding to the onset of metabolic acidosis. As exercise intensity increases, the chemical composition of the blood instigates alveolar ventilation via chemoreceptors within the aortic and carotid arteries, which relay information to the respiratory centre in the medulla.[51, 111] These mechanisms help maintain homeostatic pH levels and arterial pressures throughout different exercise intensities.[51] One study demonstrated

the influence of carotid bodies (peripheral chemoreceptors) on ventilation during intense exercise within rats. The removal of carotid bodies decreases the inspiratory and expiratory responses of the diaphragm, abdominal, and internal oblique muscles within high exercise intensities, resulting in decreases in arterial pH. This suggests that carotid bodies are involved in the respiratory compensation against high exercise intensities and potentially metabolic acidosis.[112] However, physiological responses within rats cannot be directly applied and assumed within human respiratory responses.

Conflicting theories suggest that receptors (including thermoreceptors, sensory receptors in the lungs and proprioceptors within the joints and muscles) are the primary drivers of ventilatory change, feeding back information to the medulla oblongata in response to exercise.[51, 113] Fast respiratory responses to increasing exercise intensities (especially within incremental testing using ramp protocols) suggest there is neurogenic control from the central command centres and subsequent peripheral feedback from the exercising muscles driving the increases in ventilation. The central ventilatory control originates in the medulla oblongata. Input signals to the respiratory centre regulate the activation of inspiratory and expiratory neurons that synchronize ventilatory muscles such as the diaphragm and intercostal muscles.[51, 111, 113] Ascending neural input to the cerebellum as a result of mechanical and chemical changes further regulates ventilatory responses to exercise by providing feedback to the respiratory centre.[51, 111]

2.13.1. Chemoreceptor influence

The primary theory explaining exercise-induced hyperventilation is metabolic acidosis, suggesting hyperventilation compensates for the physiological inability to maintain a blood pH of around 7.4.[8] During heavy exercise intensities, athletes incur raised blood

lactate levels, leading to metabolic acidosis. The onset of metabolic acidosis then stimulates chemoreceptors which relay signals to the respiratory centre, subsequently triggering an increase in breathing frequency. Ventilation is suggestively a primary mechanism used to regulate H^+. Below VT2, arterial pH is regulated near resting levels, so ventilation does not overcompensate. Beyond VT2, the net increase in lactate and CO_2, due to lactate buffering, instigates marked increases in ventilation to prevent further falls in pH and maintain $PETCO_2$ levels. [68] During exercise and recovery, pH homeostasis is prioritised over $PaCO_2$ regulation, suggesting pH is the main metabolic driver for ventilatory control.[114]

Central chemoreceptors and carotid bodies are significant drivers at and beyond RCP. Wasserman *et al* demonstrated that among six male subjects with surgically removed carotid bodies, hyperpnea above the anaerobic threshold was less marked and did not incur a hyperventilatory response, despite metabolic acidosis. A further study compared participants' $PaCO_2$, HCO_3 and pH levels with and without carotid bodies during different exercise intensities. At lower exercise intensities, all parameters were comparable. However, during mild to very heavy exercise intensities, large reductions in $PaCO_2$ and small changes in pH were observed within normal participants. The participants without carotid bodies had greater decreases in pH and no reduction in $PaCO_2$. Demonstrating the contribution carotid bodies have on the regulation of ventilation during metabolic acidosis.[115, 116] The study involving the removal of carotid bodies in rats (*3.13 [paragraph 2]*) reported inspiratory and expiratory changes within high exercise intensities but not low exercise intensities. *Spiller et al* concluded that carotid bodies are involved in the ‘fine tuning’ of respiratory responses to minimise

arterial gasses and pH disruptions. Therefore, during intense exercise, changes in the internal acid-base balance increase the activity of carotid bodies and subsequent respiratory responses within rats.[112]

Moreover, Meyer *et al* used sodium bicarbonate ($NaHCO_3$) to determine if a greater buffering capacity delayed the onset of hyperventilation among the participants, concluding that blood pH is a factor initiating hyperventilation during severe exercise intensities.[8] Despite this, the current understanding of the location and detection of CO_2 and H^+ chemoreceptors and how they influence hyperventilation is unknown. However, the chemoreflex associated with hypercapnia can be influenced by other neurological responses, like serotonin levels which are influenced by exercise but independent of $PaCO_2$ and the respiratory system. Moreover, the onset of hyperventilation moderates thermoregulation and blood oxygenation, widening the scope of chemoreceptors likely to contribute to hyperventilation.[117] Whilst metabolic acidosis is hypothesised to be a primary factor driving the non-linear increase in V_E during higher-severe exercise intensities [8, 118] there are likely to be numerous other non-metabolic factors regulating ventilation beyond VT2 [8, 51, 93, 111] including raised body temperature, catecholamines and occasionally arterial hypoxia within highly fit participants.[118]

Conversely, one study noted that following the supplementation of 100% O_2, the response from V_E occurred too soon to account for a reduction in blood lactate, thus suggesting an alternative metabolite stimulates V_E.[119] Ventilatory drive is likely to be a combination of neural and humoral factors. Some evidence indicates that potassium (K^+) within the interstitial fluid can stimulate muscle afferents responsible for non-

steady state changes in V_E more than $PaCO_2$, pH, lactate or osmolarity.[118-120] Hyperkalaemia, or increased blood K^+ levels in the blood, often occurs during exercise because of K^+ being lost from the working muscle and into arterial plasma during muscle contraction, which is exacerbated during high-intensity dynamic exercise. It has been estimated that up to 20-30mmol of K^+ may be lost via multiple channels from intracellular stores within working muscles. The substantial loss of K^+ is likely to contribute to skeletal muscle exhaustion.[119] Busse *et al* demonstrated the influence of K^+ on V_E within six endurance-trained men. Controls were taken for the potential effects of plasma-free fatty acid concentrations, plasma pH, or plasma bicarbonate concentration on ventilatory responses. Despite this, Busse found that substrate and acid-base changes and the relationship between plasma K^+ and V_E were unaffected.[120]

2.13.2.Peripheral influence

Non-metabolic drivers of ventilation during heavy exercise have also been investigated, for example, the activation of metaboreceptors and mechano-sensitive responses. The rapid ventilatory response to increases in exercise is potentially due to neural mechanoreceptor feedback within the musculoskeletal system and pulmonary stretch receptors.[111] Research among patients with McArdle's disease demonstrated distinct hyperventilation occurring at an average of 70-85% of their $\dot{V}O_{2Peak}$ despite blood lactate remaining at resting levels.[121]

One study demonstrated the use of surface electromyography to measure muscle activation across eight lower limb muscles in eight professional road cyclists and successfully identified two non-linear increases, with the second increase occurring at a comparable PO as the identification of VT2. This suggests there is an alteration in

motor unit recruitment during moderate to high exercise domains.[122] Mechanoreceptors relay signals in response to touch, pressure and stretching stimuli.[123] Metaboreceptors stimulate blood circulation during exercise following increased metabolic products like lactate and hydrogen ions.[90, 124] These inflexion points could stimulate mechanoreceptors and metaboreceptors, providing feedback to encourage changes in ventilation.[8, 59, 90, 121]

The use of lower-body positive pressure (LBPP) and inflated bilateral thigh cuffs has previously identified an increase in V_E during dynamic exercise, further supporting the notion of an intramuscular ventilatory stimulus. [111, 125] LBPP can decrease venous outflow, preventing the clearance of metabolites from the exercising tissue and helping to minimise metabolite-stimulated responses.[90] Dynamic exercise and positive pressure stimulated the metaboreceptors and mechanoreceptors (respectively), increasing ventilation, concluding that intramuscular responses to exercise influence V_E, independent of metabolic responses to exercise.[126] Following similar use of LBPP, a later study conducted by Smith *et al* theorised that the pressure-induced as a result of LBPP could be considered a further VE driver. However, findings suggested this pressure did not mediate an instantaneous ventilatory response. This indicates that the mechanoreceptor reflex does not actively mediate a ventilatory response in exercise. Further speculation proposes that changes in V_E are a result of peripheral chemoreceptor activation or increases in the perceived effort being relayed back to the central motor command centre.[90]

Other non-metabolic feedback instigating ventilatory responses include potential muscle reflexes and nerve endings within skeletal muscle. Early research has

investigated the influence of myelinated mechanosensitive and metabosensitive nerve endings within animals and humans on ventilatory control.[90, 127, 128] One study demonstrated the removal of dorsal roots (located within the spine and receiving afferent nerves of exercising muscles), stopped rises in blood pressure, and increases in heart rate and pulmonary ventilation during isometric exercise in cats.[127] A further study also revealed increases in ventilation when nerve endings in the gastrocnemius of an anesthetised dog were stimulated through stretching, pressing or squeezing.[128] Whilst these papers demonstrate non-metabolic control and stimulation of ventilatory responses within animals, definitive conclusions are yet to be drawn.[90, 127, 128] Moreover, further research is required to determine muscle reflexes' influence on respiratory responses within humans during high-intensity exercise.

2.13.3.Psychobiological model

Exhaustion and fatigue often go hand in hand. Fatigue limits the participant from continuing to exercise, resulting in exhaustion and task failure.[129] The reduction in reduction in power output due to exhaustion is associated with limitations of the cardiovascular, respiratory, metabolic, and neuromuscular systems.[130] However, the perception of exhaustion is currently being challenged within the surrounding literature. *Ament et al* defined exhaustion as “when the sense of effort is so intense, it topples one’s willpower to maintain the motor output and forces the participant to reduce or stop their workload”.[129] Marcora *et al* also challenged the current perspective of exhaustion during high-intensity aerobic exercise. Maximal voluntary cycling power was measured before and immediately after an exhaustive exercise cycling test within ten fit male subjects. The maximal voluntary cycling power measured immediately after time to

exhaustion was three times greater than the power output required during the time to exhaustion test, demonstrating participants had substantial neuromuscular reserve immediately following ‘exhaustion’.[130] This suggests that physiological fatigue is not the leading cause of exhaustion during maximal exercise testing.[130]

Many theories, such as; metabolic acidosis, cardio-respiratory capacity and muscle fatigue, focus on the physiological aspects of fatigue and exhaustion within endurance exercise, emphasizing metabolic and physiological capacity determining and regulating human performance.[131] A more recent model (the psychobiological model derived from Brehm’s motivational intensity theory) explores exhaustion through engagement or point of disengagement from a task relative to the potential motivation (the maximal amount of effort willing to be exerted) and motivation intensity (the actual amount of effort exerted). Disengagement occurs when the potential motivation is reached, or the task is perceived as impossible. When applied to exercise testing, disengagement is in the form of exhaustion, whereby the perceived effort required reaches potential motivation or continuation of the task is believed to be physically impossible.[130] The physiological processes associated with exertion include cardiovascular, respiratory and metabolic signals like; pulmonary ventilation, heart rate, breathing frequency and oxygen uptake. Sensory monitoring within both central and peripheral signalling occurs on a conscious and unconscious level, with alterations in breathing or respiratory discomfort being consciously monitored.[132]

It's suggested that exercise intensity based on rate of perceived exertion (RPE) values involves afferent signals from perceptual cues, thus regulating performance so that it can be completed within the biomechanical and metabolic limitations of the body.

Application of effort rating to thresholds, with scales such as the Borg scales ratings of 13 to 14 showing strong correspondence with VT1,[133] RPE can be utilised within pacing strategies.[134] This application of effort perception demonstrates athletes understanding and awareness of ventilatory responses and breathing during exercise, specifically high exercise intensities. This consciousness and cognitive association with perceived effort and fatigue could impact ventilation regulation at all intensities. However, the sensory inputs and physiological cues for perception during moderate to high exercise intensities are unclear.[130, 133]

2.14. Identifying the third ventilatory threshold (VT3)

The terms VT2 and RCP are frequently used interchangeably throughout the literature.[135] However, the two terms have a clear distinction. VT2 reflects exercise intensity tolerance when H^+ accumulates at an excess of an individual's buffering capacity. In contrast, the respiratory compensation point is described as the point of exercise-induced ventilation and should be identified via the $V_E/\dot{V}CO_2$ Slope.[10] The dramatic increase in V_E seen at RCP results from an increased drive in breathing frequency (*f*) without a rise in tidal volume. However, the physiological mechanisms driving this change in breathing are yet to be determined.[59] Despite overlapping use of the terms VT2 and RCP [19, 136] many studies claim RCP and VT2 have occurred at time points akin to each other, suggesting they are reflecting differing physiological responses at the same point of exercise-induced stress. On the other hand, RCP has been reported at a greater exercise intensity than VT2 and is subsequently a result of metabolic acidosis, not simply exceeding buffering capacity.[8, 137]

Ozkaya *et al* have suggested a third respiratory threshold. Ozkaya demonstrated a new method of combining V_E and $PETCO_2$ with time/load to identify all threshold intensities following a single incremental test. Within this graphical data, the existence of a grey phase in between the isocapnic (second) phase and hypercapnic (third) phase can be identified following a delayed rise in V_E after a clear drop in $PETCO_2$. Within this grey phase, the work rate RCP occurred at was significantly greater than the VT2 work rate when identified via the ventilatory equivalents method. Alongside this, three change points can be identified using the novel $V_E/PETCO_2$-time method, referred to as the first, second and third respiratory thresholds (RT1, RT2 and RT3, respectively). When compared with current threshold identification methods, RT1 was comparable to VT1, RT2 to VT2 and RT3 to RCP. This phenomenon was only present among well-trained athletes. Another study comparing respiratory responses and hypoxemia in well-trained athletes found that those not experiencing hypoxemia had an earlier onset of exercise-induced hyperventilation. In contrast, those that did incur exercise-induced hypoxemia had a later, delayed onset of exercise-induced hyperventilation. Conversely, the group that experienced hypoxemia had a greater training load, despite both groups having comparable $\dot{V}O_{2Max}$ and a later VT2.

The application of a ramp protocol, however, could be attributed to the separation of the two respiratory parameters (V_E and $PETCO_2$), potentially justifying the 'grey zone'. RCP and increases in V_E are associated with reduced pH, with much research suggesting that metabolic acidosis is the primary driver of exercise-induced hyperventilation.[8] The continuous nature of a ramp protocol, increasing 1W every 2 seconds, result in a rapid increase in intensity. Peripheral chemoreceptors, namely carotid bodies, are very

sensitive to changes in partial pressure of oxygen and carbon dioxide[138, 139], with a response time to exogenous H^+ being less than 1 second.[118] It is suggested that central chemoreceptors are responsible for two-thirds of ventilatory responses to CO_2 and pH and facilitate 50% to 90% of ventilatory responses during hypercapnia.[138, 139] Central chemoreceptors also have slower response times to carotid bodies.[139, 140] Concerning the ramp protocol and the subsequent ventilatory responses observed, the delayed rise in V_E following a drop in $PETCO_2$, resulting in the grey phase, could be due to the exercise intensity increasing faster than central chemoreceptors can respond. Resulting in a delayed rise in V_E. In contrast, fast-responding peripheral receptors mediate $PETCO_2$ responses to rising exercise intensity.

However, extreme respiratory responses within well trained endurance athletes at very high work rates are not so novel. One study demonstrated that well-trained athletes reached 95% of their resting maximum voluntary ventilation, a stark difference to the 60-70% increase seen among less trained participants.[118, 141] This phenomenon has been attributed to the mechanical limitation of airflow. Well-trained individuals are more likely to reach mechanical limitations for ventilation during high-intensity exhaustive exercise following optimisation of other physiological systems. Therefore, this fourth zone, following VT3, could reflect an athlete pushing and reaching maximal mechanical ventilatory capacity.[118]

2.15. Conclusions

A third threshold is a new concept; however, some evidence suggests that additional physiological responses occur beyond VT2, employed to extend exercise during heavy-severe exercise domains. Ozkaya identified the VT3 within minute ventilation,

suggesting that where RCP is not comparable to VT2, RCP is a third threshold. However, the fast increments of a ramp protocol could depict a delayed hyperventilatory response due to the slower response times associated with the employed chemoreceptors rather than defining a third threshold. Furthermore, where RCP is synonymous with exercise-induced hyperventilation through the rapid rise in V_E, such a response could also instigate rises in other respiratory parameters, suggesting VT3 could be identifiable within more established threshold identification methods. Therefore, following this literature review, Study 1 will compare methods used to identify thresholds at VT1 (V-slope and VEQ method with $PETO_2$), VT2 (VEQ method with $PETCO_2$ and $V_E/\dot{V}CO_2$ Slope) during stepwise maximal incremental cycle test. Assess the presence of additional breakpoints beyond VT2 and if trained status influences the prevalence of additional breakpoints.

3. The prevalence of a third ventilatory threshold within healthy individuals

3.1. Abstract

Overlapping terminology and a variety of methods have resulted in differing conflicting theories across the research, particularly with the application of VT2 and RCP. Whilst some research uses the terms interchangeably, others suggest RCP overestimates VT2. A recent study reports the disparity between VT2 and RCP demonstrates a third threshold beyond VT2 that is only identifiable within well-trained and elite athletes. A retrospective analysis of n=32 active males who had completed a maximal stepwise cycle were assessed. Breath-by-breath data were smoothed to identify the first ventilatory threshold (VT1), first gas exchanged threshold (GET1), second ventilatory threshold (VT2), first respiratory compensation point (RCP1), third ventilatory threshold (VT3) and second respiratory compensation point (RCP2).

VT1 and GET1 showed to be independent of each other and other thresholds (p>0.01), whilst VT2 and RCP1 reflected the same threshold and were not significantly different (p=0.99-1.00). VT3 and RCP2 was identified in 23 of the 32 participants. Compared with VT2 and RCP1, when VT3/RCP2 was identified, this occurred at a later timepoint, a greater percentage of peak power output (%PPO), absolute power output (PO) and respiratory exchange ratio (RER) (p<0.001).

Further analysis compared the performance parameters of the cyclists that did and did not present VT3/RCP2. Where VT3/RCP2 was present, participants were younger (p=0.01) and had higher power-to-weight (P:Wt), time to exhaustion (TTE) and PPO

(p=0.01-0.02). No difference was observed for $\dot{V}O_{2Peak}$ and BMI (p=0.10 and p=0.12 respectively) but moderate effect sizes (ES=0.5) were reported.

Overall, VT2 and RCP1 reflect the same transition point. Furthermore, a third threshold is identifiable via two independent methods (VT3 and RCP2), with evidence to suggest VT3/RCP2 is only present in individuals with a greater trained status.

Key words: Cycle test, Third ventilatory threshold, Respiratory compensation point, Performance.

3.2. Introduction

An athlete's performance is mainly dependent on their physiological ability to meet challenges and deal with exercise stress. The responses to exercise stress can be developed by progressive exercise training, enabling progression and improvement in sports performance. Respiratory responses, heart rate and blood lactate accumulation reflect changes in exercise intensity. Respiratory responses to exercise can be measured via gas analysers which is less intrusive than blood lactate measures and allows continuous breath-by-breath data throughout testing.

An incremental exercise test (low intensity to exhaustion) can be divided into three metabolic phases. Initially, the isotonic buffering phase occurs during low exercise intensities, where respiration and metabolism are predominantly aerobic, consisting of primarily oxidative CO_2 production. As the exercise intensity increases, the greater physiological demand instigates a transition into the isocapnic buffering phase. This transition point is where VT1 is identified. Beyond VT1, metabolic by-products continue oxidising until an equilibrium between H^+ and buffering capacity is achieved. This threshold is most applicable to endurance athletes, as training at VT1 (also associated with an equivalent of ~2mmol·L^{-1} of lactate)[25] encourages adaptations most beneficial for long-duration aerobic performance.[2, 64]

The isocapnic buffering phase reflects the period between VT1 and VT2, whereby lactate can be buffered, and pH is maintainable. VT2 then distinguishes the transition from the isocapnic buffering phase into hypercapnia. At this intensity, H^+ accumulation exceeds buffering capacity, resulting in H^+ accumulation and a decline in pH.[3] This is reflected in a visible drop in $PETCO_2$ and a dramatic VE rise. An exercise-induced

hyperventilatory response, identified via increased V_E through RCP, can also determine the transition into hypercapnia.[28, 93] However, conflicting research challenges this, suggesting that exercise-induced hyperventilation occurs beyond the second transition into the hypercapnic phase and reflects metabolic acidosis.

3.2.1. Methods of threshold identification

The two most established methods used to identify VT1 are the V-Slope method and the VEQ method (which can also be combined with $PETO_2$). The V-Slope method focuses on gas exchange through the analysis of $\dot{V}O_2$ and $\dot{V}CO_2$, whereas the VEQ method with $PETO_2$ incorporates $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ with exercise over time or work done (*Table 2*). Multiple parameters can distinguish the transition from the isotonic buffering phase into the isocapnic buffering phase; lactate of 4mmol·L of blood, rise in $\dot{V}CO_2$ output, inflexions in breathing equivalents of oxygen and carbon dioxide and reductions in $PETCO_2$.

When identifying VT2, the most established method used is the VEQ method, which, similarly to the identification of VT1, can be combined with $PETCO_2$. The identification of RCP is achieved via the $V_E/\dot{V}CO_2$ Slope *(Table 3)*, isolating the non-linear rise in V_E without $\dot{V}CO_2$. Understanding physiological responses at and beyond VT2 is well speculated. Whilst much of the research uses VT2 and RCP interchangeably, others refer to these as independent terms (*Chapter 2.10*).

Multiple approaches can be taken to identify VT1. When assessing gas exchange and ventilatory thresholds, the V-Slope and VEQ methods are the most commonly used. One study compared and combined the VEQ method, the modified V-Slope method and

the $exCO_2$ method to identify which method best identified the first threshold relative to the first lactate threshold. Gaskill *et al* demonstrated that the modified V-Slope method independently has the highest acceptance rates close to the first lactate threshold. However, combining the V-Slope and VEQ methods showed stronger correlations and minor differences.[7] Another study compared the V-Slope, VEQ, $PETO_2$, and RER=1 method, finding that the V-slope, VEQ and $PETO_2$ methods are the most comparable and reliable methods of determining VT1 with adolescents with congenital heart or lung disease.[142] Overall, a combination of VEQ, $PETO_2$ and V-Slope is preferable as it assesses the response of multiple ventilatory and gas exchange parameters, enabling a more rounded perspective of where the threshold is occurring.

When identifying VT2, the primary method seen within the literature is the VEQ method. The precision of this method can be elevated through the addition of $PETCO_2$. However, limitations of this method include a large amount of background noise associated with the VEQ method due to high respiratory chemosensitivity.[77] Alternatively, RCP identification via the $V_E/\dot{V}CO_2$ method has been used alongside or interchangeably with the VEQ method. However, Beaver *et al* and Ekkekakis *et al* found that identifying RCP is not always possible [77, 95] with others claiming RCP overestimates VT2. These discrepancies seen between VT2 and RCP could result from protocol,[102, 104, 105] data clarity and quality,[75] or the trained status of the tested participant.[10] The overarching research currently understands that whilst VT2 and RCP do not co-occur due to different underlying mechanisms, they reflect the same transition point. There are clear benefits and draw backs to both methods. Research, including

papers, suggests that utilising multiple methods to assist in evaluating a threshold could be beneficial and improve the approximation of identifying the transition point.[84, 143]

3.2.2. Ventilation beyond VT2.

The application of VT2 and RCP thresholds is debated throughout the literature. VT2 studies the interaction of gas exchange and ventilatory responses, whereas RCP is focused on the respiratory response via a rise in $V_{E.}$ Therefore, these thresholds have been used interchangeably with the assumption they reflect the same physiological turn point. However, conflicting research associates VT2 with moderate to high exercise intensities, reflective of 4mmol of lactate, or an RER of 1.[135] RCP, on the other hand, reflects a hyperventilatory response that's more readily associated with high metabolic acidosis induced by high to severe exercise intensities.[8]

Another method of threshold identification has recently been introduced to the literature by Ozkaya *et al*. This novel method plots V_E and $PETCO_2$ on the y-axis against time on the x-axis. The interaction between V_E and $PETCO_2$ is especially relevant to the transition from the isocapnic buffering phase into hypercapnia through a joint deflection of $PETCO_2$ with an inflexion in V_E. Further investigation by Ozkaya found that within well-trained and elite-level athletes, the V_E and $PETCO_2$ change points dissociate. $PETCO_2$ initially drops, followed by a rise in V_E.[10] The VEQ method demonstrates ventilatory efficiency and ventilatory control. During high exercise intensities, tidal volume often plateaus due to mechanical limitations. Therefore, rises in V_E result from an increase in breathing frequency.[2, 58, 61] Increases in V_E following rises in breathing frequency increases physiological dead space, thus, resulting in decreases in ventilatory efficiency which can be identified via rises in $VEQO_2$ and $VEQCO_{2.}$[144] Subsequently,

any rises in V_E due to hyperventilation (as reported at RCP) will increase $VEQO_2$ and $VEQCO_2$. Incorporating the VEQ method with PETCO2 should therefore report similar change points identified via the $V_E/PETCO_2$ method reported by Ozkaya *et al* above.

The reported prevalence of VT3 resulted from isolating one physiological parameter (V_E) following a ramp protocol. With the underlying mechanisms of V_E still being debated, there is some research suggesting the central chemoreceptors, which have slower response time mediate much of the ventilatory responses.[139, 140] V_E is one of the main parameters used to identify RCP, which potentially overestimates VT2 within ramp protocols. Subsequently, the rate of changing metabolic demands within a ramp protocol increasing faster could result in delayed ventilatory response times.[102, 104, 105]

Consequently the delayed identification of V_E justifying the gap reported between V_E and $PETCO_2$ and VT3 is rather a delayed reflection of VT2. It is important to establish if a gap between VT2 and RCP can be identified within a stepwise protocol. Furthermore, it is important to establish if a third threshold is identifiable via previously researched and accepted methodologies. Adapting methodologies already used within research could also help prevent further saturation and confusion within the literature.

3.2.3. Research aims

This study compares methods used to identify thresholds at VT1 (V-slope and VEQ method with $PETO_2$), VT2 (VEQ method with $PETCO_2$) and RCP1 during a maximal incremental cycle test. The secondary aim of this study is to assess the presence of additional breakpoints beyond VT2 and RCP1. The final aim is to investigate the relationship between performance and the prevalence of ventilatory thresholds.

3.3. Methods

3.3.1. Participants

This descriptive study proposes to complete a retrospective analysis of 32 male volunteers (age = 45 ± 15 years, body mass = 75 ± 11.59 kg, Body Mass Index (BMI) = 24.4 ± 2.4 $kg \cdot m^2$, TTE = 987.8 ± 169.3s, $\dot{V}O_{2Peak}$ = 51.5 ± 8.2 $ml \cdot kg^{-1} \cdot min^{-1}$). Participants were active individuals who participated in running, cycling or triathlons and were free from injury or illness. The study was approved by the University of Essex ethics committee (ethics application ETH2122-0202). Initially, written informed consent was obtained from each participant following the Declaration of Helsinki, declaring they are comfortable for their data to be used in future research. Moreover, all participants completed a physical activity readiness questionnaire (PAR-Q) before involvement in the study. All testing was conducted in controlled laboratory conditions of 20 ± 2°C temperature and 65±5% relative humidity. Participants were asked to arrive rested and abstain from alcohol and caffeine 24 hours before testing and avoid food in the final 3 hours prior. All participants were asked to come well-hydrated.

3.3.2. Procedures

Stature and body mass anthropometrics were measured upon arrival, following the completion of consent and PAR-Q forms. Participants were fitted onto the bicycle ergometer (Lode, Excalibur Sport, Ergometer, Netherlands) according to comfort. Bishop *et al* reported a warm-up of 5 minutes improves performance as it allows an athlete to begin a task with a raised VO_2, without being in a non-fatigued state.[145] Therefore, a 5-minute warm-up was completed at $1W \cdot kg^{-1}$ at a cadence of 80±10

revolutions per minute (rpm) to help prime participants for the test, optimise their performance[146], prevent injury,[147] standardised approach across all participants and familiarise with the equipment. Following the warm-up, participants could complete any static or dynamic stretches before returning to the ergometer. The incremental cycle test required participants to maintain a cadence of 80 ± 10rpm throughout. The chosen resistance for this protocol was tailored to the individual athletes, via their weight to ensure increases of resistance to be appropriate for the individual.[148] Larson et al suggested stepwise protocols, with stages lasting between 2-4 minutes were optimal to encourage a point of steady state without, limiting VO_{2MAX} values. [101, 108] Therefore, the protocol for this study started with an initial resistance for the first stage of the stepwise protocol was $1W{\cdot}kg^{-1}$ and increased by $0.5W{\cdot}kg^{-1}$ in 2-minute increments to enable a point of steady state, [101, 108] ensuring accurate threshold identification.[148] The test was terminated when the pedal rate fell below 70 rpm for more than 5 seconds or volitional termination due to exhaustion, despite strong verbal encouragement. Three of the following validation criteria were required for actual exhaustion to be accepted; $\dot{V}O_2$ plateaus ($\dot{V}O_2$ increases <2.1 $mL{\cdot}min{\cdot}kg^{-1}$), RPE of 19–20 in the Borg's 15-point scale, a respiratory exchange rtio of <1.1 and a heart rate >90% of age predicted maximum (220-age). If this criterion was not achieved, the test was disregarded.

Respiratory gases were collected continuously throughout the incremental test. Participants wore a dead-space mask with an impeller turbine assembly (Hans Rudolph, Kansas, USA) and gas concentrations were continuously sampled via a capillary line. Concentrations were determined by electrochemical (O_2) and infrared (CO_2) analysers (Vyaire CPX, Mettawa, Illinois, USA). Before each test, the gas analysers were

calibrated with gases of known concentrations (16% O_2 and 5% CO_2) and ambient air. The digital volume transducer was connected to the housing blower and calibrated automatically using high and low flow parameters. Throughout testing, a breath-by-breath analysis captured the participant's *f*, V_E, $\dot{V}CO_2$ and oxygen uptake $\dot{V}O_2$. Other breathing parameters that were calculated and recorded included $\dot{V}O_{2Peak}$, RER, VEQ ($V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$) and $PETCO_2$, $PETO_2$. Johnson et al reported averaging of data between 10 and 30-second is suitable to reduce noise within the data without removing the sensitivity of the data. [149, 150] Therefore, within this study, smoothing of the data was achieved by averaging the breath-by-breath data into 15-second intervals, alongside visual noise checks helped to reduced signal to noise ratio and identify errant breath. Heart rate responses were also collected via a wireless heart rate monitor (Polar S810i, Polar Electro, Finland). Participants also reported RPE within the final 30 seconds of each stage using Borg's 6-20 scale.[151] The $\dot{V}O_{2Peak}$ was obtained via the highest recorded $\dot{V}O_2$ value. The PPO was calculated with the following equation:

$$PPO = PO_{complete} + (t/SD * increment) \quad \text{(Equation 2).}$$

Where PO complete is the power output for the highest fully completed stage, *t* is the time (min) that the final (non-completed stage) was sustained if $t>0$, and SD is stage duration.

3.3.3. Threshold identification methods

Threshold identification was determined using a visual assessment of respiratory parameters plotted using Microsoft Excel (Excel, Microsoft, Redmond, Washington).

See *Table 4* for a list of the thresholds and identification methods. Visual assessment of the thresholds was performed by a single researcher, JT who had 2-3 years of experience working with and visually identifying ventilatory and lactate thresholds. The initial transition between the isotonic and isocapnic buffering phases was identified using two methods. Firstly, the VEQ method was combined with $PETO_2$. For this experimental chapter, thresholds identified via the VEQ method combined with $PETO_2$ will be referred to as VT1. The VEQ method identifies VT1 by the first rise in the $V_E/\dot{V}O_2$ without a rise in the $V_E/\dot{V}CO_2$.[4, 7, 77] Vincent *et al* advised that a non-linear rise in $V_E/\dot{V}CO_2$ after flattening off was the most accurate method to identify VT1.[4] Further study by Wasserman *et al* recommends the combination of the VEQ method with $PETO_2$ is preferable to avoid inaccurate identification of VT1 - a method widely adopted today.[24, 25] VT1 was therefore identified by tracing a collective rise in $V_E/\dot{V}O_2$ and $PETO_2$ without a subsequent rise in $V_E/\dot{V}CO_2$, demonstrated in *Figure 8A.*

Table 4. Summary of the thresholds and their associated method of identification.

Threshold	Method
VT1	VEQ method with $PETO_2$
GET1	V-Slope method
VT2	VEQ method with $PETCO_2$
RCP1	$V_E/\dot{V}CO_2$ Slope
VT3	VEQ method with $PETCO_2$
RCP2	$V_E/\dot{V}CO_2$ Slope

Secondly, the V-Slope method was used to determine the transition into the isocapnic phase. For the purpose of this experimental chapter, thresholds identified by the V-Slope method will be referred to as GET1. The V-Slope method depicts the interaction between two gas exchange parameters, $\dot{V}O_2$ and $\dot{V}CO_2$. This graphical display reflects the buffering of the bicarbonate system.[13] Following the modified V-Slope method, GET1 was identified through the first inflexion point due to $\dot{V}CO_2$ increasing more rapidly than $\dot{V}O_2$,[65, 77, 152] demonstrated in *Figure 8B.*

Two other methods were used when identifying the transition from isocapnic buffering to hypercapnia. Firstly, the VEQ method can be combined with $PETCO_2$ to identify the onset of hypercapnia. For the purpose of this experimental chapter, thresholds identified via the VEQ method combined with $PETCO_2$ will be referred to as VT2. The VEQ method can identify VT2 by a second rise in $V_E/\dot{V}O_2$ and the first non-linear increase in $V_E/\dot{V}CO_2$.[24, 25] Accuracy can be improved by cross-referencing this with a corresponding decrease in $PETCO_2$.[24] VT2 was therefore identified via a non-linear increase in $V_E/\dot{V}CO_2$ and a strong second increase in $V_E/\dot{V}O_2$, corresponding with a decrease in $PETCO_2$. Demonstrated in *Figure 8C.* Next, the $V_E/\dot{V}CO_2$ slope method was implemented. For the purpose of this experimental chapter, thresholds identified via the $V_E/\dot{V}CO_2$ method will be referred to as RCP1. Before reaching a state of metabolic acidosis, VE and $\dot{V}CO_2$ are closely coupled. As exercise intensity increases, there is a rise in $\dot{V}CO_2$ production following lactate buffering. Due to mechanical limitations preventing further rises in tidal volume, V_E increases to expel more CO_2 and compensate for the rise in CO_2 production.[59] An inflexion point can be identified where V_E rises out of proportion with $\dot{V}CO_2$, demonstrated in F*igure 8D.* The final transition between

a possible hypercapnic and hyperventilatory phase was identified by two different methods. First, building on VT2, the VEQ method, combined with $PETCO_{2,}$ was used to identify a later threshold. For the purpose of this experimental chapter, the threshold identified beyond VT2 via the VEQ method combined with $PETCO_2$ will be referred to as VT3. VT3 was identified via a second inflexion point in $EqCO_2$, a third increase in EqO_2, and a second corresponding decrease in $PETCO_{2,}$ demonstrated in F*igure 8E.* Lastly, the $V_E/\dot{V}CO_2$ slope method was implemented. For the purpose of this experimental chapter, thresholds identified beyond RCP1 via the $V_E/\dot{V}CO_2$ method, will be referred to as RCP2. RCP2 was identified via a second inflection point in V_E demonstrated in F*igure 8F*. Once all threshold assessments were complete the corresponding timepoint (s), power output (W), percentage of peak power output

(%PPO), and RER were determined for subsequent analysis.

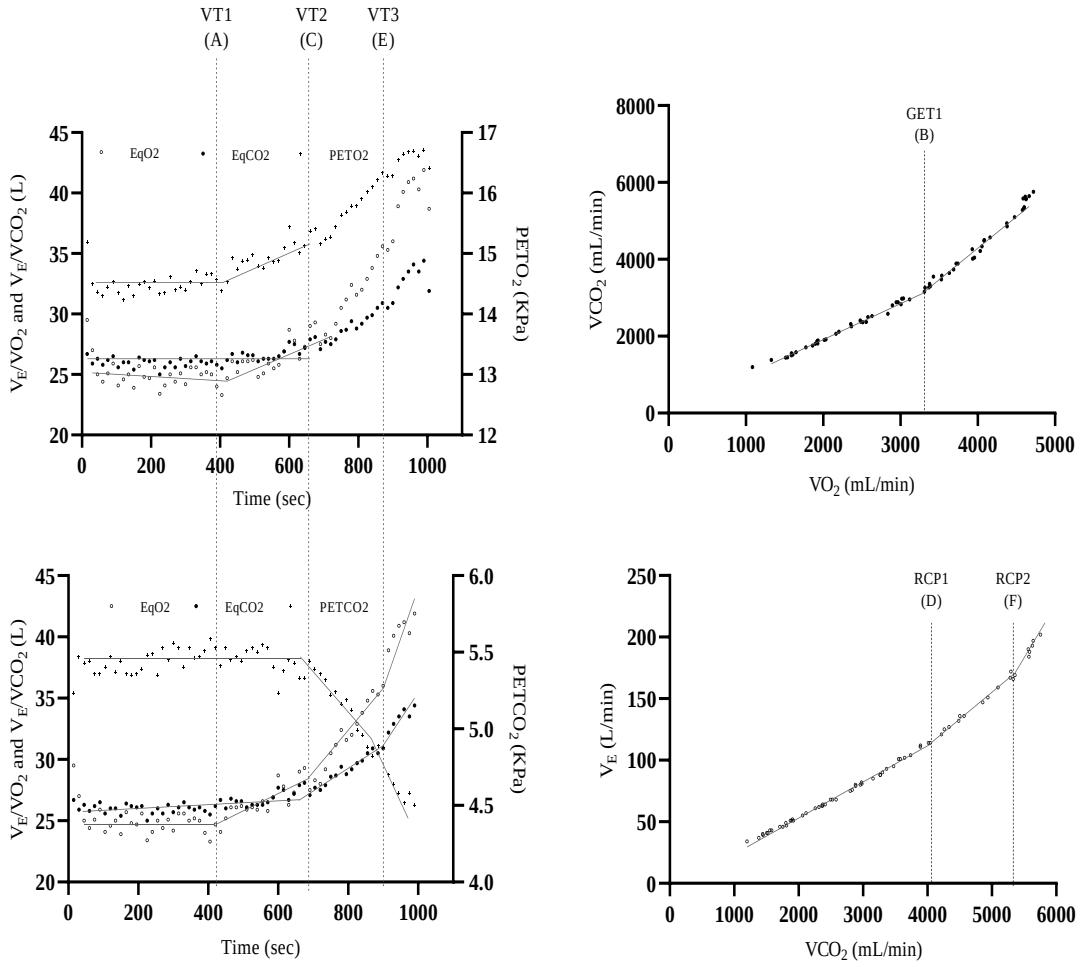

Figure 8. Gas exchange and respiratory responses to incremental stepwise cycle ergometer test graphically illustrated. A) Ventilatory equivalents (VEQ) Method combined with the end-tidal partial pressure of oxygen ($PETO_2$) identifying the first ventilatory threshold (VT1). B) V-Slope method identifying the first gas exchange threshold (GET1). C) The VEQ Method combined with the end-tidal partial pressure of carbon dioxide ($PETCO_2$) identifies the second ventilatory threshold (VT2). D) $V_E/\dot{V}CO_2$ Slope identifying first respiratory compensation point (RCP1). E) VEQ Method combined with $PETCO_2$ identifying the third ventilatory threshold (VT3). F) $V_E/\dot{V}CO_2$ Slope identifying the second respiratory compensation point (RCP2).

3.3.4. Statistical analysis

Descriptive statistics were reported as mean ± standard deviation. Initially, the Shapiro-Wilk test was applied to establish if data were normally distributed. Data for VT1, GET1, VT2, RCP1, VT3 and RCP2 were not normally distributed, and were log-transformed. Raw data values were back-transformed for illustrative purposes for graphs and tables.

Differences in the time, %PPO, power output (PO), and RER identified by each threshold identification method were assessed through a one-way within analysis of variance (ANOVA), followed by Bonferroni post hoc analysis. All data were checked for homogeneity of variance. If violations were present, they were adjusted via the Greenhouse-Geiser correction. Results with $p \leq 0.05$ were considered statistically significant. Effect sizes for time were analysed using Cohens D, defined as difference as mean/standard deviation. The Cohens D is categorised as small (0.2), medium (0.5) and large (0.8).[154]

The Bland-Altman tests include (1) a graphical representation (Bland–Altman plot) of the difference between Thresholds (VT1 vs GET1, VT2 vs RCP1 and VT3 vs RCP2) plotted against the mean of the two thresholds; (2) calculation of the mean of the difference between thresholds and 95% confidence intervals (CI); and (3) a measure of the limits of agreement (LOA) between the two thresholds, which is defined as d ± 1.96 x SDdiff, where d is the difference and SDdiff is the standard deviation of the differences. The 95% limits of agreement (95% LoA) were set at ±20W as this is deemed appropriate to distinguish biological change.[153] Heteroscedasticity of the plots

was assessed by calculating the correlation coefficient between the absolute difference and the average of threshold comparisons.

An independent t-test was run to assess performance parameters between groups that did and did not achieve either VT3 or RCP2. Equality of variance was assessed using Levene's test and effect sizes were analysed using Cohens D. All statistical analysis was completed using IBM SPSS Statistics (Version 28, Armonk, NY: IBM Corp) and the probability of a type one error was established with an alpha value $p>0.05$ to determine statistical significance.

3.4. Results

All participants satisfied at least one of the validation criteria required to confirm a state of exhaustion upon completing the incremental exercise test. VT1, GET1, VT2 and RCP1 were evident in all 32 participants. Among the 32 participants, 23 displayed either VT3 or RCP2. 19 participants presented VT3, 3 of which only presented VT3. 20 presented RCP2, 4 of which only presented RCP2.

The analysis of the cycle test data showed that there was a significant difference in time between the ventilatory thresholds as determined by one-way ANOVA ($F(5, 161) = 62.9$, $p<0.001$). A Bonferroni posthoc test revealed that VT1 occurred initially, following the onset of exercise, with GET1, VT2, RCP1, VT3 and RCP2 ($p<0.01$) occurring significantly later (*Table 5*). GET1 was determined significantly earlier than VT2, RCP1, VT3 and RCP2 ($p<0.01$). There was no significant difference between VT2 and RCP1 ($p=1.00$; $D=0.05$) or VT3 and RCP2 ($p=0.99$; $D=0.18$). However, VT2 and

RCP1 occurred significantly earlier than VT3 (p=0.01; D=1.71 and p<0.01; D=1.80, respectively) and RCP2 (p=0.04; D=1.44 and p=0.03; D=1.51, respectively).

Further analysis showed that there was a significant difference in PO between the ventilatory thresholds as determined by one-way ANOVA (F(5, 161) = 59.6, p<0.001). A Bonferroni posthoc test revealed the PO at VT1 was significantly lower than GET1, and both VT1 and GET1 occurred at a significantly lower PO to VT2, RCP, VT3, and RCP2 (p<0.01*)* (*Table 5*). There was no significant difference between VT2 and RCP1 (p=1.00) or VT3 and RCP2 (p=1.00*)*; however, VT2 and RCP1 occurred at a significantly lower PO to VT3 (p<0.001 and p<0.001 respectively) and RCP2 (p<0.01 and p<0.01 respectively).

Analysis of %PPO showed that there was a significant difference in %PPO between the ventilatory thresholds as determined by one-way ANOVA (F(5, 161) = 85.8, p<0.001). A Bonferroni posthoc test revealed the %PPO at VT1 was significantly lower than GET1, and the %PPO at VT1 and GET1 was significantly lower than VT2, RCP1, VT3, RCP2 (p<0.01*)* (*Table 5*). There was no significant difference between VT2 and RCP1 (p=1.00) or VT3 and RCP2 (p=1.00*)*. However, the %PPO at VT2 and RCP1 were significantly lower than VT3 (p<0.001) and RCP2 (p<0.001).

Lastly, the RER between ventilatory thresholds was also significantly different as determined by one-way ANOVA (F(4, 121) = 74.4, p<0.001). A Bonferroni post-hoc test revealed RER at VT1 was significantly lower than GET1, VT2, RCP1, VT3, RCP2 (p<0.001*)* (*Table 5*). GET1 was significantly lower than VT2, VT3 and RCP3 (p<0.001*)* and to RCP1 (p=0.022*).* There was no significant difference between VT2 and RCP1

(p=0.946) or VT3 and RCP2 (p=1.000*)*. however, the RER at VT2 and RCP1 were significantly lower than VT3 (p<0.001) and RCP2 (p<0.001).

Table 5. Mean (± standard deviation) time, percentage of peak power output (%PPO), power output (PO) and respiratory exchange ratio (RER) identified at the first, second and third ventilatory threshold (VT1, VT2 and VT3, respectively), the first gas exchange threshold (GET1) and the first and second respiratory compensation point (RCP1 and RCP2, respectively).

	VT1 (n=32)	GET1 (n=32)	VT2 (n=32)	RCP1 (n=32)	VT3 (n=19)	RCP2 (n=20)
Time (s)	324.9 ± 135.2*	457.4 ± 161.4[ɪ]	673.0 ± 138.4[$]	665.9 ± 131.3[$]	927.5 ± 157.8[#]	898.3 ± 172.7[#]
%PPO	40.6 ± 11.7*	52.5 ± 12.5 [ɪ]	72.0 ± 8.4[$]	71.4 ± 8.4[$]	91.1 ± 3.8[#]	89.9 ± 4.6[#]
PO (W)	144.8 ± 50.3*	186.0 ± 52.0[ɪ]	256.1 ± 51.6[$]	254.1 ± 51.3[$]	332.0 ± 34.8[#]	331.3 ± 43.3[#]
RER	0.83 ± 0.07*	0.93 ± 0.06[ɪ]	1.01 ± 0.07[$]	0.99 ± 0.07[$]	1.14 ± 0.07[#]	1.14 ± 0.09[#]

*Denotes VT1 is significantly different to GET1, VT2, RCP1, VT3, and RCP2. [ɪ]Denotes GET1 is significantly different toVT1, VT2, RCP1, VT3, and RCP2. [$]Denotes VT2 and RCP1 are significantly different to VT1, GET1, VT3 and RCP2. [#]Denotes VT3 and RCP2 are significantly different to VT1, GET1, VT2 and RCP1.

Table 6. A comparison martix demonstrating the effect size between the time thresholds were identified

	VT1	GET1	VT2	RCP1	VT3	RCP2
VT1		0.89	2.54	2.56	4.10	3.70
GET1			1.43	1.42	2.95	2.64
VT2				0.05	1.71	1.44
RCP1					1.80	1.51
VT3						0.18
RCP2						

3.4.1. Assessment of bias

There was systematic bias between VT1 and GET1 (bias = -41.2 ± 43.3: CL95% = -126.0 – 43.4), exceeding the LoA set (±20W) to distinguish differences between threshold identification methods.(*Figure 9*) This demonstrates that the PO identified at GET1 is greater than that identified at VT1. No presence of heteroscedasticity in the data from figure 9 (P=0.96). The difference between VT2 and RCP1 was substantially less than the set limits of agreement (±20W), (bias = 2.00 ± 13.9W: CL95% = -25.2 – 29.2) (*Figure 10*). No presence of heteroscedasticity in the data from figure 10 (P=0.89). There was very little difference between VT3 and RCP2, with VT3 occurring at a comparable PO to RCP2 (Bias = 0.36 ± 16.73W: CL95% = -32.41 – 33.16) (*Figure 11*). Figure 12 then shows there was no presence of heteroscedasticity in the data from Figure 11 (P=0.31).

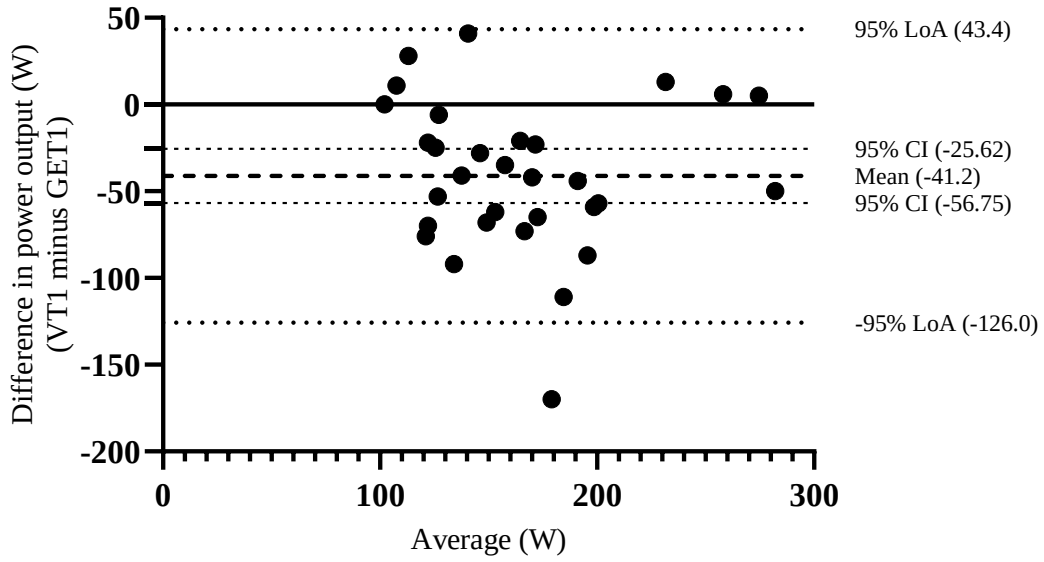

Figure 9. Bland-Altman plots for the difference between the power output (PO) at the first ventilatory threshold – the first gas exchange threshold (VT1–GET1 (n=32)). The two sets of dotted horizontal lines represent the limits of agreement (95% LoA) (1.96 SD) and 95% confidence intervals (95% CI.)

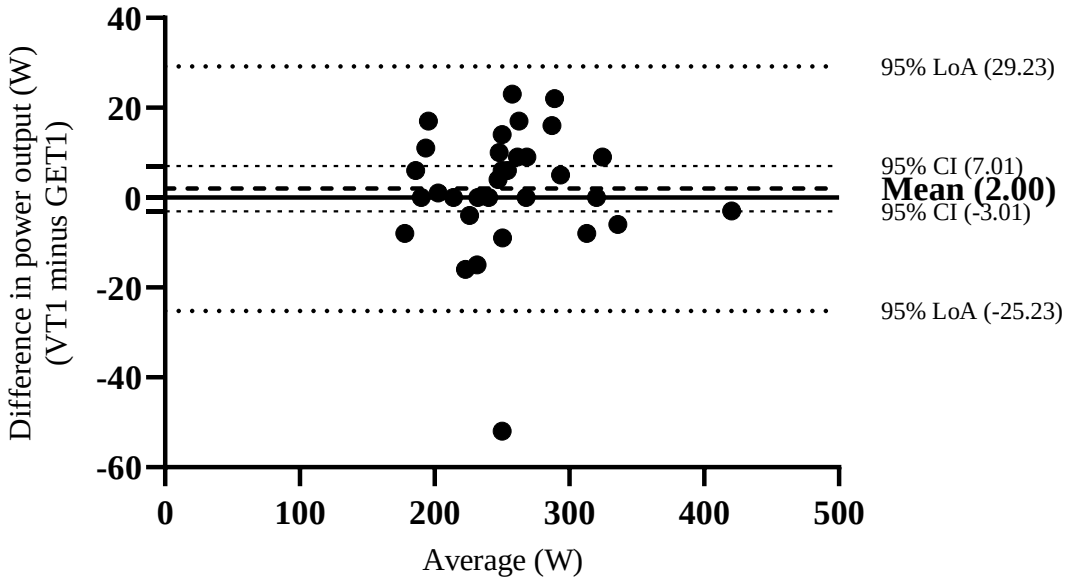

Figure 10. Bland-Altman plots for the difference between the power output (PO) at the second ventilatory threshold-first respiratory compensation point (VT2 – RCP1 (n=39)). The two sets of dotted horizontal lines represent the limits of agreement (95% LoA) (1.96 SD) and 95% confidence intervals (95% CI.)

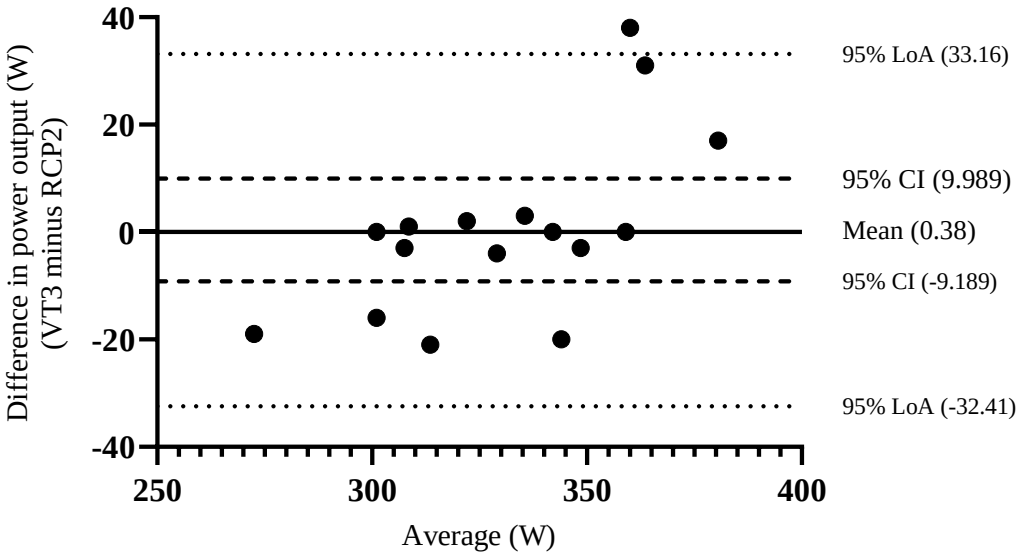

Figure 11. Bland-Altman plots for the difference between the power output (PO) at the third ventilatory threshold – the second respiratory compensation point (VT3 – RCP2 (n=20)). The dotted horizontal lines at the ends represent the limits of agreement (1.96 SD), the dashed line representing 95% confidence intervals (95%CI)

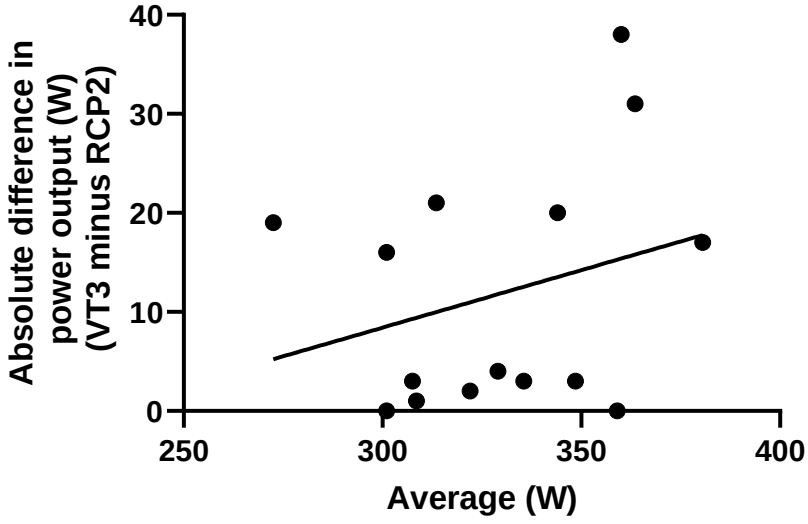

Figure 12. The heteroscedasticity plot of figure 11 for the difference in power output in watts (W) at the third ventilatory threshold – the second respiratory compensation point (VT3-RCP2).

3.4.2. Comparison of performance parameters between those with and without VT3/RCP2 present

Participant demographics and maximal performance variables, including the P:Wt, TTE, PPO, $\dot{V}O_{2Peak}$ and RER, were dichotomously split between those that presented VT3/RCP2 and those that did not. In those with VT3/RCP2 present P:Wt, TTE and PPO were greater than those without VT3/RCP2 (*Table 7).* However, no difference was observed between participants with VT3/RCP2 and those without for $\dot{V}O_{2Peak}$ and RER.

Table 7. Descriptive statistics and maximal performance variables between participants did and did not present the third ventilatory threshold or second respiraotry compensation point (VT3 or RCP2).

	Group			
	VT3/RCP2 Present	No VT3 or RCP2	p	d
N	23	9	-	-
Age (years)	41.5 ± 15.0	55.0 ± 9.4	0.01	1.0
BMI ($kg \cdot m^2$)	24.1 ± 2.5	25.2 ± 1.6	0.12	0.5
P:Wt (W·kg)	4.7 ± 0.7	4.2 ± 0.6	0.02	0.8
TTE (s)	996.5 ± 173.0	877.5 ± 148.1	0.02	0.8
PPO (W)	348.8 ± 63.3	306.9 ± 54.9	0.01	1.0
$\dot{V}O_{2Peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	51.63 ± 9.0	46.08 ± 6.8	0.10	0.5
RER	1.18 ± 0.07	1.17 ± 0.07	0.24	0.3

(Mean± SD). n=number of participants, BMI = body mass index, P:Wt = power to

weight ratio, TTE = time to exhaustion, PPO = peak power output, $\dot{V}O_{2Peak}$ = peak volume of oxygen utilisation, RER = respiratory exchange ratio.

Furthermore, comparisons between the time, PO, %PPO and RER identified at VT1, GET1, VT2 and RCP1 were compared between participants that did and did not present VT3/RCP2 (*Table 8*). For time, no difference was observed between the two groups at VT1 (p=0.17 d=0.34), GET1 (p=0.40, d=0.92), VT2 (p=0.30 d=0.19) or RCP1 (p=0.21, d=0.28). Similarly, for PO, no difference was observed between the two groups at VT1 (p=0.30 d=0.21), GET1 (p=0.39, d=0.11), VT2 (p=0.28 d=0.23) or RCP1 (p=0.24, d=0.28). The %PPO for those that presented VT3/RCP2 was significantly lower at VT1 (p=0.04, d=0.7), GET1 (p=0.05, d=0.7), VT2 (p=0.04, d=0.7) and RCP (p=0.05, d=0.7) compared to those that did not present VT3/RCP2. No difference was observed for RER at VT1 (p=0.19, d=0.4) or RCP1 (p=0.1, d=0.5). The RER at GET1 and VT2 were significantly lower for those that presented VT3/RCP2, compared to those that did not (p=0.04, d=0.7 and p=0.004, d=1.1, respectively).

Table 8. Time, power output (PO), percentage of peak power output (%PPO) and respiratory exchange ratio (RER) achieved by those that did present a third ventilatory threshold/second respiratory compensation point (VT3/RCP2) and those that did not present VT3/RCP2 at various respiratory thresholds. (Mean ± SD)

	Threshold identification method									
	VT1		GET1		VT2		RCP1		VT3	RCP2
	VT3/RCP2 present (n=23)	VT3/RCP2 not present (n=9)	VT3/RCP2 present (n=23)	VT3/RCP2 not present (n=9)	VT3/RCP2 present (n=23)	VT3/RCP2 not present (n=9)	VT3/RCP2 present (n=23)	VT3/RCP2 not present (n=9)	Present (n=19)	Present (n=20)
Time (s)	312.7±105.9	356.0±169.1	451.2±174.6	473.4±129.3	681.8±132.4	650.4±158.7	676.8±128.4	638.2±142.3	927.5±157.8	898.3±172.7
PO (W)	96.3±13.0	120.3 ±20.31	128.5±26.0	142.3±12.9	177.8±21.6	202.3±49.8	169.0±28.1	190.0±53.7	332.0±34.8	331.3±43.3
%PPO	38.3±9.4*	46.6±15.1	50.0±12.7*	58.8±10.1	70.3±7.3*	76.2±10.0	69.9±7.2*	75.3±10.2	91.1±3.8	89.9±4.6
RER	0.84±0.07	0.81±0.06	0.92±0.06*	0.96±0.05	0.98±0.06*	1.06±0.07	0.98±0.08	1.02±0.06	1.14±0.08	1.14±0.02

*Denotes significant difference between ‘VT3/RCP2 present’ vs ‘VT3/RCP2 not present’.

3.5. Discussion

3.5.1. The first ventilatory threshold and the first gas exchange threshold

The primary aim of this study was to compare the methods used to identify VT1, GET1, VT2 and RCP1 during a maximal incremental cycle test. Overall, VT1 occurred at a mean %PPO of 40.6%, with GET1 occurring at a significantly greater %PPO of 52.5%. There was no significant difference between VT2 and RCP1, but they had a significantly greater %PPO (72% and 71.4%, respectively) than VT1 and GET1. The liturature suggests that VT1 and GET1 reflect the transition from the isotonic to isocapnic buffering phase.[64, 65] This study demonstrated that %PPO, PO, timepoint and RER were greater for GET1 compared with VT1.

Whilst contradicts what is suggested within the liturature, the disparity between VT1 and GET1 is not unheard of. Previous comparisons of VT1 and GET1 with two groups of runners ($\dot{V}O_{2Peak}$ 56.4 - 72 $ml \cdot kg^{-1} \cdot min^{-1}$ and 40 - 51 $ml \cdot kg^{-1} \cdot min^{-1}$) revealed that the lower $\dot{V}O_{2Peak}$ group reported no difference between $\dot{V}O_2$, speed and HR at VT1 and GET1, demonstrating consistency across methods.[71] However, the higher $\dot{V}O_{2Peak}$ group reported a greater $\dot{V}O_2$ and speed at GET1 when compared to VT1. This suggests an increased agreement across VT1 and GET1 among individuals with lower cardiorespiratory fitness and arguably less trained.[71] The $\dot{V}O_{2Peak}$ and difference between VT1 and GET1 reported in this study are comparable to the $\dot{V}O_{2Max}$ of participants demonstrating a gap between VT1 and GET1.[71] This provides some justification for the significant difference reported in this study. Additionally, a retrospective analysis investigated the notion of a double threshold, whereby VT1 and GET1 are independent.[155] A double threshold was identified when the difference

between $\dot{V}O_2$ at VT1 and GET1 was greater than 15mL·min. 11% (51 of 476 participants) reported a double threshold. Moreover, participants with a double threshold reported a greater $\dot{V}O_2$ during the isocapnic buffering period before VT2. The increased $\dot{V}O_2$ could result from greater exercise tolerance and aerobic fitness; however, no definitive theory exists to explain this. Research often pairs VT1 and GET1 together as synonymous thresholds. Therefore, the disparity between VT1 and GET1 could result from methodological flaws (*Limitations 3.4.1*). Whilst the modified V-Slope method is a valid method to identify GET1, it has previously been reported to overestimate the first threshold compared to the computerised V-Slope method, which could also attribute to the difference between VT1 and GET1 within this study. Gaskill *et al* subsequently tested this and demonstrated a strong agreement between VT1, GET1 (using the modified V-Slope method) and lactate threshold (2mmol·lL).[7] Protocol type could also influence threshold identification accuracy.

Shimizu *et al* compared the identification of VT1 (via the VEQ method only), VT1 (via the $PETO_2$ method only) and GET1 (V-Slope) across three cycle test (two stepwise protocols of two-minute stages, one increasing by 25W and one by 50W and an individualised ramp protocol) and three different treadmill test; the Bruce (starting at 10% gradient at 1.7 miles per hour (mph) and increasing by 2% increment at 1.8mph every three minutes).[156] The modified Balke protocol (starting at two mph at 0% gradient for two minutes, increasing by 3% for the second stage, and increasing by 2.5% after that every two minutes whilst remaining at a constant speed of 2mph) and an individualised ramp protocol, lasting around 10 minutes. Overall, protocol type influenced threshold variability by 36%, but the V-Slope method was reported to be

least varied across the differing protocols. However, the V-Slope method had the highest number of un-interpretable cases across all the treadmill test and cycle tests. The VEQ method, on the other hand, was the most identifiable. Despite this, no significant difference was reported between the identification of VEQ and V-Slope methods. $PETO_2$ method occurred at significantly lower relative and absolute $\dot{V}O_2$ values. Subsequently, combining the VEQ and $PETO_2$ methods in this study could further explain the lower VT1 value identified.[157] However, this study was conducted on men with heart disease and healthy men with a mean age of 60 years. Consequently, the participant pool had a lower trained status which would have influenced the prevalence of thresholds comparatively to trained individuals. This could explain the limited variability across identification methods with other research attributing disparity between methods with those of higher trained status. [155] It is also important to note that the differences between thresholds were only identified within the treadmill test protocols. The cycle test protocols did not demonstrate any differences in threshold identification, with the treadmill test identifying VT1/GET1 significantly later than cycle tests.[157] This suggests that whilst variability is expected across threshold identification methods, identifying the first threshold is more consistent within cycle tests over treadmill test. This highlights that the thresholds are not comparable across different exercise modalities, specifically with threshold identification in a cycle test not being transferable to a treadmill test or within running.

3.5.2. The second ventilatory threshold and the first respiratory compensation point

There were high levels of agreement between VT2 and RCP1, suggesting they reflect the transition point from isocapnic to hypercapnic buffering. Both identification methods are commonly used interchangeably to distinguish this transition point.[135] There was no significant difference between the %PPO, PO, RER or the time each threshold was identified, reinforcing that the two methods identify the same threshold. VT2 and RCP1 are also closely associated with the second lactate threshold, which equates to a blood lactate level of 4mmol·L.[8] Once blood lactate levels exceed 4mmol·L, clearing and buffering systems cannot maintain the homeostatic blood pH level of around 7.4, marking the onset of metabolic acidosis. RCP1 is hypothesised to be mostly stimulated by chemoreceptors.[8] Therefore, the onset of metabolic acidosis results in the rise in V_E, instigating an exercise-induced hyperventilatory response (*Chapter 2.10 Respiratory compensation point and the third ventilatory threshold*).

Within the literature, there have been a few occurrences of RCP1 exceeding and overestimating comparable thresholds.[9] Ozkaya *et al* demonstrated a separation between the drop in $PETCO_2$ at VT2, followed by a grey phase before the rise in V_E was reported. Ten recreationally trained ($\dot{V}O_{2Max}$=43.2±3.5mL·min^{-1}·kg^{-1}) and ten well-trained (66.8±7.8mL·min^{-1}·kg^{-1}) athletes completed incremental ramp tests to exhaustion. A new computational method combining $PETCO_2$ with V_E against time (termed ‘respiratory threshold) was then applied to assess if RCP1 was an independent third threshold to VT2. Through this novel method, RT1, RT2 and RT3 were identified. RT1 was identified as akin to VT1 and GET1, RT2 was akin to VT2, and RT3 was akin

to RCP1. However, RCP1 was only observed at RT3 with well-trained athletes, suggesting RT3 is subject to trained status. It is plausible that in the present study, participants' aerobic capacity was insufficient to identify the delayed RCP1 response, hence why VT2 and RCP1 report minimal bias. Moreover, the ramp protocol implemented by Ozkaya *et al* results in a relatively fast increase in work rate of $+1W \cdot 2s^{-1}$ or $30W \cdot min^{-1}$, resulting in greater initial oxygen deficit and accelerated lactate accumulation [68, 158], intensifying ventilatory stimulation. The central chemoreceptors that stimulate changes in ventilation following CO_2 and pH also reportedly mediate 50% to 90% of ventilatory responses during hypercapnia.[138, 139] These central chemoreceptors also have significantly slower response times than peripheral chemoreceptors.[139] Therefore, a rapidly increasing protocol would promote faster lactate accumulation and result in a deferred hyperventilatory response, occurring later than other measured ventilatory parameters. However, knowledge of the mechanisms driving ventilation and hyperventilation during exercise is contested and yet to be fully understood.[159, 160] Wasserman *et al* stated that the receptor-stimulated response to pH decline increases ventilatory stimulation, increasing $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ within the VEQ method and a second rise in $PETO_2$ and a decrease in $PETCO_2$ to prevent further falls in pH. [24, 25, 68, 71] The purpose of physiological homeostasis is to maintain an optimal internal environment mainly through negative feedback initiating coordinated equal and opposite reactions. Therefore, delayed hyperventilatory responses, that usually occur alongside the drop in $PETCO_2$ and are attributed to the identification of VT2 are likely to correspond with the rise in V_E seen when identifying RCP1. However, this does not discount the notion of a later hyperventilatory response, occurring later

than VT2 and RCP1 and coordinating with the third threshold reflected in the three $PETCO_2$ inflexions. Lastly, the stepwise method applied in the present study allowed for periods of steady state, reducing the likelihood of a delayed ventilatory response, justifying why there was no difference between VT2 and RCP1. Whereas studies implementing faster-progressing protocols, comparable to Ozkaya *et als* protocol, would increase the possibility of RCP lagging behind VT2.[68, 91, 99, 138, 139] Protocol type should also be adjusted and tailored to the age, experience and type of athlete or participant being assessed. For example, a submaximal ramp protocol could be preferable within some clinical or less fit individuals, as it is perceived as less intimidating and more tolerable with the shorter duration and no sudden work rate changes.[161]

3.5.3. The third ventilatory threshold and the second respiratory compensation point

The secondary aim of this study was to assess the presence of additional breakpoints beyond VT2 and RCP1. Following applying the VEQ method with $PETCO_2$ and $V_E/\dot{V}CO_2$ slope method, 72% of participants presented additional breakpoints beyond VT2 and RCP1 via two different methods (VT3 and RCP2). Consistent threshold identification at a similar time point between the two methods supports the notion of a third breakpoint occurring within the severe exercise intensity domain. A third (respiratory) threshold was initially reported by Ozkaya *et al*, following claims of RCP1 overestimating VT2. The authors report a lag between the hypercapnic drop in $PETCO_2$ and the subsequent rise in V_E was only apparent in well-trained athletes. The well-trained athletes reported an average work rate at the third respiratory threshold (RT3)

of 324W±31.3 (~85% of PPO). By comparison, the present study reports a relative exercise intensity at VT3 of 91% and RCP2 at ~90% PPO, suggesting that RT3, previously likened to RCP1 by Ozkaya, is more closely associated with VT3 and RCP2 in the current study. Furthermore, VT2 and RCP1 in the present study occurred at ~71% PPO, a considerably lower relative intensity.

3.5.4. The prevelance of a third threshold and trained status

The final aim of this study was to investigate the relationship between performance and the prevalence of ventilatory thresholds. Those who presented VT3/RCP2 (*n*=23) demonstrated significantly greater P:Wt, TTE and PPO than those without VT3/RCP2. Conversely, $\dot{V}O_{2Peak}$ was not different between groups; however, a meaningful difference was observed of 12% in $\dot{V}O_{2Peak}$ (*d*=0.5). These results suggest that the threshold beyond RCP1 will likely be present in cyclists with a higher performance capability. An athlete's performance status can be categorised into different levels depending on the maximal parameters achieved during an incremental exercise test. An example is De Pauw *et al*, who presented a classification method offering five performance levels (1=untrained;5=professional/elite cyclists).[162] Overall, those with VT3/RCP2 present could be classified as level 3 (trained or competitive). In contrast, those without VT3/RCP2 are considered level 2 (physically active), further supporting the notion that VT3/RCP2's prevalence depends on the athlete's trained status. Athletes' performance levels result from genetic predisposition and physiological adaptations incurred from training. Those of a greater trained status is likely to have; increased mitochondrial density, capillary density, increased enzyme and protein activity, and enhanced motor recruitment of type 1 and type 2 oxidative muscle fibres, all of which

provide a physiological advantage. These adaptations and others will increase aerobic capacity and improve mechanisms that process H^+ and lactate build-up, optimising the ability to deal with exercise-induced metabolic stress.(163)

There was a significant disparity between the two dichotomously split groups of participants, with those who presented VT3/RCP2 approximately 13 years younger. Age can significantly impact participants' fitness and performance levels. Masters cyclists have previously reported a 10% and 16% decline in absolute and relative $\dot{V}O_{2Max}$ when comparing 35-44-year-olds with 45-54-year-olds. (162) Similar findings were found when comparing PPO between age groups. The authors concluded that fitness levels were closely associated with age in masters cyclists and should not be categorised in the same fashion or with similar classification standards as younger cyclists.(162) Despite the disparity in age reported between groups within the present study, the performance differences between the two groups still indicate that performance could contribute to the prevalence of VT3/RCP2.

It is also reported that %PPO and RER were different at submaximal (VT1, GET1, VT2 and RCP1) between participants with and without VT3/RCP2 present. The %PPO was lower at VT1 (8%), GET1 (9%), VT2 (6%) and RCP (5%) for those that presented a VT3/RCP2. Moreover, the RER at GET1 and VT2 were also lower, with the group presenting VT3/RCP. Lower %PPO and RER at submaximal thresholds indicate a greater physiological capacity and augmented metabolic efficiency.(51) If an athlete's %PPO is lower at a given threshold, there is a greater range beyond that threshold until maximal capacity is reached. A participant with a greater %PPO has a lower capacity beyond that threshold and will therefore reach volitional exhaustion sooner. This point

is reinforced by the lower PPO and TTE seen with those who did not present VT3/RCP2.

Similarly, exhibiting a lower RER reflects greater metabolic efficiency. At VT2, the RER for the group that presented VT3 was approximately 0.08 lower than those without VT3. Within endurance cycling, well-trained cyclists have been shown to oxidise fewer carbohydrates. They rely more on lipid oxidisation than less trained individuals at the same absolute PO or oxygen uptake [(164)] when in the presence of higher glycogen availability within the muscles.[(165)] A lower RER indicates greater metabolic efficiency, as it suggests prolonged utilisation of O_2 to metabolise substrates, which yields greater net adenosine triphosphate (ATP), with less fatiguing by-products produced, more akin to more anaerobic dominant intensities. When RER rises above 1.0, ATP production predominantly results from anaerobic metabolic pathways. Therefore, maintaining a lower RER (less than 1.0) reflects the ability to oxidise fat effectively.[(166)] Depletion of glycogen stores is a direct factor of fatigue within exhaustive cycling.[(167)] Therefore, sustaining a lower RER at higher exercise intensities indicates a reliance on lipid utilisation via oxidative phosphorylation, contributing to increased endurance capacity.

Overall, those that did not present VT3/RCP2 appear to be less trained. Within this study, the group that presented VT3/RCP2 were younger, demonstrated maximal parameters comparable with 'trained athletes' [(168)] and had a greater physiological capacity. Attaining and maintaining higher P:Wt, PPO, TTE, and $\dot{V}O_{2Peak,}$ places greater metabolic stress and pressure on physiological mechanisms to regulate homeostasis. Therefore, it could be considered that the additional VT3/RCP2 response is only achievable by those who can reach, sustain and tolerate such exercise intensities.

Suggestively, VT3/RCP2 could reflect a more extreme physiological response, instigated by more extreme exercise-induced hyperventilation as a result of a severe drop in pH and excessive CO_2 and lactate accumulation, subsequently provoking unmanageable levels of exercise stress and metabolic acidosis.

3.5.5. Limitations and future recommendations

Limitations within this study are attributed to the methods of threshold identification. The visual identification of VT1 and GET1, whilst widely accepted throughout the literature,[71, 169] is largely subjective and sometimes unreliable. Irregular breathing, minimal ventilatory stimulation, and inappropriate workload increments all increase the likelihood of inaccurate threshold identification. To avoid this, as was adopted in the present study, it is not uncommon for raw data to be smoothed and averaged rather than breath-by-breath assessment.[77] Moreover, cross-referencing the VEQ method with PET variables in the present study further informed the threshold identification point, increasing identification confidence. In future studies, applying computerised methods, in conjunction with visual analysis for all threshold analysis, could optimise threshold identification accuracy,[79] which should improve agreement between thresholds and remove subjective bias.

Overall, the findings of this study highlight a lack of agreement between the initial thresholds (VT1 and GET1); however, the identification of VT2 and RCP1 reinforces the notion that these secondary thresholds reflect the same transition point and are not two independent thresholds. Furthermore, the prevalence of a third threshold, identified via two independent methods, was repeatedly identified within cyclists, reported as VT3 and RCP2. Moreover, identification of VT3/RCP2 was seemingly dependent on age and

the trained status of the athletes, with those classified that presented VT3 or RCP2 being of a better-trained status. With regards to the interrater reliability, A 45-second interval was used to distinguish agreement between investigators when identifying thresholds. Whilst this was an arbitrary number chosen, researchers agreed that 45 seconds was justified by being less than half-stage duration, but still allowing for some variation of the transitionary nature where a threshold occurs. Future research should aim to identify an accurate agreement of time specific to the protocol implemented

Despite confidence in these initial findings, the test-retest reliability of threshold detection at RCP2 and VT3 is unknown. Therefore, such research would serve as a useful process to confirm the presence of these thresholds and inform the meaningful physiological changes required to encourage or remove the prevalence of VT3 and RCP1 following a training or de-training period.

3.6. Conclusion

Overall, this study provides important evidence of no difference between VT2 and RCP1 across all participants. However, there is evidence of a third breakpoint beyond VT2. These third breakpoints are identifiable via two different methods, drawn from previously established threshold identification methodologies. Moreover, those with a third threshold also had better performance parameters, complementing the broader literature and demonstrating contrasting physiological responses between untrained and trained populations. However, a third breakpoint was only identified by one researcher. Surrounding literature suggests the preferred protocol for visual identification involves multiple researchers independently and blindly identifying thresholds, followed by a comparison of the results. Alongside assuring interrater reliability, test-re-test reliability

is needed to determine whether the breakpoints identified are a threshold reflecting a physiological transition point or sporadic physiological response to another stressor/stimulus. Moreover, expanding the investigation into other exercise modalities would be helpful to see whether a third breakpoint is identifiable within other sports.

4. Inter-rater and test re-test reliability of ventilatory thresholds within healthy individuals

4.1. Abstract

While a third threshold has been successfully identified via multiple visual methods within cycling, its reliability, reproducibility, and prevalence in other sporting modalities have not yet been investigated. A laboratory-based analysis explored the interrater reliability and test re-test reliability of; the first ventilatory threshold (VT1), first gas exchanged threshold (GET1), second ventilatory threshold (VT2), first respiratory compensation point (RCP1), third ventilatory threshold (VT3) and second respiratory compensation point (RCP2). 20 active participants completed either two maximal stepwise CET (n=7), or TT (n=13), two to four days apart.

Following the cycle and treadmill test, moderate to excellent interclass correlation (ICC) were reported between raters after independent analysis of thresholds (r=0.69-0.99). Following collaborated analysis of discrepancies in thresholds between raters, an excellent ICC was demonstrated (r=0.96-1.00).

Of the total 14 cycle tests, VT3 and RCP2 were identified 12 and 11 times, respectively, with 6 of the 7 participants identifying a third threshold in both visits. The test re-test reliability demonstrated no significant difference between tests for all thresholds apart from RCP1 (p=0.02). The test re-test reliability between visits reported a moderate to excellent ICC (r=0.71-0.95) across all thresholds.

Of the 26 treadmill tests, VT3 and RCP2 were identified five and three times, respectively, but only repeatedly identified in two participants. The test re-test

reliability demonstrated no significant difference between tests for all thresholds apart from GET1 (p=0.04). The test re-test reliability between visits reported a moderate to excellent ICC (r=0.72-0.97) across all thresholds.

The analysis of performance parameters between participants who exhibited VT3 or RCP2 and those who did not revealed distinct differences in physiological markers. Within the cyclist population, time to exhaustion (TTE), peak power output (PPO), and power-to-weight ratio (P:Wt) demonstrated a large effect size (ES=1.5-2.6), while body mass index (BMI) and peak oxygen consumption (VO_{2peak}) showed a moderate effect size (ES=0.5 and 0.6, respectively). Runners revealed a significant difference in age between the groups (p=0.04), while VO_{2peak} demonstrated a moderate effect size (ES=0.6). However, no significant differences were reported between the groups for TTE, peak velocity at VO_{2peak}, or BMI (p=0.12-0.28).

This study demonstrates that VT3 and RCP2 can be repeatedly and reliably identified in cyclist. Whereas VT3/RCP2 are less prevalent when undertaking a treadmill test. These findings also suggest that the presence of VT3 can be associated with important differences in performance and physiological characteristics, which may have significant implications for the training and conditioning of athletes.

Key words: Reproducibility, Reliability, Third threshold, Cycling, Running, Performance.

4.2. Introduction

Endurance athletes frequently determine exercise intensity domains/zones from data obtained during laboratory testing. Specifically, assessing ventilatory thresholds via incremental exercise testing can provide valuable information for developing training programs that optimize performance. By identifying these thresholds, athletes and coaches can establish training zones that target specific energy systems and promote improvements in an endurance athlete's aerobic and anaerobic capacity. The identification and application of these thresholds must be done validly and reliably.[13] Parameters such as $\dot{V}O_{2Max}$, PO, and $\dot{V}O_2$ at VT1 have reportedly shown high test-retest reliability.[13, 170] VT1 and VT2 are considered the 'gold standard' for identifying ventilatory thresholds[11] with a wide body of literature, especially in cycling, highlighting their high levels of validity and reliability.[11, 170-172] While running and cycling are both endurance sports, they are not interchangeable. One study identified that ventilation is impaired during cycling due to the seated posture, unlike running, which is upright, and therefore improves oxygen intake efficiency.[25] The two sports require different muscle recruitment and are reportedly impacted differently by fatigue.[173] The differing demands between the two modalities could instigate different physiological responses to incremental exercise, influencing the presence of submaximal thresholds. VT3/RCP2 identified within cyclists has been associated with metabolic acidosis and extreme hyperventilatory responses at severe exercise intensities. However, the lack of similarities between cycling to running suggests that whilst a third threshold is prevalent within cycling, the same hyperventilatory response and subsequent threshold identification cannot be assumed.

The identification of ventilatory thresholds throughout the literature often employs visual analysis of thresholds and has been recommended method of threshold identification within the Guidelines on cardio pulmonary exercise testing.[143] While these guidelines recommend a minimum of one experience observer identifying these thresholds,[143] further analysis has suggested the employment of multiple researchers independently and blindly assessing the respiratory data is preferable to reduce subjective bias within the analysis.[170, 174] While modern metabolic systems automatically assign a VT value at the end of each test, the multitude of different algorithms that define VT differently mean the identification of a threshold can vary for the same participant.[174] One study compared the reliability of different assessment methods for identifying thresholds and found that using two independent evaluators was the best visual method, involving two researchers who independently evaluated VT1 and VT2 and then collaborated to identify a final threshold. That method was deemed preferable to having three fully independent evaluators, calculating the mean of the three evaluators, and having one independent research assess the data twice four months later.[170] This was corroborated by Myers *et al* who reported the three-reviewer method demonstrated unacceptably high variability, concluding the optimal method to determine thresholds reliably is through two reviewers that are both blind to the participant and blind to the other reviewer. Following corroboration between the two reviewers, a third researcher should be employed when an agreement between the original two researchers cannot be reached.[174]

Weston *et al* investigated the reproducibility of VT1 and VT2 (using computerised V-Slope and RCP analysis and double-blind visual analysis using the VEQ method between 2 evaluators) in endurance-trained cyclists following two $30 W \cdot min^{-1}$ ramp

cycle tests.[171] Overall, high levels of reproducibility (r<0.85) were reported for $\dot{V}O_{PEAK}$, peak $V_{E,}$ $\dot{V}CO_2$, heart, and work rate. Moreover, VT2 (using the VEQ method) was highly reproducible among trained endurance athletes when reported relative to $\dot{V}O_2$, heart rate, and work rate. However, for VT1 (VEQ method), only heart rate was deemed highly reproducible, with $\dot{V}O_2$ and work rate at VT1 considered less reliable. Despite this, both VT1 and VT2 demonstrated no significant differences across the repeated tests, and the reliability of work rate and $\dot{V}O_2$ at VT1 can be considered moderate and good, respectively. The reliability of $\dot{V}O_{2Peak}$ at VT1 and VT2, when expressed as an absolute value (%$\dot{V}O_{2Peak}$), was reduced to moderately reliable (r=0.67-0.7).[171] ~~Similar findings have also been reported within the surrounding literature.~~ It is important to note the above study used a mixing chamber, assessing continuously expired gas averaged over 20 second period.[171] Despite this, other studies conducted by Prud'Home *et al* and Aunola *et al* reported similar findings which are discussed below. [170, 172]

It has previously been reported that VT1 has less pronounced deflection points than VT2.[77] Furthermore, the respiratory data was collected via a mixing chamber, which is not at sensitive as breath-by-breath analysis, potentially influencing the lower reproducibility seen within VT1.[171] Prud'Home *et al* comparing the reproducibility of VT1 and VT2 between subjects completing either two test re-test cycle or treadmill test found VT1 in the treadmill test was the least reproducible. However, the researchers attributed the low reproducibility to the treadmill test protocol rather than the VT1 reproducibility. Overall, the study concluded that both treadmill test and cycle test protocols were suitable for identifying VT1 and VT2 thresholds, further highlighting the importance of protocol on test reliability.[170] A more exaggerated respiratory response to incremental exercise at VT2 may partly explain the greater reproducibility of results at this threshold. While the validity and reliability of thresholds vary slightly across threshold identification methods, different protocols, and sporting modalities, generally, the results will be reliable.[170, 172, 175]

When comparing treadmill test and cycle test, the consensus is that treadmill tests result in greater $\dot{V}O_{2Max}$ (around 7-18% greater) than cycle tests.[176] This suggests that $\dot{V}O_{2Max}$

is subject to exercise modality and that results from an incremental cycle test are not interchangeable with results of an incremental run test.[173, 176] One theory surrounding the disparity between modalities relates to the greater muscle mass recruitment during running, alongside running being a more automatic and natural movement opposed to cycling.[173] However, this reported disparity in $\dot{V}O_{2Max}$ is not always consistent. Comparisons between 10 endurance runners and nine cyclists concluded that runners performed better on the treadmill test and cyclists on the cycle test.[177] Marko *et al* justifies this by comparing elite-level junior runners, cyclists, and swimmers.[178] Runners and cyclists' $\dot{V}O_{2Peak,}$ peak heart rates, and *f* were reported to be greater within the respective modality of the runner and cyclists. However, among the swimmers, the treadmill test elicited greater $\dot{V}O_{2Peak,}$ peak heart rate, and breathing frequency values.[178] Further studies support this, suggesting that the recruitment of specialised and non-specialised muscle groups that have developed as a result of training impacts $\dot{V}O_2$ utilisation.[177-179]

Research has shown that well-trained cyclists can achieve comparable $\dot{V}O_{2Max}$ values across two different modalities (cycle test and treadmill test); the same is not true for well-trained runners, who often achieve a far lower $\dot{V}O_{2Max}$ during a cycle test over a treadmill test.[180] However, the same trend has not been reported when comparing submaximal parameters. One study involving 14 well-trained volunteers reported that 1 minute on the cycle ergometer at 250W derived 28% of its energy anaerobically. Uphill running conducted at speed comparable to the 250W, deriving a similar overall energy expenditure, derived only 17% of its energy from anaerobic energy transfer. The difference in metabolic contribution would subsequently alter the intensity VT1, and

VT2 were identified.[181] Furthermore, the identification of thresholds depends on the underlying physiological mechanism, whether neurological or peripheral.[173] Therefore, responses to exercise stress may be more prominent within different identification methods, dependent on the demands of the sport. Early comparisons between cycle tests and treadmill tests reported no significant differences between modalities regarding submaximal thresholds.[23, 177] However, ~~using unestablished protocols,~~ these papers relied on %$\dot{V}O_{2max}$ to describe the intensity of the threshold identified, which is not the most reliable parameter to reference.[170-172] In another study, six competitive cyclists were compared to six varsity cross-country runners. The cyclists achieved a comparable $\dot{V}O_{2Max}$ across the cycle test and treadmill test, whereas the runners' $\dot{V}O_{2Max}$ was significantly higher during the treadmill test than during the cycle test. The cyclists' and runners' lactate and VT occurred significantly later within the incremental test that aligned with their preferred modality.[180] The delayed onset of the lactate or VT reflects the physiological adaptations that have occurred to optimise performance within their specialised sport.[180, 182] These specialised adaptations are not optimal outside their sport, highlighting the limited transferability between different exercise modalities. Especially since cycling requires nuanced skills, different muscle recruitment patterns, and coordination, which can limit its transferability compared to running, a more familiar movement.[167, 173, 180] A study compared the ventilatory patterns of 22 trained men (runners and triathletes) following a maximal treadmill test and a maximal cycle test. The results demonstrated that the ventilatory equivalents, oxygen saturation, and end expiratory and inspiratory lung volumes were greater within the cycle test, alongside increased arterial. These findings suggest that the ventilatory

patterns differ between exercise modalities, and the difference may potentially be due to the posture required during cycling.[25] In addition, ventilatory limitations within cycling, including moments of hyperventilation being frequently reported among cyclists compared to runners.[25, 183] Furthermore, cycling derives greater fuelling from anaerobic forms of metabolism, leading to lactate build-up, pH decline, and greater pressure on buffering mechanisms.[181] Overall, within the literature, there is evidence that thresholds are inconsistent across participants of different trained statuses within the same sporting modality. Further research has demonstrated additional differences in submaximal threshold and maximal parameters between different sporting modalities, specifically between cycling and running. While VT3/RCP2 has been identified within cyclists, the more anaerobic nature of cycling is likely to invoke greater levels of fatigue and metabolic acidosis, compared to running which is more aerobically driven. Currently, it is hypothesised that VT3/RCP2 is associated with severe exercise intensities and metabolic acidosis. Thus, the more aerobic nature and differing metabolic demand of running may also impact the identification and overall prevalence of VT3 and RCP2 within treadmill test. Further research is required to assess if a third threshold can be identified within running as it can be in cycling.

In the first experimental chapter, VT3 and RCP2 thresholds were apparent among cyclists of a higher trained status (*Table 7)*. Cyclists who presented these thresholds achieved greater power output and longer time to exhaustion, indicating greater exercise intensities and levels of exercise stress. Despite this, no significant difference was found between $\dot{V}O_{2Max}$ and the prevalence of VT3 and RCP2. However, a general trend suggested that those who achieved VT3/RCP2 had a greater $\dot{V}O_{2Max}$. Therefore,

identifying these later thresholds may be more prominent within well-trained and elite runners following treadmill tests if a higher $\dot{V}O_{2Max}$ can be achieved.

4.2.1. Research aims

The primary objective is to evaluate the agreement and interrater reliability between researchers when identifying VT1, GET1, VT2, RCP1, VT3, and RCP2 during cycle test and treadmill tests. The secondary objective is to investigate each threshold's test-retest reliability and determine whether VT3 and RCP2 are consistently identified across two visits in both cycle tests and treadmill tests. The third aim of this study is to compare submaximal and maximal performance parameters between cycle test and treadmill tests and to examine the prevalence of VT3 across different endurance modalities. Finally, the study aims to evaluate the performance differences between participants who presented VT3 and those who did not in both modalities.

The study's findings will have important implications for exercise physiology, as they will help better understand the mechanisms underlying VT3 and RCP2 identification and their relationship to submaximal and maximal performance. Furthermore, the study will provide insights into the reliability and comparability of cycle test and treadmill tests and the implications of VT3 on exercise performance.

4.3. Methods

4.3.1. Participants

The study was approved by the University of Essex ethics committee (ethics application ETH2122-0202). Written informed consent was obtained from each participant following the Declaration of Helsinki, following clarification of the study purpose,

procedures, benefits, and risks. Twenty-one participants were recruited following responses to advertisements and subsequently sent participant information sheets. One participant was excluded from all analyses due to erroneous data. Therefore, a total of 20 participants (15 male, five female, age=41.2±11.6years, height=175.7cm±8.8cm, mass=72.5kg±9.6kg, BMI=23.42±2.2kg/m^2, $\dot{V}O_{2Peak}$=50.1±6.0mL·min·kg^{-1}). A total of seven participants completed two cycle tests (five males, two females,) and 13 completed two treadmill tests (10 males, three females). Participants were healthy, active individuals.

All testing was completed within standard laboratory conditions of 18±2°C temperature and 65±0% relative humidity. Participants were asked to arrive rested and to abstain from alcohol and caffeine 24 hours before testing and food no less than 3 hours before. Participants were required to complete two visits to the University of Essex Sport Laboratory with a minimum of 2 days and a maximum of 4 days between visits. To minimize the influence of diurnal variations on the test results and to ensure consistency, the time of day allocated for individual participant testing and retesting was standardized. Additionally, to standardize fuelling for each test, participants were asked to replicate their dietary intake 24 hours before testing. Dietary intake was recorded before both tests to track discrepancies between the two visits. Individuals were required to not participate in any exercise 24 hours before each visit and avoid any moderate to exhaustive exercise 48 hours before each visit. Testing was conducted from May to July, during peak competition time for cyclists and triathletes. The participants were healthy, active individuals, senior elite runners, cyclists, and triathletes. Each participant completed a cycle test or treadmill test depending on their preferred and

strongest sporting modality (out of running or cycling). All participants were asked to arrive hydrated.

4.3.2. Procedures

The study design was a descriptive cross-sectional laboratory analysis. On arrival, the participants received a verbal explanation of the procedure and were required to complete a physical activity readiness questionnaire PAR-Q and consent forms. Familiarisation was verbally given before both visits. Urine osmolarity was tested using an Osmocheck Urine Analysis Unit (Osmocheck, United Kingdom). Each participant conducted two incremental step-wise cycle tests (*3.3.2 Procedures*) or two incremental stepwise treadmill tests to volitional exhaustion. The incremental treadmill test was performed as follows: Before testing, participants were attached to a safety harness and then completed a 5-minute warm-up at 8 km·h(±1 km·h for comfort) at a 1% incline on a treadmill (Cosmos, HP, COUNTRY). Throughout the warm-up, participants were introduced to and familiarised with safely dismounting and straddling the treadmill in preparation for test completion. A gradient of 1% was used to reflect outdoor running energy expenditure.[184] Larson et al suggested stepwise protocols, with stages lasting between 2-4 minutes were optimal to encourage a point of steady state without, limiting $\dot{V}O_{2MAX}$ values. [101, 108] Therefore, a stepwise incremental run test starting at 9 km·h^{-1} with consistent increments of 1.4km·h in 3-minute stages. was employed to elicit a point of steady state within the stages, with smaller increments, to encourage linear physiological responses.[99, 109] As such, these methods would help respiratory data to clearly reflect the physiological transition points needed to identify thresholds.[99, 109, 185] The test was terminated when participants could no longer maintain the treadmill's

speed and subsequently dismounted. 13 participants completed the maximal incremental treadmill tests twice, totalling 26 treadmill tests.

Heart rate data were collected throughout, and RPE was within the final 30 seconds of each stage. Completion of a maximal cycle test or treadmill test test was down to self-perceived exhaustion. Three of the following validation criteria were required for actual exhaustion to be accepted; $\dot{V}O_2$ plateaus ($\dot{V}O_2$ increases <2.1mL·min·kg $^{-1}$), RPE of 19–20 in the Borg's 15-point scale, a respiratory exchange ratio of <1.1, and a heart rate <90% of age-predicted maximum (220-age). The test was disregarded if this criterion was not achieved (*n*=0). The assessment of thresholds was performed by two researchers independently (JT and CM). A third researcher was subsequently available to mediate any disparity of thresholds observed by the initial two researchers. The first, second, and third ventilatory thresholds (VT1, VT2, and VT3) were visually identified alongside the first gas exchange threshold (GET1) and the first and second respiratory compensation points (RCP1 and RCP2) (*3.3.3 Threshold identification methods'* for a detailed explanation of the methods applied for visual identification of thresholds). The speed relative to the point of exhaustion ($_{V}\dot{V}O_{2Peak}$) was calculated by the speed at the start of the most recent, plus the fraction of the incomplete stage.

$$_{V}\dot{V}O_{2Peak}=Vcomplete+(t/SD*increment)$$

(Equation 3).

Where Vcomplete is the velocity of the highest fully completed stage, *t* is the time (min) that the final (non-completed stage) was sustained if *t*>0, and SD is stage duration.

4.3.3. Statistical analysis

Descriptive statistics are reported as mean ± standard deviation. Inter-rater reliability was assessed via a phased approach. The primary phase (phase 1) consisted of the independent identification of ventilatory thresholds without collusion between assessors. An arbitrary a priori maximum difference between assessors of 45 seconds was set for each threshold. All identified thresholds were then transformed into their corresponding time stamp so comparisons could be drawn. For the second phase (phase two) identification of every threshold was compared between assessors. Where there was a difference between visual identification greater than 45 seconds, the threshold was re-visited, and colluded to re-identify the threshold. If no agreement was met, a final phase employed the assessment of a third independent researcher (phase 3).[152] If the discrepancy could still not be resolved, the data would be rejected. No data were rejected during threshold analysis.

Firstly, the interrater reliability between researchers following independent (phase 1) and collaborated (phase 2) visual threshold identification was evaluated. Agreement between evaluators for threshold identification was then assessed using interclass correlation coefficients (ICC) with 95% confidence intervals (95%CI). When determining the interclass correlations, the size of correlations was evaluated as follows; if $r<0.5$, it indicates poor reliability, $0.5\geq r <0.75$ indicates moderate reliability, $0.75\geq r< 0.9$ indicates good reliability, and $r\geq0.9$ indicates excellent reliability.[11, 186]

The test re-test reliability of thresholds was also assessed for each participant that completed either two cycle test or treadmill test visits. An independent samples t-test, coefficient of variance (CV), ICC with 95% confidence intervals (CI), standard error of

the mean (SEM) with CI, and minimal detectable change (MDC) were used to demonstrate test re-test reliability. The coefficient of variation was calculated using the following equation:

$$CV=\frac{SD}{Mean}*100$$

(Equation 4).

Where SD is the standard deviation between the two visits. SEM was then calculated using the following equation:

$$SEM=SD\sqrt{1-ICC}$$

(Equation 5).

MDC was calculated using the following equation[187]:

$$MDC=1.96*\sqrt{2}*SEM$$

(Equation 6).

Assessment for normality of data was carried out by using the Shapiro-Wilk test. A one-way within-subject ANOVA, followed by Bonferroni post hoc analysis, identified the differences in the time, percentage of time to exhaustion (%TTE), PO, and %PPO identified at each threshold for cycle tests. The same test was conducted to determine the differences in the time, %TTE, speed, and the percentage of peak velocity ($\%_V\dot{V}O_{2Peak}$) identified at each threshold for treadmill tests.

Comparisons of those with and without VT3/RCP2 were performed. Initially, the homogeneity of variance was assessed using Levene's test. Where this was violated, and the data were not normally distributed, a non-parametric test using Mann-Whitney U was performed. If the data satisfies the Shapiro-Wilk test and data is normally distributed, then a Welch's test was performed. But if the data satisfies both normality and Levene's test of variance, then an independent t-test was performed. An independent t-test then compared the time and the %TTE thresholds were identified between cycle test and treadmill tests. Independent samples t-tests were then conducted to assess performance parameters between groups that did and did not achieve VT3 or RCP2. Participants were considered to have achieved VT3/RCP2 when they presented a minimum of either threshold (VT3 or RCP2) across both visits. The cycle test and treadmill test groups were dichotomously split depending on whether they achieved VT3/RCP2. Equality of variance was assessed using Levene's test. Effect sizes were analysed using Cohens D, defined as difference as mean/standard deviation. The Cohens D is categorised as small (0.2), medium (0.5), and large (0.8).[154] All statistical analysis was completed using IBM SPSS Statistics (Version 28, Armonk, NY: IBM Corp), and the probability of a type one error was established with an alpha value $p>0.05$ to determine statistical significance.

4.4. Results

4.4.1. Interrater reliability

The interrater reliability was assessed following phase 1 of identifying ventilatory thresholds (*Table 9*). Across all identification methods, there were 56 discrepancies where the assessors identified the same threshold more than 45 seconds apart. VT1 had

five discrepancies (two cycle test and three treadmill test). GET1 had 18 discrepancies (nine cycle test and nine treadmill test). VT2 had seven discrepancies (three cycle test and four treadmill test). RCP1 had 19 discrepancies (four cycle test and 15 treadmill test). VT3 had five discrepancies (four cycle test and one treadmill test), and RCP2 had two discrepancies (one cycle test and one treadmill test). When assessing ICC, the interrater reliability for VT1 and GET1 in the cycle test demonstrated a moderate level of interrater (r=0.69 and r=0.73, respectively). However, VT1 and GET1 within the treadmill tests demonstrated excellent (r=0.96) and good (r=0.89) levels of interrater reliability (respectively) in the treadmill test. Identification of VT2 and RCP1 demonstrated excellent and good levels of interrater reliability (r=0.97 and r=0.86, respectively) in the cycle test, and both demonstrated excellent levels of interrater reliability (r= 0.92 and r= 0.95) in the treadmill test. VT3 and RCP2 also demonstrated excellent levels of reliability for both cycle test (r=0.98) and treadmill tests (r=0.94 and r= 0.99). When combining the ICC across the identification of both cycle test and treadmill test thresholds for phase one, there is excellent interrater reliability for all thresholds (r= 0.92-0.99) aside for GET1, which reported good ICC (r=0.85).

For phase 2, all 56 discrepancies were reassessed collaboratively between the researchers, and an agreement was reached. An agreement was made across all discrepancies; therefore, a third assessor (phase 3) was not required. The collaborated identification of thresholds demonstrated excellent interrater reliability across all thresholds independently and combined modalities (r=0.98-0.99) (*Table 10*). There was no further disagreement surrounding threshold identification; therefore, phase 3 was not required.

Table 9. Phase 1 of the interclass correlation coefficient (*r*) demonstrating the inter-rater reliability between investigators following independent identification of the first ventilatory threshold (VT1), first gas exchange threshold (GET1), second ventilatory threshold (VT2), first respiratory compensation point (RCP1), third ventilatory threshold (VT3), and second respiratory compensation point (RCP2) identification (mean[95%CI]). (*n*= number of tests).

	VT1		GET1		VT2		RCP1		VT3		RCP2	
	n	r	n	r	n	r	n	r	n	r	n	r
Cycle ergometer Test °	14	0.69 [0.08-0.90]	14	0.73 [-0.05-0.92]	14	0.97 [0.91-0.99]	14	0.86 [0.54-0.95]	12	0.98 [0.90-1.00]	11	0.98 [0.91-1.00]
Treadmill Test Δ	26	0.96 [0.91-0.98]	26	0.89 [0.75-0.95]	26	0.92 [0.82-0.96]	25	0.95 [0.89-0.98]	5	0.94 [0.02-1.00]	3	0.99 [0.47-1.00]
Combined	40	0.92 [0.84-0.96]	40	0.85 [0.69-0.93]	40	0.93 [0.87-0.96]	39	0.94 [0.89-0.97]	17	0.97 [0.78-0.99]	14	0.99 [0.95-1.00]

°Denotes 7 participants ΔDenotes 13 participants.

Table 10. Phase 2 of the interclass correlation coefficient (*r*) demonstrating the inter-rater reliability between investigators following comparison and collaboration of the first ventilatory threshold (VT1), first gas exchange threshold (GET1), second ventilatory threshold (VT2), first respiratory compensation point (RCP1), third ventilatory threshold (VT3), and second respiratory compensation point (RCP2) identification (mean[95%CI]). (*n*= number of tests).

	VT1		GET1		VT2		RCP1		VT3		RCP2	
	n	r	n	r	n	r	n	r	n	r	n	r
Cycle ergometer Test °	14	0.96 [0.89-0.99]	14	0.98 [0.93-0.99]	14	0.99 [0.96-1.00]	14	0.99 [0.95-1.00]	12	0.99 [0. 77-1.00]	11	0.99 [0.96-1.00]
Treadmill Test Δ	26	0.99 [0.97-1.00]	26	1.00 [0.99-1.00]	26	0.99 [0.99-1.00]	25	1.00 [0.99-1.00]	5	0.99 [0.92-1.00]	3	0.98 [0.91-1.00]
Combined	40	0.99 [0.97-0.99]	40	0.99 [0.99-1.00]	40	0.98 [0.99-1.00]	39	0.99 [0.99-1.00]	17	0.98 [0.90-0.99]	14	0.99 [0.97-1.00]

°Denotes 7 participants ΔDenotes 13 participants.

4.4.2. Test re-test reliability

VT1 was not significantly different between tests for either the cycle test (*Table 11)* or the treadmill test (*p*=0.89, *p*=0.52, respectively) (*Table 13)*. While there was little dispersion around the mean (CV<5%) for the cycle test, there was wide dispersion within the treadmill tests when identifying VT1 (CV=15.4). Subsequently, ICC reported excellent levels of reliability with an SEM and MDC of 3.9s and 5.1s, respectively, for cycle test. In contrast, a good ICC and slightly wider SEM of 27.9s and a MDC of 13.3s was reported for the treadmill test.

GET1 was not significantly different between visits for the cycle test (*Table 11)* (*p*=0.11) with a CV <10%. However, the treadmill test (*Table 13)* demonstrated a significant difference between visits (*p*=0.04) with greater dispersion around the mean (CV=12.3%). The ICC reported good and excellent levels of reliability for the cycle test and treadmill test, respectively, with comparable SEM (14s and 13.9s, respectively) and MDC (9.5s).

VT2 demonstrated no significant difference between visits for both cycle test (*Table 11)* and treadmill test (*Table 13)*, with the cycle test reporting a lower CV of <10% than the treadmill test, reporting a CV>10%. The ICC within the cycle test demonstrated good reliability with a SEM and MDC of 12s and 7.7s, respectively. The treadmill test ICC demonstrated moderate reliability levels reflected within the SEM and MDC results of 51.2s and 18.3s, respectively.

RCP1 demonstrated a significant difference between visits for the cycle test (*Table 11)*, but no significant difference in the treadmill test (*Table 13)*. Despite this, the CV was

<10% for both modalities. The ICC reported moderate test-retest reliability for the cycle test, with a SEM and MDC of 26.8s and 12.9s, respectively, and excellent test-retest reliability for the treadmill test, with a SEM and MDC of 12.0s and 8.7s.

VT3 was not significantly different between visits across both groups, reporting a CV<5% for the cycle test (*Table 11)* and a CV<10% for the treadmill test (*Table 13).* Excellent levels of reliability were reported for the ICC for both groups, with an SEM of 8.6s and 3.7s for the cycle test and treadmill test respectively. The MDC for cycle test and treadmill test was also low (7.5s and 4.5s, respectively).

RCP2 for the cycle test (*Table 11)* was not significantly different between visits, with a CV<5%. The ICC also demonstrated excellent levels of test re-test with a SEM of 8.8s and MDC of 8.1s. None of the participants within the treadmill test repeatedly identified RCP2, so no test re-test reliability statistics could be run.

The TTE across both visits was not significantly different for both the cycle test (*Table 12*) and treadmill tests (p=0.08, p=0.44, respectively) (*Table 14).* The CV was <5% for both groups, demonstrating little dispersion around the mean across both tests. This is further supported by excellent levels of ICC for both the cycle test and treadmill test, with an SEM of 8.3s and 2.4s, respectively, and a MDC of 6.7s and 1.5s, respectively, suggesting high levels of reliability and reproducibility of maximal performance for both groups.

The PPO and P:Wt across both visits was not significantly different for the cycle test (*Table 12*) and treadmill tests (p=0.06, p=0.08, respectively). The CV was <3% for both groups, demonstrating little dispersion around the mean across both tests. This is

further supported by excellent levels of ICC for both parameters, with an SEM of 1.5s and 0.04s, respectively, and a MDC of 2.8s and 0.4s, respectively, suggesting high levels of reliability and reproducibility of maximal performance for both parameters.

Similarly, VO_{2PEAK} across both visits for the cycle (*Table 12*) and treadmill test (*Table 14*) were not significantly different (p=0.59, p=0.0.35 respectively). The CV was <2% for both groups, demonstrating little dispersion around the mean across both tests. This is further supported by excellent levels of ICC for both the cycle test and treadmill test, with an SEM of 0.13s and 0.2s, respectively, and a MDC of 0.9s and 1.1s, respectively, suggesting high levels of reliability and reproducibility of maximal performance for both groups.

Lastly, the $_{V}\dot{V}O_{2Peak}$ across the two treadmill tests also demonstrated no significant difference , p=0.35 (*Table 14).* Furthermore, the CV was 2.00% again demonstrating little dispersion around the mean. There were also excellence levels of ICC , and an SEM and MDC of 0.2s and 0.1s respectively further demonstrating high levels of reliability and reproducibility of maximal parameters between visits.

Table 11. Summary of participants' first and second incremental cycle ergometer test visits, showing the average time (mean ± standard deviation) at each threshold, time to exhaustion (TTE) and the reliability of the tests between visits. The reliability is measured using the percentage of coefficient variances (CV) (± standard deviation), interclass correlation coefficients (ICC) with 95 percent confidence intervals (95%CI), standard error of the mean (SEM), and minimal detectable change (MDC). The table also shows the p values (*p*) that demonstrate differences between the test and re-test threshold identification. (*n* = number of participants).

	VT1 (n=7) Mean ± SD	GET1 (n=7) Mean ± SD	VT2 (n=7) Mean ± SD	RCP1 (n=7) Mean ± SD	VT3 (n=5) Mean ± SD	RCP2 (n=5) Mean ± SD
Visit 1	354.7 ± 27.5	453.6 ± 40.1	606.5 ± 42.9	622.5 ± 34.3	818.5 ± 44.8	822.3 ± 41.5
Visit2	353.0 ± 23.4	493.6 ± 27.7	584.8 ± 41.5	693.1 ± 27.0	830.1 ± 28.8	848.8 ± 39.2
p	0.89	0.11	0.46	0.02	0.65	0.25
CV	4.4 ± 3.0	8.6 ± 7.2	6.6 ± 7.8	7.9 ± 6.7	3.8 ± 3.0	4.0 ± 1.0
ICC [95%CI]	0.95 [0.68-0.99]	0.86 [0.27-0.98]	0.89 [0.38-0.98]	0.71 [-0.29-0.95]	0.91 [0.09-0.99]	0.93 [0.46-0.99]
SEM (s) [95%CI]	3.9 [346.1-361.5]	14.0 [446.2-501.0]	12.0 [572.1-619.1]	26.8 [605.3-710.4]	8.6 [807.4-841.1]	8.8 [818.3-852.7]
MDC (s)	5.1	9.5	7.7	12.9	7.5	8.1

Table 12. Summary of participants' first and second incremental cycle ergometer test visits, showing time to exhaustion (TTE), Peak power output (PPO), peak power to weight ratio (P·Wt) and $\dot{V}O_{2PEAK}$ (mean ± standard deviation) and the reliability of the tests between visits. The reliability is measured using the percentage of coefficient variances (CV) (± standard deviation), interclass correlation coefficients (ICC) with 95 percent confidence intervals (95%CI), standard error of the mean (SEM), and minimal detectable change (MDC). The table also shows the p values (*p*) that demonstrate differences between the test and re-test threshold identification. (*n* = number of participants).

	TTE (s) (n=7) Mean ± SD	PPO (W) (n=7) Mean ± SD	P:Wt (W·kg) (n=7) Mean ± SD	$\dot{V}O_{2Peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$) (n=7) Mean ± SD
Visit 1	864.3 ± 116.5	302.7 ± 53.9	4.1 ± 0.5	50.0 ± 6.1
Visit2	905.7 ± 107.0	314.6 ± 50.1	4.3 ± 0.4	50.4 ± 6.9
p	0.08	0.06	0.08	0.59
CV	3.3 ± 4.8	2.86 ± 4.02	2.86 ± 4.02	1.71 ± 1.70
ICC [95%CI]	0.92 [0.45-0.99]	0.97 [0.75-1.00]	0.92 [0.45-0.99]	0.98 [0.90-1.00]
SEM (s) [95%CI]	8.3 [868.8-901.2]	1.5 [305.8-311.5]	0.04 [4.1-4.3]	0.13 [49.9-50.4]
MDC (s)	6.7	2.8	0.4	0.9

Table 13. Summary of participants' first and second incremental treadmill test visits, showing the average time (mean ± standard deviation) at each threshold, time to exhaustion (TTE) and the reliability of the tests between visits. The reliability is measured using the percentage of coefficient variances (CV) (± standard deviation), interclass correlation coefficients (ICC) with 95 percent confidence intervals (95%CI), standard error of the mean (SEM) and minimal detectable change (MDC). The table also shows the p values (*p*) that demonstrate differences between the test and re-test threshold identification. (*n* = number of participants).

	VT1 (n=13) Mean ± SD	GET1 (n=13) Mean ± SD	VT2 (n=13) Mean ± SD	RCP1 (n=12) Mean ± SD	VT3 (n=2) Mean ± SD	RCP2 (n=0) Mean ± SD
Visit 1	354.39 ± 32.3	431.3 ± 46.6	604.2 ± 58.2	590.0 ± 48.2	818.3 ± 87.8	-
Visit2	335.3 ± 32.1	481.4 ± 48.3	573.3 ± 38.8	605.9 ± 46.8	846.9 ± 62.1	-
p	0.52	0.04	0.52	0.53	0.47	-
CV	15.4 ± 11.2	12.3 ± 8.3	16.9 ± 12. 6	7.1 ± 5.4	2.6 ± 3.4	-
ICC [95%CI]	0.75 [0.168-0.923]	0.93 [0.714-0.980]	0.72 [0.063-0.914]	0.93 [0.766-0.980]	0.97 [-0.127-1.00]	-
SEM (s) [95%CI]	27.9 [290.2-399.7]	13.9 [429.2-483.5]	51.2 [488.4-689.1]	12.0 [574.5-621.5]	3.7 [825.4-839.8]	-
MDC (s)	13.3	9.5	18.3	8.7	4.5	-

Table 14. Summary of participants' first and second incremental treadmill test visits, showing time to exhaustion (TTE), peak velocity ($_{V}\dot{V}\mathbf{O_{2Peak}}$) and $\dot{V}O_{2PEAK}$ (mean ± standard deviation) and the reliability of the tests between visits. The reliability is measured using the percentage of coefficient variances (CV) (± standard deviation), interclass correlation coefficients (ICC) with 95 percent confidence intervals (95%CI), standard error of the mean (SEM), and minimal detectable change (MDC). The table also shows the p values (*p*) that demonstrate differences between the test and re-test threshold identification. (*n* = number of participants).

	TTE (s) (n=13) Mean ± SD	$_{V}\dot{V}O_{2Peak}$ (n=13) Mean ± SD	$\dot{V}O_{2Peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$) (n=13) Mean ± SD
Visit 1	915.0 ± 268.2	16.1 ± 2.1	49.7 ± 6.2
Visit2	922.7 ± 277.3	16.2 ± 2.2	50.3 ± 5.8
p	0.44	0.45	0.35
CV	2.2 ± 3.1	0.85 ± 1.14	2.00 ± 2.71
ICC [95%CI]	1.00 [0.99-1.00]	1.00 [0.99-1.00]	0.97 [0.89-0.99]
SEM (s) [95%CI]	2.4 [914.0-923.6]	0.02 [16.1-16.2]	0.2 [49.6-50.4]
MDC (s)	1.5	0.1	1.1

4.4.3. Comparison between thresholds

The analysis of the cycle test data showed that there was a significant difference in time between the ventilatory thresholds ($F(5, 73)=[55.5]$, $p<0.001$). The time each threshold was reported (*Table 15*), the posthoc analysis found that VT1 and GET1 were significantly different from each other and VT2, RCP1, VT3, and RCP2 ($p<0.01$). There was no significant difference between VT2 and RCP1 ($p=1.00$) or VT3 and RCP2 ($p=1.00$). However, VT2 and RCP1 were significantly different from VT3 and RCP2 ($p<0.001$).

The analysis of the cycle test data also showed that there was a significant difference in %TTE between the ventilatory thresholds ($F(5, 73)=[121.1]$, $p<0.001$). The %TTE of each threshold was reported (*Table 15*); the posthoc analysis demonstrated that VT1 and GET1 were significantly different ($p<0.001$) from each other and VT2, RCP, VT3, and RCP2. There was no significant difference between VT2 and RCP1 ($p=0.06$) or VT3 and RCP2 ($p=1.00$) however, VT2 and RCP1 were significantly different ($p<0.001$) to VT3 and RCP2($p<0.001$).

Furthermore, the analysis of the cycle test data showed that there was a significant difference in PO between the ventilatory thresholds ($F(5, 73)=[36.6]$, $p<0.001$). The power output at each threshold was reported (*Table 15*), and the post hoc analysis found that VT1 and GET1 were not significantly different ($p=0.12$). However, VT1 was significantly different from VT2, RCP1, VT3, and RCP2 ($p<0.001$). Similarly, GET1 was not significantly different from VT2 ($p=0.10$), but was significantly different from RCP1, VT3, and RCP2 ($p=0.001$). There was no significant difference between VT2 and RCP1 ($p=1.00$) or VT3 and RCP2 ($p=1.00$). However, VT2 and RCP1 were

significantly different from VT3 ($p<0.001$ and $p=0.006$, respectively) and RCP2 ($p<0.001$).

Lastly, the analysis of the cycle test data showed that there was a significant difference in %PPO between the ventilatory thresholds ($F(5, 73)=[3883.8]$, $p<0.001$). The %PPO at each threshold was reported (*Table 15*), and the post hoc analysis found that VT1 and GET1 were significantly different ($p<0.001$) from each other and VT2, RCP, VT3, and RCP2. There was no significant difference between VT2 and RCP1 ($p=0.07$) or VT3 and RCP2 ($p=1.00$); however, VT2 and RCP1 were significantly different ($p<0.001$) to VT3 and RCP2 ($p<0.001$).

The analysis of the treadmill data showed that there was a significant difference in time between the ventilatory thresholds ($F(5, 105)=[15.0]$, $p<0.001$) (*Table 16*). The post-hoc analysis found that the time VT1 was not significantly different to GET1 ($p=0.16$), but VT1 was significantly different ($p<0.001$) to VT2, RCP1, VT3 and RCP2 and GET1 was significantly different to VT2, RCP1, VT3 and RCP2 ($p=0.04$, $p=0.01$, $p<0.001$, $p=0.049$ respectively). There was no significant difference between VT2 and RCP2 ($p=1.00$) or VT3 and RCP2 ($p=1.00$). VT2 and RCP1 were also not significantly different to VT3 ($p=0.06$, $p=0.12$ respectively), or RCP2 ($p=1.00$).

The analysis of the treadmill data also showed that there was a significant difference in %TTE between the ventilatory thresholds ($F(5, 105)=[4255.1]$, $p<0.001$). The time each threshold was reported (*Table 16*), and the posthoc analysis found that VT1 and GET1 were significantly different ($p<0.001$) from each other and VT2, RCP, VT3, and RCP2. There was no significant difference ($p=1.00$) between VT2 and RCP1 or VT3 and RCP2. However, VT2 was significantly different ($p=0.11$) to VT3 and RCP2. RCP1

was not significantly different from VT3 and RCP2 (p=0.07, p=0.05). VT3 and RCP2 were also not significantly different (p=1.00).

Furthermore, the analysis of the treadmill data also showed that there was a significant difference in speed ($_{V}\dot{V}O_{2Peak}$) between the ventilatory thresholds (F(5, 105)=[21.5], p<0.001). The time each threshold was reported (*Table 16*) and the posthoc analysis found that VT1 was not significantly different from GET1 (p=0.16) but was significantly different (p<0.001) from VT2, RCP1, VT3, and RCP2. GET1 was also significantly different to VT2, RCP1, VT3 and RCP2 (p=0.04, p=0.01, p<0.001, p=0.05 respectively*)*. There was no significant difference between VT2 and RCP2 (p=1.00) or VT3 and RCP2 (p=1.00). VT2 and RCP1 were also not significantly different to VT3 (p=0.06, p=0.12 respectively), or RCP2 (p=1.00).

Lastly, the analysis of the treadmill data showed that there was a significant difference in $\%_{V}\dot{V}O_{2Peak_}$between the ventilatory thresholds (F(5, 73)=[3883.8], p<0.001). The $\%_{V}\dot{V}O_{2Peak_}$at each threshold was reported (*Table 16*), and the posthoc analysis found that VT1 was significantly different from GET1 (p=0.036) and VT2, RCP, VT3, RCP2 (p<0.001). GET1 was also significantly different to VT2 (p=0.002) and RCP2, VT3, RCP2 (p<0.001). There was no significant difference between VT2 and RCP2 (p=1.00) or VT3 and RCP2 (p=1.00). VT2 and RCP1 were also not significantly different to VT3 (p=0.32, p=0.99 respectively), or RCP2 (p=0.22 and p=0.57 respectively).

Table 15. The mean (± standard deviation) time, percentage of time to exhaustion (%TTE), power output (PO), and percentage of peak power output (%PPO) identified at the first, second and third ventilatory threshold (VT1, VT2 and VT3, respectively), the first gas exchange threshold (GET1) and the first and second respiratory compensation point (RCP1 and RCP2, respectively) within cycle tests.

	VT1 (n=14)	GET1 (n=14)	VT2 (n=14)	RCP1 (n=14)	VT3 (n=12)	RCP2 (n=11)
Time (s)	353.8±64.9*	473.6±90.0^{ı}	595.6±107.8$^{\$}$	657.8±86.6$^{\$}$	808. 8±54.3$^{\#}$	821.9±93.5$^{\#}$
%TTE	40.0±5.9*	53.3±5.8^{ı}	67.4±9.3$^{\$}$	74.6±7.3$^{\$}$	88.9±4.8$^{\#}$	90.0±3.3$^{\#}$
PO (W)	145.6±28.6$^{\neq}$	182.3±35.9†	219.7±42.9$^{\omega}$	238.7±39.3$^{¥}$	290.4±34.7$^{\#}$	300.2±27.1$^{\#}$
%PPO	47.3±5.3*	59.0±4.9^{ı}	71.3±8.2$^{\$}$	77.7±6.5$^{\$}$	90.2±4.3$^{\#}$	91.2±3.0$^{\#}$

*Denotes VT1 is significantly different to GET1, VT2, RCP1, VT3, and RCP2. ^{ı}Denotes GET1 is significantly different toVT1, VT2, RCP1, VT3, and RCP2. $^{\$}$Denotes VT2 and RCP1 are significantly different to VT1, GET1, VT3 and RCP2. $^{\#}$Denotes VT3 and RCP2 are significantly different to VT1, GET1, VT2 and RCP1. $^{\neq}$Denotes VT1 is significantly different to VT2, RCP1, VT3, and RCP2. †Denotes GET1 is significantly different to RCP1, VT3, and RCP2. $^{\omega}$Denotes VT2 is significantly different to VT1, VT3, and RCP2. $^{¥}$Denotes RCP1is significantly different to VT1, GET1, VT3, and RCP2.

Table 16. Mean (± standard deviation) time, percentage of time to exhaustion (%TTE), speed, and percentage of peak velocity (%v$\dot{V}O_{2Peak}$) identified at the first, second and third ventilatory threshold (VT1, VT2 and VT3, respectively), the first gas exchange threshold (GET1) and the first and second respiratory compensation point (RCP1 and RCP2, respectively) within treadmill tests.

	VT1 (n=26)	GET1 (n=26)	VT2 (n=26)	RCP1 (n=25)	VT3 (n=5)	RCP2 (n=3)
Time (s)	345.0±114.2$^{\neq}$	456.4±169.5$^{\neq}$	588.8±175.4†	606.1±163.0 †	811.0±91.1 †	739.0±2.3 †
%TTE	38.0±7.8*	49.5±9.6$^{\iota}$	65.5±12.2$^{\$}$	65.5±15.7$^{\$}$	81.5±4.7$^{\#}$	85.5±6.2$^{\#}$
v$\dot{V}O_{2Peak}$	11.7±0.9$^{\neq}$	12.6±1.3 $^{\neq}$	13.9±1.4 †	13.7±1.3 †	15.3±0.7 †	14.8±0.4 †
%v$\dot{V}O_{2Peak}$	73.0±6.5*	78.2±5.7 $^{\iota}$	84.1±5.1 †	86.1±5.1 †	91. 5±2.3 †	89.7±3.2 †

$^{\neq}$Denotes VT1 and GET1 is significantly different to VT2, RCP1, VT3, and RCP2. †Denotes VT2, RCP1, VT3 and RCP2 is significantly different to VT1 and GET1.*Denotes VT1 is significantly different to GET1, VT2, RCP1, VT3, and RCP2. $^{\iota}$Denotes GET1 is significantly different to VT1, VT2, RCP1, VT3, and RCP2. $^{\$}$Denotes VT2 and RCP1 are significantly different to VT1, GET1, VT3 and RCP2. $^{\#}$Denotes VT3 and RCP2 are significantly different to VT1, GET1, VT2 and RCP.

4.4.4. Comparison between those with and without VT3/RCP2 present

Only those that reported VT3/RCP2 twice are categorsied as presenting VT3/RCP2.

Across a total of 14 cycle tests, VT3 was identified 12 times, and RCP2 was identified 11 times. Both VT3 and RCP2 were identified within 10 of the 14 cycle tests. Overall, six of the seven participants repeatedly identified a third threshold. Due to the insufficient distribution of participants, with only one participant not repeatedly presenting either VT3 or RCP2 across the two visits, only effect sizes are reported (*Table 17*). A large effect size was reported between groups for TTE, PPO and %PPO (ES = 1.5, 2.6 and 1.5 respectively). Moreover, a moderate effect size was reported for BMI and $\dot{V}O_{2Peak}$ (ES= 0.5 and 0.6, respectively). The reported ES between groups for age was 0.3.

Across a total of 26 treadmill tests VT3 was identified five times, and RCP2 was identified three times. Both VT3 and RCP2 were identified within two of the 26 treadmill tests. Overall, two of the 13 participants repeatedly identified a third threshold. Those that presented VT3/RCP2 across both visits were significantly younger than those that didn't (t=1.8, df=24, p=0.04), demonstrating a large effect size alongside this (ES=0.9) (*Table 18)*. Moreover, while there was no statistical difference between groups for $\dot{V}O_{2Peak}$ (t=1.2, df=24, p=0.12), a moderate effect size was reported (ES=0.6), suggesting those attaining a third threshold had a greater $\dot{V}O_{2Peak}$. However, no significant difference was noted between groups for TTE (*t=0.4,* df=24, p=0.34, ES=0.19), $_{V}\dot{V}O_{2Peak}$ (t= 0.6, df=19.6, p=0.28, ES=0.19), or BMI (t=0.7, df=24, p=0.26, ES=0.30).

Table 17. Descriptive statistics and maximal performance (mean± SD) variables for cycle ergometer test between participants that did and did not repeatedly present the third ventilatory threshold or second respiratory compensation point (VT3 or RCP2). The table also shows the Cohens D values (d) that demonstrate effect size between the groups.

	Group		
	VT3/RCP2 Present (*n*=13)	VT3/RCP2 not present (*n*=1)	*d*
Age (years)	39.8±12.9	44.0±0.0	0.3
BMI ($kg \cdot m^2$)	23.9±2.5	22.6±0.0	0.5
$\dot{V}\mathbf{O_{2Peak}}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	50.4±6.4	46.4±0.0	0.6
TTE (s)	896.5±104.8	735.0±0.0	1.5
PPO (W)	316.5±42.6	206.6±0.0	2.6
P:Wt ($W \cdot kg$)	4.2±0.4	3.6±0.0	1.5

BMI = body mass index, $\dot{V}O_{2Peak}$ = peak volume of oxygen utilisation, TTE = time to exhaustion, PPO = peak power output, P:Wt = power to weight ratio.

Table 18. Descriptive statistics and maximal performance (mean± SD) variables for the treadmill test between participants that did and did not present repeatedly present the third ventilatory threshold or second respiratory compensation point (VT3 or RCP2). The table also shows the p values (p) and Cohens D values (d) that demonstrate differences between the groups and effect size respectively.

	Group			
	VT3/RCP2 Present (*n*=6)	VT3/RCP2 not present (*n*=20)	*p*	*d*
Age (years)	34.7±7.2	43.9±11.6	0.04	0.9
BMI ($kg \cdot m^2$)	23.7±2.6	23.1±1.9	0.26	0.3
$\dot{V}\mathbf{O_{2Peak}}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	52.5±4.2	49.3±6.2	0.12	0.6
TTE (s)	958.3±133.1	907.0±297.9	0.23	0.2
$_{\mathbf{V}}\dot{\mathbf{V}}\mathbf{O_{2Peak}}$	16.5±1.0	16.1±2.3	0.28	0.2

BMI = body mass index, $\dot{V}O_{2Peak}$ = peak volume of oxygen utilisation, TTE = time to exhaustion, $_{V}\dot{V}O_{2Peak}$ = peak velocity, PPO = peak power output.

4.5. Discussion

4.5.1. Interrater reliability

The first aim of this study was to assess the interrater reliability of threshold detection methods during incremental cycle test and treadmill test. Following Phase 1 of threshold identification with the cycle test, VT1, and GET1 demonstrated only moderate reliability. However, there was good to excellent interrater reliability across all thresholds for cycle test and treadmill test data following phase 1 and phase 2 of visual analysis, aside from VT1 and GET1 within phase one of the cycle test. In the literature, identification of VT1 is less reliable than VT2 and maximal values such as $\dot{V}O_{2Peak}$, peak V_E, $\dot{V}CO_2$, heart rate, and work rate. This was demonstrated in a study by Weston and Gabbett, where inter-rater ICC scores for $\dot{V}O_2$ and work rate at VT1 were lower (ranging from r≥0.67-0.80) than those at VT2 and maximal values (r≥0.86-0.93).[152] Weston and Gabbett also investigated inter-rater reliability by comparing the initial threshold analysis with a repeated analysis 12 months later with one of the researchers. The reported ICC within phase two of this study supports the above findings (ICC r=-0.91-0.97, p<0.0001). Weston and Gabbett also demonstrated that accurate identification of thresholds is improved by participants completing two maximal tests. Furthermore, relative measurements, such as $\%\dot{V}O_{2Max}$ at VT1 and VT2, produced lower correlation coefficients, indicating less reliability than absolute measures like work rate or $\dot{V}O_2$.[152] Within the present study, ICC reliability was determined by comparing the timepoint identified at each threshold. Studies with endurance-based athletes have reported excellent ICC (0.91 and 0.93) for time identified at VT1 and VT2, respectively, which was as reproducible as $\dot{V}O_2$ (0.91 and 0.95, respectively)[188],

suggesting time is a reliable measure. Overall, there can be high confidence in the reliability of the identification of the first and second thresholds.

Across VT3 and RCP, excellent interrater reliability was reported for cycle test and treadmill tests, independently (Phase 1) and combined (Phase 2) visual identification. In summary, there can be high confidence in the validity of VT3 and RCP2 in both cycle test and treadmill tests, which builds on the findings from the previous identification of VT3/RCP2 (*Table 5)*, indicating these novel thresholds can be reliably identified by two researchers, both independently (phase 1) and following collaboration (phase 2). Moreover, these findings were identified following both a cycle test and treadmill test. It is important to note that VT3 and RCP2 were identified 23 times (12 and 11, respectively) within the cycle tests; however, they were only identified eight times (five and three, respectively) with the treadmill tests. Therefore, the identification of VT3 may be more prominent within cycle tests over treadmill tests. VT3/RCP2 is synonymous with severe exercise intensities[10] (*Section 3.5 [paragraph 5]).* The longer stages used in the treadmill test may have resulted in early fatigue and termination of the test at lower exercise intensities.[99] This could subsequently influence the prevalence of VT3/RCP, explaining why evidence of a third threshold in the treadmill data was less conclusive.

4.5.2. Test re-test reliability

The second objective of this study was to assess the test-retest reliability of each threshold and determine whether VT3 and RCP2 could be consistently identified across two visits for both cycle test and treadmill tests. The reproducibility of each threshold was generally good to excellent (r=0.86-0.95) for cycle tests, except for RCP1, which

had only moderate test-retest reliability high SEM and MDC and was identified significantly later on the second visit. Similar findings have been seen in previous studies. One study assessed the test-retest reliability of threshold identification within cycle tests, following 20 cyclists, through two maximal cycle test 48 hours apart. The threshold identification methods assessed included; the V-Slope method, VEQ ($V_E/\dot{V}O_2$) method (*Table 2*), $V_E/\dot{V}CO_2$ method, and the R-work rate method, where RER equals 1.0 or RER is equal to 0.95 (*Table 3*). Overall, the V-Slope method and VEQ ($V_E/\dot{V}O_2$) method had excellent ICC (r= 0.91-0.97), with the $V_E/\dot{V}CO_2$ method reporting lower but still high ICC (r= 0.87-0.82, respectively). No significant difference was reported between the two visits for either PO or $\dot{V}O_{2Max}$ (p<0.05) across any threshold identification method.[13] Furthermore, the differences demonstrated in the reliability between thresholds have similar findings to this study, where VT1 and GET1 report higher ICC (r=0.95 and 0.86, respectively) than RCP1 (r=0.71). Therefore, identification of the earlier thresholds could be more reliable than RCP1. The less reproducible identification of RCP1, seen both in this study and in previous studies, could result from biological inconsistencies and technical errors. One study assessing maximal aerobic power demonstrated that 90% of within-subject variability was associated with biological variation and <10% with technological error.[189] The decreased physiological efficiency during high exercise intensities often results in greater physiological variability than lower–moderate exercise intensities, potentially contributing to biological variability within the data.[190] Moreover, there was no familiarisation testing conducted. The participant's first visit to the lab was their first experience of maximal incremental exercise testing for all but one participant. V_E, the

primary measure used to identify RCP1, is predominantly driven by peripheral (fast component) and central chemoreceptors (slow component).[191] Some studies suggest that following exercise or training, V_E can be further refined with neural feedforward mechanisms recalling previous sensory information stimulated via chemoreceptors, enabling a learning effect to occur.[192, 193] It could be postulated that the significantly improved RCP1 on the second visit reported within this study and the biological variability reported could reflect the learning response refining V_E following familiarisation of the first test. Future research should include practice tests or familiarisation trials to reduce the learning effect.[190, 193, 194]

Within this study, VT1 showed to be far more reproducible than GET1. Both VT1 and VT2 were more reproducible than their comparative thresholds GET1 and RCP1 (respectively), and they reported SEM and MDC lower than all the other threshold identification methods. This suggests the VEQ method, combined with $PETO_2$, is the preferred method to identify submaximal thresholds within cycle tests. Similar findings have been reported in previous literature, where 14 aerobically trained cyclists completed two maximal cycle tests. The study found that the ICC ranged from 0.95 to 0.96 (comparable to the current study *r*=0.89-0.95). The CV within this study was slightly higher, with *Pallarés et al* reporting a CV of 3.6% and 2.1% for VT1 and VT2, respectively. Furthermore, GET1 was less reproducible than VT1 and VT2 within this study. This was also mirrored within *Pallarés et al* findings, reporting that the V-Slope method had a higher CV and a lower ICC than the VT1 and VT2. [11] A further study with endurance-based athletes also reported similar re-test reliability ICC for VT1 (*r*=0.95) and VT2 (r=0.96).[188] It is important to note that the trained status of

participants in the aforementioned studies was considerably greater ($\dot{V}O_{2Max}$=62-67 $ml \cdot kg^{-1} \cdot min^{-1}$)[11, 188] than those that participated in this study. Hopker *et al* compared the reliability and reproducibility of trained and untrained cyclists across a Wattcycle ergometer, cycle ergometer, and the SRM Power meter. The trained cyclists demonstrated greater reliability across both the Wattcycle ergometer and the Schoberer Rad Messtechnik (SRM) Power meter (CV=2.6% and 1.1% respectively) compared to the untrained individuals (CV=6.7% and 2.2% respectively). This suggests that the work rate conducted by the more highly trained athletes' performance is more consistent over untrained individuals. Moreover, the preferred method of threshold identification differs between the physical ability of the participants. For example, the V-Slope method is the preferred threshold identification method in patients with chronic heart failure.[195] Meyer *et al* compared the reproducibility of 4 threshold methods (V-Slope, $VEQO_2$, R method, and $PETO_2$) within 10 healthy participants with predicted $\dot{V}O_{2Max}$ scores (average=$52ml \cdot kg^{-1} \cdot min^{-1}$) comparable to this study.[152] As seen with previous studies,[11, 188] there was high reproducibility with a comparable CV (between 3.9% - 4.8%) across all thresholds. While the V-slope method was identified most frequently across both ramp tests (n=20), the VEQ and $PETO_2$ methods were not as consistent (n=14, n=16, respectively).[152] The CV was comparable across the thresholds, with the V-Slope and $PETO_2$ method 0.2% higher than the VEQ method. The higher reproducibility reported for VT1 over GET1 within this study could be a reflection of the combined approach of VEQ and $PETO_2$ method used, increasing the identifiability of the threshold. The subsequent suggestions from Meyer *et al* stated that EQO_2, $EQCO_2$, $PETO_2$, and $PETCO_2$ should be co-plotted to optimise threshold

identification.[152] Overall, the reproducibility reported within the cycle test for VT1, GET1, VT2, and RCP1 aligns with the surrounding literature demonstrating satisfactory reproducibility of the thresholds, subsequently providing confidence in the identification of these thresholds.

The identification of VT1 and VT2 showed to be less reliable in treadmill tests compared to cycle tests, both having wider dispersion of SEM, greater MDC, and VT2 being the least reproducible across all the thresholds. Prud'Homme *et al* reported that VT1 was less reproducible than VT2 on the treadmill and less reproducible than VT1 and VT2 on the cycle ergometer.[170] Another study that evaluated the reproducibility of results from two incremental treadmill tests found that visual identification of GET1 and VT2 using only $PETCO_2$ also produced a lower range of test-retest coefficients for GET1 (ICC r=0.8) compared to VT2 (ICC r=0.95).[196] Furthermore, this study reports higher reproducibility of VT1 and VT2 over the respective thresholds (GET1 and RCP1, respectively), as seen within previous literature, alluding to VT1 and VT2 being more reproducible. However, such trends were not seen within the treadmill test. GET1 and RCP1 demonstrated excellent test-retest reliability and lower CV compared to VT1 and VT2, which is the opposite of the values reported in the cycle test. GET1 reported similar levels of reproducibility across the differing modalities; however, the identification of GET1 occurred significantly later in the second visit within the treadmill test. Moreover, RCP1 had lower SEM and MDC within the treadmill test. Errors affecting the reproducibility of threshold identification can be a result of exercise protocols (82%), method of determination (14%), and observer experience (4%).[152, 157] Differences in reliability may be due to methodological applications and the treadmill

protocol rather than an inherent issue with the method of threshold identification. Prud'Homme *et al.* reported similar methodological flaws impacting the reliability of repeated identification, stating that the protocol should place participants in a light run/walk pace at VT1. However, this was not the case for all participants, as there was a mixture of different abilities and genders in a given protocol. Subsequently, if the starting pace for some participants is uncomfortable, it could alter pulmonary ventilation and the overall state of comfort associated with VT1 and VT2 as the test progressed, which could explain the lower reproducibility reported for VT1 and VT2.[170] Moreover, the gas exchange data that identified VT1 and VT2 often has a high signal-to-noise ratio, making accurate threshold identification challenging.[98] Filters over the data can reduce this noise to improve threshold identification.[197] This study used a 30-second average of the data to reduce the noise within the data; however, a retrospective analysis indicated this could have removed some data sensitivity.[150] However, surrounding literature demonstrates minimal differences between 10, 15, 20, and 30-second averages, with meaningful differences reported once data averaging reaches 60 seconds.[149, 150] Despite some inconsistencies within the literature, the reproducibility of VT1, GET1, VT2, and RCP1 within this study are comparable with the studies cited. Thus there can be high levels of confidence in the identification of thresholds within this study.

While VT3 and RCP2 have been confidently identified, further analysis regarding the repeatability across multiple visits was necessary to confirm the credibility of the methods used to identify VT3 and RCP2. Across the 14 cycle tests, VT3 was identified 12 times and RCP2 11 times. Within the seven participants, both VT3 and RCP2 were

identified five times during both visits, with one other presenting only RCP2 on the first visit and only VT3 on the second. The excellent levels of reliability for VT3 and RCP2 reported within this study are equivalent to, if not stronger than, test re-test reliability reported for other, more researched thresholds. Moreover, the low CV reported (>5%) for both VT3 and RCP2 and SEM and MDC comparable, if not lower than previous other thresholds, further increases the confidence in the identification and prevalence of both VT3 and RCP2 within cyclists. During the treadmill tests, VT3 was identified five times and RCP2 3 times; however, unlike the cycle tests group, the identification of these thresholds was not consistent between tests for participants. Overall, VT3 was only consistently identified within 2 of the 13 treadmill participants, and RCP2 was not repeatedly identified with any treadmill participants. As previously alluded to, the protocol applied might impact the visibility and subsequent identification of VT3 and RCP2 (*3.5.1 Limitations and Future Recommendations*). While only a small number identified a third threshold, where VT3 was present, there was excellent ICC between tests and low CV% and comparable SEM and MDC, indicating high test re-test reproducibility and low variability between visits. Further research is required to investigate the prevalence of VT3 and RCP2 during treadmill tests.

4.5.3. The prevelance of a third threshold in cyclists and runners

The third aim of this study was to assess the prevalence of VT3 across different exercise modalities. The comparison of thresholds across mean time, %TTE and %PPO were consistent with the previous chapter, where VT1 and GET1 significantly differed from each other and every other threshold (*Table 5 and Table 15*). Parllares *et al* also reported the workload identified at VT1, maximal lactate steady state, and VT2 were

all significantly different from GET1 (via the V-Slope method).[11] The separation between VT1 and GET1 was also demonstrated by another study, suggesting the independent identification of VT1 and GET1 could indicate the participants' fitness level.[71] This notion was reinforced by a retrospective analysis identifying a “double threshold” in 51 participants' respiratory data, identifying those with the double threshold spent longer in the isocapnic buffering period.[155] However, the ‘double threshold’ was not consistently identified throughout all measures, with PO presenting no difference between VT1 and GET1. Further explanations for the prevalence of a ‘double’ threshold could result from protocol flaws or discrepancies in the identification of either VT1 or GET. (*Discussion 3.4 [Paragraph 1]*). PO at GET1 and VT2 were also not significantly different within the cycle test; however, there is no evidence to explain this overlap. PO has shown to be more sensitive to different protocols than other measures, such as $\dot{V}O_2$. Therefore, the application of other measures, less influenced by the protocol, could be preferable to PO.[99]

As seen with the cycle test, time, %TTE and %PPO at VT1 and GET1 were significantly different, However, the run test did not reflect the same differences between thresholds as time and ${}_V\dot{V}O_{2Peak}$ showed no difference between VT1 and GET1. While a difference in VT1 and GET1 has been identified within the literature, it is not always the case[155] as much of the surrounding liturature associate both VT1 and GET1 with the transition from the isotonic to isocapnic buffering phase.[64, 65] Two groups of runners categorised by trained status reported different results when comparing VT1 and GET1. Participants with a lower $\dot{V}O_{2Max}$ [40-51ml·kg^{-1}·min^{-1}] reported no difference between VT1 and GET1, whereas those with a higher $\dot{V}O_{2Max}$ [56.4-72 ml.kg^{-1}.min^{-1}])

demonstrated a difference between VT1 and GET1, with GET1 occurring at a significantly higher intensity.[71] The mean $\dot{V}O_{2Max}$ of the treadmill runners within the present study was 50.0 $ml \cdot kg^{-1} \cdot min^{-1}$, therefore on the upper boundary of the lower $\dot{V}O_{2Max}$ group. It is plausible that in the current study, the trained status of the runners might not be high enough to demonstrate the disparity between the two thresholds. Alongside this, a retrospective analysis conducted by Rovai *et al* found that the 11% of participants that had a difference between VT1 and GET1 also could tolerate a greater volume of exercise and prolong the duration spent within the isocapnic buffering period before acidosis and exercise-induced hyperventilation.[155] Identification of VT3 and RCP2 was not consistent in the treadmill test. The previous chapter suggests that the third threshold is only attainable for those of a higher trained status. While the data around VT3/RCP2 within the cycle test cannot be explored; the gap identified between VT1 and GET1 within the cycle test (but not consistently within the treadmill test) can be explored. While maximal and submaximal parameters cannot be compared between modalities, the gap between VT1 and GET1 could indicate that the cycling group had greater exercise tolerance, and increased capacity to deal with fatigue.[155] Subsequently, the gap between VT1 and GET1 could be indicative the cyclists being of a greater trained status, justifying the abundant prevelance of VT3/RCP2 cycle test group and not the treadmill test group. Equally, the contrasting findings between cycling and running at respective thresholds could also reflect the different physiological demands underpinning each modality.[181, 198] Subsequently, the different metabolic demands across different modalities will stimulate different physiological responses, altering

how VT1 and GET1 are reflected through ventilatory and gas exchange responses and subsequently identified.

There has been a wide debate within the literature as to whether RCP1 is an independent threshold to VT2, occurring at a higher exercise intensity, or if they are interchangeable methods used to identify a second threshold. Within this study and the first experimental chapter, VT2 and RCP1 have not been significantly different across a number of variables for both cycle test and treadmill tests (*Table 5, Table 15, and Table 16, respectively)*. This reinforces the theory that VT2 and RCP1 reflect the same transition point from isocapnic to hypercapnic buffering in the populations and protocols implemented here (*Discussion 3.4 [Paragraph 3-6]).* Initially, it was theorised that VT2/RCP1 occurs between 50-75% $\dot{V}O_{2Max}$.[70] The identification of VT2 and RCP1 within this study (*Table 15 and 13)* and the previous study (*Table 5)* occurred between 71-77% PPO, which is consistent in other research conducted on participants of a comparable trained status (60-82% PPO).[94, 199] Identification of VT2/RCP1 consistent with the literature also increases the confidence in the identification of VT3/RCP2, as it is less likely VT2/RCP1 was mistakenly identified as a third threshold. Furthermore, it potentially justifies why previous research reported differences between VT2 and RCP, as the assumption that only one transition point would occur beyond moderate exercise intensities may have interfered with the threshold identification. VT3 and RCP2 have been consistently and repeatedly identified at a significantly greater time, PO, and %PPO than VT2 and RCP1. Across Chapter 2 and the present study, the mean %PPO for the identification of VT3 and RCP2 was between 89.9%-91.2%. If VT2/RCP1 was identified earlier within this study, (at lower %PPO for example), it

would suggest subjective bias of threshold identification to promote the identification of VT3, as the earlier identification of VT2/RCP1 would have allowed more time and opportunity to see a third threshold. However, this not being the case, further increases confidence in the identification of VT3/RCP2. The wider research also supports it, with speculation of a third threshold beyond VT2 at a severe exercise intensity close to exhaustion.

However, the same confidence was not reflected within the treadmill test. Within the wider research, VT2 and RCP1 can be identified between 80-84% $_{V}\dot{V}O_{2Peak}$ during treadmill tests,[25, 200] which is comparable to the 84-86 % $_{V}\dot{V}O_{2Peak}$ VT2 and RCP1 were identified within this study. However, in elite athletes and runners, VT2 has been reported at greater exercise intensities (87.7±4.1%$_{v}\dot{V}O_{2Peak}$),[200] a percentage which starts to overlap with where VT3/RCP2 theoretically occurs. VT3/RCP2 reflects a physiological response to exercise beyond the transition from the isocapnic phase into hypercapnia, reflected by VT2. VT3/RCP2 has been demonstrated to occur at 89.7-91.5%$_{v}\dot{V}O_{2Peak}$. Therefore, if VT2/RCP1 occurs at intensities adjacent to VT3/RCP2, it is more likely the novel third threshold is a continuation of physiological responses to hypercapnia, rather than representing an additional transitional phase in response to severe exercise. Consequently, with VT2, RCP1, VT3 and RCP2 not significantly different from each other across all variables other than %TTE a third threshold cannot be conclusively identified within running.

Treadmill testing is known to result in higher maximal parameters compared to cycling unless the cycle test is performed by trained cyclists [173, 176, 201, 202]. This is due to the increased oxygen demand from engaging more muscles, including those in the torso

and arms, which increases $\dot{V}O_2$ demand and $\dot{V}CO_2$ production, leading to higher ventilation rates, and more efficient lactate clearance.[203] In Study 1, it was found that the presence of VT3 was associated with significantly higher TTE, PPO, and P:Wt, and a trend of higher $\dot{V}O_{2Peak}$. Therefore, it is tempting to assume that a third threshold might be identified through a treadmill test, as it is associated with a response to exercise-induced stress resulting from higher exercise intensity. However, if VT3/RCP2 is a physiological by-product of exercise intensity and metabolic acidosis, then it could be expected for VT3/RCP2 to be present in running over cycling as it results in a greater $\dot{V}O_{2Max}$. However, a study found that the magnitude and duration of excess post-exercise oxygen consumption (EPOC) following moderate cycling or running was not significantly different between modalities.[204] Another study comparing cycling and running found that lactate concentrations at submaximal (30%, 60%, 80%) and max intensities reported higher lactate concentrations and RER values during cycling exercise than running.[198] These findings were supported by Scott *et al*, who demonstrated that at heavy to severe exercise intensity domains during running and cycling, there is a greater flux of anaerobic rapid glycolytic ATP re-synthesis during cycling over running.[181] The higher reliance on anaerobic metabolism and subsequently increased lactate is likely due to a smaller volume of muscle mass being recruited to generate a comparable power output to the larger volume of muscle recruited within running.[198] Furthermore, lactate clearance is significantly faster during running over cycling, with.[205] While these findings are reflective of short bouts of exercise, further research is needed applying longer durations of exercise[181](*4.1 Introduction [Paragraph 5]*). Subsequently, the resultant lactate accumulation leading

to localised fatigue and metabolic acidosis could contribute to the prevalence of VT3/RCP2 and why it is more prevalent in cycling.[198]

Much of the discussion surrounding a third threshold has been associated with metabolic acidosis being the main driver of exercise-induced hyperventilation and other responses identified at and beyond VT2.[8] It could therefore be suggested that the physiological demands of running do not create the extreme acidic environment seen with cycling that is required to drive an additional third response. Moreover, within this study, the mean $\dot{V}O_{2Max}$ with the cycling and running group was comparable ($50.2\pm6.3 ml.kg^{-1}min^{-1}$ and $50.01\pm5.9 ml.kg^{-1}.min^{-1}$). However, while cyclists' $\dot{V}O_{2Max}$ was often comparable across modalities, the $\dot{V}O_{2Max}$ for runners differ between modalities [202] with runners likely to achieve a lower $\dot{V}O_{2Max}$ score on a cycle test compared to a treadmill test. Therefore, it could be postulated that if VT3 is identifiable within a treadmill test, the relative fitness required to achieve it might be greater to attain the level of exercise stress and metabolic acidosis required to achieve VT3. Furthermore, with endurance athletes adaptations vastly tailored to optimise aerobic performance and prevent metabolic acidosis. Therefore, it should be considered that the prevalence of a third threshold would be more evident within well-trained athletes more familiar with both anaerobic and aerobic demands of exercise, i.e. an intermittent invasion sport athlete such as football. Like cyclists, such athletes will be better equipped to work anaerobically for longer, potentially simulating the metabolic environment required to achieve VT3/RCP2.

4.5.4. Trained status and the prevelance of a third threshold in cyclists and runners

The final aim of this study was to compare the performance of participants who showed VT3 in both cycling and treadmill modalities. The results showed that in the cycle test, there was no significant difference in TTE and P:Wt between those who exhibited VT3/RCP2 and those who did not. With only one participant not consistently identifying VT3 or RCP2 across both visits, the results do not harbour enough power to make conclusive findings. However, effect sizes suggest a trend within the data whereby TTE, PPO, P:Wt, and $\dot{V}O_{2Peak}$ is generally higher within those that presented VT3/RCP2. This supports previous research that showed a relationship between higher maximal values for parameters such as $\dot{V}O_{2Peak}$, TTE, PPO, and P:Wt and the trained status of an athlete.[168] Furthermore, this is consistent with the previous chapter, which also presented significant differences between groups that did and did not present VT3/RCP2 for age as well as P:Wt, and TTE, complementing the large effect sizes reported for TTE, P:Wt and PPO and moderate effect sizes for $\dot{V}O_{2Peak}$. Results of the treadmill test demonstrated no difference between BMI, $\dot{V}O_{2Peak,}$ TTE, and $_{V}\dot{V}O_{2Peak}$ between those that did and did not achieve VT3/RCP2. However, there was a significant difference between the age of the two groups (*Table 18)*. As discussed in the previous study (3.4 *Discussion [paragraph 8])*, the impact of age on cyclists' fitness means that master cyclists should be independently categorised to younger cyclists.[162] Research suggests that ageing results in decreases in training intensity and volume alongside decreased maximal oxygen consumption and running speed at the lactate thresholds within masters athletes.[206] A further study comparing age in running, cycling, and

swimming performance found that decrements in running were more prominent than in cycling or swimming.[207] During long-distance running (marathon running, 10,000m and 5000m), performance declined up to 14% between the ages of 35-45 years, declining a further 12%-19% by 50 years. In comparison, long-distance cycling declined up to 11% between 35-45 years and 6%-14% at 50 years. At the age of 85, long-distance running performance declined by 98%-294%, whereas swimming declined by 90-107%.[207] Declines in running performance are particularly prominent, as this modality evokes greater impact and 'wear and tear', with greater risk of injury than other endurance sports like cycling or swimming.[206-208] Therefore, it is less likely for runners to continue competing and training as intensely, resulting in reductions in performance capability earlier. However, the limited number of participants who demonstrated VT3/RCP2 during the treadmill test means that the interpretation of these results should be approached with caution, and the findings related to age should be further explored through additional research.

4.5.5. Limitations and future recommendations

A possible limitation associated with methods this study used to identify thresholds, is application of visual identification rather than computerised methods. The reason for using the visual method of threshold identification over less subjective, automated methods was because previous research has shown no significant difference between the visual and computerised methods. Moreover, the visual method has demonstrated high test-retest reliability.[171, 188] However, one study found that while there was a high correlation between visual and computerised assessments of variables at VT2, there was less agreement between the two methods at VT1.[171] We can therefore be confident that

the present study implemented an acceptable approach to threshold detection and reports excellent interrater reliability. Despite this, in light of the usefulness and objectivity of computerized methods, automated detection of VT3/RCP2 should be considered in future research.

The present study split participants into dichotomous groups depending on the presence of VT3. In doing so, the number of participants is divided into smaller groups, reducing the power of the results. To power the study further, including those with a higher aerobic capacity(161) and therefore of a better trained status would help to understand further the relationship between VT3/RCP2 and trained status.

A further consideration of the current study is the underrepresentation of females. While maximal parameters will differ between males and females, the prevalence of submaximal thresholds relative to maximal exertion does not differ between genders.[209] However, there was no consideration for how the menstrual cycle might impact cardiopulmonary responses to exercise. Therefore no data was collected to determine what phase of the menstrual cycle the female participants were in during testing. A pairwise meta-analysis found that performance outcomes were reduced in the early follicular phase during both endurance and strength-based tests compared to other phases of the menstrual cycle.[210] It is conceivable that female participants between visits one and two could have been between the follicular phase and subsequently influenced physiological response during incremental exercise. However, other research has demonstrated that the performance and prevalence of lactate and ventilatory thresholds do not fluctuate significantly throughout the menstrual cycle.[211] Although researchers did report an increased ventilatory drive during the luteal phase

compared with the follicular phase, which could also influence the identification of submaximal thresholds.[211]

Lastly, the prevalence of VT3/RCP2 in the treadmill test could be related to the protocol implemented. Different treadmill protocols have been shown to influence the presence of submaximal thresholds, [157] with one study demonstrating that small speed increments during treadmill tests are preferable to optimise reproducible identification of VT1, RCP1, and $\dot{V}O_{2Max.}$[212] The type of protocol used, should be adjusted and tailored to the age, experience and type of the individual being assessed. Many participants reportedly felt uncomfortable during the later stages of the test due to the stepped increase in speed and fear of not keeping up with the treadmill. In the future ramp protocol could be preferable within some clinical or less fit individuals, as it is perceived as less intimidating and more tolerable with the shorter duration and no sudden work rate changes however this might not be optimal for data collection. Each participant completed the test to exhaustion with a harness, having practised getting into the safety position. A stepped increment in the speed is more challenging due to the physical coordination needed to adjust. As such, this protocol might have instigated premature termination of the test. Applying smaller steps or a ramp protocol would be preferable in future to accommodate the participant's comfort and encourage maximum exhaustion. However, contrary to this suggestion, all participants in the current study satisfied the a-priori determinants for maximal exertion. Furthermore, ramp protocols elicit greater levels of exercise stress, higher minute ventilation, and $\dot{V}O_{2Max}$ values. If the prevalence of VT3/RCP2 is respective of exercise stress and metabolic acidosis, a ramped protocol could be more applicable to aid the identification of this threshold.

However, if the prevalence of VT3/RCP2 depends on protocol, further discussion is needed to justify whether VT3/RCP2 should be classified as a threshold or circumstantial homeostatic response. More research is needed to see if individualised protocol selection is preferable. However, this was beyond the scope of the current study aim, and a standard test was implemented with the view of identifying the presence of a third threshold.

4.6. Conclusion

Overall, this study reinforces the first study's findings, demonstrating that a third threshold can be repeatedly and reliably identified within cyclists via two independent and novel methods, with VT3 and RCP2 occurring at a significantly greater exercise intensity than VT2 and RCP1. However, the application of a third threshold within running did not present the same findings. There is some indication of VT3 and RCP2 being prevalent within running through synonymous identification of both thresholds across researchers. However, the test re-test reliability and overall reproducibility of VT3/RCP2 were not strong enough to warrant conclusive identification of a third threshold within runners. Furthermore, the comparison of the exercise intensity showed that the second and third thresholds were not significantly different across all of the measures, thus reducing the likelihood that an additional third transition point had been identified. Despite this, further research employing protocols with shorter stage durations and among well-trained and elite-level runners would be beneficial to investigate further the prevalence of VT3/RCP2 within running. Lastly, further exploration surrounding the prevalence of VT3 and trained status demonstrated that those achieving VT3/RCP2 also attained a greater TTE, PO, and P:Wt. However, the

limited sample size reduces the power of these findings. Therefore, further research comparing untrained and well-trained/elite cyclists or runners and the prevalence of VT3/RCP2 would be beneficial to explore this aim further.

5. Summary chapter

5.1. Thesis discussion/summary chapter

Endurance athletes often use ventilatory and gas data to determine personalised training thresholds for prescribing training zones. However, the wide scope of research has resulted in overlapping and conflicting terminology, leading to a lack of consistency in the literature when discussing specific thresholds. Furthermore, understanding the physiological and metabolic underpinning of the thresholds identified during moderate to severe exercise intensities is widely speculated, with few conclusive findings. Within this, there has been controversy surrounding the VT2 and RCP. Some suggest the two thresholds can be used interchangeably to describe the transition from the isocapnic buffering phase into hypercapnia. At the same time, other studies claim RCP overestimates VT2 and therefore is not a reliable parameter. Further studies suggest the disparity between the thresholds results from a third threshold occurring during severe exercise intensities and reflects a third physiological transition point. Moreover, the discrepancy between the two thresholds was only identified within well-trained/elite endurance athletes, suggesting that a third threshold's prevalence is only attainable due to training adaptations. Subsequently, the overarching aim of this thesis was to draw comparisons between VT2 and RCP and investigate the prevalence of additional breakpoints beyond the second threshold.

Chapter 1 discusses the literature related to training prescription, depicting the different methodologies used to identify ventilatory thresholds and the physiological underpinnings of each threshold. The literature review focused on understanding what is currently understood about each threshold, identifying the established methodologies

used to classify the first and second thresholds, and exploring the conflicting literature around VT2 and RCP1. Within this chapter, VT3 and RCP2 were successfully identified at a significantly greater exercise intensity than VT2 and RCP1. Moreover, the trained status of the participants that demonstrated VT3/RCP2 was also of a greater trained status compared to those that did not achieve a third threshold. These findings then informed the methodology and aims of the first experimental chapter.

The first experimental chapter measured breath-by-breath gas and respiratory responses (V_E, $\dot{V}O_2$, $\dot{V}CO_2$, EQO_2, $EQCO_2$, $PETO_2$, and $PETCO_2$) to maximal incremental cycle test. These responses were plotted and visually inspected to identify inflexion points within the data. Commonly used methods were employed to identify thresholds, including VEQ with $PETO_2$, the V-Slope method to determine the first threshold (VT1 and GET1, respectively), and VEQ with $PETCO_2$ and $V_E/\dot{V}CO_2$ slope to identify the second threshold (VT2 and RCP1, respectively). Two novel methods were then adopted to investigate the prevalence of additional thresholds, coined VT3 and RCP2, using adaptations of the methods used to identify VT2 and RCP1. Contrary to Ozkaya *et al* findings, the first experimental study demonstrated that VT2 and RCP1 were not significantly different in cyclists, suggesting the thresholds represent the same physiological transition point. At the same time, the lack of difference between VT2 and RTC1 could be attributed to the participants within this study having a lower trained status ($\dot{V}O_{2Peak}$=51.5±8.2ml·kg^{-1}·min^{-1}) compared to the participants within Ozkaya *et al* study ($\dot{V}O_{2Max}$=66.8±7.8mL·min^{-1}·kg^{-1}) that demonstrated the grey zone between VT2 and RCP1. The subsequent application of the novel methods used to identify a third threshold successfully identified VT3 and RCP2 within 72% of participants, which

complements Ozkayas overall suggestions regarding a third threshold. Subsequently, this study builds on Ozkaya *et al* hypothesis, confirming the presence of a third threshold while demonstrating that multiple respiratory and gas exchange parameters reflect the third threshold and are not a response isolated to RCP1 or V_E. Furthermore, within this study, while the participants that did present VT3/RCP2 were not considered "elite", they were also of greater trained status than those that didn't. These findings complement Ozkaya *et al* research, demonstrating that above a particular trained status (potentially trained–well-trained status), cyclists have a different or additional physiological response to severe exercise intensities,[2, 8-10] presenting as a third transition point. However, the exact trained status required is yet to be established. A single researcher's visual identification of the thresholds provided little control for subjective identification. These findings inspired the second study, which analysed both interrater reliability of threshold identification and test re-test reliability of the thresholds to reduce the chances of inaccurate subjective analysis. Study two also investigated whether VT3/RCP2 can be identified during a treadmill test within active individuals, runners and triathletes.

The experimental study in Chapter 3 implemented the same visual methodologies and cycle test protocol as Chapter 2 to allow for a comparison of findings across the discussion and conclusion of the thesis. The inclusion of a double-blind identification of ventilatory thresholds by multiple researchers, followed by a collaborated agreement, found that the visual identification of all the thresholds was valid and reliable. In addition, the test re-test reliability of the thresholds was evaluated. Good-excellent reproducibility of commonly used thresholds (VT1, GET1, VT2, RCP1) was reported,

with the results comparable to the surrounding literature. In addition, the novel identification and test re-test reliability of VT3/RCP2 was also deemed to be excellent within the cycle test. There was some evidence to suggest VT3/RCP2 can be visually identified in a treadmill test; however, only a small number of participants repeatedly presented the third threshold. As there were confident levels of interrater reliability for VT3/RCP2 within a treadmill test, the lack of reproducibility of the third threshold within participants could be due to unsuitable protocol choice, with the longer stage durations potentially limiting the exercise intensity and need for anaerobic respiration.[212] Alongside this, due to the more aerobic nature of running, participants of higher trained statuses were needed to achieve a greater intensity to stimulate the physiological response that reflects a third transition point.[198] Despite this, Chapters 2 and 3 exhibit trends suggesting that individuals of a better-trained status are more likely to report a third threshold via VT3 and/or RCP2. While no definitive trained status has been identified, this study suggests a third threshold is identifiable in those categorised at least as trained/well-trained.[162]

5.2. Limitations and recommendations for future research

Despite the visual evidence supporting a third threshold, modern methods of threshold analysis involving computational identification methods could be preferable as they remove subjective bias and are more time efficient. Despite the documented problems of visual identification methods, they have been considered the “gold standard” alongside lactate threshold measures, providing acceptable and reliable threshold identification.[171] Literature comparing visual and computational methods of threshold identification demonstrate no significant differences between methods, with highly

correlated results.[171, 188] Throughout the literature, there are multiple computerised methods that can be applied. One study compared nine different regression-based computerised methods to identify the first threshold. Overall, there was little agreement across the methods, suggesting a combination of methods would likely be preferable to reliably identify the first threshold, concluding that automated threshold identification should be used as an aide – but not as a definitive result.[95] Moreover, computerised methods, like linear regressions, require a prior assumption of the number of breakpoints, i.e. bi-segmental or tri-segmental analysis,[96] which re-introduces a forced identification of breakpoints during analysis. Alternatively, applying AI and computer learning methodologies could provide a reliable, objective, and quick method of identifying thresholds. Machine learning automatically identifies thresholds through neural networks that identify non-linear relationships between variables.[98, 213] Accuracy of identification improves by adding new data, and unlike regression analysis, can handle multiple variables at a time. A couple of studies demonstrated the neural network's ability to competently identify VT1 and VT2 to a level of accuracy comparable to the current "gold standard" visual identification methodology.[98, 213] However, it is important to note, the prevalence of thresholds is different dependent on the trained status of the athlete, protocol, exercise mode and more. Therefore there will likely need to be specific neural networks for various categories and connotations.[98] Currently, databases are being built, refining AI technology with the aim for physiologists to have access to AI beyond the laboratories.[98]

There is contradictory evidence regarding the preferable protocols to apply when identifying submaximal thresholds. While the ramp protocol is preferable to optimise

maximal parameters and achieve a higher $\dot{V}O_{2Max}$ (*3.12. Stepwise vs ramp protocol*), research indicates it also overestimates and reduces the reliability of submaximal threshold identification.[99, 109, 214] Further literature also suggests that smaller speed increments, especially within treadmill tests, can be preferable to determine ventilatory and gas exchange thresholds. The gradual increments in exercise allow time for metabolic adjustments to exercise intensity.[212] Some studies suggest that protocols with stages lasting more than 3 minutes lower ṾO2Max values within treadmill test and reduce VT1 and RCP consistency.[107, 212] While a stepwise protocol was used to ensure no lag or delay in physiological responses due to rapid increments, shorter duration step increments could be beneficial in improving the identification of VT3/RCP2 within the treadmill tests.[212] The 3-minute stages, while allowing for adjustment between increments, likely meant the participants did not reach a severe enough exercise intensity before stopping. Future research could compare the identification of VT3/RCP within different stepwise and ramp protocols to assess which is more reproducible and reliable. Further research could compare the prevalence and reliability of VT3/RCP2 within cycle tests, treadmill tests and other modalities such as rowing, swimming, and arm crank.

Within this thesis's participant pool, we confidently identified a third threshold via two visual methods (VT3/RCP2). However, there was little diversity among the trained status and gender of the participants in both studies, which limited the deeper understanding of the third threshold. Some of the findings support the hypothesis stating VT3/RCP2 is dependent on trained status, which is also referenced in the wider literature. Additional confidence in this hypothesis could be gained by comparing

cardiopulmonary data from untrained, trained, and elite athletes. Furthermore, independent analysis of the prevalence of VT3 in males and females could further develop the understanding surrounding the novel third threshold and potential implications of the menstrual cycle in females (3.4.1 Limitations and future recommendations [paragraph 3]).

Lastly, subsequent research could investigate the physiological and metabolic responses during severe exercise intensities, which may provide insights into the mechanisms underlying the exercise-induced hyperventilatory response. Particularly, suppose metabolic acidosis and exercise tolerance are precursors for the prevalence of a third threshold. In that case, future research should further explore this hypothesis by measuring lactate, pH, and potentially HCO_3 responses alongside the attainment of VT3/RCP2.

5.3. Practical applications

Throughout both studies, the prevalence of a third threshold has been consistently identified via two independent methods. Further research is required to assess what variables contribute to this threshold's prevalence. Whether it be trained status, physiological responses to severe exercise-induced stress, or metabolic acidosis, understanding the third threshold underlying mechanisms would help focus the practical application of VT3/RCP2. This present thesis has unpacked the presence of a third threshold, and the exercise domain (high–severe) VT3/RCP2 occurs. Exercise at severe intensities, ~90% $\dot{V}O_{2Max,}$ (equivalent to VT3/RCP2), is synonymous with high intensity interval training (HIIT). HIIT training has been defined "as short bursts of vigorous activity, interspersed by periods of rest or low-intensity exercise for recovery".[63] The

exercise intensity ranges from between 70%-90% PPO[215-217] to maximal exertion[218] or even supramaximal intensities up to 170% of an individual's $\dot{V}O_{2Max.}$

Research suggests HIIT invokes improvements in buffering capacity, ventilatory thresholds, fat oxidation, and anaerobic capacity.[219] A study using well-trained individuals demonstrated improvements in $\dot{V}O_{2Peak}$, VT1, VT2, and anaerobic capacity with the participant's ability to tolerate greater volumes of lactate following four weeks of various forms of HIIT programmes.[220] Currently, when conducting HIIT training, there is no identified optimal prescribed intensity, with most HIIT training being completed at an arbitrary percentage of someone's $\dot{V}O_{2Max.}$ or a voluntary maximal intensity. Application of arbitrary units was not specific enough to optimise training adaptations (*2.3 Threshold testing and training, [paragraph 3]*); thus, prescribed training thresholds corresponding with VT1, GET1, VT2, and RCP1 personalise and optimise training for athletes. Moreover, Meyer *et al* established that when a constant workload is set, the higher the percentage of $\dot{V}O_{2Peak,}$ the greater the metabolic strain varies between participants, further reinforcing that percentages alone may not be sufficient to elicit optimal, targeted metabolic responses to training.[35] VT3/RCP2 exercise intensity is akin to that described in HIIT research. Therefore, VT3/RCP2 could provide individualised training at severe exercise intensities, optimising performance and promoting marginal gains within elite endurance athletes. Moreover, prescribing training at VT3/RCP2 could significantly reduce overtraining through non-specific training programmes, which is even more important when training at severe exercise intensities that elicit greater levels of exercise stress.

A further measurable parameter, critical power (CP), is also associated with heavy-severe exercise intensity domains, attributing CP with VT3/RCP2. CP aims to determine the highest work rate that can be maintained without fatigue and can be depicted on a power/velocity-time curve. Throughout the literature, CP has been associated with multiple other parameters; VT1, GET1, VT2, RCP1, and maximal lactate steady state.[94, 191] A systematic review and meta-analysis identified that maximal lactate steady state consistently under-estimated CP on average by 11%, while VT2 and RCP overestimate CP by 6-21%.[191] Furthermore, studies suggest that when exercising at CP, participants attained a steady state within pulmonary gas exchange, ventilation, and blood lactate.[221] Such physiological attributes of VT3/RCP2 are more consistent with responses that occur above CP, such as an exercise-induced hyperventilatory response,[8, 10] and rising $\dot{V}O_2$ values.[221] With the differing reported physiological responses of the two thresholds, and the surrounding research suggesting CP occurs equivalent to or before VT2/RCP1, it is likely CP is not comparable to VT3/RCP2, despite being described as reflecting heavy-severe exercise intensities.

5.4. Conclusion

A third threshold (VT3 and RCP2) was repeatedly identified in cyclists at ~90% PPO by two novel visual identification methods. A similar trend was also reflected within incremental treadmill tests, with VT3 and RCP2 identifiable at a comparable $\%_V\dot{V}O_{2Peak}$ (~91% $_V\dot{V}O_{2Peak}$). However, a third threshold during treadmill exercise was only identified repeatedly in a small sample of participants. Therefore further research is needed to explore the prevalence of VT3/RCP2 during this modality. Additional findings suggest that the VT3/RCP2 is more likely prevalent in trained, well-trained,

elite athletes. This notion is supported by wider research regarding VT3/RCP2 and other submaximal thresholds. Further research should address the determinants of achieving a third threshold, its prevalence in well-trained runners, and whether this threshold can benefit the personalised approach to training prescription.

6. References

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