

**Effect of carbohydrate ingestion during prolonged exercise on  
durability of the moderate-to-heavy intensity transition and severe-  
intensity performance**

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# Abstract

**Purpose:** ‘Durability’ refers to resilience to the effect of prolonged exercise on physiological profiling variables, such as power output at the moderate-to-heavy intensity transition. The effect of carbohydrate ingestion on durability of the moderate-to-heavy intensity transition has not been studied. Therefore, the purpose of this investigation was to determine the effect of carbohydrate ingestion during prolonged exercise on durability of the moderate-to-heavy intensity transition and severe-intensity performance.

**Methods:** Twelve well-trained cyclists and triathletes (10 males, 2 females;  $\dot{V}O_{2peak}$ ,  $59 \pm 5$  mL kg<sup>-1</sup> min<sup>-1</sup>; training volume,  $14 \pm 5$  h week<sup>-1</sup>) underwent a characterisation trial and three experimental trials. Participants performed an incremental test and 5-min time-trial (TT) without prior exercise (PRE), and after 150-min of moderate-intensity cycling, with (POST<sub>CHO</sub>) and without (POST<sub>CON</sub>) carbohydrate ingestion.

**Results:** Power output at the moderate-to-heavy intensity transition was lower in POST<sub>CHO</sub> ( $225 \pm 36$  W,  $\Delta -7 \pm 3$  W,  $\Delta -3 \pm 2\%$ ,  $P = 0.0265$ ) and POST<sub>CON</sub> ( $216 \pm 35$  W,  $\Delta -13 \pm 9$  W,  $\Delta -6 \pm 4\%$ ,  $P = 0.0011$ ) than PRE ( $229 \pm 37$  W), and lower in POST<sub>CON</sub> than POST<sub>CHO</sub> ( $\Delta -7 \pm 9$  W,  $\Delta -3 \pm 4\%$ ,  $P = 0.0186$ ). The prolonged exercise-induced reduction in metabolic power was greater in POST<sub>CON</sub> than POST<sub>CHO</sub> ( $P = 0.0101$ ). Mean power output in the 5-min TT was lower in POST<sub>CHO</sub> ( $351 \pm 53$  W,  $\Delta -15 \pm 12$  W,  $\Delta -4 \pm 3\%$ ,  $P = 0.0251$ ) and POST<sub>CON</sub> ( $328 \pm 63$  W,  $\Delta -35 \pm 38$  W,  $\Delta -10 \pm 10\%$ ,  $P = 0.0272$ ) than PRE ( $363 \pm 55$  W), but POST<sub>CHO</sub> and POST<sub>CON</sub> were not significantly different ( $\Delta 25 \pm 37$  W,  $\Delta 9 \pm 13\%$ ,  $P = 0.1859$ ).

**Conclusion:** These data suggest that durability of the moderate-to-heavy intensity transition is improved with carbohydrate ingestion. Athletes are therefore able to maintain higher power outputs in relation to their intensity domain transitions during prolonged exercise when ingesting carbohydrate. Additional research is necessary to further understand the underlying mechanisms.

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# Attestation of Authorship

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.”

# Co-Authored Works

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# Ethics Approval

All experimental procedures of this thesis were approved by the Auckland University of Technology Ethics Committee on 3<sup>rd</sup> August 2023 (23/197).

# Chapter 1: Introduction

Athlete profiling is an important component for coaches or physiologists when working with endurance athletes. Profiling methods vary from field- to laboratory-based assessments, which aim to determine particular physiological landmarks, or specific external output measures. The outcomes of these profiling assessments can then be used to inform general and specific training prescription, as well as understanding performance capabilities. Importantly, identifying additional variables that might alter profiling outcome measures should be further studied to improve the validity and reliability of athlete profiling.

This thesis explores the effect of carbohydrate ingestion during prolonged exercise on durability of the moderate-to-heavy intensity transition and severe-intensity performance. In this chapter, traditional endurance performance metrics and athlete profiling methods will be discussed, followed by how these metrics and profiling methods are influenced by physiological durability and carbohydrate ingestion. Due to this thesis being presented in Format 2: manuscript structure, there is some repetition between the literature review in Chapter 2, and the introduction of the manuscript in Chapter 3.

The outline of this thesis is shown in Table 1. Following this short introduction, Chapter 2 is a substantial literature review which describes physiological profiling, and how durability is often overlooked in physiological models of endurance. Chapter 3 is an experimental study, which addresses the following research question: does carbohydrate ingestion during prolonged exercise partially mitigate the decline in power output at the moderate-to-heavy intensity transition and severe intensity performance? Chapter 4, will further discuss the results of Chapter 3, placing them in an applied context and offering directions for further research.

Table 1: Thesis outline.

Chapter	Title	Purpose
1	Introduction	General introduction to the thesis
2	Durability in the context of physiological profiling	Narrative literature review on physiological profiling in endurance sport, and the implications of durability
3	Effect of carbohydrate ingestion during exercise on durability of the moderate-to-heavy intensity transition	A randomised, counterbalanced, crossover study assessing the effect of carbohydrate ingestion during exercise on durability of the moderate-to-heavy intensity transition, and severe intensity performance
4	Summary and future directions	Overall discussion in which the results of Chapter 3 are applied to practice, and future research directions are offered

# Chapter 2: Durability in the context of physiological profiling

## Intensity domains

Exercise is characterised by discrete exercise intensity domains. Exercise performed in the moderate-, heavy-, and severe-intensity domains elicits distinct metabolic, cardiovascular, respiratory, and neuromuscular responses, which ultimately influences the amount of work achievable, and the accumulated physiological stress (Black et al., 2017; Jones et al., 2008). Work outputs at the boundaries between the intensity domains—the so-called ‘thresholds’—are used in applied settings for training load monitoring, training programming, within-session intensity regulation, monitoring adaptations to training, and predicting performance (Maunder et al., 2021) (Figure 1).

The lactate threshold defines the boundary between the moderate- and heavy-intensity domains. Exercise below the lactate threshold, in the moderate-intensity domain, can be sustained for multiple hours and elicits subtle perturbations in cardiovascular, respiratory, and muscle metabolic responses which quickly establish a steady state (Black et al., 2017; Seiler & Kjerland, 2006; Sylta et al., 2014). Blood and muscle lactate concentrations and pH remain unchanged from resting values, while oxygen consumption ( $\dot{V}O_2$ ) and plasma  $K^+$  concentration increase gradually but reach a steady state within minutes (Black et al., 2017; Goodwin et al., 2007; Roston et al., 1987). Exercise in the moderate-intensity domain results in modest changes in muscle metabolites, such as ATP and PCr concentrations, while muscle glycogen becomes depleted following multiple hours of moderate-intensity exercise (Black et al., 2017; Coyle et al., 1986; Gonzalez et al., 2015; Jeukendrup et al., 1999). Blood glucose concentration will also progressively decline during multiple hours of moderate-intensity exercise, as liver glycogen stores are depleted, and this may be linked to fatigue as hypoglycaemia develops (Gonzalez et al., 2015; Noakes, 2022). Additionally, the moderate-to-heavy intensity transition is a threshold for autonomic nervous system (ANS) disturbance, whereby post-exercise heart rate variability (HRV) remains suppressed for potentially multiple hours following exercise in the heavy-intensity domain, while very little disturbance in HRV is seen following exercise in the moderate-intensity domain (Seiler et al., 2007).

The maximum metabolic steady state (MMSS) defines the boundary between the heavy- and severe-intensity domains (Faude et al., 2009; Jones et al., 2019; Maunder et al., 2021). Within the heavy-intensity domain, plasma  $K^+$  concentrations rise more noticeably due to intensified muscle contractile activity, while lactate production surpasses clearance capacity, leading to accumulation in blood and muscle lactate and a subsequent decline in pH (Black et al., 2017; Poole et al., 2016). However, plasma  $K^+$  and muscle and blood lactate are still able to stabilise after ~10-min of heavy-intensity exercise, hence exercise in the heavy-intensity domain

elicits a delayed steady state (Black et al., 2017). In the heavy-intensity domain, steady state ATP and PCr concentrations, and muscle and blood pH, will be lowered compared to prolonged exercise performed in the moderate-intensity domain (Black et al., 2017; Jones et al., 2008).

During exercise in the severe-intensity domain, muscle metabolic homeostasis cannot be attained and therefore an unsustainable cardiovascular and metabolic response occurs that is characterised by the absence of a steady state (Jones et al., 2008). In the severe-intensity domain, even at a constant external work rate, muscle efficiency declines (Poole et al., 2016). This induces the  $\dot{V}O_2$  slow component, or a gradual upward drift in  $\dot{V}O_2$  until the attainment of the individual's maximum rate of oxygen consumption ( $\dot{V}O_{2max}$ ) at task failure (Colosio et al., 2020; Jones et al., 2008, 2011). Additionally, blood and muscle lactate and plasma  $K^+$  concentrations progressively increase until task failure, while muscle ATP and PCr concentrations and pH progressively decline until failure (Black et al., 2017; Jones et al., 2008). These variables will result in similar peaks or nadirs at task failure in the severe-intensity domain, regardless of whether exercise is performed at the upper (~15 min) or lower (~3 min) end of external work rates in the severe-intensity domain (Black et al., 2017; Jones et al., 2008). Thus, it has been suggested that fatigue in the severe-intensity domain occurs due to achievement of the maximum tolerable muscle metabolic disturbance (Black et al., 2017; Jones et al., 2008). Interestingly, the magnitude of autonomic disturbance following exercise performed in the severe-intensity domain does not greatly differ from that observed in the heavy-intensity domain, possibly due to the shorter sustainable duration of exercise in the severe-intensity domain (Seiler et al., 2007). Fatiguing severe-intensity exercise also results in more modest reductions in muscle glycogen stores than fatiguing moderate- or heavy-intensity exercise, as exercise typically terminates prior to the depletion of muscle glycogen stores (Black et al., 2017; Seiler et al., 2007).

Therefore, exercise performed in the moderate-, heavy-, and severe-intensity domains elicits distinct physiological response kinetics, characterised by achievement of either a rapid steady state, delayed steady state, or no steady state, respectively. These clear differences in the physiological response to exercise performed in the different intensity domains have implications for the type and degree of stress induced by the exercise, as evidenced by the different autonomic disturbances. These distinctions have clear implications for training load monitoring, training intensity regulation, and predicting performance.

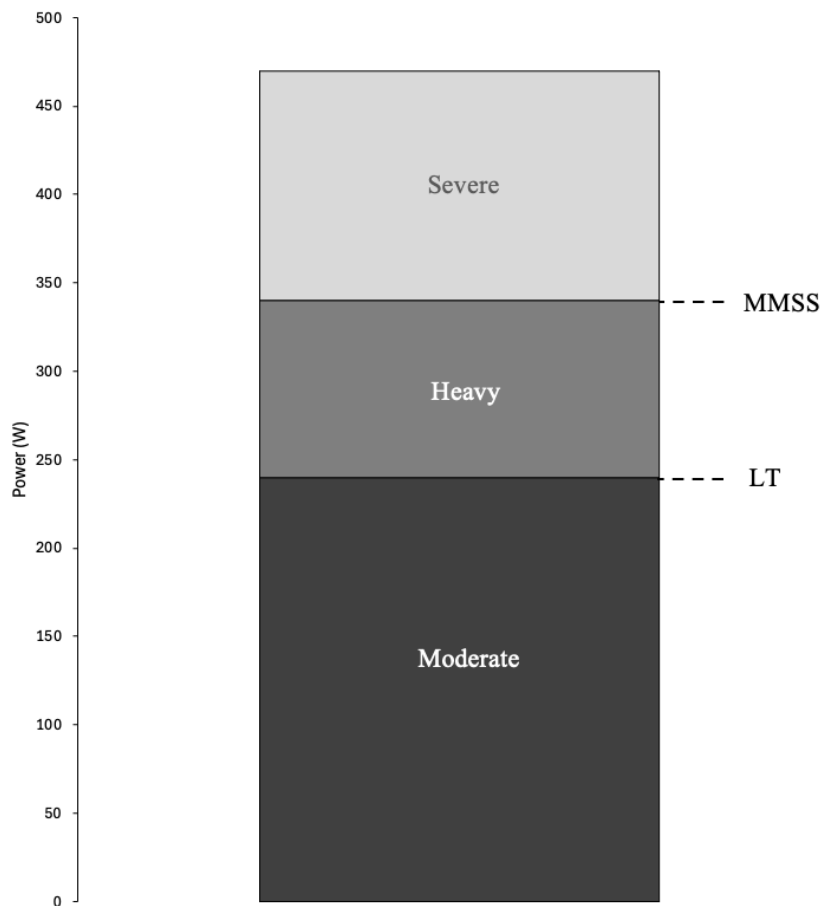


Figure 1: Boundaries between the moderate-, heavy-, and severe-intensity domains are defined by the lactate threshold (LT) and maximal metabolic steady state (MMSS).

## Application to practice

One of the fundamental roles of applied performance physiologists working with endurance athletes is to profile the work rates at which the intensity domain transitions, or the lactate threshold and maximum metabolic steady state, occur. Knowledge of the work outputs at the intensity domain transitions allows for more physiologically-informed training programming and load monitoring, as well as performance prediction. For example, a coach may wish to prescribe a low-stress training session to increase training volume, with little recovery required before subsequent sessions. In this instance, by prescribing the intensity of the session below the power/pace associated with the lactate threshold ensures that the athlete can increase training volume, while remaining under the threshold for increased autonomic disturbance (Seiler & Kjerland, 2006). Elite endurance athletes often conduct the majority of their training volume below this threshold, particularly during the preparatory phases of a season, to sustain high training volumes (Stöggl & Sperlich, 2014). Depending on the phase within a race season/macrocycle, coaches will also prescribe more, or fewer, sessions within the heavy-, or severe-intensity domains (Seiler, 2010; Stöggl & Sperlich, 2014). Knowledge of the intensity domain transitions is therefore important for programming training and accurately quantifying training intensity distribution, so that specific variations in training styles, such as polarised, threshold, or

pyramidal training, can be executed. Additionally, knowledge of the intensity domain transitions also allows athletes to regulate intensity within a session to ensure they complete the session with the desired physiological responses (Seiler & Kjerland, 2006; Sylta et al., 2014).

Monitoring changes in power output at the lactate threshold and MMSS is also useful to help coaches understand if an athlete is responding positively to training, or whether they are overreaching or detraining (Halson, 2014). Monitoring these changes may allow coaches and exercise physiologists to better predict performance, as power outputs at the intensity domain transitions are positively correlated with performance outcomes (Goodwin et al., 2007; Jones & Vanhatalo, 2017; Joyner, 1991).

Therefore, knowledge of the intensity domain transitions is useful for applying a more physiologically informed approach to training programming and monitoring. Many more physiological variables are also routinely profiling in athlete profiling assessments.

## Other physiological profiling variables

In addition to the intensity domain transitions, there are other physiological variables that are commonly measured when profiling endurance athletes. The  $\dot{V}O_2\text{max}$  refers to the maximum rate at which oxygen can be consumed, and therefore defines the aerobic capacity of an athlete (Poole & Jones, 2017). The  $\dot{V}O_2\text{max}$  is primarily limited by the ability of the cardiorespiratory system to deliver oxygen to the working muscle, as opposed to skeletal muscle oxygen extraction (Mortensen et al., 2005). As  $\dot{V}O_2$  can be calculated via the Fick equation ( $\dot{V}O_2 = \text{cardiac output} \times \text{arterial-venous } O_2 \text{ difference}$ ) (Levine, 2008), changes in  $\dot{V}O_2\text{max}$  are primarily achieved through changes in maximum cardiac output, through adaptations such as ventricular hypertrophy and the oxygen-carrying capacity of blood, such as increased blood and plasma volume (Bassett & Howley, 2000). The  $\dot{V}O_2\text{max}$  is one of the most common performance variables used to profile endurance athletes due to its sensitivity to training stimuli (Lundby et al., 2023), and ease of measurement through indirect calorimetry in the laboratory (Kaviani et al., 2018), and now in the field with emerging technologies (Montoye et al., 2020). Due to the high rates of oxidative metabolism required during endurance events,  $\dot{V}O_2\text{max}$  in heterogenous cohorts is positively correlated with endurance performance (Carlsson et al., 2013; McLaughlin et al., 2010). Accordingly, profiling  $\dot{V}O_2\text{max}$  is useful for monitoring adaptations to training and predicting performance capabilities.

Anaerobic capacity can also be measured in routine physiological profiling assessments and is useful for monitoring adaptations and pacing efforts in the severe-intensity domain. Anaerobic capacity refers to the work capacity from non-oxidative energy sources, such as the phosphocreatine and glycolytic energy systems (Hargreaves & Spriet, 2020). Anaerobic energy sources provide energy at a rapid rate, however alactic energy

stores such as PCr are quickly depleted and take time to replenish once exhausted. To sustain workloads in the severe-intensity domain, appreciable contributions from anaerobic metabolism are required, and thus the greater an athlete's anaerobic work capacity, the greater the ability to sustain workloads in the severe-intensity domain. Variables such as  $W'$  (work capacity above critical power, discussed below) or the anaerobic power reserve (power between power at  $\dot{V}O_{2\max}$  and peak power output) can be used to quantify the anaerobic capacity, which is important for predicting performance in the severe-intensity domain.

Movement economy refers to the metabolic cost of producing a given external work output (Barnes & Kilding, 2015). Being more economical is highly advantageous for endurance athletes, as less oxygen consumption and metabolic energy expenditure is required to produce a given speed or power output. Similar to  $\dot{V}O_{2\max}$ , movement economy can also be quantified via indirect calorimetry, as steady state  $\dot{V}O_2$  and  $\dot{V}CO_2$  can be used to estimate rates of energy expenditure (Barnes & Kilding, 2015). In cycling, movement economy is commonly quantified as gross efficiency, or the percentage of metabolic energy expenditure converted into mechanical power (Moseley & Jeukendrup, 2001). In running, movement economy is typically referred to as running economy and expressed as  $\dot{V}O_2$  or energy expenditure at predetermined running velocities (Barnes & Kilding, 2015; Fletcher et al., 2009). Movement economy can be altered by metabolic, cardiorespiratory, biomechanical, and neuromuscular efficiency and has a strong positive relationship with performance in endurance sports (Barnes & Kilding, 2015; Horowitz et al., 1994; Joyner & Coyle, 2008; Passfield & Doust, 2000). Therefore, profiling an athlete's movement economy is useful for monitoring adaptations to training, and predicting performance capabilities.

Physiological profiling assessments may also be used to provide insight into an athlete's propensity to oxidise fat and carbohydrate. This is also determined by indirect calorimetry through the respiratory exchange ratio ( $RER = \dot{V}CO_2/\dot{V}O_2$ ), which provides insight into which substrates are being oxidised to produce energy (Farinatti et al., 2016; Ramos-Jiménez et al., 2008). Carbohydrate oxidation has an RER of  $\sim 1.0$ . In contrast, fat metabolism results in an RER of  $\sim 0.7$ , since more  $O_2$  is consumed relative to  $CO_2$  produced due to the higher hydrogen to carbon ratio in fatty acids (Farinatti et al., 2016; Ramos-Jiménez et al., 2008). At moderate-heavy intensities, RER typically remains  $< 1.0$  when fasted, as fat oxidation provides a significant contribution to ATP synthesis (Achten et al., 2002; van Loon et al., 2001). Carbohydrate oxidation rises as exercise intensities increase, and contributes solely to oxidative metabolism beyond the MMSS, with RER exceeding 1.0 (Romijn et al., 1993; van Loon et al., 2001). Carbohydrate metabolism is limited by finite storage within the body (Murray & Rosenbloom, 2018). For example, in a 65 kg person with 12% body fat,  $\sim 110$  g of carbohydrate are stored in the liver and  $\sim 500$ g are stored as muscle glycogen. Meanwhile, they possess an essentially unlimited, in an exercise context, store of fat ( $\sim 7800$ g) in subcutaneous and visceral adipose tissue cells (Melzer, 2011). Accordingly, during exercise of sufficient intensity and duration, muscle and liver glycogen stores can be depleted to very low concentrations, and hypoglycaemia may develop, which may be linked to fatigue. Therefore, improving the ability to oxidise fat at higher intensities may be a target for endurance athletes, to reduce the burden on glycogen stores. An athlete's capacity for fat oxidation can be

achieved by measuring an athlete's peak fat oxidation (PFO) rate during an incremental test (Maunder et al., 2018, 2021). PFO has been related to fat oxidation rates during prolonged exercise and endurance performance and therefore can be used to monitor adaptations to certain training interventions (Maunder et al., 2018, 2022).

## Profiling methods

Different profiling assessment methods have been developed to assess the various physiological variables routinely profiled in performance physiology. These assessment methods, while generally associated with similar distinct physiological landmarks, use a variety of laboratory- or field-based assessment techniques (Maunder et al., 2021).

### *Critical power*

The critical power model is an assessment method based on the power vs. sustainable duration relationship and involves a series of 3-5 constant-work rate trials to exhaustion lasting 2-15-min (Jones & Vanhatalo, 2017; Leo et al., 2022). This model identifies the asymptote in the curvilinear power-duration relationship, known as critical power (Jones & Vanhatalo, 2017; Leo et al., 2022) (Figure 2). Critical power is equivalent to the maximum metabolic steady state, or the highest work rate at which a heavy-intensity domain physiological response is observed (Jones et al., 2019). Specifically, exercise above the critical power results in rapid depletion of high energy phosphates and accumulation of metabolites in the working muscles, while during exercise below critical power, depletion occurs before a delayed steady state is reached (Jones et al., 2007). The power-duration relationship is also used to quantify the finite work capacity above critical power, known as  $W'$  (Jones & Vanhatalo, 2017) (Figure 2). The  $W'$  is determined as the curvature constant in the power-duration curve (Poole et al., 2016). The  $W'$  is a fixed work capacity, and task failure during severe-intensity exercise occurs when  $W'$  has been depleted (Jones & Vanhatalo, 2017; Leo et al., 2022). Therefore,  $W'$  is a measure of an athlete's anaerobic work capacity.

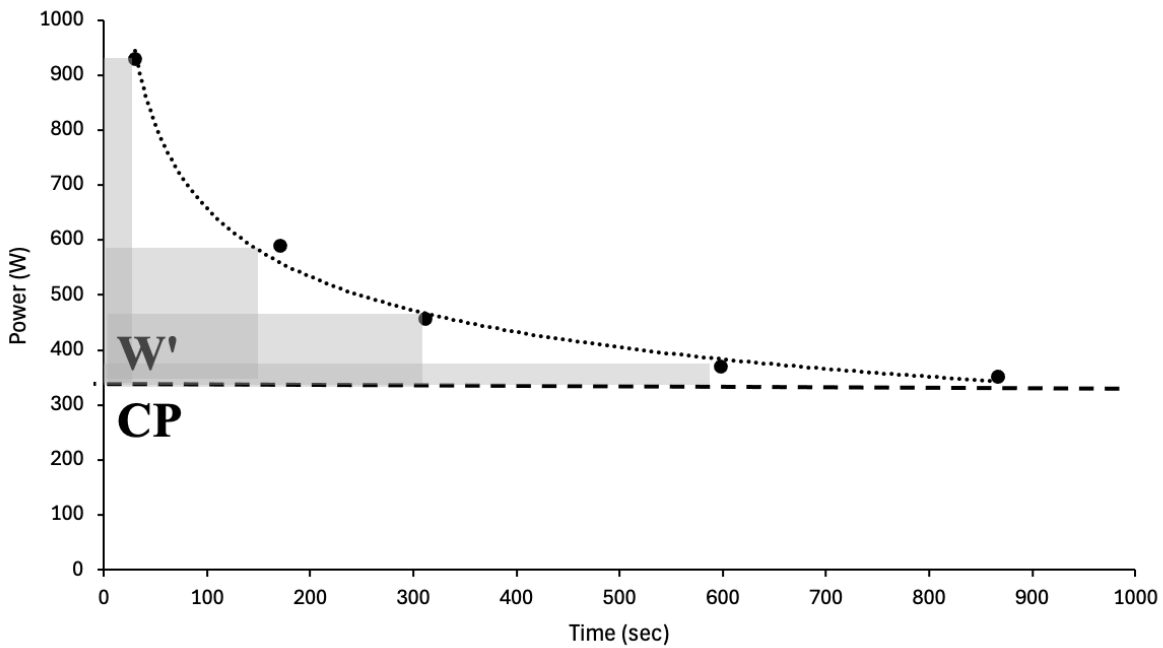


Figure 2: Curvilinear power-duration relationship, identifying critical power (CP) and  $W'$ .

### *Maximal lactate steady state*

The heavy-to-severe intensity transition can also be estimated using the maximal lactate steady state (MLSS). The MLSS is defined as the highest work rate at which blood lactate concentration does not rise by  $>1 \text{ mmolL}^{-1}$  between 10- and 30-min of exercise (Beneke & von Duvillard, 1996) (Figure 3). Therefore, assessment of the maximal lactate steady state involves a series of square-wave exercise trials, during which blood lactate concentration is measured every 5-10-min (Jones et al., 2019) (Figure 4C). However, some authors suggest that the MLSS does not precisely determine the boundary between the heavy and severe intensity domains (Jones et al., 2019), as evidenced by arbitrary defining parameters that are not necessarily associated with a physiological landmark, and studies observing physiological responses characteristic of the heavy-intensity domain, such as steady-state  $\dot{V}O_2$  during exercise above the MLSS (Iannetta et al., 2018; Mattioni Maturana et al., 2016; Nixon et al., 2021). Accordingly, some studies have reported that the power output associated with the MLSS is on average  $\sim 7\%$  lower than critical power (Dekerle et al., 2003; Mattioni Maturana et al., 2016; Pringle & Jones, 2002; Smith & Jones, 2001). Therefore, critical power may serve as a better estimate of the heavy-to-severe intensity transition, due to research showing intensity domain-specific responses above and below critical power, compared to the arbitrary defining parameters of the MLSS.

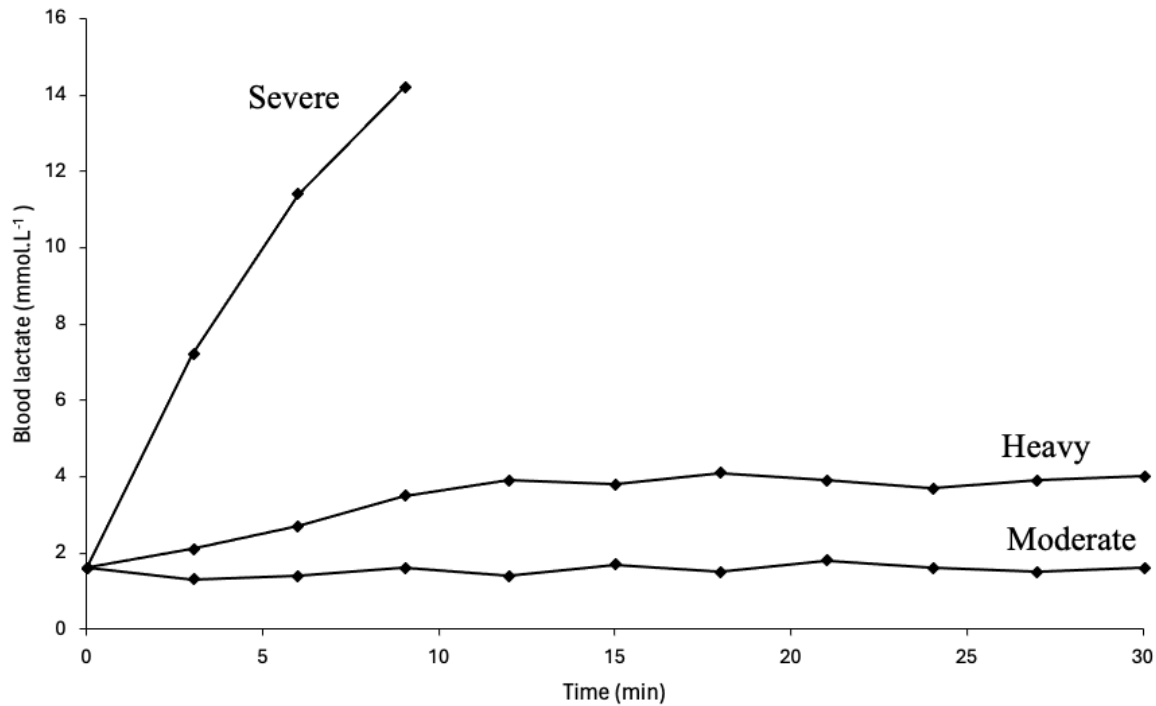


Figure 3: Blood lactate response to exercise performed within the moderate-, heavy-, and severe-intensity domain.

### *Functional threshold power*

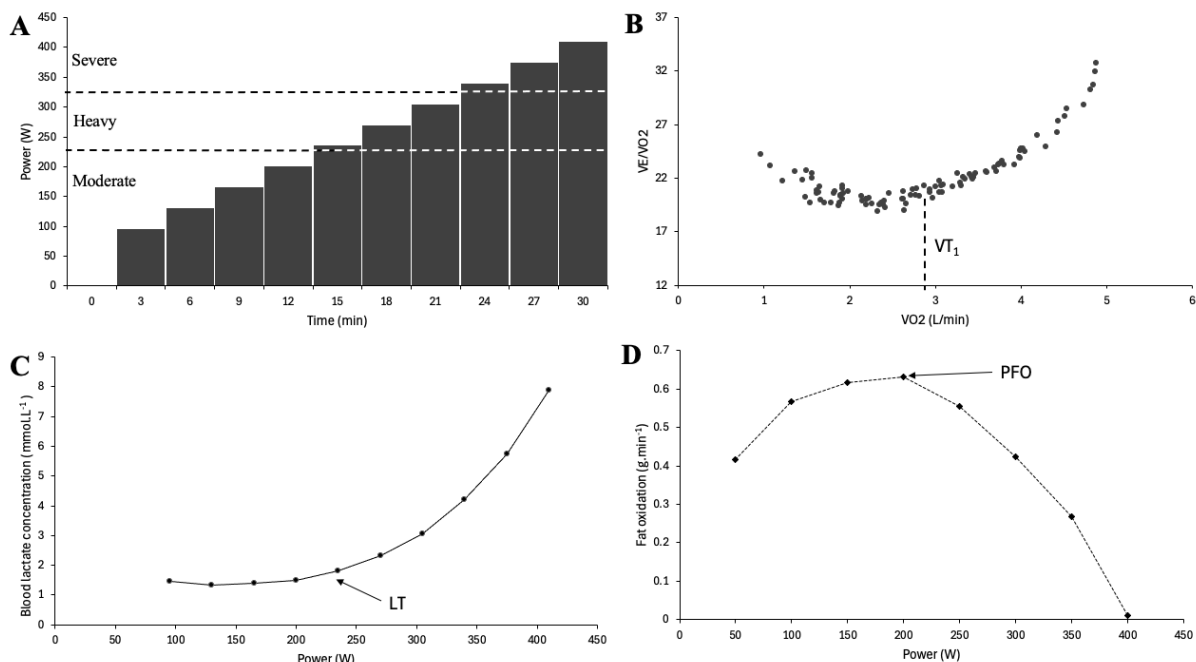
A popular profiling method used by amateur coaches and cyclists is functional threshold power (FTP). The FTP represents a surrogate measure of maximum 60-min power and is commonly measured using 95% of an athlete's maximum effort power over 20-min (Allen & Coggan, 2006). To measure FTP, athletes are only required to perform a 20-min maximal effort and have access to an accurate power meter, which has contributed to its mass adoption by amateur coaches and cyclists. The FTP attempts to predict the heavy-to-severe intensity transition, however many authors who have compared FTP to more physiologically robust estimates of MMSS, such as critical power, have shown largely poor agreement between models (Inglis et al., 2020; Klitzke Borszcz et al., 2019; Morgan et al., 2019; Valenzuela et al., 2018). It therefore appears that while the FTP test may be a reliable performance assessment method (Klitzke Borszcz et al., 2020; McGrath et al., 2019), it does not provide a valid estimate of the heavy-to-severe intensity transition.

### *Incremental exercise test*

Finally, incremental exercise tests (IET) are used to identify a greater number of key profiling variables compared to the previously mentioned models, including substrate utilisation, movement economy, lactate threshold, MMSS, and  $\dot{V}O_2\text{max}$ . An IET involves physiological measurements, such as heart rate, blood lactate concentrations, and expired gas, across a range of exercise work rates, such that a profile of these variables

against intensity can be ascertained in a single laboratory visit. Therefore, an IET begins with exercise at a low exercise work rate, and the work rate is increased every 30-s–4-min, until failure or attainment of a particular threshold (Bentley et al., 2007) (Figure 4A).

The  $\dot{V}O_2$  and  $\dot{V}E$  measured during each stage of an IET can be used to estimate the first ventilatory threshold ( $VT_1$ ), which is strongly associated with the lactate threshold (Loat & Rhodes, 1993; Ramos-Campo et al., 2017). The  $VT_1$  is determined as the  $\dot{V}O_2$  associated with the breakpoint in the ventilatory equivalent for oxygen ( $\dot{V}E/\dot{V}O_2$ ) vs.  $\dot{V}O_2$  relationship (Figure 4B). During an IET,  $\dot{V}O_2$  will increase linearly with exercise intensity, however, beyond  $VT_1$ , the  $\dot{V}E$  disproportionately rises, relative to  $\dot{V}O_2$ . This hyperventilation is likely caused by the metabolic perturbations associated with exercise in the heavy-intensity domain, such as lactate and  $H^+$  accumulation, which stimulates metaboreceptors and peripheral chemoreceptors that stimulate respiratory centres in the brain to increase  $\dot{V}E$  (Loat & Rhodes, 1993). Substrate oxidation rates and energy expenditure can be estimated for each stage during an IET using steady state  $\dot{V}O_2$  and  $\dot{V}CO_2$  measurements, through stoichiometric equations (Jeukendrup & Wallis, 2005). Additionally, quantifying substrate utilisation from these equations at each stage of an IET can also determine peak fat oxidation and the power output associated with the peak (Figure 4D).



**Figure 4:** **A** Incremental increase in power output during an incremental step test (IET), **B** Change in  $VE/VO_2$  with increasing intensity, identifying the first ventilatory threshold ( $VT_1$ ), **C** Blood lactate response to increasing exercise intensity, identifying the lactate threshold (LT), **D** Change in fat oxidation rate relative to exercise intensity, identifying the peak fat oxidation (PFO) rate.

## *Summary: Profiling methods*

The critical power model uses the power-duration relationship to determine the highest sustainable power output (CP) and the work capacity above CP ( $W'$ ). This power output is strongly associated with the MMSS and therefore can determine the heavy-to-severe intensity domain and the anaerobic work capacity, which is useful for monitoring adaptations to training, intensity regulation, load monitoring, and predicting performance. The MLSS uses a series of square-wave exercise trials to determine the highest intensity that can sustain stable blood lactate concentrations. While this threshold is linked to the heavy-to-severe intensity transition, some authors suggest the MLSS underestimates the MMSS, and therefore CP should be used in practice. Functional threshold power is a popular profiling method which represents maximal 60-min power. This threshold attempts to predict the heavy-to-severe intensity transition, however, many studies suggest poor agreement with CP. Incremental exercise tests involve recording a range of physiological variables as exercise intensity increases. Collection of expired gases throughout each stage enables the identification of a wide range of physiological variables such as substrate utilisation, movement economy, lactate threshold, MMSS, and  $\dot{V}O_2\text{max}$ , and therefore is highly practical by providing a more detailed athlete profile compared to the other commonly used profiling methods.

## Durability

A major issue with the aforementioned athlete profiling assessments is that they are typically performed when athletes are 'fresh,' and therefore do not account for how these variables change during prolonged exercise (Maunder et al., 2021). Recent studies have reported significant deteriorations in physiological profiling variables over time during prolonged exercise, such as power output at the moderate-to-heavy (Gallo et al., 2024; Hamilton et al., 2024; Stevenson et al., 2022) and heavy-to-severe (Clark et al., 2018; Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019) intensity transitions, and movement economy (Stevenson et al., 2022). Importantly, these studies have reported large between-athlete variability in the changes of these profiling variables (Hamilton et al., 2024; Stevenson et al., 2022). These observations have implications for the application of data from physiological profiling assessments to prolonged training sessions and racing scenarios, as the values measured when fresh may not reflect the values after multiple hours of exercise. 'Durability' has been defined as the time of onset and magnitude of deterioration in physiological-profiling characteristics over time during prolonged exercise (Maunder et al., 2021). Durability therefore has implications in practice for training programming, load monitoring, within-session intensity regulation, monitoring adaptations to training, and predicting performance.

Initial research demonstrated that power output at the heavy-to-severe intensity transition (CP) significantly declined following 2-h of cycling in the heavy-intensity domain, while no difference was observed when participants only performed 40-, or 80-min of cycling in the heavy intensity domain (Clark, Vanhatalo, Thompson, Joseph, et al., 2019). Following these studies, research from another group concluded that durability of the heavy-to-severe intensity transition could be predicted by traditional physiological metrics ( $\dot{V}O_2$ peak, gross efficiency, and peak fat oxidation) assessed in a rested state, thus suggesting a simplified, dependent relationship with these traditional metrics (Spragg et al., 2023). However, this understanding was subsequently contradicted following more recent work by Valenzuela and colleagues (2023), who showed that traditional endurance metrics were not associated with an athlete's durability of 20-min time-trial (TT) performance following a bout of prolonged exercise. Whether due to methodological differences or interpretations of their respective findings, these differing results highlight the importance of identifying the mechanisms behind durability.

Experimental data shows that power output at the moderate-to-heavy intensity transition declines by on average ~10% following a ~2.5-h period of prolonged cycling in the moderate-intensity domain (Hamilton et al., 2024; Stevenson et al., 2022). The reduction in power output at the moderate-to-heavy intensity transition is non-linear, with a significant degree of variability in the onset of decline between individuals (Gallo et al., 2024). The decline in power output was attributed to both a decline in gross efficiency, as well as a decline in metabolic power (Stevenson et al., 2022). This means that the energetic cost of producing a given power output increased, but also that the rate of metabolic energy expenditure associated with the transition from moderate-to-heavy intensity exercise decreased.

Plausibly, the reduction in power output at the moderate-to-heavy and heavy-to-severe intensity domain transitions following prolonged exercise could be related to decreased carbohydrate substrate availability (Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Stevenson et al., 2022). Muscle glycogen stores are progressively depleted during prolonged exercise (Bergström & Hultman, 1966, 1967; Hermansen et al., 1967; Karlsson et al., 1974), with the depletion of intramyofibrillar glycogen being proposed as a potential mechanism of fatigue (Nielsen et al., 2009, 2011, 2014; Ørtenblad et al., 2013). Experimental data shows that force production is reduced when in-vitro muscle samples are subjected to a lower glycogen environment (Cairns & Renaud, 2023). This was due to a greater percentage of inexcitable fibres, and thus less fibres contributing to total force production. The mechanism behind this is likely the reliance on intramyofibrillar glycogen for sarcoplasmic reticulum  $Ca^{2+}$  release, as demonstrated by a strong association between muscle glycogen and cytoplasmic free  $Ca^{2+}$  (Nielsen et al., 2014). The order in which these fibres become inexcitable has since been shown in a recent study (Nielsen et al., 2024). The authors demonstrated that as moderate-intensity exercise progresses, the oxidative capacity of the pool of excitable fibres declines, suggesting that the most oxidatively-capable type I fibres were depleted first, and thus the first to lose excitability. At moderate intensities, type I fibres are preferentially stimulated, and therefore less efficient type II fibres are subsequently stimulated once the type I fibres become inexcitable.

Therefore, decreased muscle glycogen during prolonged exercise may partially explain the reduction in power output achieved at intensity domain transitions after periods of prolonged exercise.

Regardless of the mechanism behind durability, recent data suggest durability may be a performance determinant. Severe-intensity performance outcomes following a 2.5-h fatiguing protocol were significantly associated with durability of the moderate-to-heavy intensity transition ( $r_s = 0.719$ ,  $P = 0.007$ ), suggesting that highly glycolytic end-spurt performance is hindered to a greater extent in athletes with poorer durability (Hamilton et al., 2024). The onset of decline in power output at the moderate-to-heavy intensity transition was also positively correlated with exercise capacity (Gallo et al., 2024).

The record power profiles of professional cyclists have also recently been used to provide insights into durability in elite cycling. In these studies, durability is quantified by using individual power data of best efforts of varying durations (~10-s–30-min) during racing, when fresh and after a fixed amount of work had been completed. These studies have reported a positive relationship between durability of record power profiles and performance outcomes (Leo et al., 2021; Mateo-March et al., 2022; Muriel et al., 2022; van Erp et al., 2021). In a fresh state, the power profiles of World and Pro Tour riders are not significantly different, however World Tour riders and the most successful riders in their respective categories appeared to be the most durable and displayed a significantly reduced decline in efforts ranging from 30-s to 30-min after a given period of work (15-45 kJ·kg<sup>-1</sup>) (Mateo-March et al., 2022; Muriel et al., 2022). Similarly, van Erp and colleagues (2021) showed that more successful climbers and sprinters displayed smaller reductions in 5-min and 20-min, and 10-sec sprint power respectively, compared to less successful climbers and sprinters. Therefore, durability of best effort power profiles appears to be related to performance, however a major limitation with comparing power profiles is it's not known whether the efforts recorded were maximal efforts.

Durability has specific implications for training load monitoring and intensity regulation. For example, if an athlete's power output at the moderate-to-heavy intensity transition was initially ~240 W, a prolonged training session designed to elicit low levels of physiological stress and contribute to a high overall training volume within a polarised or pyramidal training plan may be programmed at ~220 W. However, if after ~2-h of exercise the athlete's moderate-to-heavy intensity transition power output has declined to ~200 W, 220 W is now within the heavy-intensity domain (Figure 5). This intensity domain drift may impact the physiological response to exercise, autonomic disturbance associated with the session, and recovery required following the session. This domain drift may also cause inaccuracies in the calculation of training load and training intensity distribution.

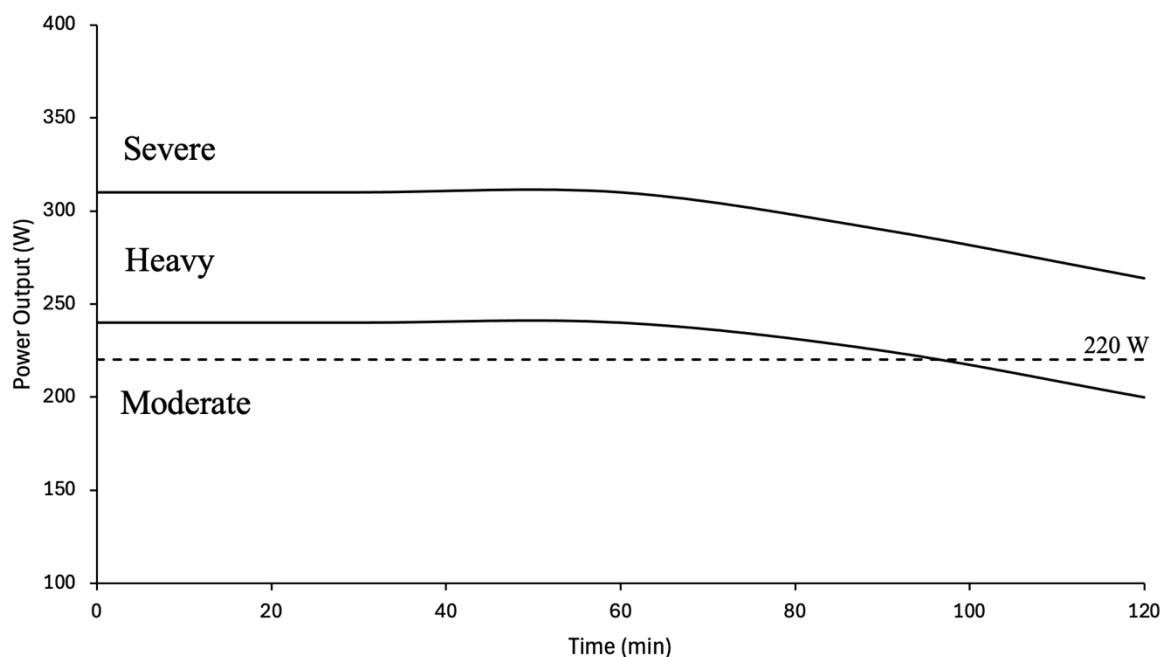


Figure 5: Non-linear decline in power output at moderate-to-heavy, and heavy-to-severe intensity domain transitions over time.

In summary, the application of physiological profiling data to practical settings is limited by the deterioration of physiological profiling variables over time during prolonged exercise. ‘Durability’ refers to an individual athlete’s resilience to the effects of prolonged exercise on physiological profiling variables, and recent research suggests that durability is variable between athletes and related to performance outcomes. Durability therefore has implications for training load monitoring, training intensity regulation, monitoring adaptations to training, and predicting performance.

## Carbohydrate Ingestion

During prolonged exercise, combustion of carbohydrate and fat provide the primary source of energy to sustain muscle activity. Large amounts of energy are stored in adipose tissue, muscle, and plasma as triglycerides (>50000 kcal), while significantly less energy is stored as endogenous carbohydrate (~2500 kcal) (Horowitz & Klein, 2000; Murray & Rosenbloom, 2018). Glycogen is stored in muscle, where it is oxidised to support muscle energy metabolism during exercise. In contrast, liver glycogen is broken down to glucose and released into the circulation, from where it can be transported into muscle and used to support muscle energy metabolism (Hargreaves & Spriet, 2020; Murray & Rosenbloom, 2019). When exogenous carbohydrates are ingested during exercise, mouth and gastrointestinal enzymes break down the ingested carbohydrate into monosaccharides such as glucose, which is then absorbed across the small intestine and transported to the liver, where it then enters the bloodstream, and is then taken up by muscle cells (Malone et al., 2021; Rollo et al., 2020).

The contribution to aerobic ATP synthesis from fat and carbohydrate depends largely on relative exercise intensity (Hargreaves & Spriet, 2020; Romijn et al., 1993; van Loon et al., 2001). At low intensities, fat oxidation from whole-body fat stores and plasma free fatty acids, makes a substantial contribution to ATP synthesis (van Loon et al., 2001). However, as exercise intensity increases, beyond approximately ~55-65%  $\dot{V}O_2\text{max}$ , the contribution of carbohydrate oxidation from liver and muscle glycogen, markedly increases while fat oxidation decreases, proportionally to the increase in intensity (Romijn et al., 1993; van Loon et al., 2001). The reduction in fat oxidation appears not to be associated with free fatty acid availability at higher intensities, but rather is associated with a reduction in pH or free carnitine availability (van Loon et al., 2001). Free carnitine availability is essential in facilitating long chain fatty acid transport across the inner mitochondrial membrane, and thus limiting fat oxidation (van Loon et al., 2001).

Substrate utilisation is also altered with exercise duration. During the initial phases of exercise, glycogen stored in muscle cells, in particular intramyofibrillar glycogen, is rapidly broken down to glucose-6-phosphate by hexokinase in a process known as glycogenolysis (Murray & Rosenbloom, 2019). Muscle glycogenolysis however is influenced by the quantity of glycogen stored in the muscle, and therefore will progressively decline over time (Hargreaves et al., 1995). As exercise continues, liver glycogen becomes increasingly important for carbohydrate oxidation and maintaining blood glucose levels. Liver glycogenolysis releases glucose into the bloodstream, where it can maintain blood glucose levels and be taken up by active muscles (Hargreaves & Spriet, 2020; Murray & Rosenbloom, 2019). Additionally, the depletion of muscle glycogen stores will also increase the rate of fat oxidation (Watt et al., 2002).

Following a bout of prolonged exercise (>90-min), the finite endogenous carbohydrate stores eventually become depleted, resulting in a decline in blood glucose, potentially leading to hypoglycaemia (Felig et al., 1982), and reduced excitability of type I muscle fibres, and therefore reduced contractile function (Nielsen et al., 2024). Understandably, if hepatic glycogen stores are depleted, and gluconeogenic metabolism from non-carbohydrate substrates is insufficient to match the rate of glucose disappearance, then blood glucose concentration falls. This is another proposed mechanism of fatigue, whereby an inability to maintain blood glucose, a process that is tightly regulated by the brain at all times, initiates a strong neural drive to terminate or significantly reduce exercise intensity (Noakes, 2022).

When exogenous carbohydrate is ingested at sufficiently high rates (>30  $\text{g h}^{-1}$ ), the contribution of glucose from the liver to total glucose appearance in the blood is suppressed, therefore preserving finite hepatic glycogen stores (Coyle et al., 1992; Gonzalez et al., 2015; Jentjens, Achten, et al., 2004; Jentjens, Moseley, et al., 2004; Jentjens & Jeukendrup, 2005; Wallis et al., 2005). Therefore, increasing carbohydrate availability also allows for better maintenance of blood glucose concentration (Coggan & Coyle, 1991; Gonzalez et al., 2015; Rollo et al., 2020). The impact of exogenous carbohydrate ingestion on the preservation of muscle glycogen however remains inconclusive. Surprisingly, multiple studies have found that the rate of muscle glycogen depletion was not different between prolonged exercise trials with and without carbohydrate

ingestion (Coyle et al., 1986; Jeukendrup et al., 1999; Gonzalez et al., 2015), while another group in two separate studies found that the rate of glycogen utilisation was ~25-28% less when participants ingested carbohydrate during prolonged exercise (Tsintzas et al., 1995, 1996). While no conclusions can currently be drawn on whether carbohydrate ingestion does or does not spare muscle glycogen, perhaps a stable supply of glucose, derived from a sufficient blood glucose concentration, is enough to maintain excitability of muscle fibres and thus maintain contractile function.

Plausibly, carbohydrate availability may be mechanistically related to durability. If athletes maintain blood glucose concentration and preserve hepatic glycogen stores, muscle excitability and neural drive may also be maintained, and thus mitigate the reduction in power output at the intensity domain transitions seen over time during prolonged exercise. These observations were present in an early durability study, which demonstrated a ~9% decline in the heavy-to-severe intensity transition following 2-h of prolonged exercise in the heavy-intensity domain, however the decline was then completely mitigated with exogenous carbohydrate ingestion ( $60 \text{ g}\cdot\text{h}^{-1}$ ) during the 2-h preload (Clark, Vanhatalo, Thompson, Joseph, et al., 2019). However, no work has yet been published on the effect of carbohydrate ingestion during prolonged moderate-intensity exercise on durability of the moderate-to-heavy intensity transition, a physiological landmark important for ultra-endurance racing.

## Summary

Physiological profiling is a method used in performance physiology to inform training load monitoring, training programming, within-session intensity regulation, monitoring adaptations to training, and predicting performance. Accordingly, a number of physiological profiling variables, such as power output at the intensity domain transitions, are routinely assessed. However, the application of physiological profiling data to practice is limited by the deterioration in physiological profiling variables that occurs over time during prolonged exercise. 'Durability' refers to time of onset and magnitude of deterioration in physiological-profiling characteristics over time during prolonged exercise. Durability therefore has clear implications for applied practice.

Power output at the moderate-to-heavy intensity transition decreases in a non-linear fashion during prolonged exercise. Durability of the moderate-to-heavy intensity transition has been linked to prolonged exercise capacity and severe-intensity end-spurt performance. Plausibly, durability of the moderate-to-heavy intensity transition could be related to carbohydrate availability, as glycogen depletion reduces excitability of muscle fibres, and thus contractile force. Preservation of hepatic glycogen stores and maintenance of blood glucose concentration through carbohydrate ingestion during exercise may improve durability via increased neural drive and muscle excitability. Indeed, ingestion of carbohydrates during prolonged heavy-intensity exercise

has been shown to mitigate the decline in power output at the heavy-to-severe intensity transition. However, this effect on the moderate-to-heavy intensity transition has not yet been studied.

Accordingly, the purpose of the study conducted for this thesis was to investigate the effect of carbohydrate ingestion during prolonged exercise on durability of the moderate-to-heavy intensity transition and severe-intensity time-trial performance. Addressing this aim was intended to improve the understanding of the mechanisms behind durability of the moderate-to-heavy intensity transition, and to have implications for applied practice in performance physiology.

# Chapter 3: Effect of carbohydrate ingestion during exercise on durability of the moderate-to-heavy intensity transition

Chapter 3 presents a randomised, counterbalanced, crossover experimental study which addresses the primary aim of this thesis, to investigate the effect of carbohydrate ingestion during prolonged exercise on durability of the moderate-to-heavy intensity transition, and severe-intensity time-trial performance. This Chapter is presented in manuscript format, and therefore contains a standalone introduction, followed by a methods, results, and discussion section. This manuscript is prepared for submission to the European Journal of Applied Physiology.

The data presented in Chapter 3 suggest that carbohydrate ingestion during prolonged exercise enhances durability of the moderate-to-heavy intensity transition, with the effects on durability of severe-intensity time-trial performance less certain. These results have implications for our understanding of the mechanisms that underpin durability, for how durability is considered in training load monitoring and programming, and for performance. These implications are discussed in detail in the discussion section of Chapter 3, and then placed into further practical context in Chapter 4.

## Introduction

Physiological variables profiled in endurance athletes include peak oxygen uptake ( $\dot{V}O_{2peak}$ ), movement efficiency, and work output at the transitions between the moderate-, heavy-, and severe-intensity domains (Coyle et al., 1991; Joyner & Coyle, 2008; Maunder et al., 2021). These attributes are used by physiologists and coaches for training programming and load monitoring, intensity regulation, monitoring training adaptations, and predicting performance (Maunder et al., 2021). Importantly, power output at the intensity domain transitions decreases during prolonged exercise, with large between-athlete variability (Clark et al., 2018; Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Hamilton et al., 2024; Stevenson et al., 2022). ‘Durability’ refers to an individual’s resilience to prolonged exercise-induced degradation in the three previously mentioned physiological parameters (Maunder et al., 2021). Therefore, it has been suggested that durability or ‘physiological resilience’ should be considered as a fourth component in endurance athlete profiling (Jones, 2023; Maunder et al., 2021).

Plausibly, the reduction in power output at the moderate-to-heavy and heavy-to-severe intensity domain transitions following prolonged exercise could be related to decreased carbohydrate substrate availability (Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Stevenson et al., 2022). Muscle glycogen progressively declines during exercise (Bergstrom & Hultman, 1966, 1967; Hermansen et al., 1967; Karlsson et al., 1974), and depletion of the inter- and intramyofibrillar glycogen

stores has been linked with preserving function of the sarcoplasmic reticulum  $\text{Ca}^{2+}$  ATPase and  $\text{Na}^+$ ,  $\text{K}^+$  ATPase, and thus has been proposed as a fatigue mechanism (Nielsen et al., 2009, 2011, 2014, 2022; Ørtenblad et al., 2013). As muscle glycogen declines with prolonged exercise, individual muscle fibres become inexcitable, rendering them unable to contribute to force production (Cairns & Renaud, 2023). As the most oxidative fibres are preferentially recruited during prolonged moderate and heavy exercise, the glycogen stores of type I fibres are depleted fastest (Nielsen et al., 2024). Consequently, the active, excitable fibre pool becomes progressively less oxidative during prolonged exercise, and power production increasingly requires activation of type II fibres (Nielsen et al., 2024). Therefore, decreased muscle glycogen during prolonged exercise may partially explain the reduction in power output achieved at intensity domain transitions during prolonged exercise.

In line with this hypothesis, prolonged heavy-intensity exercise reduces power output at the heavy-to-severe intensity transition, but this effect is mitigated when carbohydrate is ingested during exercise (Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019). Prolonged heavy-intensity exercise results in activation of glycolytic type II muscle fibres (Altenburg et al., 2007), high carbohydrate oxidation rates (van Loon et al., 2001), and thus significant glycogen depletion (Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Vigh-Larsen et al., 2022). Therefore, the positive effect of carbohydrate ingestion on durability of the heavy-to-severe intensity transition could plausibly be ascribed to muscle glycogen sparing (Tsintzas et al., 1995, 1996). However, undermining this hypothesis, most papers suggest carbohydrate ingestion during prolonged cycling does not spare muscle glycogen (Coyle et al., 1986; Jeukendrup et al., 1999; Gonzalez et al., 2015), although no studies have assessed the effect of carbohydrate ingestion during prolonged exercise on intramyofibrillar glycogen stores specifically. In contrast, the evidence for endogenous liver glycogen sparing with carbohydrate ingestion during exercise is more conclusive, with exogenous carbohydrate ingestion during exercise consistently shown to reduce liver glucose output and preserve liver glycogen stores (Bosch et al., 1994; Gonzalez et al., 2015; Hargreaves et al., 1995; Jeukendrup et al., 1999). The supply of glucose from the liver is important for the maintenance of blood glucose concentration, which provides an additional source of glucose for contracting muscles and prevents hypoglycaemia (Coyle et al., 1986). Preventing hypoglycaemia is crucial for avoiding the neuroprotective downregulation of motor unit recruitment that occurs when blood glucose drops below the tightly regulated normal range (Elghobashy et al., 2024; Glace et al., 2019; Nybo, 2003). Therefore, it is possible that carbohydrate ingestion during prolonged exercise promotes durability of the moderate-to-heavy intensity transition via effects on either muscle glycogen or hepatic glycogen sparing.

No work has yet been published on the effect of carbohydrate ingestion during prolonged moderate-intensity exercise on durability of the moderate-to-heavy intensity transition, a physiological landmark important for ultra-endurance racing and an intensity below which forms a significant portion of endurance training (Seiler, 2010). Exercise at this intensity can be maintained by more lipolytic (type I) muscle fibres, and therefore has reduced reliance on carbohydrate metabolism (Alghannam et al., 2021; Helge et al., 1999). However, a

reduction in power output at the moderate-to-heavy intensity transition is still observed following a period of prolonged cycling (Gallo et al., 2024; Hamilton et al., 2024; Stevenson et al., 2022), and this may plausibly be related to carbohydrate availability, although this has not been studied directly.

The relationship between durability of physiological profiling characteristics and performance outcomes is not well-characterised. Retrospective analysis of power profiles in professional cyclists has determined that durability of these power profiles distinguishes performance outcomes (Leo et al., 2021; Mateo-March et al., 2022; Muriel et al., 2022; van Erp et al., 2021). These studies show that higher ranked cyclists displayed smaller differences in personal record power outputs over durations of ~10-s–30-min when fresh, compared to after a fixed amount of work has been completed (15-45 kJ·kg<sup>-1</sup>). However, there are many limitations to using power profiles as performance durability measures, as it is unknown whether efforts were truly maximal, or if an arbitrarily-defined duration captures the whole effort (Leo et al., 2022). In more recently published work, a strong relationship was established between durability of the moderate-to-heavy intensity transition and prolonged exercise capacity (Gallo et al., 2024), as well as a relationship between durability of the moderate-to-heavy intensity transition and resilience to the effects of prolonged exercise on severe-intensity performance (Hamilton et al., 2024). Plausibly, athletes with superior durability of the moderate-to-heavy intensity transition will spend less time in the heavy-intensity domain during prolonged exercise, due to reduced intensity domain drift (Stevenson et al., 2022). Consequently, endogenous glycogen stores will be depleted at a slower rate, which in turn may preserve fibre excitability and therefore maintain contractile function for greater end-spurt performance. Similarly, interventions that enhance durability might be expected to promote high-intensity performance following prolonged exercise, but this has not been studied.

Accordingly, the purpose of this study was to assess the effect of carbohydrate ingestion during prolonged exercise on durability of the moderate-to-heavy intensity transition and severe-intensity time-trial performance. We hypothesised that carbohydrate ingestion during exercise would partially mitigate the reduction in power output at the moderate-to-heavy intensity transition, and during a 5-min time-trial, following prolonged exercise.

## Methods

### Participants

Twelve well-trained cyclists and triathletes were recruited to take part in this investigation (ten males, two females; age, 31 ± 6 years; height, 179.9 ± 5.5 cm; mass, 75.6 ± 6.4 kg;  $\dot{V}O_{2peak}$ , 59.3 ± 5.2 mL kg<sup>-1</sup> min<sup>-1</sup>; training volume, 14 ± 5.4 h week<sup>-1</sup>; habitual energy intake, 2502 ± 608 kcal day<sup>-1</sup>) (Table 2). The participants ranged from 20 to 55 years old, were free of illness and musculoskeletal injury (>3 months), had never had cardiovascular disease, self-reported training >8 hours week<sup>-1</sup>, and had a peak oxygen uptake ( $\dot{V}O_{2peak}$ ) >55 mL·kg<sup>-1</sup>·min<sup>-1</sup> for males and >48 mL·kg<sup>-1</sup>·min<sup>-1</sup> for females (determined in the first visit) and self-reported best-

effort 20-min power output of  $>3.5 \text{ W}\cdot\text{kg}^{-1}$ . A priori sample size estimation indicated a total of 12 participants were required to detect a large magnitude ( $d = 0.8$ ) effect of carbohydrate ingestion on durability of the moderate-to-heavy intensity transition with 80% power and a type I error rate of 0.05, using a one-tailed test. A large effect size was used as previous research on the effect of carbohydrate ingestion during exercise on durability of the heavy-to-severe intensity transition reported a large effect (Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019). All participants completed a general health screening and provided written informed consent. This study was performed in accordance with the standards of the Declaration of Helsinki, 2013, and the Auckland University of Technology Ethics Committee approved all procedures (23/197).

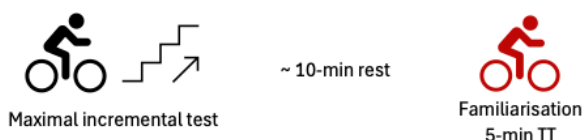
Table 2: Mean physical characteristics of participants

<i>N = 12</i>	<i>Male (N = 10)</i>	<i>Female (N = 2)</i>
<i>Age (years)</i>	34	37
<i>Height (cm)</i>	$181.9 \pm 3.2$	$170.0 \pm 2.3$
<i>Mass (kg)</i>	$76.9 \pm 5.2$	$69 \pm 9.8$
<i><math>\dot{V}O_2</math>peak (<math>\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}</math>)</i>	$60.7 \pm 4.2$	$52 \pm 5.1$
<i>Training volume (<math>\text{h}\cdot\text{week}^{-1}</math>)</i>	$14.3 \pm 5.7$	$14.0 \pm 5.7$
<i>Habitual energy intake (kcal)</i>	$2636 \pm 494$	$1899 \pm 920$
- Carbohydrate (%)	$47 \pm 10$	$22 \pm 3$
- Fat (%)	$34 \pm 9$	$46 \pm 12$
- Protein (%)	$19 \pm 3$	$33 \pm 15$

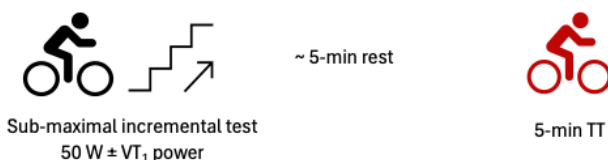
## Study design

This study involved four laboratory visits, adopting a randomised, Latin Square counterbalanced, double-blind, cross-over design, with one characterisation trial and three experimental trials (Figure 6). The first visit was used to assess eligibility, to provide an initial estimate of power output at  $VT_1$  and peak oxygen uptake ( $\dot{V}O_2$ peak), to measure the peak fat oxidation (PFO) rate and gross cycling efficiency, and to familiarise the participants with the 5-min time-trial used in subsequent trials. Visits 2-4 were the experimental trials, and took place in a randomised, counterbalanced order. Each experimental trial began in the morning following the consumption of a standardised breakfast and involved the estimation of  $VT_1$  using a five-step incremental test and 5-min time-trial performance (i) without prior cycling (PRE), (ii) following 150-min of cycling at 90% of the initial estimate of  $VT_1$ , with consumption of carbohydrate at  $60 \text{ g}\cdot\text{h}^{-1}$  (POST<sub>CHO</sub>), and (iii) following 150-min of cycling at 90% of the initial estimate of  $VT_1$ , without carbohydrate (POST<sub>CON</sub>). In POST<sub>CON</sub>, participants consumed a calorie-free, taste-matched placebo drink. Participants and researchers were blinded to the condition (POST<sub>CHO</sub> vs. POST<sub>CON</sub>).

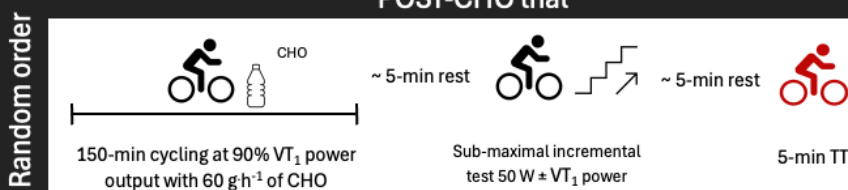
## Characterisation and familiarisation trial



## PRE trial



## POST-CHO trial



## POST-CON trial

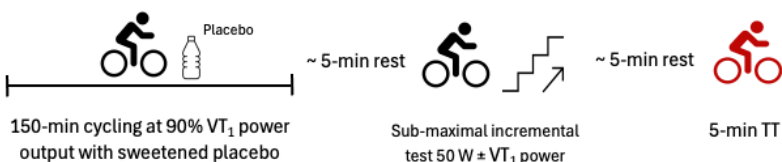


Figure 6: Schematic of study design. TT, time-trial; VT<sub>1</sub>, first ventilatory threshold; CHO, carbohydrate.

## Characterisation trial

### Maximal incremental test and familiarisation time-trial

Participants arrived in the laboratory having fasted overnight for ~10 h, for accurate quantification of substrate utilisation, and having ingested ~1-2 L of water in the morning before arrival. Before beginning the session, the height and body mass of participants was measured. Cycling then commenced on personal road bicycles mounted to a direct-drive smart indoor trainer (Kickr, Wahoo Fitness, GA, USA) at 95 W, with the work rate increased by 35 W every 3 min. Expired gases and heart rate were collected continuously during exercise (TrueOne 2400, ParvoMedics, UT, USA; Polar H10, Polar Electro Oy, Kempele, Finland). Upon confirmation of the second ventilatory threshold (VT<sub>2</sub>), the work rate was increased by 35 W every minute until volitional exhaustion. The  $\dot{V}O_{2peak}$  was accepted as the highest 15-s average  $\dot{V}O_2$ , and VT<sub>1</sub> was determined by identifying the  $\dot{V}O_2$  at the first breakpoint in the  $\dot{V}O_2$  vs.  $\dot{V}_E \dot{V}O_2^{-1}$  relationship. This  $\dot{V}O_2$  was subsequently converted into a power output via simple linear regression of  $\dot{V}O_2$  vs. power output during the 3-min stages,

using the average  $\dot{V}O_2$  in the last minute of each stage. The last minute of expired gas data in each 3-min stage was used to quantify whole-body fat oxidation using standard equations (Eq. 1-2, Jeukendrup & Wallis, 2005).

(Eq. 1) Whole body fat oxidation rate ( $\text{g}\cdot\text{min}^{-1}$ )

$$= 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2 \text{ where } \dot{V}O_2 \text{ and } \dot{V}CO_2 \text{ are in } \text{L}\cdot\text{min}^{-1}.$$

(Eq. 2) Whole body rate of energy expenditure ( $\text{kcal}\cdot\text{min}^{-1}$ )

$$= 0.550 \times \dot{V}CO_2 + 4.471 \times \dot{V}O_2 \text{ where } \dot{V}O_2 \text{ and } \dot{V}CO_2 \text{ are in } \text{L}\cdot\text{min}^{-1}.$$

The highest observed rate of whole-body fat oxidation was identified as PFO (Maunder et al., 2022). After completion of the incremental test, participants rested for ~15-min prior to completing a 5-min performance time-trial. Participants were instructed that the time-trial was to be performed with maximum effort, with the goal of achieving the highest possible average power output. Participants were blinded to power output, heart rate, and cadence, but able to see elapsed time. Expired gases were collected during the time-trial, with the highest 30-s average  $\dot{V}O_2$  accepted as the time-trial  $\dot{V}O_{2\text{peak}}$ . Following the time-trial, participants were instructed on how to accurately record exercise for the week prior and dietary intake for the day prior to the second trial using a smartphone application (Easy Diet Diary Connect, Xyris Software, QLD, Australia). Habitual dietary macronutrient intake was subsequently quantified (Easy Diet Diary, Xyris Software, QLD, Australia). Participants were asked to replicate their exercise and food intake in advance of the third and fourth trials.

## Experimental trials

*PRE, POST-CHO, and POST-CON assessments of the moderate-to-heavy intensity transition and severe-intensity time-trial performance*

Participants returned to the laboratory 5-14 days later to complete the first of the three experimental trials. Participants arrived having consumed a breakfast of their choosing containing ~1  $\text{g}\cdot\text{kg}^{-1}$  of carbohydrate, which was replicated for experimental trial, and ~800 mL of water one hour beforehand. Participants were fitted with a heart rate monitor such that heart rate was recorded continuously throughout the trial. All trials began with a 5-min warm-up at 100 W. Following the warm-up, participants cycled for 150-min at 90% of the  $VT_1$  power output estimated in the characterisation trial in the  $POST_{CHO}$  and  $POST_{CON}$  trials, but not the  $PRE$  trial, with expired gases collected for 4-min every 15-min. Expired gas data was used to quantify rates of whole-body energy expenditure, carbohydrate oxidation, and fat oxidation during the 150-min preload. Heart rate data was separated into 15-min bins during the 150-min preload. In the  $POST_{CHO}$  and  $POST_{CON}$  trials, participants consumed 150 mL of water every 20-min during the first 120-min of the 150-min preload. In  $POST_{CHO}$ , water was mixed with a calorie-free electrolyte mix (Electrolytes, Musashi, Vitaco Health, Auckland, New Zealand)

and maltodextrin (Pure Maltodextrin, Reactiv, Auckland, New Zealand) such that carbohydrate was consumed at a rate of 60 g·h<sup>-1</sup> during the first 120-min of the 150-min preload. In POST<sub>CON</sub>, water was mixed with the same calorie-free electrolyte mix with added sweetener (Sugar Fix'd Natural Sweetener, Natvia, VIC, Australia) to match the taste of the POST<sub>CHO</sub> beverage but be devoid of carbohydrate. Additionally, a fingertip capillary blood glucose measurement was obtained using a blood glucose analyser (Freestyle Optimum Neo, Abbott, Berkshire, United Kingdom) at rest and every 30-min during the 150-min preload in POST<sub>CHO</sub> and POST<sub>CON</sub>. Participants and researchers were blinded to trial allocation.

Subsequently, the moderate-to-heavy intensity transition was estimated precisely using a five-step incremental test, with continuous collection of expired gases. The first step was 50 W below the VT<sub>1</sub> power output estimated in the first laboratory visit, and the power output was increased by 25 W every 4-min, such that the fifth and final step was 50 W above the VT<sub>1</sub> power output estimated in the first laboratory visit. The moderate-to-heavy intensity transition power output was estimated using the methods previously described for determining VT<sub>1</sub> in the first laboratory visit, but with greater precision given the denser cluster of datapoints around the transition. Following the five-step incremental test, participants cycled at 100 W for 5-min before completing a 5-min performance time-trial according to the procedures described above.

Mathematically, loss of power output at VT<sub>1</sub> can be attributed to: (i) reduced mechanical power output at a given rate of metabolic energy expenditure (reduced energetic efficiency) and (ii) reduced rate of metabolic energy expenditure at VT<sub>1</sub> (reduced metabolic power). To determine the contributions of changes in energetic efficiency and metabolic power to changes in power output at VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub> vs. PRE, the rate of energy expenditure associated with the power output at VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub> were calculated using linear regression of the power output vs energy expenditure relationship, using the last minute of expired gas data in each 4-min stage. Subsequently, the rate of energy expenditure associated with VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub> was converted to power output using linear regression of the power output vs. energy expenditure relationship in the PRE trial (denoted POST-CON<sub>EE</sub>PRE<sub>Eff</sub>, POST-CHO<sub>EE</sub>PRE<sub>Eff</sub>). Therefore, POST-CON<sub>EE</sub>PRE<sub>Eff</sub> and POST-CHO<sub>EE</sub>PRE<sub>Eff</sub> identifies the power output that the rate of metabolic energy expenditure measured at VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub> would have produced with the same level of gross cycling efficiency as in the PRE trial. Accordingly, the contributions to changes in power output at VT<sub>1</sub> made by changes in energetic efficiency and metabolic power, in POST<sub>CON</sub> and POST<sub>CHO</sub>, were calculated (Eq. 3).

Eq. 3) Contribution of change in energetic efficiency to change in power output at VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub> =  
 $POST_{CON} - POST-CON_{EE}PRE_{Eff} = POST_{CHO} - POST-CHO_{EE}PRE_{Eff}$

Contribution of change in metabolic power to change in power output at VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub> =  
 $POST-CON_{EE}PRE_{Eff} - PRE, POST-CHO_{EE}PRE_{Eff} - PRE$

Where POST-CON<sub>EE</sub>PRE<sub>Eff</sub>, POST-CHO<sub>EE</sub>PRE<sub>Eff</sub> is the theoretical power output produced with the metabolic energy expenditure at VT<sub>1</sub> in POST<sub>CON</sub> and POST<sub>CHO</sub>, respectively, with the gross cycling efficiency in PRE.

## Statistical analyses

Data are expressed as mean  $\pm$  standard deviation and were analysed using GraphPad Prism Version 9.3.1. Statistical significance is inferred when  $P \leq 0.05$ . The normality of datasets was assessed using the Shapiro-Wilk test. Power output at VT<sub>1</sub> and 5-min time-trial performance were compared between-trials using mixed-effects analyses due to missing data. Variance was located post-hoc using Bonferroni corrected paired *t*-tests. The  $\dot{V}O_2$ peak was compared between-trials using a one-way repeated measures ANOVA. Percentage changes were calculated to determine the practical magnitude of differences. Contributions to changes in power output at VT<sub>1</sub> by changes in energetic efficiency and metabolic power were compared between-trials using paired *t*-tests. Whole-body rates of energy expenditure, carbohydrate oxidation, fat oxidation, gross efficiency, blood glucose concentration, and heart rate during the 150-min preload in POST<sub>CON</sub> and POST<sub>CHO</sub> were compared using two-way repeated measures analyses of variance, with trial and time as factors. Bonferroni corrected *t*-tests were used to locate variance. Relationships between habitual dietary macronutrient intake and PFO and durability of power output at VT<sub>1</sub>, 5-min time-trial performance, and  $\dot{V}O_2$ peak were assessed using Pearson's or Spearman's rank-order correlation coefficients (depending on normality) and expressed with 95% confidence intervals.

## Results

### Characterisation trial

The  $\dot{V}O_2$ peak measured in the characterisation trial was  $4.48 \pm 0.55$  L $\cdot$ min<sup>-1</sup>. The estimated power output at VT<sub>1</sub> was  $226 \pm 38$  W, and therefore a power output of  $215 \pm 36$  W was used during the preload phase of the prolonged experimental trials. The estimated power output at VT<sub>2</sub> was  $290 \pm 38$  W and peak fat oxidation was  $0.63 \pm 0.16$  g $\cdot$ min<sup>-1</sup>.

### Prolonged phase

One participant did not complete the POST<sub>CHO</sub> trial due to an injury not related to the study, hence the data presented below is  $N = 12$  for POST<sub>CON</sub> and  $N = 11$  for POST<sub>CHO</sub>. During the prolonged phase, there was an effect of time for blood glucose concentration ( $P = 0.0288$ , Figure 7E) and heart rate ( $P < 0.0001$ , Figure 7F). No other effects of time were observed during the prolonged phase. There was an effect of trial for blood glucose concentration ( $P = 0.0054$ ), and a trial-by-time interaction for carbohydrate oxidation ( $P = 0.0179$ ). No other effects of trial or trial-by-time interactions were observed during the prolonged phase. Carbohydrate oxidation rate was significantly greater in POST<sub>CHO</sub> than POST<sub>CON</sub> at 150-min ( $P = 0.043$ ). Blood glucose concentration was significantly greater in POST<sub>CHO</sub> than POST<sub>CON</sub> at 120- and at 150-min (Figure 7E).

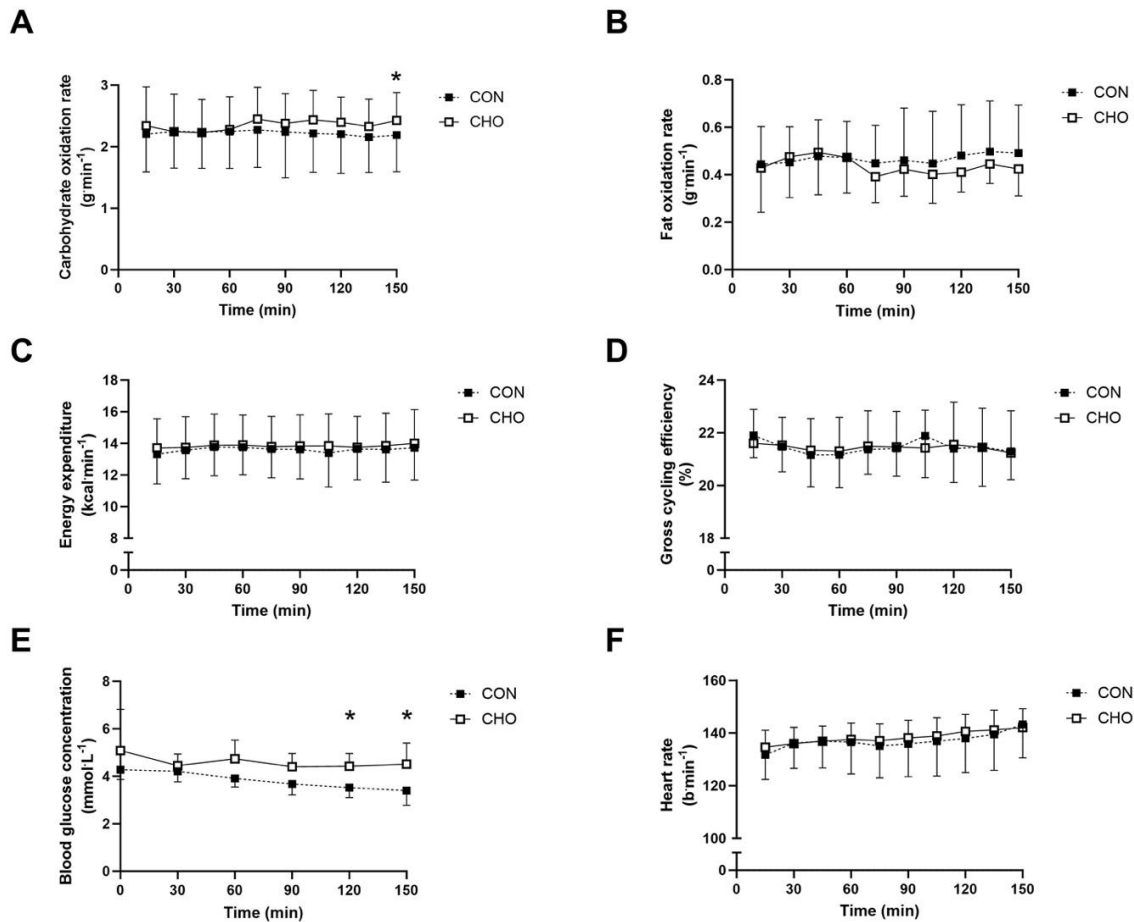


Figure 7: **A** Carbohydrate oxidation rate, **B** fat oxidation rate, **C** energy expenditure, **D** gross cycling efficiency, **E** blood glucose concentration, **F** heart rate during the preload phase of POSTCHO and POSTCON. \* denotes  $P \leq 0.05$ .

## Effects of prolonged exercise and carbohydrate ingestion

There was a significant effect of trial on power output at  $VT_1$  ( $P = 0.0163$ ). Power output at  $VT_1$  was significantly lower in  $POST_{CHO}$  ( $225 \pm 36$  W,  $\Delta -7 \pm 3$  W,  $\Delta -3 \pm 2\%$ ,  $P = 0.0265$ ) and  $POST_{CON}$  ( $216 \pm 35$  W vs.  $229 \pm 37$  W,  $\Delta -13 \pm 9$  W,  $\Delta -6 \pm 4\%$ ,  $P = 0.0011$ ) than PRE ( $229 \pm 37$  W) (Figure 8A). Power output at  $VT_1$  was significantly greater in  $POST_{CHO}$  than  $POST_{CON}$  ( $225 \pm 36$  W vs.  $216 \pm 35$  W,  $\Delta 7 \pm 9$  W,  $\Delta 3 \pm 4\%$ ,  $P = 0.0186$ ). The contribution to loss of power output at  $VT_1$  following prolonged exercise made by reduced metabolic power was significantly greater in  $POST_{CON}$  than  $POST_{CHO}$  ( $P = 0.0101$ ) (Figure 8B). This difference was not significant for loss of energetic efficiency ( $P = 0.3131$ ).

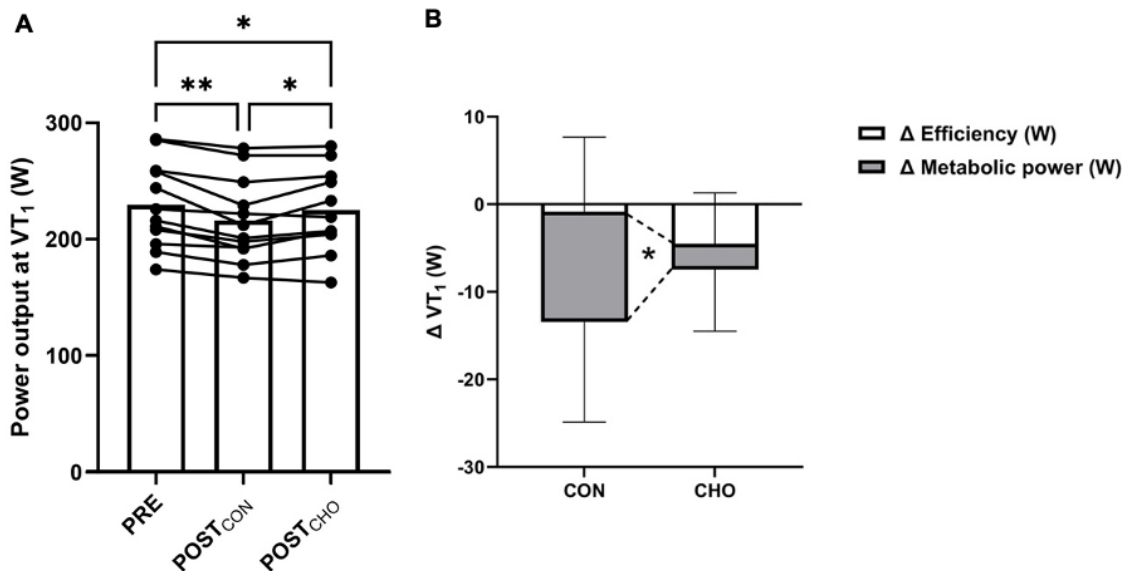


Figure 8: **A** Power output at the first ventilatory threshold (VT<sub>1</sub>) in the PRE, POST<sub>CON</sub>, and POST<sub>CHO</sub> trials, **B** Contribution to changes in the power output at the first ventilatory threshold (VT<sub>1</sub>) by losses in energetic efficiency and metabolic energy expenditure at the threshold after a period of prolonged exercise in the POST<sub>CON</sub> and POST<sub>CHO</sub> trials. \* denotes  $P \leq 0.05$ , \*\* denotes  $P \leq 0.01$ .

There was a significant effect of trial on 5-min time-trial performance ( $P = 0.0266$ ). Mean power output in the 5-min time-trial was significantly lower in POST<sub>CHO</sub> ( $351 \pm 53$  W,  $\Delta -15 \pm 12$  W,  $\Delta -4 \pm 3\%$ ,  $P = 0.0251$ ) and POST<sub>CON</sub> ( $328 \pm 63$  W,  $\Delta -35 \pm 38$  W,  $\Delta -10 \pm 10\%$ ,  $P = 0.0272$ ) than PRE ( $363 \pm 55$  W) (Figure 9A). Mean power output in the 5-min time-trial was not significantly different between POST<sub>CHO</sub> and POST<sub>CON</sub> ( $\Delta 25 \pm 37$  W,  $\Delta 9 \pm 13\%$ ,  $P = 0.1859$ ). There was no significant effect of trial on  $\dot{V}O_{2peak}$  measured during the 5-min time trial ( $P = 0.0831$ ) (Figure 9B).

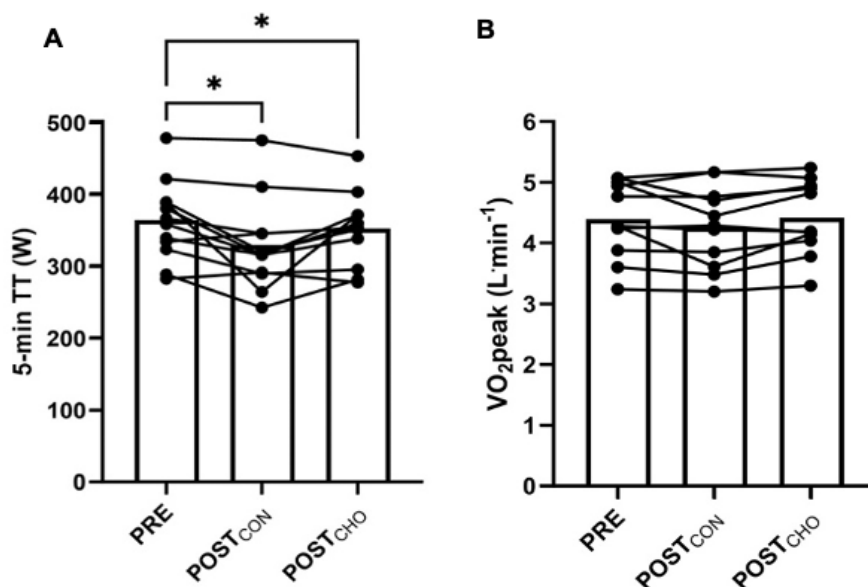


Figure 9: **A** 5-min time trial (TT) performance in the PRE, POST<sub>CON</sub>, and POST<sub>CHO</sub> trials, **B** Peak rate of oxygen consumption (VO<sub>2peak</sub>) in the PRE, POST<sub>CON</sub>, and POST<sub>CHO</sub> trials. \* denotes  $P \leq 0.05$ .

## Discussion

The primary aim of this study was to determine the effect of carbohydrate ingestion during prolonged moderate-intensity cycling on durability of the moderate-to-heavy intensity transition and severe-intensity time-trial performance. We hypothesised that carbohydrate ingestion during prolonged cycling would partially mitigate the decline in power output at the moderate-to-heavy intensity transition and 5-min time-trial performance. Our primary observations were that: (i) carbohydrate ingestion during prolonged cycling partially mitigated the decline in power output at the moderate-to-heavy intensity transition and (ii) carbohydrate ingestion did not significantly influence the decline in severe-intensity time-trial performance following prolonged exercise. These data therefore suggest that durability of the moderate-to-heavy intensity transition is related to carbohydrate availability.

In line with our hypothesis, carbohydrate ingestion during prolonged exercise in POST<sub>CHO</sub> partially mitigated the decline in power output at the moderate-to-heavy intensity transition seen in POST<sub>CON</sub> (Figure 8A). Specifically, power output at the moderate-to-heavy intensity transition was ~6% lower in POST<sub>CON</sub> vs. PRE, but this difference was only ~3% in POST<sub>CHO</sub>. The reduction in power output at the moderate-to-heavy intensity transition following prolonged exercise aligns with previous research concerned with both the moderate-to-heavy (Hamilton et al., 2024; Gallo et al., 2024; Stevenson et al., 2022), and heavy-to-severe (Clark, Vanhatalo, Thompson, Joseph, et al., 2019; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Spragg et al., 2024) intensity transitions. Additionally, these results align with previous work reporting that the decline in power output at the heavy-to-severe transition was mitigated with carbohydrate ingestion during exercise (Clark, Vanhatalo, Thompson, Joseph, et al., 2019).

In the present study, whole-body carbohydrate oxidation rates were ~11% greater at the 150-min timepoint during the prolonged phase in POST<sub>CHO</sub> compared to POST<sub>CON</sub> (Figure 7A). This modest increase in total carbohydrate oxidation with carbohydrate ingestion is in line with the predictions of a recent meta-regression (Rothschild et al., 2022). Studies using stable isotope tracers have reported carbohydrate ingestion during prolonged exercise decreases endogenous carbohydrate oxidation rates, due to oxidation of the ingested carbohydrate (Gonzalez et al., 2015; Jentjens, Achten, et al., 2004; Jentjens, Moseley, et al., 2004; Jentjens & Jeukendrup, 2005; Wallis et al., 2005). The reduction in endogenous carbohydrate oxidation seen with carbohydrate ingestion, reduces liver glucose output and spares liver glycogen stores (Bosch et al., 1994; Jeukendrup et al., 1999; McConell et al., 1994; Gonzalez et al., 2015). It is therefore likely that carbohydrate ingestion in POST<sub>CHO</sub> reduced liver glucose output and spared liver glycogen stores compared to POST<sub>CON</sub>. However, as we did not partition exogenous and endogenous carbohydrate oxidation, we cannot determine the effect of carbohydrate ingestion on liver glycogen stores in the present study. Confounding influences on carbohydrate oxidation such as diet and prior exercise were controlled for as exercise and food intake was kept consistent by participants between trials.

Liver glycogen stores have an important role in the regulation of blood glucose concentrations (Hargreaves & Spriet, 2020; Murray & Rosenbloom, 2019). It is therefore possible that the positive effect of carbohydrate ingestion on durability of the moderate-to-heavy intensity transition was mediated by improved regulation of blood glucose concentration, due to liver glycogen sparing. In support of this, blood glucose concentration was maintained in POST<sub>CHO</sub>, but declined over time in POST<sub>CON</sub> (Figure 7E). Mechanistically, hypoglycaemia may induce, and carbohydrate ingestion may mitigate the development of neuromuscular fatigue during prolonged exercise (Noakes, 2022). Previous studies have reported that hypoglycaemia attenuates central nervous system activation and induces central fatigue, however this effect is mitigated with carbohydrate ingestion during exercise (Elghobashy et al., 2024; Glace et al., 2019; Nybo, 2003). Hypoglycaemia-induced central fatigue and consequent impaired motor unit recruitment may therefore have impaired the coordination of muscle contractile function, and thus lowered the power output at which participants transitioned from a moderate to heavy-intensity response. However, we did not measure neuromuscular fatigue or motor unit recruitment in this study, and so cannot confirm this hypothesis. We suggest that future studies investigating the mechanisms by which carbohydrate ingestion during exercise promotes durability of the moderate-to-heavy intensity transition should assess effects on central fatigue and motor unit recruitment. Therefore, it is possible that the positive effect of carbohydrate ingestion during prolonged exercise was related to improved regulation of blood glucose concentrations, but future studies should interrogate this mechanism further.

It was also observed that the contribution made by reduced metabolic power to the reduction in power output at the moderate-to-heavy intensity transition was significantly greater in POST<sub>CON</sub> compared to POST<sub>CHO</sub> (Figure 8B). Metabolic power is referred to as the rate of whole-body metabolic energy expenditure that is achieved at the moderate-to-heavy intensity transition. It is plausible that the greater reduction in metabolic power seen in POST<sub>CON</sub> might be related to effects of hypoglycaemia on neural drive and motor unit recruitment, or reduced fibre excitability due to greater depletion of intramyofibrillar glycogen stores. Muscle glycogen is a crucial energy substrate for various muscle contractile processes (Nielsen et al., 2022). Inter- and intramyofibrillar glycogen availability has been linked to Ca<sup>2+</sup> release from the sarcoplasmic reticulum through Ca<sup>2+</sup> ATPase, and intramyofibrillar glycogen is implicated in Na<sup>+</sup>, K<sup>+</sup> ATPase and the regulation of ionic balance during muscle contraction (Nielsen et al., 2022). Recent work suggests glycogen-depleted fibres become inexcitable when exposed to high K<sup>+</sup> concentrations (Cairns & Renaud, 2023). An elevated interstitial K<sup>+</sup> concentration may occur due to impaired Na<sup>+</sup>, K<sup>+</sup> ATPase function, due to intramyofibrillar glycogen depletion (Nielsen et al., 2022). Therefore, the reduced metabolic power and overall reduction in power output at the moderate-to-heavy intensity transition following prolonged exercise in the present study could plausibly have been related to effects of muscle glycogen depletion on muscle contractile function and sarcolemmal excitability.

Furthermore, as the most aerobic, type I fibres are preferentially activated during prolonged exercise, glycogen depletion during prolonged exercise occurs most rapidly in type I fibres (Jensen et al., 2020; Tsintzas et al.,

1996). Consequently, assuming individual fibres become inactive at a threshold level of glycogen depletion, it is possible that the active fibre pool may progressively become less aerobic as prolonged exercise progresses, as recently proposed (Nielsen et al., 2024). Accordingly, a progressive reduction in the oxidative power of the active muscle fibre pool may have contributed to the reduction in metabolic power seen following prolonged exercise in the present study (Figure 8B). Therefore, if carbohydrate ingestion during POST<sub>CHO</sub> slowed glycogen depletion in individual fibres, fibre excitability may have been better maintained, preserving metabolic power and contributing to the positive effects of carbohydrate ingestion on durability of power output at the moderate-to-heavy intensity transition (Figure 8B). However, undermining this hypothesis, most studies suggest that carbohydrate ingestion during prolonged exercise does not preserve muscle glycogen stores (Coyle et al., 1986; Fielding et al., 1985; Flynn et al., 1987; Gonzalez et al., 2015; Hargreaves & Briggs, 1988; Jeukendrup et al., 1999; Noakes et al., 1988). However, to our knowledge no studies have determined the impact of carbohydrate ingestion during prolonged exercise on intramyofibrillar glycogen depletion, which may have specific implications for maintenance of muscle contractile function during prolonged exercise (Nielsen et al., 2022). Future studies could explore the mechanisms for the positive effect of carbohydrate ingestion on durability of the moderate-to-heavy intensity transition by assessing effects on neural drive and muscle excitability, with measurements of liver and muscle glycogen content.

Secondly, in contrast to our hypothesis, carbohydrate ingestion in POST<sub>CHO</sub> did not significantly mitigate the decline in severe-intensity time trial performance seen in POST<sub>CON</sub> (Figure 9A). Specifically, 5-min time trial performance was reduced by ~4% in POST<sub>CHO</sub> and ~10% in POST<sub>CON</sub>, but the difference between POST<sub>CHO</sub> and POST<sub>CON</sub> was not significant (Figure 9A). Previous research using a very similar experimental design reported a strong, positive association between durability of the moderate-to-heavy intensity transition and durability of 5-min time trial performance (Hamilton et al., 2024). It was hypothesised that this effect was related to having spent less time in the heavy-intensity domain during the prolonged phase (Hamilton et al., 2024). Carbohydrate ingestion did improve durability of the moderate-to-heavy intensity transition (Figure 8A), so it is therefore surprising that 5-min time trial performance was not improved in POST<sub>CHO</sub> vs. POST<sub>CON</sub> (Figure 9A). It is possible that the lack of statistical difference in 5-min time trial performance between POST<sub>CHO</sub> and POST<sub>CON</sub> was due to the relatively small sample size and/or heterogeneity in the level of performance decrement associated with the prolonged exercise protocol (range, +11 W to -124 W from PRE to POST). As some participants saw very little effect of prolonged exercise on 5-min time trial performance in POST<sub>CON</sub> (Figure 9A), the prolonged exercise protocol may have been inadequately fatiguing in some participants, therefore masking potential benefits of carbohydrate ingestion. Therefore, it may be necessary to reexamine this hypothesis in a study with a larger sample size and/or more homogenous cohort undertaking a more consistently-fatiguing prolonged phase.

These data add to our understanding of durability, and have implications for training load monitoring, within-session intensity regulation, and training programming. These data show that carbohydrate ingestion during prolonged exercise allows athletes to maintain a higher power output in relation to their intensity domain

transitions. However, inter-individual variability in the effect of prolonged exercise on power output at the moderate-to-heavy intensity transition, and effects of nutrition status, suggests generalised estimates cannot be used when working with individual athletes to account for durability in training programming and quantification of training load. In this study, the effect of the prolonged exercise protocol on power output at the moderate-to-heavy intensity transition ranged from -32 W (-13%) to -3 W (-2%) in POST<sub>CON</sub> and -11 W (-6%) to -3 W (-2%) in POST<sub>CHO</sub> (Figure 8A). Therefore, future studies should look to develop tools for monitoring power output at the intensity domain transitions in real-time, that are sensitive to the variable effects of both prolonged exercise carbohydrate ingestion. Development of these tools would allow the intensity of training to be regulated in real-time in accordance with dynamic intensity domain transitions, and allow more accurate, physiologically-based quantification of training load.

In conclusion, these data demonstrate that carbohydrate ingestion during prolonged exercise partially mitigates the decline in power output at the moderate-to-heavy intensity transition. The positive effect of carbohydrate ingestion on durability of the moderate-to-heavy intensity transition occurred with better maintenance of blood glucose concentrations, and greater preservation of metabolic power at the moderate-to-heavy intensity transition. However, in contrast to our hypothesis, carbohydrate ingestion during prolonged exercise did not mitigate the decline in 5-min time trial performance. This may have been due to the large variability in responses between-participants, as prolonged exercise had little effect on 5-min time trial performance in some participants, and this may have masked positive effects of carbohydrate ingestion. Our data further highlight the importance of monitoring durability at an individual level due to the large variability between-athletes and the additional complexity that nutritional interventions add to durability responses. This has direct implications for training load monitoring, training intensity regulation, and predicting performance, even with carbohydrate ingestion. Future studies should explore the effect of carbohydrate ingestion on durability of the moderate-to-heavy intensity transition with measurements of muscle and liver glycogen, neural drive, and muscle excitability.

## Chapter 4: Summary and future directions

The primary aims of the study presented in this thesis were to determine the effect of carbohydrate ingestion during prolonged moderate-intensity cycling on durability of the moderate-to-heavy intensity transition and severe-intensity time-trial performance. We hypothesised that carbohydrate ingestion during prolonged cycling would partially mitigate the decline in power output at the moderate-to-heavy intensity transition and 5-min time-trial performance. The key findings were that: (i) carbohydrate ingestion during prolonged cycling partially mitigated the decline in power output at the moderate-to-heavy intensity transition and (ii) carbohydrate ingestion did not significantly influence the decline in severe-intensity time-trial performance following prolonged exercise. This chapter will discuss the primary practical applications of this thesis, and then discuss the recommendations for future research directions.

## *Applications to practice*

The results from the experimental study presented in this thesis further support recent work regarding the behaviour of the moderate-to-heavy intensity transition during prolonged exercise (Gallo et al., 2024; Hamilton et al., 2024; Stevenson et al., 2022). That is, that power output at the moderate-to-heavy intensity transition declines with prolonged cycling, with large variability in the magnitude of this decline between participants. The novel finding in this investigation was that this decline was mitigated by carbohydrate ingestion during prolonged exercise. These findings are important to endurance sport practitioners as carbohydrate ingestion may reduce the stress associated with prolonged training sessions and allow greater power output to be sustained during training sessions programmed according to intensity domain transitions. Additionally, the positive effect of carbohydrate ingestion on the behaviour of the moderate-to-heavy intensity transition may need to be considered in training load monitoring, when training load is physiologically-based and, again, calculated according to intensity domain transitions. Additionally, these data further support the need to develop tools that allow coaches and athletes to monitor changes intensity domain transitions in real-time.

Another important application to practice as a result of these findings, is the need to consider durability assessment as part of the physiological profiling of endurance athletes. Again, these data, along with those published recently, suggest that due to the large between-athlete variability in durability, durability needs to be considered at an individual level. Therefore, it is recommended that coaches and Performance Physiologists that work with endurance athletes implement individual durability monitoring assessments as part of their routine athlete profiling assessments. This might include implementing a second profiling assessment that is performed after a prolonged, controlled training session that can be replicated in subsequent assessments. Importantly, based on these findings, carbohydrate intake during these assessments should be controlled to account for its effect on durability. Durability can therefore be assessed by measuring traditional endurance performance metrics, through a variety of profiling assessments methods, which have been discussed in detail in this thesis, in both a fresh and fatigued state while also adhering to typical training or race-day nutrition strategies. The difference between variables when measured with and without prior prolonged exercise can then be used to quantify durability. This assessment method has practical application in elite sport settings, as work output can be monitored and controlled easily using smart indoor trainers.

## *Future research directions*

To further advance our understanding of durability and the influence of carbohydrate ingestion on durability, it is recommended that future studies should further investigate the mechanisms behind the positive effect of carbohydrate ingestion during prolonged exercise. Specifically, to assess the role of carbohydrate availability on durability, future research should manipulate glycogen levels, prior to prolonged exercise, then assess the effect of prolonged exercise on durability. In this study design, participants are likely to finish the prolonged

exercise bout with different glycogen levels. If durability is related to endogenous carbohydrate availability, power output at the moderate-to-heavy intensity transition should be lower in the trial that commenced with lowered glycogen. Additionally, compartmental muscle glycogen utilisation should also be measured, to determine glycogen utilisation and provide insight into the potential mechanisms of fatigue. Another direction that could also be explored is the neural effect of carbohydrate ingestion on improved durability, as opposed to the glycogen sparing effect. Multiple studies have shown performance benefits from carbohydrate mouth rinses (Carter et al., 2004; Chambers et al., 2009), likely via effects on improved central drive and thus muscle unit recruitment. Furthermore, this research could be expanded by manipulating pre-exercise carbohydrate availability in participants with different levels of 'fat-adaptation,' or a high and low ability to oxidise whole-body fat stores and plasma free fatty acids. Therefore, future studies could explore the effect of carbohydrate mouth rinsing during prolonged exercise on durability of the moderate-to-heavy intensity transition, as this approach may isolate the neural effect of carbohydrate ingestion on durability, and provide insight into the differences in central fatigue under low glycogen availability with different levels of fat-adaptation.

It is also recommended that future studies should look to develop tools for monitoring power output at the intensity domain transitions in real-time, that are sensitive to the variable effects of both prolonged exercise and carbohydrate ingestion. Development of these tools would allow the intensity of training to be regulated in real-time in accordance with dynamic intensity domain transitions, and allow more accurate, physiologically-based quantification of training load. This may require the integration of multiple physiological variables during exercise, such as heart rate, minute ventilation, and breathing frequency.

## *Conclusion*

In conclusion, the key findings of this thesis were that carbohydrate ingestion during prolonged exercise partially mitigated the decline in power output at the moderate-to-heavy intensity transition, and that statistically significant effects of carbohydrate ingestion on durability of severe-intensity time-trial performance were not observed. Accordingly, the primary recommendations for the application of these results to applied practice are that: (i) coaches and Performance Physiologists should consider the effect of carbohydrate ingestion on the stress of prolonged training, and therefore its impact on training load, (ii) that durability should be monitored at an individual level as part of athlete profiling assessments, and (iii) that these assessments are conducted with the implementation of similar training or race-day nutrition strategies, to account for the effect of carbohydrate ingestion on the measured variables. The primary recommendations for future research are that: (i) the mechanisms underlying durability are investigated, by manipulating pre-exercise glycogen levels, and measuring the compartmentalised utilisation of muscle glycogen during prolonged exercise, or (ii) by isolating the neural contribution of carbohydrate ingestion with a carbohydrate mouth rinse and investigating a fat-adapted cohort that may respond differently with altered pre-exercise carbohydrate availability, and (iii) that future studies should look to develop tools to assess power output at

the intensity domain transitions in real-time, in order to more accurately quantify training load. These additional research findings will provide a deeper understanding of what makes athletes more durable, and enhance the practical application of durability research to improve endurance performance.

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