Durability of the moderate-to-heavy intensity transition is related to the effects of prolonged exercise on severe-intensity performance

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Abstract

Purpose: Physiological characteristics such as power output at the moderate-to-heavy intensity transition degrade during prolonged exercise. Resilience to this has been termed 'durability'. The relationship between durability and performance has not been well-characterised. Therefore, the purpose of the present investigation was to assess the relationship between durability and the effect of prolonged exercise on severe-intensity performance, and explore intramuscular correlates of durability.

Methods: Thirteen well-trained endurance cyclists and triathletes (11 males, 2 females, $\dot{V}O_2$peak 57.3 ± 4.8 mL·kg^{-1}·min^{-1}, training volume 12 ± 2.1 h·week^{-1}) performed four laboratory visits. On separate days, an incremental test and 5-min time-trial (TT) was performed to determine power output at the first ventilatory threshold (VT_1) and severe-intensity performance, with (POST) and without (PRE) 150-min of prior moderate-intensity cycling. A resting *vastus lateralis* microbiopsy was also obtained.

Results: Prolonged exercise reduced power output at VT_1 (211 ± 40 W vs. 198 ± 39 W, Δ -13 ± 16 W, Δ -6 ± 7%, P = 0.013) and 5-min TT performance (333 ± 75 W vs. 302 ± 63 W, Δ -31 ± 41 W, Δ -9 ± 10%, P = 0.017). The reduction in 5-min TT performance was significantly associated with durability of VT_1 (r_s = 0.719, P = 0.007), but not PFO. Durability of VT_1 was not related to *vastus lateralis* carnosine content, citrate synthase activity, or complex I activity.

Conclusion: These data provide the first direct support for the hypothesis that durability of the moderate-to-heavy intensity transition is an important endurance performance parameter.

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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

- Kate, 80%; conceived and designed the research, conducted experiments and collected the data, analysed the data, drafted the manuscript.
- Ed, 15%; conceived and designed the research, assisted with data collection, read and approved the manuscript.
- Andrew, 5%; assisted with the conception of the research, read and approved the manuscript.

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Ethics Approval

All experimental procedures of this dissertation were approved by the Auckland University of Technology Ethics Committee on 18th July 2022 (*22/163)*

Chapter One: Physiological Profiling in Endurance Sport

Introduction

One of the fundamental roles of a Performance Physiologist working with endurance athletes is to undertake physiological profiling assessments. These assessments are performed to help understand the individual athlete's physiological responses to exercise, and adaptations to training. Routine physiological profiling assessments involve controlled exercise tests with concurrent measurement of various physiological responses. A routine physiological profiling assessment typically involves the completion of an incremental exercise test. An incremental test requires an athlete to exercise at progressively increasing intensities, such that physiological measurements can be made across a range of workloads. Accordingly, a profile of the relationship between exercise intensity and an athlete's physiology is constructed, and a number of specific physiological attributes relevant to endurance performance and training are estimated (Jamnick et al., 2020).

The physiological attributes identified in routine physiological profiling assessments are subsequently relayed back to coaches and athletes to allow more informed decisions to be made regarding training and competition. For example, physiological profiling assessments and attributes are often used to assess current performance capabilities, and for use in optimising training programming, monitoring training load and adaptations to exercise, and for within-session intensity regulation. The classic physiological profiling attributes routinely assessed in physiological profiling assessments and used for these purposes are the peak rate of oxygen consumption ($\dot{V}O_2$peak), movement economy, and the work output at intensity domain transitions, or 'thresholds'.

Peak rate of oxygen consumption

The $\dot{V}O_2$peak is a physiological profiling attribute first introduced by A.V. Hill in the 1920s (Hill et al., 1924; Hill & Lupton, 1923). The $\dot{V}O_2$peak refers to the highest rate of oxygen uptake and utilisation achievable by an individual during intense maximal exercise, where further increases in work rate do not elicit additional rises in $\dot{V}O_2$. An individual's $\dot{V}O_2$peak is determined by their capacity for oxygen delivery to working muscle, and skeletal muscle oxidative capacity, as demonstrated in the Fick equation ($\dot{V}O_2$ = cardiac output x arterial-venous O_2 difference) (Stickland et al., 2012). During dynamic exercise involving activation of a large muscle mass, it appears that $\dot{V}O_2$peak is limited by oxygen delivery (Mortensen et al., 2005).

Given the requirement for high rates of oxidative metabolism to maintain high absolute work rates during endurance performance, it is unsurprising that a high $\dot{V}O_2$peak is a common characteristic amongst elite endurance athletes (LuciA et al., 2002; Sanders & Heijboer, 2019; Sylta et al., 2014; Tønnessen et al., 2014). Many studies have identified positive relationships between $\dot{V}O_2$peak and endurance performance, particularly among heterogeneous populations (Carlsson et al., 2013; Costill et al., 1973; Craig et al., 1993). The $\dot{V}O_2$peak is also responsive to training (Lundby et al., 2023; Rønnestad et al., 2022; Rønnestad et al., 2021), with many studies seeking to determine the optimal intervention to enhance $\dot{V}O_2$peak and therefore performance (Milanović et al., 2015). Therefore, quantifying $\dot{V}O_2$peak in routine physiological profiling assessments is useful for monitoring training adaptations relevant to performance, programming the intensity of interval training sessions, and understanding pacing for those who compete in stochastic endurance events.

Movement economy

Movement economy refers to the metabolic cost of a given external work rate. In runners, movement economy is typically quantified as running economy, or the energy expenditure required to cover a given distance at a given speed (Barnes & Kilding, 2015; Fletcher et al., 2009). Due to constraints associated with quantifying speed during indoor cycling, the movement economy of cyclists during routine physiological profiling assessments is typically expressed as gross efficiency, or the percentage of total metabolic energy expenditure that is translated into mechanical power output (MacDougall et al., 2022). Therefore, cyclists with greater gross cycling efficiency produce more mechanical power output at a given metabolic rate. Accordingly, movement economy is identified during routine physiological profiling assessments as a marker of performance capabilities, and to quantify adaptations to training.

Intensity domain transitions

Physiological responses to acute exercise can be grouped into distinct exercise intensity domains (Black et al., 2017; Jones et al., 2019). Exercise performed in the moderate intensity domain is characterised by low and stable blood lactate concentrations, whereas exercise in the heavy intensity domain elicits blood lactate concentrations that stabilise over time at elevated concentrations. During exercise in the severe intensity domain, a muscle metabolic steady state cannot be attained, and therefore phosphocreatine concentrations and pH progressively fall, while inorganic phosphate concentrations and $\dot{V}O_2$ progressively rise until the limit of tolerance is reached or the work rate is reduced (Black et al., 2017). Accordingly, exercise performed in the different

intensity domains elicits markedly different levels of physiological stress and therefore required recovery (Stanley et al., 2013), and so knowledge of the work outputs at the intensity domain transitions is used in training programming and load monitoring (Maunder et al., 2021).

The work rates at which the moderate-to-heavy and heavy-to-severe intensity transitions occur in an individual athlete can be assessed during routine physiological profiling assessments using a range of methods, including pulmonary gas exchange (the ventilatory thresholds) and blood lactate concentrations (the lactate thresholds). Various indices of the work rates achieved at the intensity domain transitions have been correlated with endurance performance (Bentley et al., 2001; Bishop et al., 1998), and identification of these thresholds is therefore used in training programming and load monitoring (Jamnick et al., 2020).

Application of physiological profiling attributes to prolonged exercise scenarios: Durability

Importantly, the physiological profiling attributes quantified during routine assessments are often applied to prolonged exercise scenarios. These assessments are typically performed in well-rested athletes, and the attributes profiled progressively degrade over time during prolonged exercise. For example, in cyclists, power output at the moderate-to-heavy (Stevenson et al., 2022) and heavy-to-severe (Clark et al., 2018; Clark, Vanhatalo, et al., 2019b; Clark, Vanhatalo, Thompson, Wylie, et al., 2019) intensity transitions are reduced following prolonged exercise. Importantly, there appears to be substantial inter-individual variability in the magnitude of the effect of a given bout of prolonged exercise on physiological profiling attributes. Recently, ‘durability’ has been used to refer to an individual’s resilience to the effects of prolonged exercise on physiological profiling attributes (Maunder et al., 2021).

Durability has implications for the use of routinely assessed physiological profiling attributes in within-session intensity regulation, training programming, and training load monitoring. For example, when undertaking a long, low intensity training session that is designed to elicit low levels of physiological stress, an initially moderate-intensity power output may drift into the heavy intensity domain after multiple hours of exercise, and therefore elicit an unintended stress response. Therefore, appreciating individual durability characteristics may improve the application of physiological profiling data to real-world endurance training programming and monitoring.

It is also plausible that the of physiological profiling attributes has significant implications for performance outcomes in prolonged endurance events, although these have not been well-characterised. In stochastic events such as road cycling, performance outcomes are often determined by an athlete's ability to produce high work outputs in the severe-intensity domain following multiple hours of moderate-intensity exercise (Fernández-García et al., 2000; Sanders & Heijboer, 2019; Sanders & van Erp, 2020). As athletes with the same well-rested physiological profile may show differences after multiple hours of exercise (Clark et al., 2018; Clark, Vanhatalo, et al., 2019b; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Stevenson et al., 2022), durability characteristics may determine performance capabilities at the end of road cycling events, and therefore performance outcomes. Therefore, durability may be an influential determinant of performance in endurance sport. To establish if durability of specific physiological profiling attributes are related to endurance performance outcomes, controlled assessment of relationships between durability and relevant endurance performance outcomes is warranted.

Similarly, the physiological mechanisms that explain inter-individual differences in durability have not been identified. Many mechanisms could plausibly be related to durability, such as the ability to maintain endogenous carbohydrate availability, and the oxidative properties of muscle. For example, possessing a high capacity to oxidise fatty acids to fuel energy metabolism during prolonged exercise may preserve finite endogenous intramyofibrillar glycogen stores, and therefore contractile function (Nielsen et al., 2014; Nielsen et al., 2009; Ørtenblad et al., 2011; Ørtenblad et al., 2013), and thus resilience to duration-induced decrements in exercising physiology. Similarly, mitochondrial protein content may also influence durability characteristics, as a larger mitochondrial pool may spread the oxidative burden of demanding exercise and therefore reduce the damage that occurs to individual mitochondria during prolonged exercise (Layec et al., 2018; Lewis et al., 2021; Trewin et al., 2017). Therefore, assessment of relationships between durability and various potential underlying mechanisms is warranted. Understanding the mechanistic determinants of durability may allow for the development of targeted interventions to improve it.

Purpose of this dissertation

Accordingly, the study conducted for this dissertation was designed to answer the following research questions:

1. Is durability of the moderate-to-heavy transition and/or $\dot{V}O_2$peak related to the magnitude of prolonged exercise-induced reductions in severe-intensity time-trial performance?

2. Is there a relationship between prolonged exercise-induced reductions in severe-intensity time-trial performance and PFO?
3. Are oxidative properties of skeletal muscle related to durability of the moderate-to-heavy intensity transition?

In the next chapter, I present an original research study conducted to answer these research questions in manuscript format. In the subsequent chapter, I summarise the main implications of the research.

Chapter Two: Durability of the moderate-to-heavy intensity transition is related to the effects of prolonged exercise on severe-intensity performance

Introduction

Several physiological profiling characteristics are estimated in well-rested athletes during routine laboratory assessments and used for the assessment of performance capabilities, within-session intensity regulation, and monitoring training load and adaptation (Maunder et al., 2021; Seiler, 2010). Prolonged exercise elicits progressive physiological changes such as increased core and muscle temperature (Febbraio et al., 1994), depletion of endogenous fuel stores (Areta & Hopkins, 2018; Gonzalez et al., 2016; Stokie et al., 2023), accumulation of muscle damage (Stevens et al., 2018), and cellular stress (Peake et al., 2017). Consequently, physiological profiling characteristics such as work output at the moderate-to-heavy (Stevenson et al., 2022) and heavy-to-severe intensity transitions (Clark et al., 2018; Clark, Vanhatalo, et al., 2019b; Clark, Vanhatalo, Thompson, Wylie, et al., 2019), gross cycling efficiency (Passfield & Doust, 2000), the peak rate of oxygen consumption ($\dot{V}O_2$peak) (Brownstein et al., 2022), and movement economy (Moseley et al., 2004; Moseley & Jeukendrup, 2001) degrade during prolonged exercise. 'Durability' is defined as an individual's resilience to deteriorations in physiological profiling characteristics during prolonged exercise, and has been proposed as a key endurance performance parameter (Maunder et al., 2021). However, the influence of durability of physiological profiling parameters on performance outcomes has not been well-characterised.

Performance outcomes in stochastic endurance events such as road cycling are often determined by the ability to produce high work outputs in the severe-intensity domain following multiple hours of primarily moderate-intensity exercise (Fernández-García et al., 2000; Sanders & van Erp, 2020). Like physiological profiling characteristics, severe-intensity performance decreases with prolonged exercise (Clark et al., 2018; Clark, Vanhatalo, et al., 2019b; Clark, Vanhatalo, Thompson, Wylie, et al., 2019). It might be expected that durability of the intensity domain transitions promotes resilience to the effects of prolonged exercise on severe-intensity performance. As power output at the moderate-to-heavy intensity transition decreases during prolonged exercise (Stevenson et al., 2022), an initially moderate-intensity power output may drift into the heavy-intensity domain. Heavy-intensity exercise elicits distinct physiological responses compared to the moderate-intensity domain, such as greater extracellular K^+ accumulation (Black et al., 2017). As extracellular K^+ accumulation impairs muscle contractile function (Cairns et al., 1997; De Paoli et al., 2007), greater time spent in the heavy domain may result in reduced subsequent capacity for work outputs in the severe-intensity domain. Therefore,

better durability of the moderateo-heavy intensity transition may promote resilience to the effects of prolonged exercise on severe-intensity performance. However, the relationship between these variables has not been assessed.

The effect of prolonged exercise on severe-intensity performance capabilities may also be related to glycogen depletion. Glycogen-depleted muscle is more sensitive to severe-intensity exercise-induced K^+ disturbances (Cairns & Lindinger, 2008; Lindinger & Cairns, 2021), and depletion of endogenous intramyofibrillar glycogen stores impairs muscle contractile function (Nielsen et al., 2014; Nielsen et al., 2009; Ørtenblad et al., 2011; Ørtenblad et al., 2013). Therefore, it is plausible that athletes capable of oxidising fat at high rates to preserve glycogen during submaximal exercise may be better able to maintain severe-intensity performance following prolonged exercise. The propensity for fat oxidation during exercise has been quantified using the peak fat oxidation rate (PFO) observed during an incremental exercise test (Maunder et al., 2018). The PFO has previously been related to endurance performance outcomes (Frandsen et al., 2017; Maunder et al., 2018; Maunder et al., 2022). However, the relationship between PFO and the effect of prolonged exercise on severe-intensity performance has not been assessed.

Similarly, the physiological determinants of durability are not well-explored. Plausibly, skeletal muscle fibre type composition may influence durability, as type I fibres are more fatigue-resistant (Thorstensson & Karlsson, 1976). Likewise, a larger mitochondrial pool may spread the oxidative burden of a given exercise work rate, and therefore reduce the damage induced to individual mitochondria during prolonged exercise at a specific work rate (Layec et al., 2018; Lewis et al., 2021; Trewin et al., 2017). This may promote durability by extending the time over which individual mitochondria are fully functional, and therefore preserve the work capacity of muscle. Understanding the mechanistic determinants of durability may allow for the development of targeted interventions to improve it.

Therefore, the primary aims of the present investigation were to: (i) determine if durability of the moderate-to-heavy transition and/or $\dot{V}O_2$peak is related to the magnitude of prolonged exercise-induced reductions in severe-intensity time-trial performance, (ii) assess the relationship between prolonged exercise-induced reductions in severe-intensity time-trial performance and PFO, and (iii) quantify relationships between various intramuscular characteristics and durability of the moderate-to-heavy intensity transition. We hypothesised that cyclists who have greater durability of the moderate-to-heavy intensity transition would be more resilient to the effect of prolonged exercise on severe-

intensity time-trial performance, that resilience to the effects of prolonged exercise on severe-intensity time trial performance would be related to PFO, and that durability of the moderate-to-heavy intensity transition would be related to various oxidative properties of skeletal muscle.

Methods

Ethical approval

The study was performed in accordance with the Declaration of Helsinki, 2013. The Auckland University of Technology Ethics Committee approved all procedures (22/163), and all participants provided written informed consent prior to participation. This study was not registered in a database. Raw data is available upon request.

Participants

Thirteen well-trained endurance cyclists and triathletes took part in the present investigation (11 males, 2 females, age 29 ± 7, height 182.6 ± 8 cm, mass 78.1 ± 11.7 kg, $\dot{V}O_2$peak 57.3 ± 4.8 mL·kg^{-1}·min^{-1}, training volume 12 ± 2.1 h·week^{-1}). *A-priori* calculations indicated that 15 participants were required to detect a significant bivariate correlation of r = 0.6, assuming a null hypothesis correlation of r = 0, and a one-tailed test, with 80% statistical power and a type I error rate of 0.05. All participants were free of recent (<3 months) illness and musculoskeletal injury, free of cardiovascular disease, training >8 hours·week^{-1} in endurance cycling with a peak oxygen uptake >55 mL·kg^{-1}·min^{-1}, and self-reported best-effort 20-min power output of >3.5 W·kg^{-1}. All participants provided written, informed consent. Three participants dropped out after the first visit due to the inclusion criteria of remaining free of illness for >3 months.

Study design

The present study adopted a cross-sectional design (Figure 1). Participants visited the laboratory on four separate occasions, ~5-14 days apart, at ~6:00am. The first visit was a characterisation trial involving an incremental cycling test for estimation of power output at the first ventilatory threshold (VT_1) for use in subsequent trials and peak oxygen uptake ($\dot{V}O_2$peak), and a familiarisation 5-min time-trial. The second and third visits took place in random, counterbalanced order, and involved either (i) PRE: a submaximal incremental test to determine the moderate-to-heavy intensity transition, and performance and $\dot{V}O_2$peak in a 5-min time-trial, or (ii) POST: 150 min of cycling at 90% of the first ventilatory threshold (VT_1) power output estimated in the characterisation trial, followed by the submaximal incremental test to determine the moderate-to-heavy intensity transition, and performance and $\dot{V}O_2$peak in a 5-min time-trial. The remaining visit was for a resting *vastus lateralis* microbiopsy.

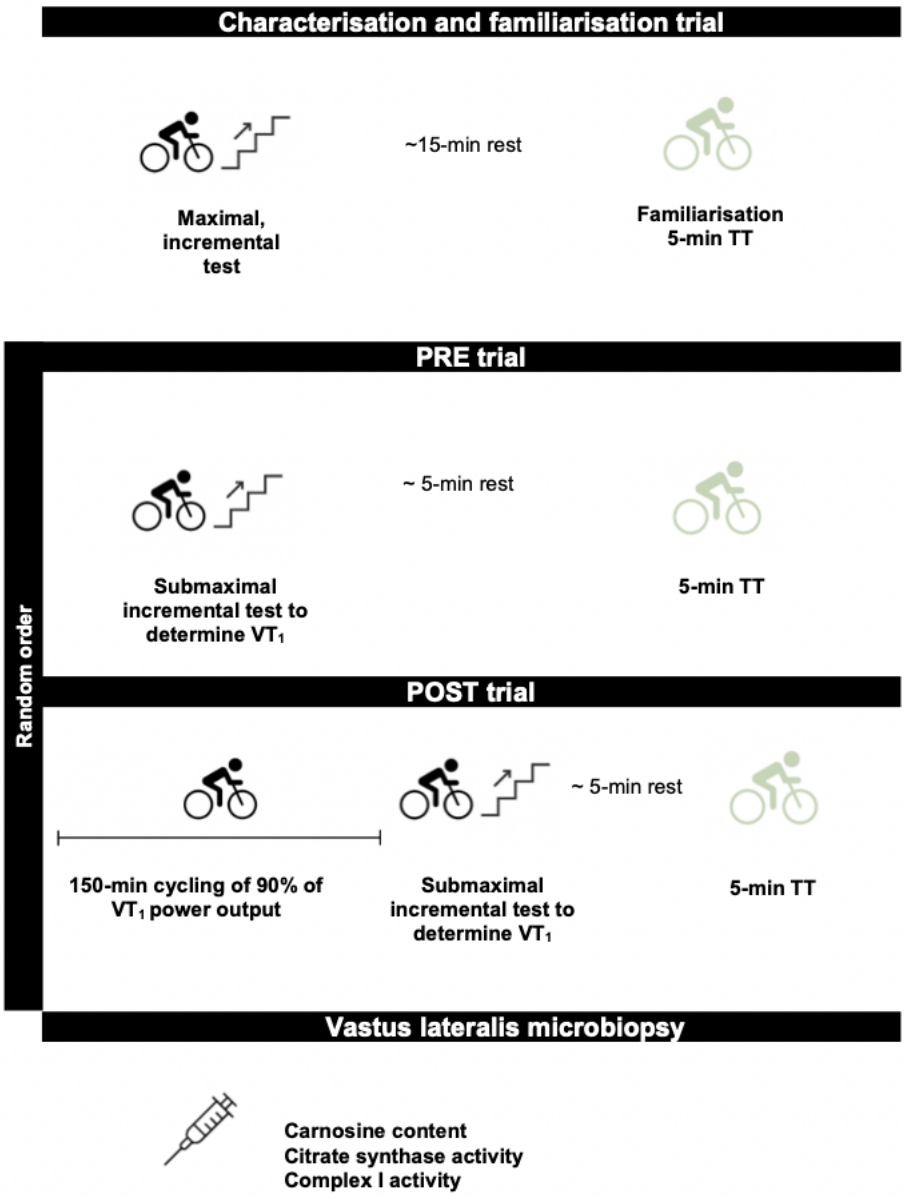

Figure 1. Schematic of the study design. TT, time trial; VT_1, first ventilatory threshold.

Characterisation trial

Participants reported to the laboratory for an initial incremental cycling test and 5-min familiarisation time-trial, after having fasted overnight for ~10 h and having ingested 1-2 L of plain water before arrival. After providing written informed consent, height and mass were determined. Cycling commenced with a 5-min warm-up at 100 W on personal road bicycles mounted to a direct-drive smart indoor trainer (Kickr, Wahoo Fitness, GA, USA). Subsequently, the incremental cycling test began at 95 W, with the work rate increasing

by 35 W every 3 min. Expired gases and heart rate were collected continuously using indirect calorimetry (TrueOne 2400, ParvoMedics, UT, USA) and a chest-strap heart rate monitor (Polar Electro Oy, Kempele, Finland). When the respiratory exchange ratio (RER) reached 1.0, power output was increased by 35 W every minute until volitional exhaustion. The $\dot{V}O_2$peak was accepted as the highest 15-second average $\dot{V}O_2$, and VT_1 was identified as the first $\dot{V}O_2$ breakpoint in the $\dot{V}O_2$ vs. $\dot{V}_E{\cdot}\dot{V}O_2^{-1}$ relationship. This $\dot{V}O_2$ was converted to power output by linear regression of the $\dot{V}O_2$ vs. power output relationship, using the last minute of $\dot{V}O_2$ data from each 3-min stage. The last minute of expired gas data in each 3-min stage was also used to quantify whole-body rates of carbohydrate and fat oxidation using standard equations (Eq. 1) (Jeukendrup & Wallis, 2005). The highest observed rate of whole-body fat oxidation was identified as the peak fat oxidation rate (PFO) (Maunder et al., 2022).

$$\text{Carbohydrate oxidation rate } (g{\cdot}min^{-1}) = 4.210 \times \dot{V}CO_2 - 2.962 \times \dot{V}O_2$$
$$\text{Fat oxidation rate } (g{\cdot}min^{-1}) = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2$$

Eq. 1 where $\dot{V}O_2$ and $\dot{V}O_2$ are in $L{\cdot}min^{-1}$.

Following completion of the incremental test, participants rested for 15 min before completing a 5-min performance time-trial at maximal effort, with the goal of achieving the highest possible average power output. Expired gases and heart rate were collected throughout, however, participants were blinded to all data other than elapsed time. Verbal encouragement was not provided. The highest 15-s average of $\dot{V}O_2$ was accepted as the time trial $\dot{V}O_2$peak. If this value exceeded the $\dot{V}O_2$peak achieved during the maximal, incremental cycling test, it was used as the characterisation trial $\dot{V}O_2$peak. Following the time trial, participants were provided with blank 7-d exercise and 2-d diet record sheets, which were completed in advance of the second trial, and replicated in advance of the third trial.

Visits two and three: PRE and POST assessments

Participants returned to the laboratory 5-14 days following the characterisation trial to complete the first of the two subsequent trials. Participants arrived having consumed a standardised breakfast containing ~2 $g{\cdot}kg^{-1}$ of carbohydrate and ~800 mL of water one hour beforehand. Participants were fitted with a chest-strap heart rate monitor, and a wireless near-infrared spectroscopy device for estimation of muscle oxygenation (S_mO_2) on their right leg (Moxy Monitor, Fortiori Design LLC, Hutchinson, MN, USA). The device

was placed over the mid-belly of the *vastus lateralis*, half the distance between the tibial tuberosity and greater trochanter. The precise site of the device was recorded such that it could be repeated in the remaining trial. Heart rate and muscle oxygenation were measured continuously throughout the trial.

The PRE and POST trials began with a 5-min warm-up at 100 W. Following warm-up, participants cycled for 150 min at 90% of their estimated VT_1 power output in the POST but not PRE trial, with expired gases collected for 4 min every 15 min. Expired gases were used to quantify rates of whole-body rates of energy expenditure, carbohydrate oxidation, and fat oxidation during the 150-min preload. In POST, participants consumed 150 mL of water every 15 min in a solution made with electrolyte mix (LMNT) containing 125 mg Na^+, 25 mg K^+, and 7.5 mg Mg^{2+} during the first 120 min of the 150-min preload.

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 began 50 W below the VT_1 power output estimated in the first laboratory visit, and increased by 25 W every 4 min, such that the fifth and final step was 50 W above the VT_1 power output estimated in the first laboratory visit. The moderate-to-heavy intensity transition power output was estimated using the methods described for determining VT_1 in the first laboratory visit, but with greater precision given the greater density of data around the transition. This method has been used to estimate the moderate-to-heavy intensity transition previously (Stevenson et al., 2022). 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. The effect of prolonged exercise on the moderate-to-heavy intensity transition power output, 5-min time-trial performance, and $\dot{V}O_2$peak during the 5-min time-trial was determined by subtracting PRE from POST values. We used this exercise protocol as we previously observed reduced power output at the moderate-to-heavy intensity transition after 150 min of initially moderate-intensity cycling (Stevenson et al., 2022), and to simulate a road cycling event, in which a severe-intensity effort near the finish may follow a longer period of lower-intensity cycling (Fernández-García et al., 2000; Sanders & van Erp, 2020).

Visit four: Resting vastus lateralis microbiopsy

Approximately 5-14 days following the third visit, participants returned to the laboratory having consumed breakfast and having refrained from vigorous exercise. A ~15-30 mg resting microbiopsy sample was obtained from the mid-belly of the *vastus lateralis* of the dominant leg, ~10-15 cm above the patella. Local anaesthesia was applied to the skin and superficial muscle fascia. A microbiopsy needle was then inserted into the mid-belly

of the *vastus lateralis* to a depth of ~2 cm to recover ~20-40 mg of tissue using a spring-loaded mechanism (14G Ultimate Biopsy Needle, Zamar Care, Croatia). Muscle tissue was immediately frozen on dry ice and stored at -80°C until further analysis.

Muscle analyses

Frozen muscle was cut and rinsed using cold phosphate-buffered saline (PBS) and then suspended to ~25 $mg \cdot mL^{-1}$ in PBS. Samples were then ground manually and thoroughly using a pre-cooled Dounce homogeniser. Homogenate was solubilised in extraction buffer (ab260490, Abcam®) to ~5 $mg \cdot mL^{-1}$ and incubated on ice for 20 min prior to centrifugation at 16000 g for 10 min at 4°C. Supernatant was extracted and stored at -80°C prior to further analysis. Supernatant was thawed and assayed in duplicate for carnosine concentration (MBS721162, MyBioSource®; coefficient of variation [CV], 12.6%), citrate synthase enzyme activity (ab119692, Abcam®; CV, 11.4%), and complex I enzyme activity (ab109721, Abcam®; CV, 12.1%). All outcome measures were expressed relative to sample protein concentration using a Bradford assay, performed in triplicate (ab102535, Abcam®; CV, 6.1%).

Statistical analyses

Data are expressed as mean ± standard deviation, unless otherwise stated. Normality of datasets was assessed using Shapiro-Wilk tests. Simple PRE vs. POST comparisons of the moderate-to-heavy intensity transition power output, $\dot{V}O_2peak$, and time trial performance were made using paired *t*-tests or Wilcoxon signed-rank tests, depending on normality, and used to verify the effect of prolonged exercise on these parameters. The effect of time on whole-body rates of energy expenditure, carbohydrate oxidation, fat oxidation, S_mO_2, and heart rate during the 150 min preload in POST was analysed using one-way repeated measures analyses of variance.

Bivariate relationships between (i) the magnitude of the PRE vs. POST change in moderate-to-heavy intensity transition power output and the magnitude of the PRE vs. POST change in time trial performance, (ii) the magnitude of the PRE vs. POST change in $\dot{V}O_2peak$ and the magnitude of the PRE vs. POST change in time trial performance, (iii) the magnitude of the PRE vs. POST change in time trial performance and PFO, (iv) the magnitude of the PRE vs. POST change in moderate-to-heavy intensity transition power output and skeletal muscle characteristics were assessed using Pearson's or Spearman's rank-order correlation coefficients (depending on normality), and expressed with 95% confidence intervals. Additionally, participants were separated into low and high durability subgroups based on the magnitude of their individual PRE to POST

change in moderate-to-heavy intensity transition power output. The low and high durability subgroups consist of the six participants with the largest and smallest reductions in moderate-to-heavy intensity transition power output from PRE to POST, respectively. The PRE to POST changes in time trial performance and skeletal muscle characteristics of the low and high durability subgroups were compared using unpaired *t*-tests or Mann-Whitney-U tests, depending on normality. The PRE and POST values for S_mO_2 at the moderate-to-heavy intensity transition were compared using paired *t*-tests or Wilcoxon signed-rank tests, depending on normality, and intra-class correlation coefficients and coefficient of variation statistics were computed. All analyses were performed in GraphPad Prism Version 9.3.1. Significance was inferred when $P \leq 0.05$.

Results

Prolonged exercise phase

The estimated power output at VT_1 in the initial assessment was 208 ± 30 W. The 150-min prolonged phase in POST was therefore completed at 187 ± 27 W. During the prolonged phase, there was an effect of time on heart rate, EE, carbohydrate oxidation, and fat oxidation ($P < 0.05$). Significant effects of time on $\dot{V}O_2$ and S_mO_2 were not observed (Figure 2).

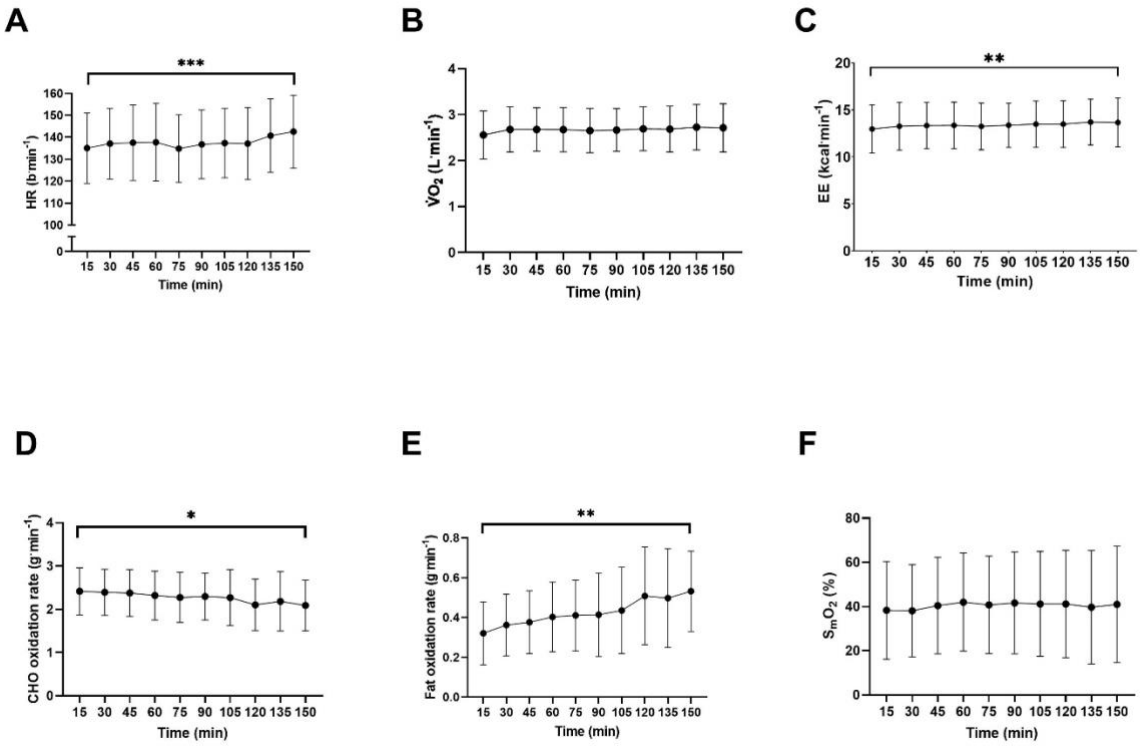

Figure 2. **A** Heart rate (HR), **B** rate of oxygen consumption ($\dot{V}O_2$), **C** energy expenditure (EE), **D** carbohydrate (CHO) oxidation rate, **E** fat oxidation rate, and **F** muscle oxygen saturation (S_mO_2) during the prolonged phase of the POST trial. * denotes $P \leq 0.05$, ** denotes $P \leq 0.01$, *** denotes $P \leq 0.001$.

Effects of prolonged exercise

Power output at VT_1 (211 ± 40 W vs. 198 ± 39 W, Δ -13 ± 16 W, Δ -6 ± 7%, $P = 0.013$) and 5-min time-trial performance (333 ± 75 W vs. 302 ± 63 W, Δ -31 ± 41 W, Δ -9 ± 10%, $P = 0.017$) significantly decreased from PRE to POST. The $\dot{V}O_2$peak (4.37 ± 0.85 vs. 4.28 ± 0.78 $L \cdot min^{-1}$, $P = 0.252$) and S_mO_2 at VT_1 (37 ± 13 vs. 40 ± 16%, $P = 0.139$) were

not significantly different between PRE and POST (Figure 3). The within-subject CV for S_mO_2 at VT_1 in PRE and POST was 13.0%, with an intraclass correlation of 0.874.

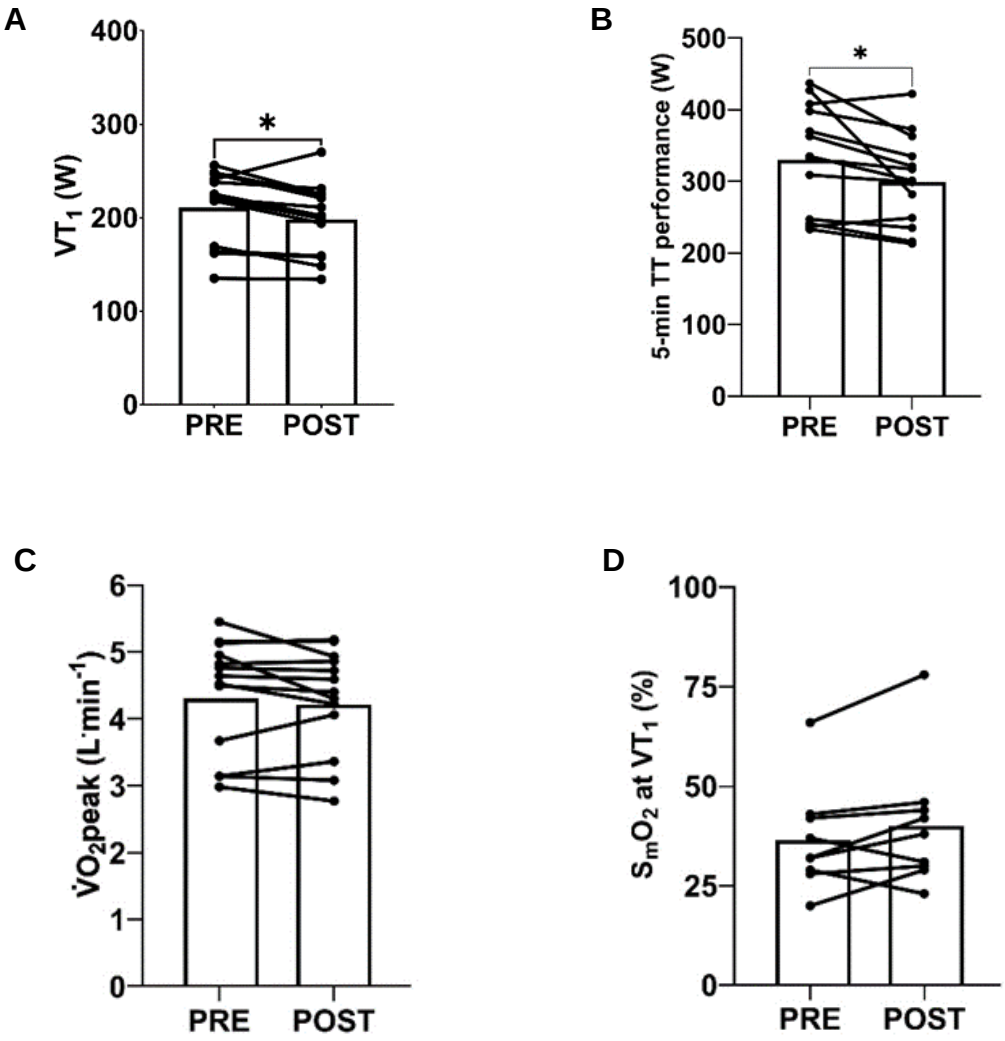

Figure 3. **A** Power output at the first ventilatory threshold (VT_1), **B** 5-min time-trial (TT) performance, **C** peak rate of oxygen consumption ($\dot{V}O_2$peak), and **D** muscle oxygen saturation (S_mO_2) at VT_1 in the PRE and POST assessments. * denotes $P \leq 0.05$.

Correlational analyses

The change in power output at VT_1 from PRE-to-POST was significantly associated with PRE-to-POST changes in 5-min time-trial power output and $\dot{V}O_2$peak ($P < 0.05$). The PRE-to-POST change in $\dot{V}O_2$peak was not significantly associated with the change in 5-min time-trial power output, nor was PFO (Figure 4). No significant relationships were observed between the PRE-to-POST change in power output at VT_1 and *vastus lateralis* carnosine concentration (24.0 ± 12.2 $\mu g \cdot g^{-1}$ protein), CS activity (16.1 ± 5.1 $\mu mol^{-1} min^{-1} \cdot mg^{-1}$ protein), or Complex I activity (5.8 ± 3.2 $\mu mol^{-1} min^{-1} \cdot mg^{-1}$ protein) (Figure 5).

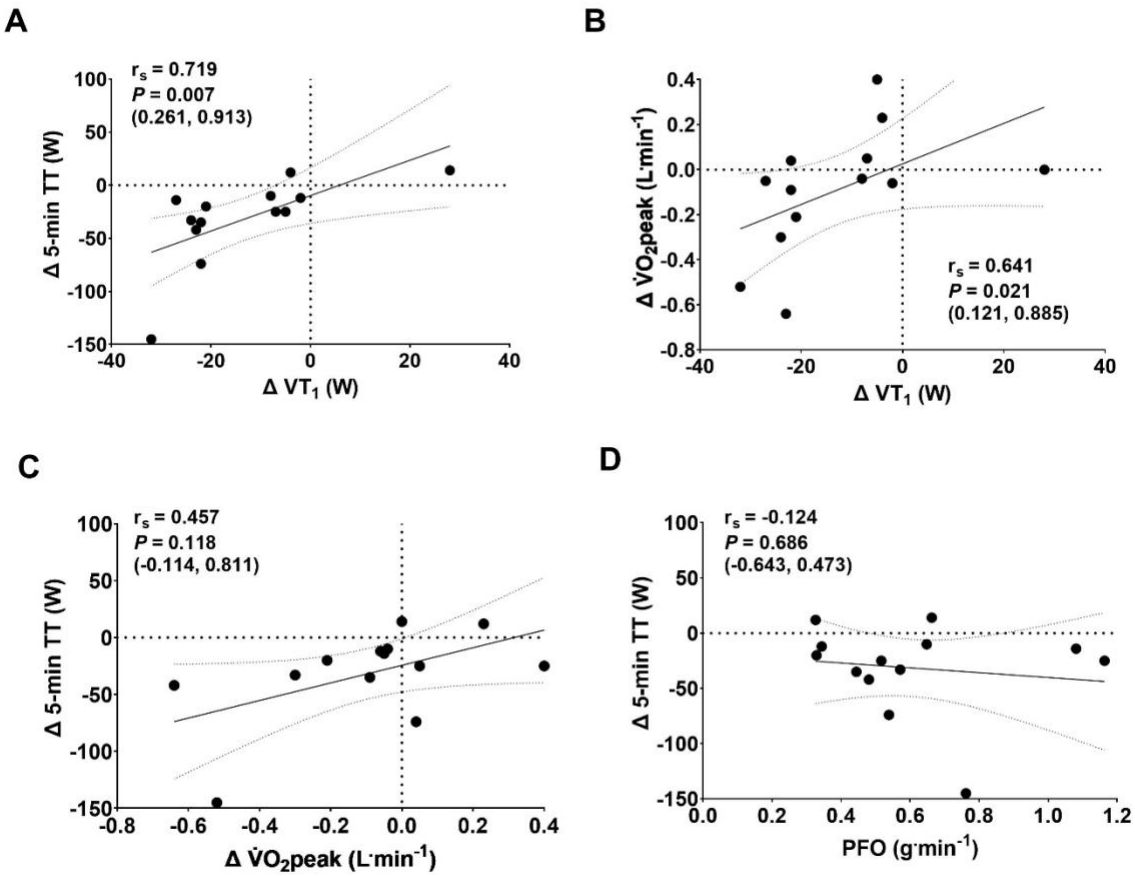

Figure 4. Relationships between **A** PRE-to-POST changes in VT_1 and 5-min time-trial (TT) performance, **B** PRE-to-POST changes VT_1 and $\dot{V}O_2$peak, **C** PRE-to-POST changes in $\dot{V}O_2$peak and 5-min TT performance, and **D** peak fat oxidation rate (PFO) and PRE-to-POST changes in 5-min TT performance. Data are presented as Spearman's rank-order correlation coefficients (r_s) with *P* values and 95% confidence intervals.

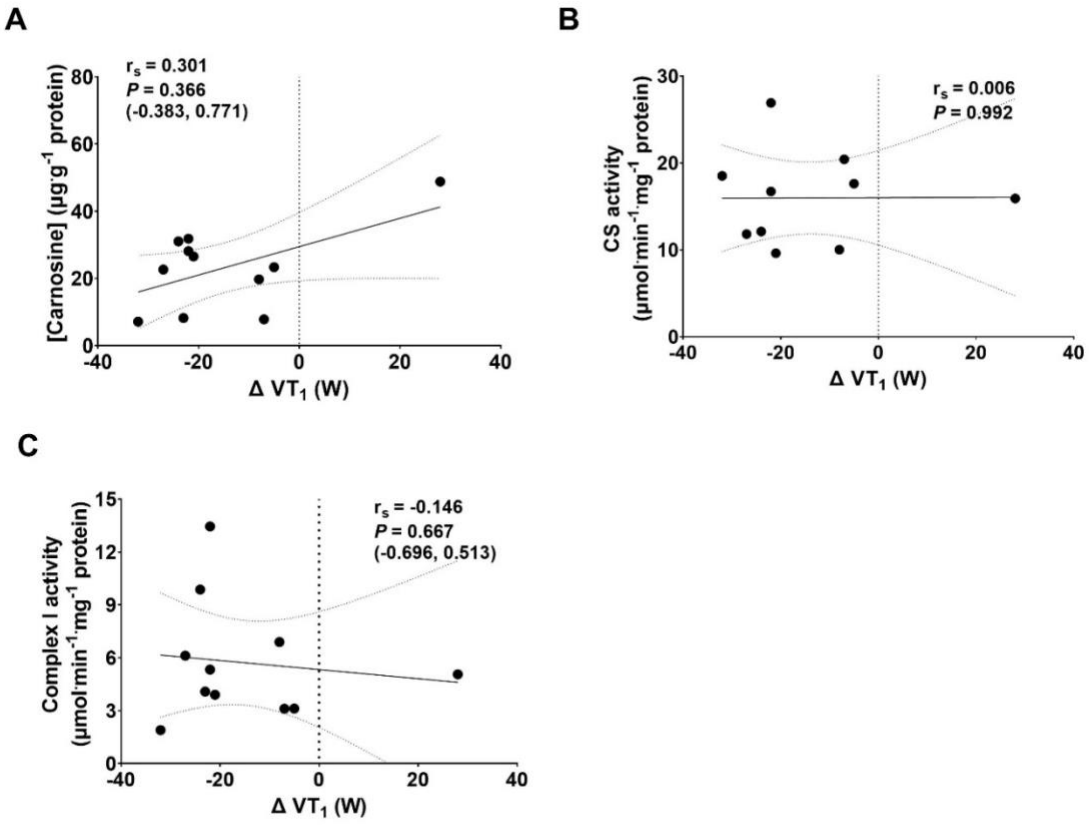

Figure 5. Relationships between the PRE-to-POST change in VT_1 power output and *vastus lateralis* **A** carnosine concentration, **B** citrate synthase (CS) activity, and **C** Complex I activity. Data are presented as Spearman's rank-order correlation coefficients (r_s) with *P* values and 95% confidence intervals.

Subgroup analyses

The six most durable athletes (HIGH: those with the smallest absolute reduction in VT_1 power output from PRE-to-POST) had significantly lower PRE-TO-POST reductions in 5-min TT power output and $\dot{V}O_2$peak than the six least durable athletes (LOW: those with the largest absolute reduction in VT_1 power output from PRE-to-POST). Between-subgroup differences were not observed for PFO or intramuscular variables (Figure 6).

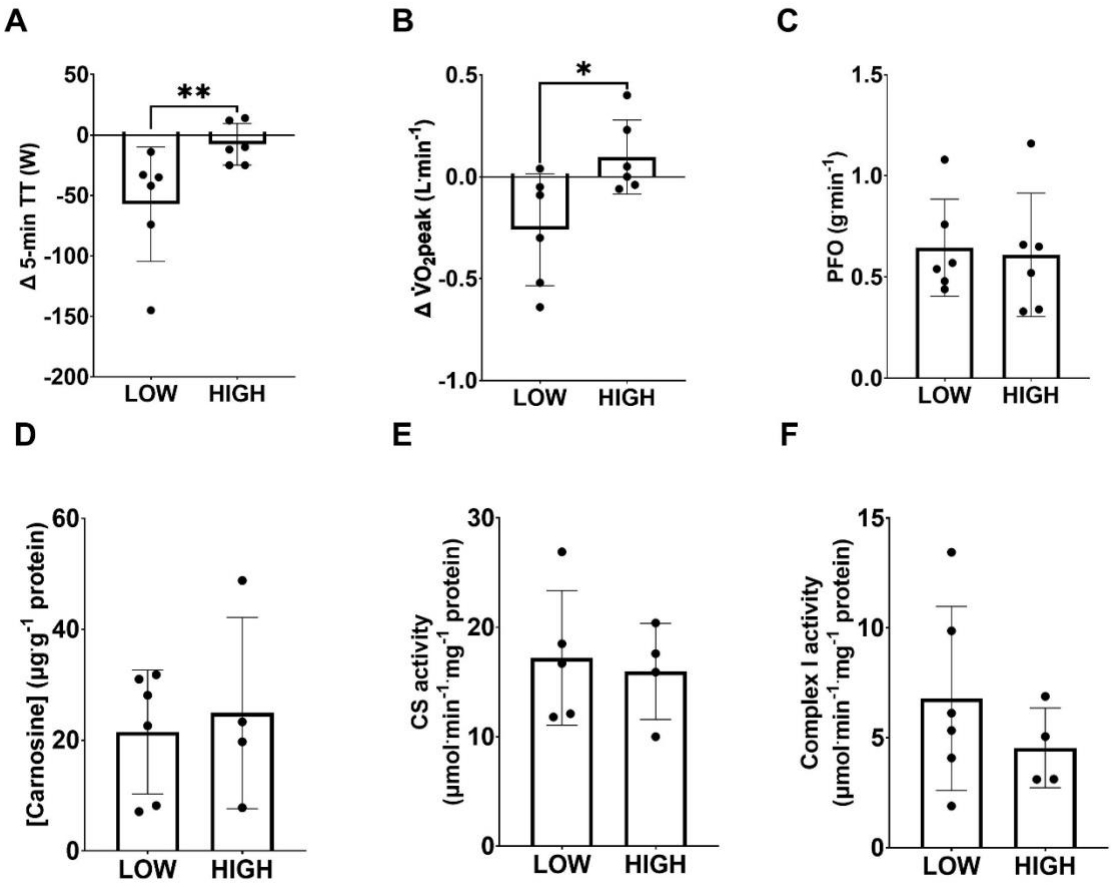

Figure 6. Subgroup comparisons between the least (LOW: those with the largest absolute reduction in VT_1 power output from PRE-to-POST) and most (HIGH: those with the smallest absolute reduction in VT_1 power output from PRE-to-POST) durable athletes for **A** PRE-to-POST change in 5-min time trial (TT) performance, **B** PRE-to-POST change in $\dot{V}O_2$peak, **C** peak fat oxidation (PFO), **D** *vastus lateralis* carnosine concentration, **E** *vastus lateralis* citrate synthase (CS) activity, and **F** *vastus lateralis* Complex I activity. Bars indicate mean values and lines indicate individual responses. * denotes $P \leq 0.05$, ** denotes $P \leq 0.01$.

Discussion

The primary aims of this study were to: (i) determine if durability of the moderate-to-heavy transition and/or $\dot{V}O_2$peak is related to the magnitude of prolonged exercise-induced reductions in severe-intensity time-trial performance, (ii) assess the relationship between prolonged exercise-induced reductions in severe-intensity time-trial performance and PFO, and (iii) quantify relationships between various intramuscular characteristics and durability of the moderate-to-heavy intensity transition. Our primary observations were that: (i) prolonged exercise-induced reductions in severe-intensity time trial performance were related to durability of the moderate-to-heavy intensity transition, but not durability of $\dot{V}O_2$peak, (ii) prolonged exercise-induced reductions in severe-intensity time-trial performance were not related to PFO, (iii) no relationships were observed between *vastus lateralis* CS activity, Complex I activity, or carnosine concentration and durability of the moderate-to-heavy intensity transition. These observations provide the first direct support for the hypothesis that durability of the moderate-to-heavy intensity transition is an important endurance performance parameter, and further support that durability of the moderate-to-heavy intensity transition might be monitored at an individual level.

In line with our hypothesis, prolonged exercise led to a reduction in power output at the moderate-to-heavy intensity transition and 5-min time-trial performance (Figure 3). These observations are in line with our previous study reporting a reduction in moderate-to-heavy intensity transition power output following 2.5 h of moderate-intensity cycling (Stevenson et al., 2022), and reduced total work done, or performance, in a three-minute all-out test following 2 h of heavy-intensity cycling (Clark et al., 2018; Clark, Vanhatalo, et al., 2019a; Clark, Vanhatalo, Thompson, Wylie, et al., 2019). Our novel observation is that the magnitude of these reductions was related; that is, those exhibiting larger reductions in power output at the moderate-to-heavy intensity transition with prolonged exercise exhibited the largest reductions in 5-min time-trial performance (Figure 3). This correlational analysis is further supported by our subgroup analysis, which demonstrated that the high durability subgroup had a smaller reduction in 5-min time-trial power output and $\dot{V}O_2$peak than the low durability subgroup (Figure 6). These data are therefore the first to demonstrate that durability of the moderate-to-heavy intensity transition is related to performance capabilities relevant to stochastic endurance events such as road cycling, and therefore support the assessment of durability as a key endurance performance parameter.

The strong relationship between the effects of prolonged exercise on the moderate-to-heavy intensity transition and 5-min time-trial performance could plausibly be

mechanistically related. Those athletes who saw greater reductions in power output at the moderate-to-heavy intensity transition likely spent more time in the heavy domain during the prolonged phase. Heavy-intensity exercise results in greater extracellular K^+ accumulation than moderate-intensity exercise (Black et al., 2017). Extracellular K^+ accumulation has been shown to depress muscle force production and therefore induce fatigue *in vitro* (Cairns et al., 1997; De Paoli et al., 2007). It is therefore plausible that the more durable athletes were better able to maintain 5-min time-trial performance due to better ability to maintain K^+ homeostasis during the prolonged phase. We suggest that this mechanism is interrogated in studies with measurement of plasma and interstitial K^+ concentrations.

Secondly, prolonged exercise-induced reductions in 5-min time-trial performance were not related to PFO (Figure 4d). This contrasts our hypothesis, which was that resilience to the effects of prolonged exercise on 5-min time-trial performance would be related to PFO. More specifically, we hypothesised that athletes who are capable of oxidising fat at high rates during the prolonged phase would better preserve glycogen for use during the subsequent severe-intensity time-trial. Muscle glycogen is an important fuel for high-intensity exercise (Van Loon et al., 2001), and muscle glycogen utilisation is autoregulated by muscle glycogen availability (Hargreaves et al., 1995). Additionally, glycogen-depleted muscle appears to be more sensitive to K^+ disturbances induced by severe-intensity exercise (Cairns & Lindinger, 2008; Lindinger & Cairns, 2021). Therefore, maintaining muscle glycogen availability during the prolonged phase may be favourable for mitigating the fatiguing effects of K^+ disturbances during the subsequent 5-min time-trial. Despite the lack of association between the effect of prolonged exercise on 5-min time-trial performance and PFO, it remains possible that glycogen availability does mediate the effects of prolonged exercise on severe-intensity performance. Although PFO during incremental exercise relates to fat oxidation rates during prolonged exercise (Maunder et al., 2022), PFO is not a direct measure of glycogen utilisation or availability. We therefore recommend that future studies interrogate the relationship between the effects of prolonged exercise on severe-intensity performance and muscle glycogen availability using direct measures of glycogen content.

In contrast to our hypothesis, durability of the moderate-to-heavy intensity transition was not related to *vastus lateralis* carnosine concentration, citrate synthase activity, or complex I activity (Figure 5). Similarly, differences in these intramuscular characteristics were not observed between LOW and HIGH durability subgroups (Figure 6). Carnosine concentrations, as assessed by ^{1}H-magnetic resonance spectroscopy, have been related to fibre type profile (Baguet et al., 2011). Lower carnosine concentrations are

observed in type I compared to type II skeletal muscle fibres (C. Harris et al., 1998). We therefore hypothesised that greater carnosine concentrations would be associated with larger prolonged exercise-induced reductions in power output at the moderate-to-heavy intensity transition, given greater type I fibre composition has been related to fatigue resistance (Cairns et al., 2017). Similarly, CS and Complex I activities are related to mitochondrial protein content (Larsen et al., 2012). We therefore hypothesised that CS and Complex I activities concentrations would be related to durability of the moderate-to-heavy transition, as a greater mitochondrial pool may spread the oxidative burden of a given exercise work rate, and reduce mitochondrial damage during prolonged exercise (Layec et al., 2018; Lewis et al., 2021; Trewin et al., 2017). The absence of relationships between these variables and durability of the moderate-to-heavy intensity transition may indicate that these variables are not mechanistically related, or may be due to the variability in these outcome measures, relatively low sample size, and/or relatively homogenous participant group. Another intramuscular variable that may be related to durability is heat shock protein 70 (HSP70) availability. HSP70 is an intracellular chaperone involved in managing protein aggregation and cellular stress (Krüger et al., 2019). Greater HSP70 abundance may therefore augment the capacity to manage the cellular stress generated during prolonged exercise, and therefore promote durability. We did attempt to measure HSP70 in our muscle samples, but unfortunately this assay did not produce usable results. Due to budgetary constraints, we were unable to repeat the assay. We recommend that the relationship between intramuscular HSP70 abundance and durability is assessed in future studies.

As power output at the moderate-to-heavy intensity transition declines during prolonged exercise, identification of a marker that can be viewed in real-time during prolonged exercise and used to assess proximity to the moderate-to-heavy intensity transition would be useful for within-session training intensity regulation (Maunder et al., 2021). Muscle oxygenation (S_mO_2) reflects the balance between local oxygen use and supply (Wittekind et al., 2012; Yogev et al., 2023). Here we measured S_mO_2 using a non-invasive, wireless near-infrared spectroscopy device that could theoretically be used for this purpose. The exercise intensity domains show distinct S_mO_2 responses to prolonged exercise (Kirby et al., 2021), and the S_mO_2 response to incremental exercise can be used to identify intensity domain transitions (Batterson et al., 2023). We found that the S_mO_2 coincident with the moderate-to-heavy intensity transition was not systematically different between PRE and POST (Figure 3d). This supports the live-monitoring of S_mO_2 for within-session intensity regulation, as estimates of the S_mO_2 associated with the moderate-to-heavy intensity transition derived in routine physiological profiling assessments appear to hold over time during prolonged exercise. However, the within-

subject CV for S_mO_2 at the moderate-to-heavy intensity transition was ~13%, which suggests these measurements should be applied to prolonged exercise with caution. This variability may be due to movement of the device and therefore measurement site (Crum et al., 2017). Nevertheless, our data supports further exploration of how S_mO_2 can be applied to within-session intensity regulation during prolonged exercise.

This study is limited by the sample size. We may not have been sufficiently powered to detect relationships between durability and the intramuscular variables, given the known variability in the assays performed. In a between-subject analysis, this variability is further exacerbated by minor between-subject differences in the biopsy site, given previous research showing variability in intramuscular parameters within an individual at different sites along a muscle tissue (Horwath et al., 2021). Furthermore, we cannot determine if the results observed within this study readily translate to elite athletes, to prolonged exercise with carbohydrate ingestion, or during stochastic-intensity prolonged exercise protocols, that may be more reflective of real-world road cycling events (Sanders & van Erp, 2020). We therefore recommend that the implications of durability for endurance performance are studied using a range of prolonged exercise protocols and athlete populations to provide a more detailed understanding of how this physiological profiling characteristic influences real-world endurance performance outcomes.

In conclusion, we observed that durability of the moderate-to-heavy intensity transition was related to the effect of prolonged exercise on severe-intensity time trial performance. However, we were unable to identify intramuscular variables that related to durability of the moderate-to-heavy intensity transition. This study provides the first direct support that durability of the moderate-to-heavy intensity transition is an important endurance performance parameter, and therefore that individual monitoring of durability of the moderate-to-heavy intensity transition may be valuable.

Chapter Three: Summary and future directions

The primary aims of the study presented in this dissertation were to: (i) determine if durability of the moderate-to-heavy transition and/or $\dot{V}O_2$peak is related to the magnitude of prolonged exercise-induced reductions in severe-intensity time-trial performance, (ii) assess the relationship between prolonged exercise-induced reductions in severe-intensity time-trial performance and PFO, and (iii) quantify relationships between various intramuscular characteristics and durability of the moderate-to-heavy intensity transition. The main findings were that: (i) prolonged exercise-induced reductions in severe-intensity time trial performance were related to durability of the moderate-to-heavy intensity transition, but not durability of $\dot{V}O_2$peak, (ii) prolonged exercise-induced reductions in severe-intensity time-trial performance was not related to PFO, and (iii) no relationships were observed between *vastus lateralis* CS activity, Complex I activity, or carnosine concentration and durability of the moderate-to-heavy intensity transition. In this chapter, I will summarise firstly the main practical recommendations yielded from this dissertation, and then the main future research directions.

Implications for applied practice

As discussed, the findings from this study provide the first direct support for the hypothesis that durability of the moderate-to-heavy intensity transition is an important endurance performance parameter, and further support for the suggestion that durability of the moderate-to-heavy intensity transition might be monitored at an individual level. Therefore, I recommend that Performance Physiologists working with endurance athletes consider the assessment of durability characteristics within their routine practice. Specifically, I recommend that physiological profiling assessments are performed not only in the well-rested state, as is typically the case, but repeated during assessments performed after the completion of controlled training sessions that can be easily repeated in subsequent assessments. This approach will allow for the quantification of durability characteristics, calculated as the difference between physiological profiling attributes estimated when well-rested and post-prolonged exercise. As demonstrated in the present dissertation, durability of the moderate-to-heavy intensity transition may provide insights into endurance performance capabilities relevant to events such as road cycling. In an applied setting, this approach is practically feasible, given the popularity of indoor training devices such as smart indoor trainers that can be used to perform controlled exercise bouts.

A second key practical recommendation arising from the present dissertation relates to the use of non-invasive, wireless measures of muscle oxygenation for within-session

intensity regulation. The loss of power output at the intensity domain transitions observed here (211 ± 40 W vs. 198 ± 39 W), and elsewhere (Clark et al., 2018; Clark, Vanhatalo, et al., 2019b; Clark, Vanhatalo, Thompson, Wylie, et al., 2019; Stevenson et al., 2022), makes using the power output at intensity domain transitions derived from physiological profiling assessments to regulate intensity during prolonged exercise sessions challenging. Specifically, using real-time power output data to assess proximity to a given intensity domain transition is limited by the dynamic nature of the intensity domain transitions over time during prolonged exercise. This calls for the identification of physiological variables that remain static in relation to intensity domain transitions, rather than power output, during prolonged exercise, and that can be monitored in real-time during prolonged exercise to allow for continuous assessment of proximity to the intensity domain transitions. This need is particularly pressing given the recent observation that the upward drift in heart rate relative to power output was disproportionate to the downward drift in power output at the moderate-to-heavy intensity transition, meaning that the heart rate associated with the transition increased during prolonged exercise (Stevenson et al., 2022). Here we found that S_mO_2, derived from a non-invasive, wireless near-infrared spectroscopy device, may be a candidate measure for this purpose. The S_mO_2 associated with the moderate-to-heavy intensity transition was not systematically different between PRE and POST, despite the reduction in power output (Figure 3d). This suggests that Performance Physiologists working with endurance athletes might consider adding S_mO_2 data to routine physiological profiling assessments, and quantifying the S_mO_2 associated with the moderate-to-heavy intensity transition in these assessments. I do recommend that these data are interpreted with caution, given the substantial variability in S_mO_2 measures, and the need for careful, consistent placement of the device to allow S_mO_2 data derived from physiological profiling assessments to be applied to training sessions (Crum et al., 2017).

Future research directions

I have identified several future research directions that are justified based on the data presented in this thesis. Firstly, the mechanisms underpinning athlete durability characteristics warrant further consideration, particularly given I was unable to identify relationships between durability of the moderate-to-heavy intensity transition and any of the intramuscular variables assayed. Specifically, I recommend that future studies consider assessing the relationship between intramuscular HSP70 and durability. I also recommend that future studies exploring durability take compartmental muscle glycogen measures to explore the hypothesis that durability is related to the maintenance of intramyofibrillar glycogen availability. Lastly, I recommend that future studies of durability assess relationships with extracellular K^+ concentrations, to explore the hypothesis that

more durable athletes see lower extracellular K^+ accumulation during prolonged exercise.

Secondly, the current study was limited by its sample size, and may not have been sufficiently powered to detect relationships between intramuscular outcome measures and durability of the moderate-to-heavy intensity transition. This is particularly relevant given that assessment of these relationships needs to overcome technical and day-to-day variability in both measures; that is, technical and day-to-day variability in intramuscular markers and durability. A larger study involving a more heterogenous group of athletes may be required to elucidate these relationships. These relationships may also be explored in longitudinal studies, in which changes in intramuscular markers and durability are concomitantly assessed.

Thirdly, the outcome measures assessed here need to be studied using a range of prolonged exercise protocols to better understand durability-related responses to diverse exercise scenarios. For example, using a prolonged, intermittent or stochastic exercise protocol with that mimic road cycling events provide a deeper understanding of how durability relates to real-world endurance performance outcomes. Specifically, studies that delineate the impact of prior exercise intensity, duration, and total work completed are warranted. Related to this, a deeper real-world understanding of durability would be achieved with studies of elite and female athletes, featuring carbohydrate feeding during exercise, and in different environmental conditions.

Conclusion

In summary, the primary novel observation of this dissertation was that durability of the moderate-to-heavy intensity transition was significantly associated with the effect of prolonged exercise on severe-intensity time-trial performance. My primary recommendations for applied practice in performance physiology are that: (i) physiological profiling assessments are performed not only in the well-rested state, but are repeated during assessments performed after the completion of controlled training sessions that can be easily repeated, to allow for quantification of durability characteristics, and (ii) practitioners consider use of wireless, non-invasive measures of muscle oxygenation for within-session intensity regulation and training load monitoring. My primary recommendations for future research are that: (i) larger studies with more heterogenous populations explore relationships between intramuscular characteristics, including HSP70, and durability, (ii) future studies of investigating the mechanisms that explain durability outcomes measure intramyofibrillar glycogen content and extracellular K^+, (iii) durability outcomes are assessed following intermittent, stochastic prolonged

exercise protocols and using experimental designs that delineate the effects of prior exercise intensity, duration, and total work completed on durability outcomes, and (iv) durability outcomes are assessed in elite and female athlete populations, and during exercise in different environmental conditions. Collectively, these data would provide a more complete understanding of durability responses, and the application of durability research to real-world endurance exercise.

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