The effect of lowered carbohydrate availability on power output at intensity domain transitions in endurance-trained females

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Abstract

Aim:

'Durability' refers to an individual's resilience to the effects of prolonged exercise on power output at the intensity domain transitions. Carbohydrate availability is reduced during prolonged exercise, and sustaining prolonged exercise is closely linked to the availability of muscle glycogen stores. The aim of this study was to investigate the effects of lowered carbohydrate availability on power output at intensity domain transitions, muscle activation, and gross cycling efficiency in endurance-trained female cyclists.

Methods:

Nine well-trained female cyclists completed a randomised, counterbalanced crossover study consisting of two conditions. Each participant completed five trials: (1) an incremental test, (2) glycogen-depleting exercise #1, (3) experimental trial #1 (submaximal incremental test and a maximal 3-min all-out test), (4) glycogen-depleting exercise #2, (5) crossover experimental trial #2. Participants consumed ≥9 $g \cdot kg^{-1} \cdot day^{-1}$ (HIGH) or ≤1 $g \cdot kg^{-1} \cdot day^{-1}$ (LOW) carbohydrate between the glycogen-depleting exercise and experimental trial to manipulate carbohydrate availability.

Results:

Lowered carbohydrate availability significantly reduced power output at the first ventilatory threshold (VT_1, 133 ± 24 vs. 152 ± 28 W, Δ -19 ± 14 W, $P = 0.011$), but did not affect the lactate threshold (148 ± 29 vs. 142 ± 26 W, Δ 6 ± 19 W, $P = 0.331$). Gross cycling efficiency during submaximal cycling was lower in the low-carbohydrate condition ($P = 0.003$). Median power frequency of the vastus lateralis (VL, $P = 0.025$) and vastus medialis (VM, $P = 0.007$) was higher in the low-carbohydrate condition during submaximal cycling, however there was no effect of condition on EMG amplitude in the VL ($P = 0.232$) and VM ($P = 0.655$). There was no significant effect of condition on critical power (227 ± 34 vs. 226 ± 34 W in LOW and HIGH respectively, Δ 1 ± 12 W, $P = 0.748$). There was no significant effect of condition for EMG amplitude or median power frequency in the VL and VM during maximal cycling.

Conclusion:

These data suggest lowered carbohydrate availability reduced power output at the moderate-to-heavy transition (VT_1), likely due to increased recruitment of higher-threshold motor units to compensate for glycogen-depleted fibres, which impaired gross cycling efficiency. These suggest that the maintenance of carbohydrate availability is likely important in athlete durability, and that nutritional status should be considered when conducting physiological profiling and programming training intensities.

Table of Contents

List of Figures and Tables

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.”

Signed:

Co-authorship Contributions

STUDENT AND SUPERVISOR APPROVALS

By signing you are confirming that the co-author contributions stated in the table(s) below are accurate.

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Ethics Approval

All experimental procedures of this thesis were approved by the Auckland University of Technology Ethics Committee on 16th September 2024 (24/247).

Chapter 1: Introduction

Endurance athletes routinely undertake physiological assessments that profile key variables such as peak oxygen uptake ($\dot{V}O_2$peak), gross cycling economy, and power output at the intensity domain transitions. These attributes inform physiologically-based training programming and load monitoring, support intensity regulation during extended training sessions or competitions, and allow the tracking of adaptations pertinent to performance. Typically, these variables are assessed in a well-rested state. However, physiological profiling characteristics, such as power output at the moderate-to-heavy and heavy-to-severe intensity transitions, gross cycling efficiency and $\dot{V}O_2$peak may deteriorate during prolonged exercise. An individual's resilience to the effect of prolonged exercise on physiological profiling characteristics has been termed 'durability'. Durability has implications for the application of physiological profiling data to prolonged training and competition. As prolonged exercise reduces carbohydrate availability, investigating the impact of carbohydrate availability on physiological profiling could generate insights into the role of carbohydrate availability in durability.

This thesis explores the effects of lowered carbohydrate availability on physiological profiling measures in endurance trained females. Muscle glycogen depletion likely contributes to this decline by impairing contractile function, and reducing energetic efficiency, ultimately lowering power output at intensity domain transitions. Assessing this effect has implications for our understanding of the mechanisms behind the loss of power output at the intensity domain transitions that occur during prolonged exercise. This study included a female-only cohort to address the underrepresentation of females in exercise science research. Testing females is important to ensure that findings in endurance physiology are relevant across sexes, as performance guidelines and mechanistic insights should be informed by data from both males and females.

The structure of this thesis is outlined in Table 1. Following this general introduction, Chapter 2 is a substantial literature review that covers key aspects of physiological profiling in endurance sports, including intensity domains and transition markers, and explores how carbohydrate availability may influence durability and muscle recruitment. Chapter 3 is an experimental study, which investigates whether lowered carbohydrate availability reduces power output at intensity domain transitions in endurance-trained females. Finally, Chapter 4 discusses the findings in an applied context and outlines directions for future research.

Table 1. Outline of each chapter within this thesis.

Chapter	Title	Purpose
1	Introduction	General introduction to the thesis
2	A literature review on physiological profiling and a proposed role for carbohydrate availability in the deterioration in physiological profiling attributes during prolonged exercise	Narrative literature review on physiological profiling, and how carbohydrate availability may influence durability and muscle activity
3	The effect of lowered carbohydrate availability on power output at intensity domain transitions in endurance-trained females	A randomised, counterbalanced, crossover study assessing the effect of lowered carbohydrate availability on power output at intensity domain transitions in endurance-trained females
4	Summary and future directions	Overall discussion, in which the findings from Chapter 3 are applied to practice and directions for future research are proposed.

Chapter 2: A literature review on physiological profiling and a proposed role for carbohydrate availability in the deterioration in physiological profiling attributes during prolonged exercise

The main focus of this thesis is to investigate the effects of lowered carbohydrate availability on physiological profiling attributes in endurance-trained females. As prolonged exercise reduces carbohydrate availability and deteriorates physiological profiling attributes, investigating the impact of carbohydrate availability on physiological profiling could generate insights into the role of carbohydrate availability in 'durability', or resilience to the effects of prolonged exercise on physiological profiling attributes. Therefore, in this literature review chapter, I begin by outlining physiological profiling in endurance sports, highlighting the key attributes typically profiled. I then explore the exercise intensity domains and the methods used to determine the transitions between them. Following this, the concept of durability is examined, focusing on the potential role of carbohydrate availability. Finally, I consider how carbohydrate availability may influence motor unit recruitment.

Physiological profiling

A series of physiological attributes relevant to endurance training and performance are routinely quantified in physiological profiling assessments (1). These attributes are subsequently used for monitoring training adaptations relevant to performance, training programming and load monitoring, intensity regulation during prolonged training sessions or competitions, and the prediction of exercise performance. The main attributes typically assessed include maximal oxygen uptake ($\dot{V}O_2max$), movement economy, substrate oxidation rates, and intensity domain transitions. The specific profiling method used depends on the attribute of interest, with different tests targeting different components of endurance performance. In the next section, I define these attributes, discuss their use in applied practice, and describe how they are measured.

Maximum oxygen uptake

Maximum oxygen uptake ($\dot{V}O_2max$) refers to the maximum rate at which an athlete can consume oxygen in aerobic metabolism during exercise, and is therefore a marker of an athlete's aerobic capacity (2, 3). The $\dot{V}O_2max$ is a product of the integrative functioning of the pulmonary, cardiovascular, and muscular systems. The Fick equation ($\dot{V}O_2$ = cardiac output × arterial-venous O_2 difference) demonstrates that $\dot{V}O_2max$ is determined by the ability of the heart to deliver oxygen in the blood to the working muscles (cardiac output), and the working muscles' capacity to extract and utilise oxygen transported in the blood (a-vO_2 difference).

The main factor limiting $\dot{V}O_2max$ is oxygen delivery to the working muscle rather than skeletal muscle oxygen extraction (4, 5). This is supported by findings showing that muscle-specific $\dot{V}O_2max$ is significantly higher during isolated muscle exercise than whole-body exercise (5). This suggests that during maximal whole-body exercise, the working muscles can consume more oxygen than is delivered, likely because cardiac output must be distributed across a larger active muscle mass. Given the need to sustain high rates of aerobic metabolism to support high work outputs in endurance sports, a positive relationship between $\dot{V}O_2max$ and endurance performance has been observed (6, 7).

Importantly, the $\dot{V}O_2$max is responsive to training and detraining (8). Exercise training can induce cardiac hypertrophy or increased heart muscle mass and volume, particularly of the left ventricle (9, 10). This allows for greater stroke volume, and therefore maximal cardiac output (11). Plasma and red blood cell volume also increase, enhancing ventricular filling and stroke volume, and therefore maximal cardiac output, and oxygen-carrying capacity (12–15). Together, these adaptations raise maximal cardiac output. Additionally, endurance training increases capillary density in skeletal muscle and enhances oxygen extraction (3, 16). Collectively, these adaptations support increased oxygen delivery, and therefore $\dot{V}O_2$max.

Therefore, routine measurements of $\dot{V}O_2$max are made to track training adaptations. Measurements of $\dot{V}O_2$max are typically completed in laboratory settings using indirect calorimetry, in which expired gases are analysed to estimate oxygen consumption (2). The incremental exercise test protocol is frequently utilised to assess the $\dot{V}O_2$max, and involves increasing the external work rate until the participant reaches voluntary exhaustion (17). Additionally, exhaustive severe intensity exercise, such as the 3-minute all-out test, can be used to determine $\dot{V}O_2$max as this elicits maximal oxygen uptake before volitional exhaustion is reached (18).

Movement economy

The metabolic cost of producing a specific external work rate is referred to as movement economy. In runners, running economy can be measured in units of either energy expenditure or oxygen consumption per distance travelled (19). Gross efficiency, or the proportion of total energy expenditure converted to mechanical power, is a common measurement of movement economy in cycling (20, 21). Movement economy can be influenced by factors such as muscle fibre type, neuromuscular coordination, biomechanics and substrate utilisation (20, 22, 23). For endurance athletes, being more economical is beneficial because less metabolic work is required to produce a certain speed or power output (24, 25). Therefore, many studies have reported a positive relationship between movement economy and endurance performance (25, 26). Accordingly, movement economy is routinely measured in physiological profiling to track training adaptations. Like $\dot{V}O_2$max, movement economy can be measured via indirect calorimetry, where steady-state $\dot{V}O_2$ and $\dot{V}CO_2$ are used to estimate energy expenditure at controlled work rates (19).

Substrate oxidation rates

During endurance exercise, carbohydrate and fat are the primary substrates oxidised to fuel energy metabolism (27). Carbohydrate is stored as glycogen in skeletal muscle and the liver, and total body glycogen stores amount to 500–600 g (28). In contrast, even lean individuals have substantially greater fat reserves; for example, a 70-kg athlete with 10% body fat stores has ~68,000 kcal of energy stored as fat (29). Therefore fat availability is effectively unlimited during exercise. As exercise intensity increases, shifts in energy substrate mobilisation and utilisation occur (30). Fat oxidation peaks during moderate-intensity exercise, and then decreases at higher exercise intensities (31, 32). In contrast, carbohydrate oxidiation continues to rise as exercise intensity increases (27, 32). The shift towards carbohydrate metabolism at high exercise intensities occurs as carbohydrates are more oxygen efficient and can support faster rates of ATP regeneration (33). After endurance training, fat oxidation rates at given intensities increase (34, 35), which is a favourable adaptation given that glycogen depletion has

been linked to the development of fatigue . Enhancing fat utilisation may therefore help preserve limited glycogen stores during prolonged exercise. An athlete's propensity for fat oxidation has been associated with a higher proportion of type I muscle fibres, greater capillary density, elevated mitochondrial protein content, greater expression of proteins involved in the transport of fatty acids across the sarcolemmal and mitochondrial membranes, and increased activity of enzymes involved in β-oxidation and intramuscular triacylglycerol lipolysis (36–38) as well as endurance performance (39).

Accordingly, substrate utilisation profiles can be used to track adaptations to targeted interventions aimed at enhancing an athlete's fat utilisation during competition (40). Substrate oxidation rates during exercise are assessed using indirect calorimetry via the respiratory exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$) (41, 42). Specifically, an athlete's fat oxidation capacity can be assessed by measuring their peak fat oxidation (PFO) rate during an incremental test (40). The PFO is linked to fat oxidation during prolonged exercise and endurance performance, making it a valuable metric for tracking adaptations to specific training interventions (39, 43). As PFO is highly sensitive to pre-trial diet and recent exercise, these factors should be standardised to ensure reliable and reproducible results (44). Therefore, profiling an athlete's capacity to oxidise fat and carbohydrates provides valuable data for optimising training and performance.

Intensity domains

Physiological responses to prolonged exercise can be categorised into discrete exercise intensity domains. The moderate-, heavy-, and severe-intensity domains are characterised by rapid attainment of a steady state in muscle and whole-body metabolic homeostasis, delayed attainment of a steady state in muscle and whole-body metabolic homeostasis, and inability to achieve muscle and whole-body metabolic homeostasis, respectively (45, 46), as shown in Figure 1. Therefore, exercising in the moderate-, heavy-, and severe-intensity domains invokes differences in oxidative and nonoxidative energy supply, muscle and blood biochemistry, cardiovascular responses, fatigue processes, and perceived effort, which, if repeated continually, would be expected to promote different physiological adaptations (35, 45, 46). Accordingly, the stress imposed, and therefore the recovery cost, varies between moderate, heavy and severe intensity exercise (47).

Exercise in the moderate domain can be sustained for a long duration with little change in cardiovascular, respiratory, and muscle metabolic responses, which quickly establish a steady state (45). Muscle metabolic perturbation is slight; blood and muscle lactate concentrations and pH remain at values similar to rest. Following several hours of exercise in the moderate-intensity domain, muscle glycogen becomes depleted (45, 48, 49). Liver glycogen stores become depleted during prolonged moderate-intensity exercise; thus, blood glucose levels gradually decline (50, 51). This reduction in circulating glucose may contribute to fatigue, particularly if blood glucose levels fall below 4 mmol/L, resulting in hypoglycemia (49, 52–55). The development of peripheral fatigue within this domain is likely related to the considerable reduction of muscle glycogen and impaired neuromuscular function (56–58). In summary, moderate-intensity exercise can be sustained for long durations with minimal disturbance to muscle metabolic homeostasis, which supports a role for depletion of carbohydrate energy stores in fatigue within this domain.

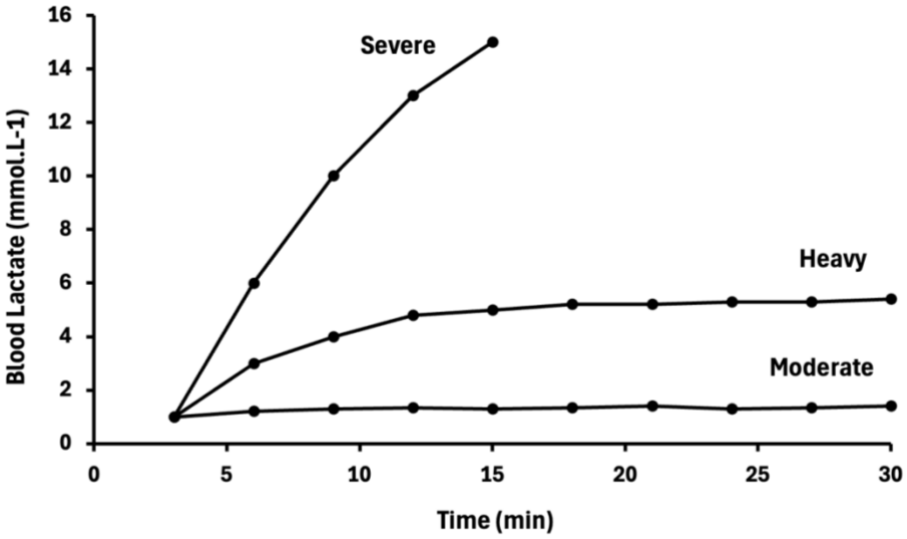

Figure 1. Blood lactate response during a constant load exercise in the moderate, heavy and severe exercise domains.

During exercise in the heavy domain, a steady state in muscle metabolites is typically reached within ~10 minutes, and there is limited muscle metabolic perturbation (46). Similarly, variables such as blood lactate concentration will stabilise at concentrations above baseline, unlike the moderate intensity domain (46, 59, 60). When exercising within this domain, the lactate production rate initially exceeds the lactate removal rate, and plasma K^+ concentrations increase rapidly; concurrently, a decline in pH occurs due to H^+ accumlation from ATP hydrolysis (45). However, these variables eventually stabilise at elevated levels, allowing exercise to be sustained. Athletes are often able to complete ~20-60 minutes of exercise within this domain. Fatigue during heavy-intensity exercise is related to disruptions in muscle membrane excitability, accumulation of muscle metabolites, and decreased muscle glycogen (45). Together these factors impair excitation-contraction coupling and contribute to performance decline (45).

The severe-intensity domain is defined by the inability to achieve a steady-state in key physiological variables, such as VO_2, blood lactate, and pH (46, 61). Muscle efficiency decreases in the severe-intensity domain, even when the external work rate remains constant (61). This is reflected in the development of the $\dot{V}O_2$ slow component, which drives $\dot{V}O_2$ to its maximum value at the tolerance limit (62). In contrast to exercise in the heavy-intensity domain, where metabolite levels stabilise, muscle ATP and PCr concentrations, and pH progressively decline, indicating that no metabolic steady state can be reached (45, 46). Furthermore, the concentrations of plasma K^+, and blood and muscle lactate gradually rise. Therefore task failure within this domain is linked to the decline of muscle PCr and ATP concentrations and pH and an increase in muscle and blood lactate, Pi, H_2O_4- and plasma K^+ concentrations (45, 46). It has therefore been proposed that exhaustion in the severe-intensity domain is caused by the attainment of a maximum tolerable level of muscle metabolic disturbance. Due to exercise typically ending before muscle glycogen stores are depleted, fatiguing severe-intensity exercise causes less reduction in muscle glycogen stores than fatiguing moderate or heavy-intensity exercise (45, 63).

In summary, exercise in the moderate, heavy, and severe-intensity domains elicits distinct physiological responses and kinetics, which has implications for fatigue mechanisms and training stress management. Therefore, identifying the work rates at which the transitions between intensity domains occurs is useful when working with endurance athletes, such that training can be programmed and monitored in accordance with a physiologically-based stress model.

Intensity domain transitions

The moderate-to-heavy intensity transition

The boundary between the moderate- and heavy-intensity domains is estimated using the lactate (LT) and first ventilatory (VT_1) threshold. Accordingly, the moderate-to-heavy intensity transition is assessed via blood lactate and gas exchange measures (Figure 2). Research has shown a strong agreement between the LT and VT_1 across a range of populations and exercise modalities (64–68).

The lactate threshold is the exercise intensity at which blood lactate concentrations first rise above baseline, indicating a transient imbalance between lactate appearance and disappearance from the circulation (65, 69). Blood lactate appearance in the circulation rises due to a greater demand for use of the pyruvate-to-lactate pathway for ATP synthesis, and therefore muscle lactate production (70). Lactate is produced when pyruvate, the end product of glycolysis, is converted to lactate instead of entering the mitochondria as acetyl CoA for aerobic metabolism (71). This supports more rapid ATP production during high-intensity exercise, but comes at a cost of lower efficiency and accumulation of byproducts (e.g., H^+). Typically, blood lactate concentrations measured during an incremental exercise test are used to fit a relationship between exercise intensity and blood lactate concentrations. The typical procedures used are visual inspection, where lactate is plotted against exercise intensity to identify the first rise above baseline; the log-log method, which mathematically detects the first breakpoint using a logarithmic transformation of lactate and work rate data; and the +0.5 $mmol \cdot L^{-1}$ method, which defines the lactate threshold as the point at which lactate increases by at least 0.5 $mmol \cdot L^{-1}$ above baseline (66). These methods are highly correlated (ICC ~ 0.98) (72). However, despite high correlations, the visual inspection point has shown poor reliability, the baseline +0.5$mmol.L^{-1}$ has favourable reliability, and the reliability of the log-log LT is uncertain (64, 67, 72). The log-log LT and baseline +0.5$mmol.L^{-1}$ methods are least impacted by the incremental exercise test protocol design (73).

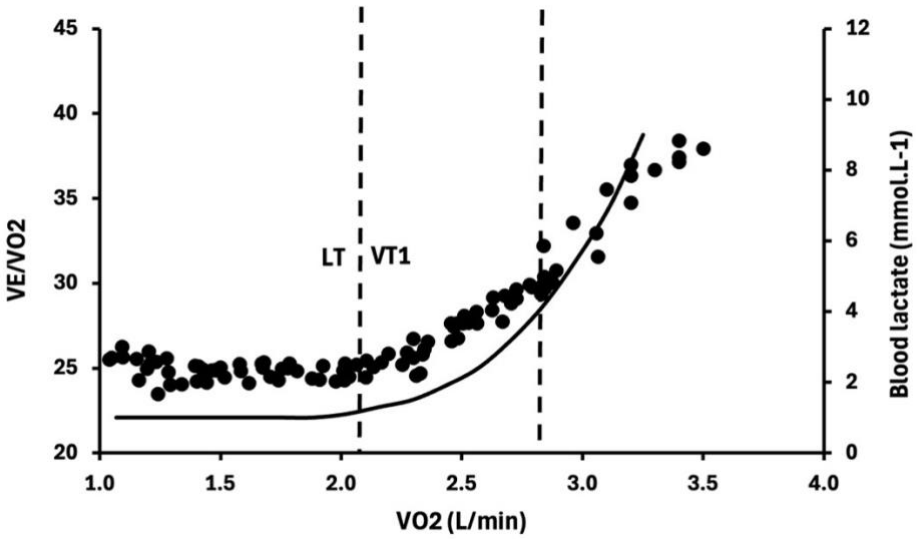

Figure 2. The moderate-to-heavy transition is demarcated by the lactate threshold (LT) and first ventilatory threshold (VT_1) as shown by the dotted line. The dark circles are pulmonary data points of minute ventilation ($\dot{V}_E$) relative to oxygen uptake ($\dot{V}O_2$). The solid line represents the fitted blood lactate curve.

The moderate-to-heavy intensity transition can also be estimated using gas exchange measures. The VT_1 is closely related to the lactate threshold and was first referred to as the anaerobic threshold (74, 75). Specifically, the VT_1 represents a breakpoint in the relationship between ventilation ($\dot{V}_E$) and oxygen uptake ($\dot{V}O_2$) during incremental exercise (66, 76). In our laboratory, and elsewhere, VT_1 is determined as the $\dot{V}O_2$ associated with the breakpoint in the ventilatory equivalent for oxygen ($\dot{V}E/\dot{V}O2$) vs. $\dot{V}O2$ relationship (66, 77) (Figure 2). During an incremental exercise test, $\dot{V}O_2$ increases linearly with exercise intensity; however, above VT_1, the $\dot{V}_E$ disproportionately rises relative to $\dot{V}O_2$. This hyperventilation is likely caused by the metabolic perturbations related to exercise within the heavy-intensity domain, such as lactate and H^+ accumulation, which stimulate metaboreceptors and peripheral chemoreceptors, which in turn stimulate respiratory centres in the brain to increase $\dot{V}_E$ (74). Unlike LT, which relies solely on lactate measurements, VT_1 is responsive to multiple metabolic signals that affect respiratory drive, including changes in pH, CO_2, K^+, and H^+ concentrations (74). Therefore, VT_1 is a useful tool for evaluating the moderate-to-heavy transition as it offers a sensitive, non-invasive measure of the metabolic response to exercise intensity (64).

The heavy-to-severe intensity transition

The heavy-to-severe intensity transition is the highest work rate at which steady-state values are observed for physiological variables such as blood lactate concentrations, oxygen uptake, and muscle H^+ concentration (65, 78, 79). The heavy-to-severe intensity transition can be assessed in a number of ways, including the maximal lactate steady state (MLSS) and critical power (CP) (80).

The MLSS is the maximum work rate at which blood lactate concentration does not increase by >1 $mmol \cdot L^{-1}$ between 10 and 30 minutes of exercise (81). To determine MLSS, athletes usually complete a series of constant-load trials, with blood lactate concentrations measured at regular intervals (e.g., every 5–10 minutes) during each bout (80). Researchers have questioned whether the MLSS accurately reflects the boundary between the heavy-to-severe intensity domains. This is largely due to its reliance on arbitrary thresholds rather than clear physiological breakpoints, as well as observations of steady-state oxygen uptake during workloads above the MLSS (82, 83). Studies have found power output at MLSS is often about ~7% less than the CP (79, 84), which is increasingly seen as a better measure of the heavy-to-severe intensity transition (80).

The heavy-to-severe intensity transition can be estimated via the power-duration relationship. The hyperbolic relationship between power output and its sustained time has been well described (60, 85–87) and is a fundamental property of exercise performance in humans. The work-rate asymptote of this hyperbolic relationship has been termed critical power (CP). The CP can be established via three to four or more high-intensity exercise tests on different days, during which an athlete sustains a fixed power output until exhaustion. The power outputs are intended to produce times to exhaustion in a minimum of ˜2 minutes and a maximum of ˜15 minutes (88). The time to task failure is recorded at each of these power outputs. When power output is plotted against time, it can be observed that the sustainable power output falls as a function of the exercise duration and that it levels off or asymptotes (Figure 3A). This asymptote is CP, which is measured in watts (W). At the same time, the curvature of the power-time relationship represents the work capacity available above CP (*W'*), which is measured in kilojoules (kJ). Several studies have validated CP as a reliable marker between heavy- and severe-intensity exercise domains. Poole et al. (1988) showed that exercise below CP led to a stable VO_2 and lactate values, while exercise just above CP elicited a continuous rise in VO_2 and a continuous accumulation of blood lactate. Similarly, studies confirmed that CP describes the highest intensity at which metabolic homeostasis can be maintained (88, 89). These findings establish CP as a performance metric and a boundary between intensity domains.

As measurement of CP requires multiple exhaustive severe-intensity trials, the 3-minute all-out test was developed as a single-visit assessment of the heavy-to-severe intensity transition (18). It has been shown that the end-test power (EP) in a 3-minute all-out test produced results similar to CP determined from repeated exhaustive exercise tests (90) (Figure 3B). The principle of the test is that once the finite anaerobic energy stores are exhausted, the remaining power output must be sustained by aerobic metabolism alone (18, 46). The CP is therefore calculated as the average power output during the final 30 seconds, during which power output typically plateaus (91). The 3-minute all-out test therefore provides a practical and physiologically meaningful assessment of an athlete's performance profile within a single visit (18, 90, 92).

In summary, knowledge of the intensity domain transitions is important for programming training, regulating intensity within a session to ensure it is completed with the desired physiological responses (93), and accurately quantifying training load and intensity distribution (94). Further, monitoring power output at the intensity domain transitions is useful for predicting performance capabilities, given strong relationships with endurance performance (1). Accordingly, monitoring changes in power output at the intensity of dominant transitions can be used to determine if an athlete is responding positively to training or are overreaching or detraining (95).

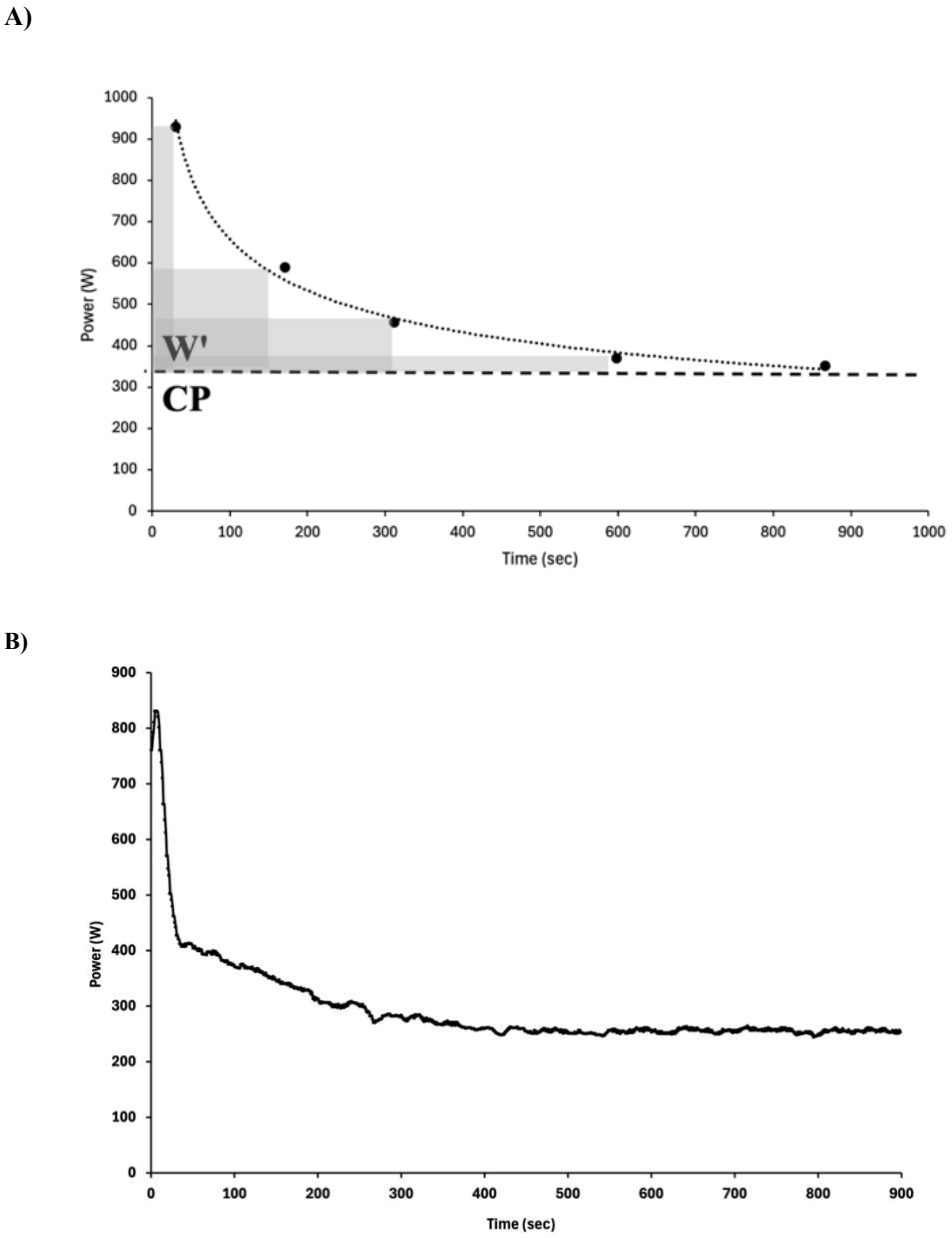

Figure 3. A Hyperbolic relationship between power output (y-axis) and time (x-axis), where the critical power is indicated by the power asymptote and the W' is the curvature constant. **B** Power output (W) over time (sec) during a 3-min all-out cycling test. The mean power over the final 30 seconds is used to estimate CP.

Durability: An additional physiological profiling attribute relevant to endurance performance

Physiological profiling attributes are routinely assessed in well-rested athletes. These data are then applied to the prolonged exercise performed in training and competition. Therefore, a weakness of the physiological profiling models and methods described above is that they fail to account for exercise-induced deteriorations in physiological profiling attributes (1). It has been shown that prolonged exercise induces substantial declines in power output at the moderate-to-heavy transition, heavy-to-severe transition, $\dot{V}O_2max$, and movement economy. An individual's resilience to reductions in physiological-profiling characteristics caused by prolonged exercise has been termed 'durability' (1).

Specifically, a series of recent studies has shown that prolonged cycling (~1.5-3 h) decreases (~10%) power output at the moderate-to-heavy intensity transition, marked by VT_1 (77, 96–100) (Figure 4). These reductions in power output have been shown to decline in a non-linear fashion (98). Mechanistically, the decline in power output can be attributed to decreased cycling efficiency and/or reduced metabolic energy expenditure at the transition (so-called 'metabolic power'), with previous studies finding that the reduction was primarily attributable to reduced metabolic power (77). Reduced energetic efficiency refers to an impaired conversion of metabolic energy expenditure to mechanical power output, whereas reduced metabolic power refers to a reduction in the rate of metabolic energy expenditure at which the transition from moderate- to heavy-intensity exercise occurs (77). Carbohydrate intake during prolonged exercise mitigated this decline by ~3%, likely via preservation of blood glucose (97), given evidence suggesting the carbohydrate intake during prolonged exercise does not slow the rate of muscle glycogenolysis (48). Importantly, durability of the moderate-to-heavy intensity transition appears to be an important performance attribute, as more durable athletes exhibited smaller reductions in 5-min time trial performance following prolonged exercise (99).

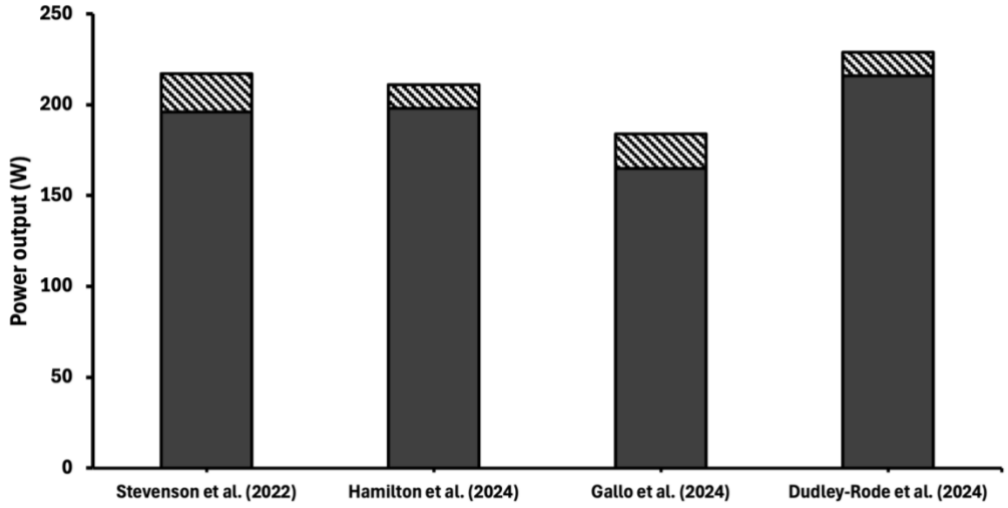

Figure 4. Solid bars represent the mean power output at the moderate-to-heavy intensity transition measured following prolonged exercise, while the striped area represents the corresponding value when fresh, in a series of recent studies *(77, 97–99)*.

Power output at the heavy-to-severe transition also decreases following prolonged cycling, with CP and the work capacity above CP (*W′*) significantly reduced (101–103). Specifically, it was shown that CP (~11%) and *W′* (~20%) declined significantly following 2 h of heavy-intensity cycling (103). A follow-up time course study reported that CP was maintained following 40 and 80 minutes of heavy-intensity cycling, but decreased by ~9% following 2 hours, while W′ declined progressively (102). Notably, carbohydrate ingestion at 60 g/h preserved CP with a ~2% decline following 2 hours of exercise (102). Supporting this, a 10% decline in 6-min time trial power and 6% drop in peak power output after 4 h. of intermittent cycling with a high carbohydrate intake at 100 g/h was observed in recent research (104). These reductions align with findings from previous studies involving elite cyclists subjected to prolonged exercise (105, 106).

Studies have highlighted significant between-participant variability in durability (77, 99). Specifically, Clark and colleagues reported reductions in CP of ~1% to ~31% in their cohort. Gallo et al. (2024) reported substantial inter-individual variability in the exercise time at which power output at VT_1 had decreased by 5% (21–279 min), highlighting the individual nature of this response. These data suggest that durability is an individual physiological attribute, and the onset and magnitude of deterioration differ remarkedly between participants. Therefore, its inclusion in physiological profiling may enhance the precision of training load prescription and performance prediction for long-duration events (98).

Durability has important implications for managing training load and exercise intensity. Coaches may schedule training sessions based on baseline intensity domain values, which is problematic, as significant drops in power output at the intensity domain transition during exercise could affect the desired physiological response, and therefore impact autonomic stress and the required recovery time. Additionally, it adds the possibility of errors in training load estimation and classifying training intensity distribution.

In summary, the decline of physiological profiling variables over time during prolonged exercise limits the use of physiological profiling data in real-world situations. "Durability" is the term used to describe an athlete's resilience to the impacts of prolonged exercise on physiological attributes. According to recent research, durability varies among athletes and is associated with performance outcomes. As a result, durability affects performance prediction, training load monitoring, training intensity regulation, and training adaptation monitoring.

Is carbohydrate availability a determinant of durability?

Durability of the intensity domain transitions could plausibly be related to glycogen availability. Glycogen is a glucose polymer and the major source of readily available energy in mammalian skeletal muscle. It acts as a storage form for glucose that is present in a variety of tissues, although it is mainly stored in skeletal muscles and the liver. During exercise, glycogen stored in the muscle is oxidised to support muscle energy metabolism. Glycogen stored in the liver is broken down to glucose and released into the circulation, where it can be transported into muscle and utilised to support energy metabolism (33). Glycogen plays a key role in muscle function, as demonstrated by the inability to sustain prolonged high-intensity exercise when glycogen stores are depleted (107, 108). Numerous studies have verified these findings, and it is now well accepted that muscle glycogen levels and fatigue resistance are closely related during both prolonged and high-intensity intermittent exercise (109–113).

Muscle glycogen is stored in distinct subcellular areas of the muscle cell and in pools within fibres (114, 115). The three distinct pools are subsarcolemmal glycogen, found just beneath the sarcolemma; intermyofibrillar glycogen, located between the myofibrils; and intramyofibrillar glycogen found in the myofibril. The intermyofibrillar glycogen pool makes up around 75% of the total glycogen reserve, while intramyofibrillar and subsarcolemmal glycogen make up 5–15% each (116). Studies have demonstrated a larger relative utilisation of intramyofibrillar glycogen during exercise, which is preferentially depleted compared to intermyofibrillar and subsarcolemmal glycogen (115, 117). Additionally, intramyofibrillar glycogen has been shown to correlate most strongly with time to exhaustion (56, 118).

It has been shown that there is a link between the slower release of Ca^{2+} in sarcoplasmic reticulum (SR) vesicles and the decrease in intramyofibrillar glycogen levels, indicating a connection between low glycogen and fatigue (57, 115, 119). Decreased intramyofibrillar glycogen may be related to reduced sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) activity (120). Research shows that the Na^+/K^+-ATPase function appears to be reliant on intramyofibrillar glycogen (121), and intramyofibrillar glycogen depletion has been associated with impaired muscle contractile function (57, 116). Furthermore, work done in mouse muscle showed lowered muscle glycogen together with increased K^+ interactively reduces muscle force, which may impair performance (122). This was due to an increased number of inexcitable muscle fibres. As a result, a smaller pool of excitable fibres is available to generate force. To compensate, the neuromuscular system may increase motor unit recruitment and firing frequency to maintain force output (123, 124). Accordingly, there is strong mechanistic rationale for the proposed link between muscle glycogen depletion and muscle contractile function.

Liver carbohydrate stores eventually become depleted following prolonged exercise, causing a drop in blood glucose levels, which may lead to hypoglycaemia (125). If liver glycogen stores are depleted and gluconeogenic metabolism from non-carbohydrate substrates is insufficient to match the rate of glucose disappearance, then blood glucose levels will decline. This is another proposed mechanism of fatigue; when the body is unable to maintain blood glucose levels, a process that is tightly regulated by the brain, it can cause a strong neural response to terminate or significantly reduce power output. When carbohydrate is ingested during exercise, the liver reduces glucose output, preserving finite liver glycogen stores (112). The glucose supplied from the liver is crucial in regulating blood glucose levels, as this provides an additional source of glucose for working muscles and prevents hypoglycaemia (48). Therefore, the positive effect of carbohydrate ingestion on the durability of the intensity domain transitions may be linked to better blood glucose regulation (33, 97, 102, 126).

During prolonged exercise, slow-twitch muscle fibres, associated with low-threshold motor units, are preferentially recruited and often exhibit greater glycogen depletion compared to fast-twitch fibres (118, 127–129). Low-threshold motor units have adapted to metabolise energy substrates effectively by having a high maximal oxidative power, which is indicated by the cellular mitochondrial volume percentage (130). Recently, researchers have suggested that there is progressive decline in muscle oxidative power as exercise progresses, due to the preferential depletion of glycogen in slow-twitch fibres, which have the highest oxidative capacity, and therefore become inactivated first (131). As slow-twitch fibres are preferentially recruited, less effective fast-twitch fibres are then activated once the slow-twitch fibres become inexcitable due to glycogen depletion, which results in a less-oxidative active fibre pool (131).

Plausibly, the effect of prolonged exercise on carbohydrate availability has implications for durability. If muscle glycogen stores are depleted during prolonged exercise, particularly in the preferentially-activated slow-twitch fibres, the power output at which the transition from moderate-to-heavy, and heavy-to-severe, intensity occurs may be reduced. This hypothesis is supported by the the favourable effect of carbohydrate ingestion on durability of the intensity domain transitions reported previously (77, 97, 102). However, no studies have systematically assessed the effect of altered glycogen stores on the power output at the intensity domain transitions and physiological profiling variables. Assessing this effect has implications for our understanding of the mechanisms behind the loss of power output at the intensity domain transitions that occur during prolonged exercise.

The effects of carbohydrate availability on muscle recruitment

Electromyography (EMG) may be a useful tool for exploring the hypothesis that durability is linked to carbohydrate availability. EMG is a popular non-invasive method for evaluating muscle function, neuromuscular activation, and fatigue (132–135). EMG captures the electrical activity of skeletal muscle at the skin's surface. EMG signals represent the combined electrical potentials of active motor units and can provide insight into motor unit recruitment strategies throughout various forms of muscle contraction (Viitasalo & Komi, 1977). In particular, EMG amplitude and frequency characteristics are important for analysis. These EMG measurements can be used to assess how the nervous system alters the pattern of muscle fibre activation in response to various metabolic and mechanical variables, including fatigue and substrate availability (136, 137).

During prolonged or repeated submaximal contractions, EMG amplitude increases due to increased motor unit recruitment and increased motor unit firing rate to sustain force output (138). This is especially evident at lower intensities, where greater amplitude reflects increased motor unit recruitment to compensate for declining force-generating capacity (139). As exercise intensity increases, more type II fibres are recruited (140). In contrast, during maximal efforts, EMG amplitude may decrease over time as all motor units are already active and firing rates decline due to the muscle's reduced ability to sustain high firing rates.

A decrease in the median power frequency (MPF) has been shown to reflect neuromuscular fatigue (136). Slower motor units are typically made up of type I fibres, which twitch at a lower frequency, while larger, faster motor units are generally comprised of type II fibres, which have a higher twitch frequency (137). Motor unit action potential conduction velocity, which is impacted by muscle fibre type and cross-sectional area, determines the MPF (138). Therefore, during submaximal contraction an increase in MPF suggests an increase in motor unit action potential conduction velocity, and therefore greater type II fibre recruitment. During maximal contractions, it is assumed that all motor units are active; therefore, MPF may decline as muscle fibers become fatigued and conduction velocity is reduced (135). Thus, MPF is a valuable addition to amplitude when assessing muscle fibre recruitment patterns. Lowered muscle glycogen impairs sarcoplasmic reticulum Ca^{2+} release (57) and reduces the energy available to support Na^{+}/K^{+}-ATPase activity (120) important for muscle excitability; therefore there is an increase in higher threshold motor units. During submaximal exercise, if glycogen depletion leads to the inactivation of type I muscle fibres, necessitating increased recruitment of type II motor units to maintain a fixed power output, then greater EMG amplitude and MPF is expected. In contrast, during maximal contractions under glycogen-depleted conditions, a greater decline in EMG amplitude and MPF could be observed. This is likely due

to the accelerated fatigue of muscle fibres resulting from limited glycogen availability, which compromises their ability to sustain forceful contractions.

Specifically, research using surface EMG has reported that EMG amplitude is significantly elevated when performing high-intensity cycling under glycogen-depleted conditions, particularly when cycling above 70% $\dot{V}O_2$max (141). These data suggest that there is an increased neural activation to compensate for the reduced carbohydrate availability. In protocols involving sustained isometric contractions, glycogen-depleted muscles exhibited higher EMG amplitudes and reached task failure faster than normal glycogen content muscles, which suggests glycogen depletion causes a faster onset of neuromuscular fatigue (123). Osborne and Schneider (2006) found that surface EMG MPF increased more rapidly during heavy cycling exercise following glycogen reduction, which is suggestive of greater type II fibre recruitment and heightened neuromuscular activation to sustain performance. These results may be interpreted as evidence of greater central motor drive and increased recruitment of type II fibres to maintain force output when muscles are depleted of glycogen. Together, these findings support the idea that under low glycogen availability, there is a shift toward earlier and more pronounced motor unit recruitment, reflected in increased EMG amplitude and an increase in MPF.

The observed increases in EMG amplitude and MPF under glycogen depleted conditions suggests a shift toward an earlier and greater recruitment of type II motor units to maintain power output. This shift is likely caused as reduced carbohydrate availability leads to an earlier depletion of glycogen in type I muscle fibres, which are recruited first due to their high oxidative capacity during submaximal exercise (118, 131). As these fibres fatigue and become less excitable, the neuromuscular system compensates by recruiting higher-threshold, type II fibres to maintain force output. As type II fibres are more glycolytic and less fatigue-resistant, their early recruitment increases the energetic cost of exercise and accelerates fatigue development (116). This mechanism may partly explain why there is a reduction power output at the moderate-to-heavy and heavy-to-severe transitions following prolonged exercise (77, 96–99, 102, 103). Therefore, the EMG data showcasing motor unit recruitment patterns provides mechanistic support for the idea that carbohydrate availability may be a key determinant of durability.

Summary

For endurance athletes, physiological profiling is essential for guiding training and predicting performance. Important indicators that provide information on aerobic capacity, efficiency, and intensity regulation include $\dot{V}O_2$max, movement economy, substrate utilisation, and intensity domain transitions. These measurements, however, are usually taken while the subject is fresh, thus they might not accurately represent the physiological stress brought on by prolonged activity. Recent research has demonstrated that these variables deteriorate over time after prolonged activity. The term "durability" describes the rate and degree of decline in physiological profiling characteristics over an extended period of activity. The mechanisms behind durability are poorly understood; however, growing evidence suggests that carbohydrate availability plays a key role.

Specifically, muscle glycogen depletion has been linked to reduced muscle excitability, impaired contractile function, and altered neuromuscular recruitment patterns. To maintain power output under glycogen-depleted conditions, the neuromuscular system may compensate by recruiting higher-threshold, less efficient type II fibres,

accelerating fatigue. These changes contribute to the decline in power output at intensity domain transitions. Carbohydrate ingestion during prolonged exercise mitigates these effects, likely by preserving blood glucose and supporting central and peripheral drive. Therefore, investigating the role of carbohydrate availability in durability may improve the application of physiological profiling to real-world endurance performance.

Accordingly, the purpose of the study conducted for this thesis was to investigate the effect of lowered carbohydrate availability on power output on the intensity domain transitions. Addressing this aim was intended to improve the understanding of the mechanisms behind durability and to have implications for applied practice in performance physiology.

Chapter 3: The effect of lowered carbohydrate availability on power output at intensity domain transitions in endurance-trained females

Introduction

Physiological responses to exercise are characterised by distinct moderate, heavy, and severe intensity domains (61, 80, 92). The moderate, heavy, and severe intensity domains are characterised by the attainment of a rapid steady state, delayed steady state, or absence of a steady state in a range of metabolic variables such as phosphocreatine (PCr) and pH, blood lactate concentrations, and whole-body oxygen uptake ($\dot{V}O_2$), respectively (46, 89, 142). Power output at the intensity domain transitions is routinely assessed in endurance athletes during physiological profiling assessments, which are typically conducted in a well-rested state and used to inform physiologically-based training programming and load monitoring, intensity regulation during extended training sessions or competitions, and assessment of adaptations pertinent to performance (1, 143). However, power output at the intensity domain transitions decreases during prolonged exercise (96, 98, 99, 102, 103, 144) in a non-linear fashion (10). Resilience to the effect of prolonged exercise on power output at the intensity domain transitions has been termed 'durability' (1). Durability has implications for the application of physiological profiling data to training programming, intensity regulation, and load monitoring, as exercise performed in the moderate, heavy, and severe intensity domains elicits distinct metabolic, autonomic, and adaptive responses (45, 145).

The physiological mechanisms that contribute to the reduction in power output at the intensity domain transitions during prolonged exercise are not established. Durability of the intensity domain transitions could plausibly be related to carbohydrate availability. The ability to sustain prolonged work is linked to the availability of muscle glycogen stores (109, 131). Several studies have shown that muscle glycogen levels decrease progressively during exercise (107, 108), and that muscle glycogen depletion is associated with exercise-induced fatigue (57, 116, 146). Lowered muscle glycogen has been linked to key fatigue mechanisms, including impaired Na^+/K^+-ATPase activity, disrupted Ca^{2+} handling, and reduced muscle contractile function (57, 116, 119, 147). Type I fibres are preferentially activated during prolonged exercise, and are therefore depleted of glycogen fastest (131). As a result, after prolonged exercise, the pool of active, excitable fibres are less oxidative, and increased type II fibre activation is required to sustain power output (131). Thus, muscle glycogen depletion necessitates recruitment of higher threshold motor units, or increased firing rate of active motor units, to maintain a give work rate (148). Importantly, type II fibres have poorer energetic efficiency than type I fibres, which means that more metabolic energy expenditure is required to produce a given power output if type II fibre recruitment is increased (21, 26). Therefore, exercise-induced muscle glycogen depletion may contribute to the decrease in power output at intensity domain transitions during prolonged exercise via impairment of muscle contractile function, increased type II motor unit recruitment, and reduced energetic efficiency. However, no studies have systematically assessed the effect of altered muscle glycogen stores on power output at the intensity domain transitions. Supporting the role of carbohydrate availability in durability, carbohydrate ingestion during exercise mitigated the loss of power output at the moderate-to-heavy and heavy-to-severe intensity transitions during prolonged cycling (97, 102, 103).

Accordingly, the primary aims of the present investigation were to determine the effect of reduced carbohydrate availability on power output at the intensity domain transitions in well-trained female cyclists, and to explore effects on muscle activation and gross efficiency during submaximal cycling. Our secondary aim was to determine

the impact of carbohydrate availability on $\dot{V}O_2$peak and the finite capacity for work in the severe-intensity domain. We hypothesised that lowered carbohydrate availability would reduce power output at intensity domain transitions, increase recruitment of type II fibres at fixed power outputs and, therefore, increase muscle activation, resulting in reduced submaximal gross efficiency.

Methods

Participants

Nine well-trained female endurance cyclists and triathletes participated in the present investigation. A priori sample size calculation determined that five participants are required to detect an effect size of 1.7 with 80% statistical power and an alpha value of 0.05. This effect size was based on the effect of prolonged exercise on power output at the moderate-to-heavy intensity transition (144). We assumed that the effect of lowered glycogen would be smaller than prolonged exercise, given prolonged exercise elicits a number of physiological effects that could influence power output at the intensity domain transitions beyond lowered glycogen. A sample size of nine is sufficient to detect an effect size of 1.1 with 80% statistical power and an alpha value of 0.05. All participants were free of recent (<3 months) illness and musculoskeletal injury and free of cardiovascular disease. Participants met two out of the following three criteria: training >5 $h \cdot week^{-1}$ in endurance cycling, peak oxygen uptake ($\dot{V}O_2peak$) >48 $mL \cdot kg^{-1} \cdot min^{-1}$, and self-reported best-effort 20-min power output of >3.0 $W \cdot kg^{-1}$. All participants provided written informed consent and completed a general health screening. The menstrual cycle phase and oral contraceptive use were not controlled for; however, recent evidence suggests that these factors may not significantly influence physiological profiling outcomes in trained females (149–151).

Study design

An overview of the randomised, crossover study design is shown in Figure 5. Participants arrived for each visit having fasted overnight for ~10 h and having refrained from alcohol and caffeine consumption for ~18 h. Participants visited the laboratory on five occasions, involving: (1) a characterisation trial, which included an incremental cycling test to determine participant eligibility, and a familiarisation to the three-minute all-out test, (2) glycogen-depleting exercise #1, (3) an experimental trial including a comprehensive physiological profiling assessment of VT_1, critical power, $\dot{V}O_2peak$, and cycling economy #1, (4) glycogen-depleting exercise #2, (5) the crossover experimental trial. Between the glycogen-depleting exercise bouts and the experimental trials, for ~24 hours participants consumed a low (<1 g CHO·kg of $BM^{-1} \cdot day^{-1}$) or high (>9 g CHO·kg of $BM^{-1} \cdot day^{-1}$) carbohydrate diet to produce two distinct glycogen storage conditions (152).

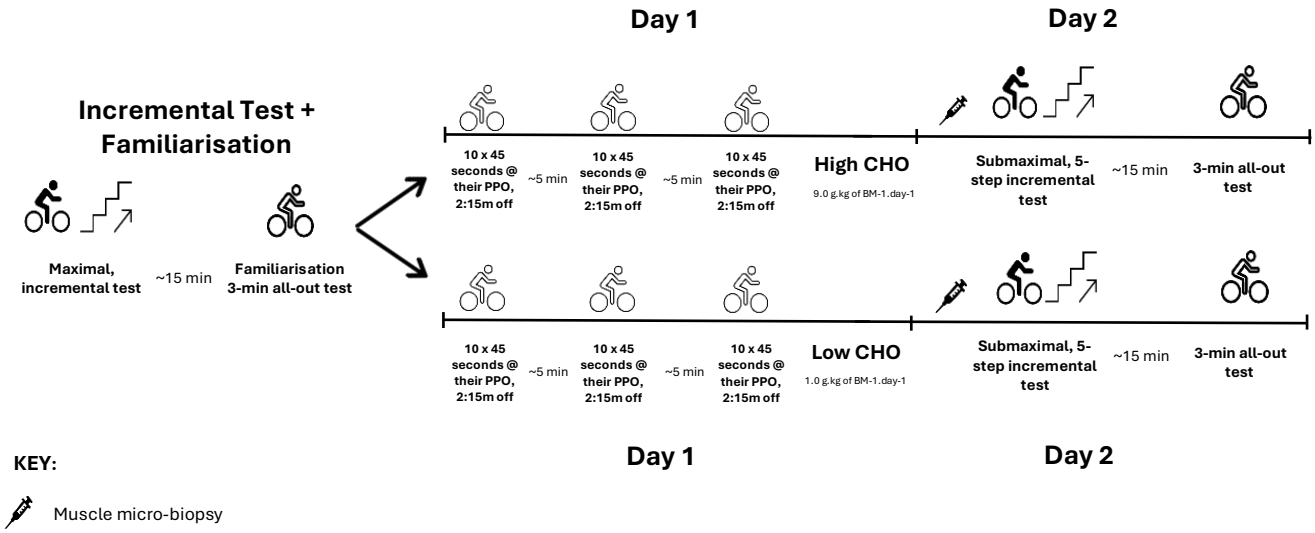

Figure 5. Schematic overview of the study design. High CHO, high carbohydrate diet; low CHO, low carbohydrate diet. PPO, peak power output achieved during the maximal, incremental test.

Visit one: Characterisation trial

Incremental test

Participants reported to the laboratory for an initial incremental cycling test and 3-min all-out test familiarisation after having fasted overnight for ~10 h and having refrained from alcohol and caffeine consumption for ~18 h. After providing written informed consent, height and body mass were determined. Cycling commenced with a 5-minute warm-up at 50 W on an electromagnetically-braked cycle ergometer (Lode; Excalibur sport, Groningen, The Netherlands). Subsequently, the incremental cycling test began at 75 W, with the power output increasing by 25 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 clear signs of increased $\dot{V}_E \cdot \dot{V}O_2^{-1}$ emerged, the power output was increased by 25 W every minute. The $\dot{V}O_2$peak was accepted as the highest 15-s average $\dot{V}O_2$, and VT_1 was identified as the first 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 carbohydrate and fat oxidation rates using standard equations (Eq. 1-3) (76). The highest observed rate of whole-body fat oxidation was identified as the peak fat oxidation rate (PFO) (39).

(Eq. 1) Whole body rate of energy expenditure ($kcal \cdot min^{-1}$) = $0.550 \times \dot{V}CO_2 + 4.471 \times \dot{V}O_2$, where $\dot{V}O_2$ and $\dot{V}CO_2$ are in $L \cdot min^{-1}$.

(Eq. 2) Whole body carbohydrate oxidation rate ($g \cdot min^{-1}$) = $4.210 \times \dot{V}CO_2 - 2.962 \times \dot{V}O_2$, where $\dot{V}O_2$ and $\dot{V}CO_2$ are in $L \cdot min^{-1}$.

(Eq. 3) Whole body fat oxidation rate ($g \cdot min^{-1}$) = $1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2$, where $\dot{V}O_2$ and $\dot{V}CO_2$ are in $L \cdot min^{-1}$.

3-min all-out test familiarisation

After completing the incremental test, participants rested for ~15 min before completing a familiarisation protocol for the 3-min all-out test. The 3-minute all-out test began with participants performing a warm-up at 100 W for 5 min. Participants then rested for 30 seconds and, during the last 5 s, were asked to increase their cadence to approximately 120 rev·min^{-1}. Participants then cycled all-out for 3 minutes. Instructions were given to reach peak power output as quickly as possible and to maintain an all-out effort throughout the test. Loud verbal encouragement was given throughout the test, but the subjects were not informed of the elapsed time. The resistance to pedalling during the test was set so that participants attained a power output of 50% of the difference between VT_1 and $\dot{V}O_2$peak at their preferred cadence, using the linear factor of the Lode ergometer (linear factor = power/cadence^2) (18, 90). An incorrect formula was used to determine the participants linear factor. The linear factor was designed to adjust to each participant's preferred cadence, however the cadence remained fixed at 67.5 rpm for all participants. Expired gases and heart rate were collected throughout the test. Critical power was estimated as end test power, or mean power output in the last 30-s of the 3-min all-out test. End-test power has been shown to be a valid measure of the heavy-to-severe intensity transition (90). Following the 3-min all-out test, the participants were given the carbohydrate intake required for their first condition. A consultation occurred in which their preferred foods were discussed to create a draft meal plan, and they were made familiar with the smartphone application (Easy Diet Diary), which was used to track macronutrient intake.

Visits two and four: Glycogen depletion exercise

Participants returned to the laboratory 5-14 days following the characterisation trial to complete the glycogen depletion exercise. Participants arrived having fasted overnight for ~10 h and having refrained from alcohol and caffeine consumption for ~18 h. Cycling began with a 5-min warm-up at 75 W. Following the warm-up, the participants completed a series of high-intensity cycling intervals designed to deplete glycogen in slow and fast twitch fibres. The protocol consisted of 10 repetitions of 45-s intervals at the highest power achieved during the incremental test in the characterisation trial, with 135-s of passive recovery between each repetition. After completing the 10 repetitions, participants were given a 5-min passive rest period. This sequence was repeated two further times for a total of 30 repetitions (153). The total duration of the this visit was ~90 min.

Following the glycogen depletion exercise, either a low carbohydrate (<1.0 g·kg of BM^{-1}·day^{-1}) or a high carbohydrate (>9 g·kg of BM^{-1}·day^{-1}) diet was followed (152). The order of these two diets was randomised. This diet was followed until the subsequent experimental session (~24 h). Total energy intake for both conditions was standardised based on the high-carbohydrate intake. In both trials, the energy intake was 100-133% of the calories provided by 9 g·kg of BM^{-1}·day^{-1} of carbohydrate.

Visits three and five: Experimental sessions

Muscle microbiopsy

On the morning of the experimental session, participants returned to the laboratory, having consumed a light breakfast containing of 0.2 g·kg of BM^{-1}·day^{-1} carbohydrates. This breakfast was replicated in both experimental sessions. A resting microbiopsy muscle sample was obtained from the mid-belly of the vastus lateralis of the dominant leg. 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 ~15-30 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.

Measurement of muscle activity

Muscle activity was measured throughout the exercise via surface electromyography (Quattrocento, OT Bioelettronica S.r.l., Torino, Italy, sampled at 2048 Hz and bandpass filtered 20-500 Hz). Before the start of the test, location of the motor point of the VL and VM muscles was estimated using a stimulation pen, which was used to deliver single rectangular pulse stimuli (duration = 0.2 ms, intensity = 20 mA) by a high-voltage constant current stimulator (DS7AH; Digitimer, Welwyn Garden City, UK). This location was determined as the point that provoked the strongest twitch of the muscle, as demonstrated by visual inspection by the researcher and the contraction sensation of the participants. A self-adhesive 5 × 10 cm rectangle electrode was placed on the gluteal fold. The disposable electromyography (EMG) electrodes (Norotrode, Myotronics Inc., WA, USA) were placed such that the proximal electrode was over the motor point and the electrodes were oriented in the direction of the muscle fibres. The ground electrode was placed on the bony region of the shin. Before placing the electrodes, the skin was shaved and abraded with alcohol. The EMG electrode placement locations were outlined with a marker to replicate the placements during the second experimental trial.

Experimental trial

Participants were fitted with a heart rate monitor, and their heart rate was recorded throughout the exercise trial. Expired gases were collected continuously during the exercise trial. The exercise trial began with a five-step incremental test, where the first step was at a power output 20% below the VT_1 power output estimated in the characterisation trial. The power output was then increased by 10% of the previously estimated VT_1 every 4 minutes, such that the fifth and final step was 20% above the VT_1 power output estimated in the first visit. A finger-prick capillary blood sample was obtained in the last 30 s of each stage for lactate analysis via a hand-held lactate analyser (Lactate Pro, Akray, Japan). Raw EMG signals were recorded throughout the test for the VL and VM. Following the five-step incremental test, participants rested for 15 min before completing the three-minute all-out test to estimate critical power according to the procedures described in the characterisation trial. Pulmonary gas exchange was measured throughout the test and recorded in 30-s intervals. Heart rate was recorded continuously throughout the test. Loud verbal encouragements were given throughout. Raw EMG signals were recorded throughout the test for the VL and VM. These experimental procedures were repeated for the other condition ~7 days later.

Data analysis

5-step test

The moderate-to-heavy intensity transition power output was calculated using the previously described methods for determining VT_1 during the first laboratory visit, but with enhanced precision due to the denser clustering of data points around the transition. This method was previously used to estimate the moderate-to-heavy intensity transition, producing similar results to blood lactate-derived measurements (77). The final sample size for this measure was N = 7, as two participants did not reach VT_1. To estimate lactate threshold (LT), blood lactate concentrations during the five-step test were plotted against power output, and the breakpoint in the curve was identified using the LoglogLT method (73). Expired gas data was used to quantify rates of whole-body energy expenditure, carbohydrate oxidation, and fat oxidation using the last minute of expired gas in each 4-min stage and standard equations standard equations (Eq. 1-3) (76). Gross cycling efficiency was calculated as the percentage of metabolic energy expenditure converted to mechanical power using the energy expenditure calculated in the last minute of each stage.

3-min all-out test

Critical power was estimated as end test power, or mean power output in the last 30-s of the 3-min all-out test. The work completed during the test above end power (WEP) was also calculated (154). Peak power output was the highest power output achieved during the first 10 s of the test. The $\dot{V}O_2$peak was accepted as the highest 15-s average $\dot{V}O_2$. Total work completed during the test was calculated in kilojoules.

Muscle activation

Custom programs in MATLAB (The MathWorks, Inc., Natick, MA, USA) were used to analyse the EMG data. All EMG data were band-pass filtered (Butterworth 4^{th} order, 20-450 Hz) and partitioned into five bins for the 5-step test (i.e., five stages), and into six bins for the 3-min all-out test (i.e., 30-s bins). For the 5-step test, only data from the last-minute each stage were analysed. EMG data were available for seven participants (N = 7) due to data loss from a system disconnection and a detached ground electrode.

The filtered data were full-wave rectified, and root mean squared (RMS) with a 25-ms moving window. Based on Özgünen et al. (2010), we used 35% of the mean value calculated over the whole RMS envelope during the 5-step test and 3-min all-out test to identify active bursts in the VL and VM during the respective tests (156). The EMG data under the burst threshold were excluded from the analyses. Median power frequency (MPF) was calculated as the frequency that divides the power spectral density (PSD) into two equal halves, using the periodogram-based method.

To normalise the RMS and MPF data, peak values were obtained from the 3-min all-out test. The 3-min all-out test data were partitioned into 1-s bins and the highest RMS and MPF values from the first 10 s (i.e., period where peak power output is typically reached) were used for normalisation. Stage-specific RMS and MPF values from the 5-step test and 30-s bin values from the 3-min all-out test were then expressed relative to these peak values.

Muscle glycogen analysis

Frozen muscle tissue samples were homogenised in a buffer containing 100 mM Tris–HCl, 5 mM EGTA, and 5 mM EDTA (Sigma Aldrich, MO, USA), supplemented with Halt protease and phosphatase inhibitor cocktail (ThermoFisher Scientific, Waltham, MA). The protein concentration of the homogenates was determined in triplicate using a non-modified Lowry assay (Peterson, 1977). Glycogen concentration was measured by digesting an aliquot of the crude homogenate with amyloglucosidase (Sigma-Aldrich, St. Louis, MO, USA) at 50°C for 60 min in a buffer containing 0.1 M sodium acetate, pH 6.0. Following centrifugation at 16,000 g for 2 min, the supernatant was removed, and glucose levels were determined in triplicate using a two-enzyme, colorimetric glucose assay (Sigma-Aldrich, St. Louis, MO, USA). Glycogen levels were measured in glucose units (nmol), normalised to protein content (mg, determined by the non-modified Lowry assay), and presented as relative levels for comparative purposes.

Statistical analysis

Data are expressed as means ± standard deviation. The Shapiro-Wilk test was used to confirm a normal Gaussian distribution. Power output at the moderate-to-heavy (VT_1 and LT) and heavy-to-severe (critical power) intensity transitions, muscle glycogen content, $\dot{V}O_2$peak, WEP, PPO, and total work done during the 3-min all-out test were compared between-trials using paired t-tests. Linear mixed models were used to assess gross efficiency, fat oxidation rates, and muscle activation (RMS and MPF). Condition, stage and their interaction were included as fixed effects, and participant ID was included as a random effect to account for within-subject variability. The α for all statistical tests was set at $P < 0.05$. All analyses were conducted using JASP (Version 0.19.3).

Results

Diet and muscle glycogen content

Participants consumed significantly more carbohydrate and total energy, with less protein and fat, between glycogen-depleting exercise and the experimental trial in HIGH than LOW (Table 2). Muscle glycogen content normalised to protein was significantly lower in LOW than HIGH (447 ± 157 vs. 731 ± 174 nmol glycosyl units·mg^{-1} protein, P = 0.005, Figure 6A). Muscle glycogen content normalised to dry weight was significantly lower in LOW than HIGH (248 ± 97 vs. 406 ± 110 nmol glycosyl units·mg^{-1} dw, P = 0.005, Figure 6B).

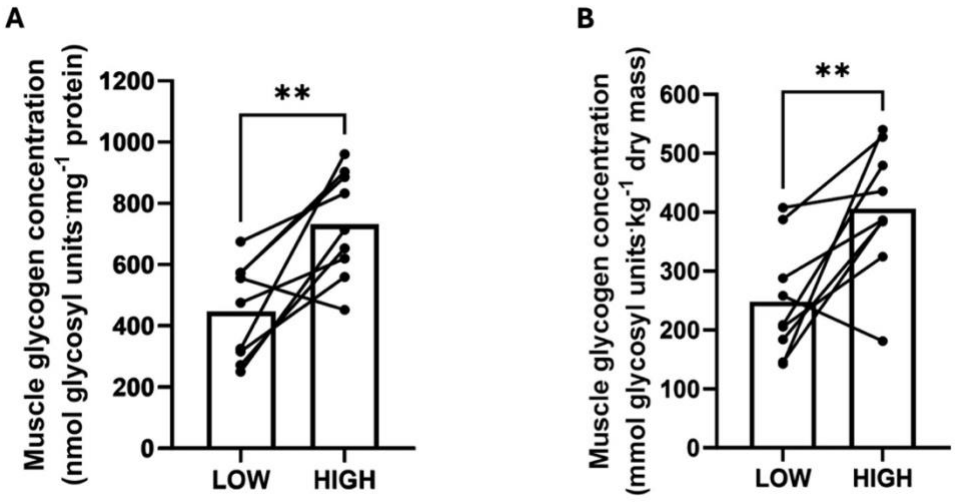

Figure 6. A Muscle glycogen content of participants in the LOW trial and the HIGH trial normalised to protein. **B** Muscle glycogen content of participants in the LOW trial and the HIGH trial normalised to dry weight. Bars indicate mean values and lines indicate individual responses. N = 9. ** denotes P ≤ 0.005

Table 2. Dietary intake during LOW and HIGH carbohydrate diets. Means ± SD, (N = 9) are shown for total energy intake, absolute macronutrient intake, energy contributed by each macronutrient, percentage of total energy, and carbohydrate intake relative to body mass.

	LOW	**HIGH**	**P**
Total energy intake (kcal)	2605.8 ± 538.5	3248.7 ± 636.3	0.002
Macronutrient intake (g)			
CHO	74.1 ± 20.9	469.9 ± 140.5	<.001
Fat	167.6 ± 47.5	98 ± 43.2	0.003
Protein	200.3 ± 46.3	121.8 ± 37	0.003
Energy (kcal)			
CHO	296.4 ± 83.5	1879.6 ± 562.1	<.001
Fat	1508 ± 427.7	882 ± 388.7	0.003
Protein	801.3 ± 185.1	487.1 ± 147.8	0.003
Energy (%total cal)			
CHO	11.5 ± 2.9	57.8 ± 12.5	<.001
Fat	57.3 ± 7.4	27 ± 10.6	<.001
Protein	31.2 ± 6.4	15.1 ± 4.0	<.001
CHO per BM (g·kg of BM^{-1})	1.2 ± 0.3	7.6 ± 2.2	<.001

Intensity domain transitions

Power output at VT_1 was significantly lower in LOW than HIGH (133 ± 24 vs. 152 ± 28 W, Δ -19 ± 14 W, P = 0.011, Figure 7A). Power output at the lactate threshold was not significantly different between LOW and HIGH (148 ± 29 vs. 142 ± 26 W, Δ 6 ± 19 W, P = 0.331, Figure 7B). The rate of energy expenditure at VT_1 was not significantly different between conditions (9.1 ± 1.5 vs. 9.8 ± 1.5 kcal·min^{-1} in LOW and HIGH respectively, Δ 0.7 ± 0.8, P = 0.067). The change in power output at VT_1 from HIGH to LOW was attributable to decreased energetic efficiency (-7 ± 8 W) and rates of metabolic energy expenditure at the transition (-12 ± 15 W). The contribution made by decreased efficiency and metabolic power to the decrease in power output at VT_1 was not significantly different (P = 0.525).

There was no significant effect of condition on EP (227 ± 34 vs. 226 ± 34 W in LOW and HIGH respectively, Δ 1 ± 12 W, P = 0.748, Figure 7C). Similarly, there was no significant effect of condition on WEP (8.6 ± 2.2 vs. 9.3 ± 2.3 kJ in LOW and HIGH respectively, P = 0.232), $\dot{V}O_2$peak (2.8 ± 0.3 vs. 2.8 ± 0.4 L·min^{-1} in LOW and HIGH respectively, P = 0.434), peak power output (846 ± 194 W vs. 864 ± 169 W in LOW and HIGH respectively, P = 0.554), or total work done during the three-minute all-out test (49.7 ± 6.97 kJ vs. 50.2 ± 6.94 kJ in LOW and HIGH respectively, P = 0.192).

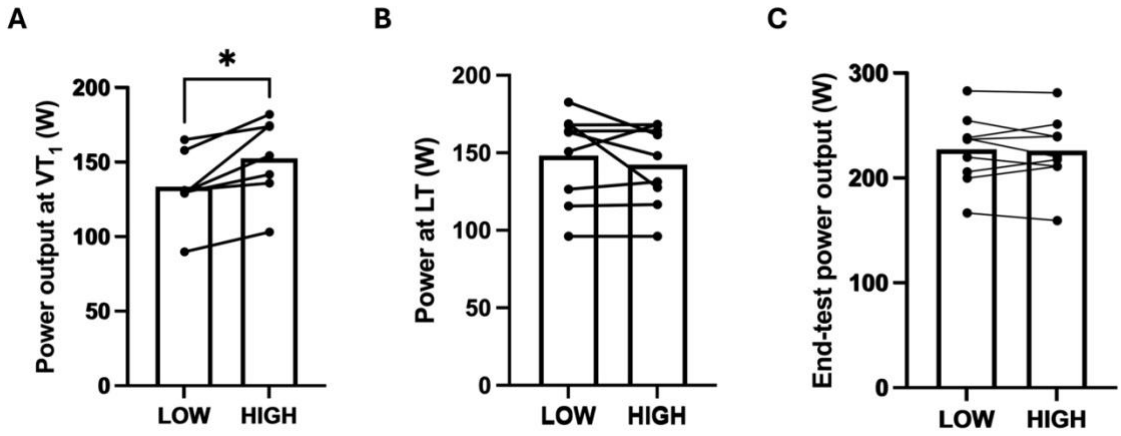

Figure 7. Power output at the moderate-to-heavy intensity transition as determined by the **A** first ventilatory threshold (VT_1) (N = 7) and **B** lactate threshold (LT) (N = 9) in a low carbohydrate (LOW) and high (HIGH) carbohydrate state. **C** End test power (EP) during the three-minute all-out test in a low (LOW) and high (HIGH) carbohydrate state (N = 9). Bars indicate mean values and lines indicate individual responses. * denotes P ≤ 0.011

Muscle activity

During the five-step test, median power frequency of the vastus lateralis (VL, P = 0.025) and vastus medialis (VM, P = 0.007) was significantly higher in LOW than HIGH, with no condition-by-stage interaction for VL (P = 0.375) or VM (P = 0.749) (Figure 8A, 8B). There was no effect of condition on amplitude in the VL (P = 0.232) and VM (P = 0.655) during the five-step test, and there were no condition-by-stage interactions for VL (P = 0.809) or VM (P = 0.830) (Figure 8C, 8D). There was no significant effect of condition for amplitude or median power frequency in the VL and VM during the three-minute all-out test (data not shown).

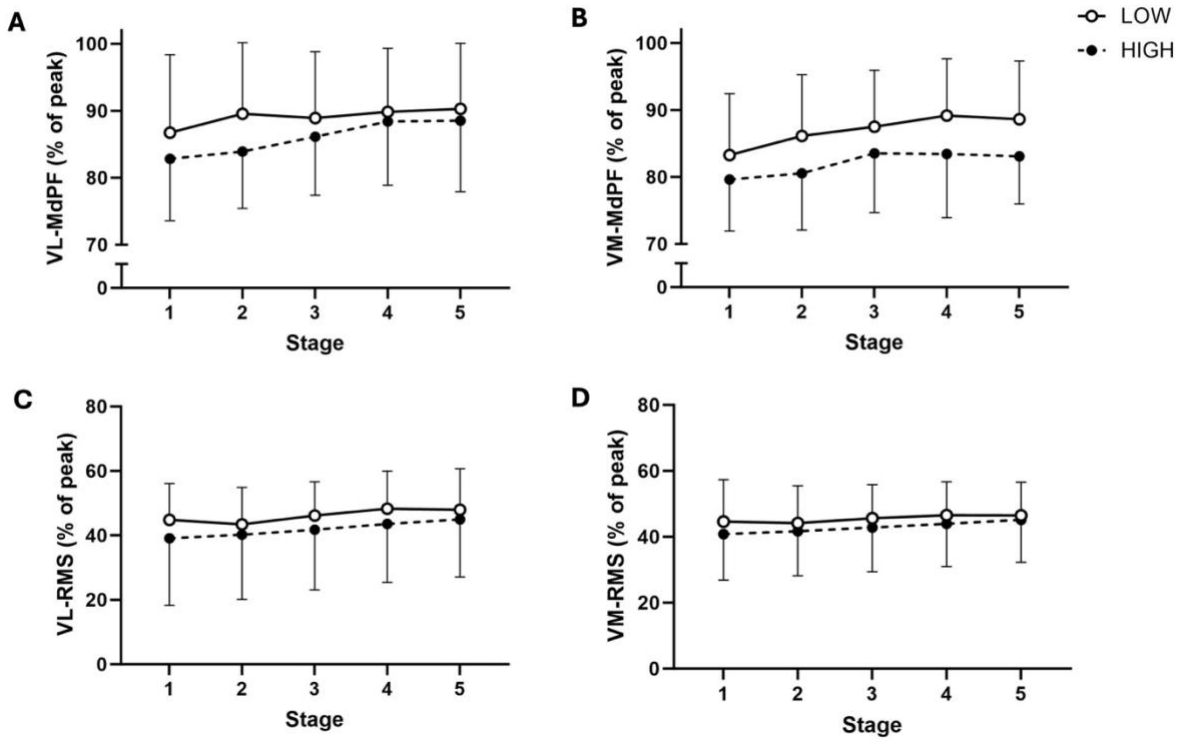

Figure 8. Muscle activity during the 5-step test was determined by **A** Vastus lateralis median power frequency (MPF), **B** vastus medialis median power frequency (MPF), **C** vastus lateralis amplitude (RMS), **D** vastus medialis amplitude (RMS) during the five-step test. Data are normalised to the peak value achieved during the corresponding three-minute all-out test. The dots indicate raw means, and the error bars indicate SD. N = 9.

Substrate oxidation

Gross cycling efficiency during the five-step test was significantly lower in LOW than HIGH ($P = 0.003$), and there was a condition-by-stage interaction whereby effects were abolished in the fourth and fifth stages (Figure 9A). Fat oxidation rates during the five-step test were significantly higher in LOW than HIGH ($P = 0.006$), but there was no condition-by-stage interaction ($P = 0.799$, Figure 9B).

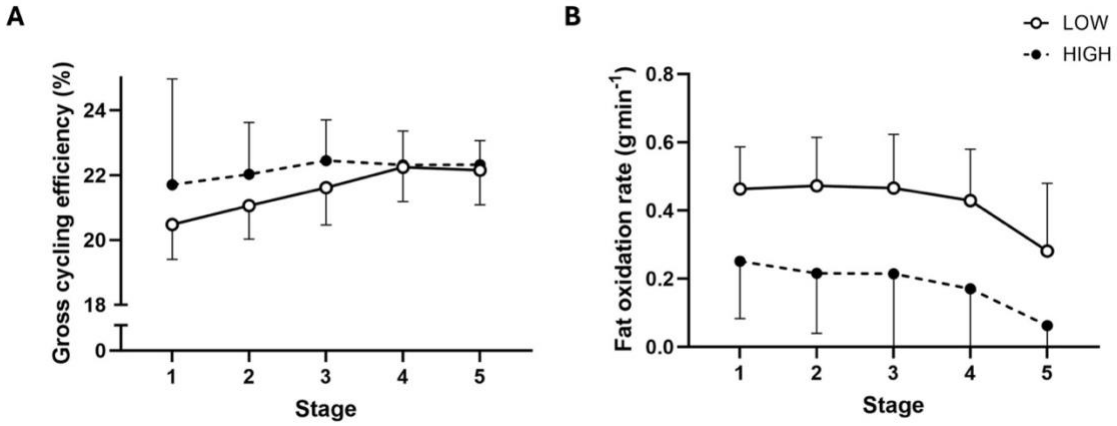

Figure 9. A Gross efficiency, **B** fat oxidation rates during the five-step test. The dots indicate raw means, and the error bars indicate SD. N = 9.

Discussion

The primary aims of this study were to determine the effect of lowered carbohydrate availability on power output at the intensity domain transitions, and to explore effects on muscle activation patterns and gross efficiency during submaximal cycling, in endurance-trained females. We hypothesised that lowered carbohydrate availability would reduce power output at the intensity domain transitions, increase recruitment of higher threshold motor units at fixed power outputs and, therefore, increase muscle activation amplitude and median power frequency, resulting in reduced submaximal gross efficiency. The primary findings were that lowered carbohydrate availability: (i) reduced power output at VT_1, but not the lactate threshold or critical power, (ii) increased the median power frequency of the VL and VM during submaximal cycling, reflecting greater activation of higher threshold motor units, and (iii) reduced gross cycling efficiency during submaximal cycling. Collectively, these data suggest that lowered carbohydrate availability may reduce power output at the moderate-to-heavy, but not heavy-to-severe, intensity transition due to alterations in motor unit recruitment and impaired gross cycling efficiency. These observations are consistent with the hypothesis that the progressive loss of carbohydrate availability during prolonged exercise could be a mechanistic cause of the loss of power output at the moderate-to-heavy intensity transition during prolonged exercise.

Mechanistic explanation for loss of VT_1 with reduced carbohydrate availability

In line with our hypothesis, lowered carbohydrate availability reduced power output at VT_1 by ~12% (Figure 7A). Our data are consistent with our proposed mechanism, where the reduction in power output at VT_1 with lowered muscle glycogen may be attributable to inactivation of specific muscle fibres, which are compensated for through activation of higher threshold motor units, resulting in impaired gross cycling efficiency. Specifically, we observed significantly higher VL and VM median power frequency in LOW (Figure 8A, 8B). The median power frequency is determined by the motor unit action potential conduction velocity, which is affected by the type of muscle fibre and cross-sectional area (138). Therefore, during submaximal contractions, an increase in median power frequency indicates an increase in motor unit action potential conduction velocity. Activation of higher-threshold motor units, which have faster muscle fibre conduction velocities, results in a shift in the electromyographic power spectrum toward higher frequencies, which in turn increases the median power frequency (157, 158). However, shifts in conduction velocity likely reflect the progressive activation of higher-threshold motor units along a continuum of fibre characteristics, rather than a discrete switch from type I to type II fibres (159). This aligns with the "onion skin" model, which suggests that low-threshold motor units are recruited first and fire at higher rates, followed by the progressive recruitment of higher-threshold units as fatigue develops (160). Therefore, the elevated VL and VM median power frequency at submaximal power outputs in LOW likely reflects an increased activation of higher threshold motor units.

Interestingly, although we observed an increase in median power frequency in LOW, there was no effect of reduced carbohydrate availability on VL and VM amplitude (Figure 8C, 8D). Our amplitude measure during submaximal cycling reflects the number of active motor units (136, 137), and therefore the absence of a between-trial difference suggests there was no significant increase in motor unit recruitment in LOW. High-threshold motor units or type II fibres are highly-active during intense, but not moderate-intensity submaximal exercise (161, 162).

Therefore, the increase in median power frequency but unchanged amplitude in LOW suggests that lowered carbohydrate availability shifted the muscle fibres that were activated, rather than recruited additional fibres.

Also in line with our hypothesis, gross cycling efficiency at submaximal power outputs was impaired in LOW (Figure 9A). Individuals with a higher percentage of type I fibres have greater cycling efficiency (21, 26), which is consistent with the notion that type I fibres are more energetically efficient than type II fibres. It has been shown that moderate carbohydrate intake (4 g/kg/day) after glycogen-depleting exercise reduces intramyofibrillar glycogen density (163), and in the current study participants consumed even less carbohydrate in following glycogen-depleting exercise LOW (1 g/kg/day), which collectively suggests that intramyofibrillar glycogen stores were substantially reduced in LOW. Depletion of intramyofibrillar glycogen has been shown to impair excitation-contraction coupling by reducing calcium release, therefore limiting the force generating capacity of the affected fibres (57, 119). Low-threshold fibres were likely depleted preferentially during the glycogen-depleting exercise, reducing their function and increasing reliance on less-efficient fast-twitch fibres (131). Therefore, the participants may have been more reliant on less efficient high-threshold motor units to sustain power output, which would contribute to the observed reduction in efficiency. These impairments likely increased the metabolic load on the remaining active fibres to maintain force production, further contributing to the decline in gross cycling efficiency observed in the LOW condition. This is further supported by the increased VL and VM median power frequency, which is consistent with increased recruitment of high-threshold motor units. Taken together, these results are consistent with the hypothesis that lowered carbohydrate availability reduced power output at VT_1 due to greater activation of higher-threshold motor units in compensation for impaired function of glycogen-depleted individual fibres, resulting in impaired gross cycling efficiency.

Explanation for the disparity in the effects of lowered carbohydrate availability on VT_1 and LT

Interestingly, reduced carbohydrate availability lowered power output at VT_1, but not at the lactate threshold (Figure 7B). The lactate threshold and VT_1 are both used as markers of the moderate-to-heavy intensity transition (66), hence we had anticipated that these markers would respond similarly to reduced carbohydrate availability. The disparity in the effect of reduced carbohydrate availability on VT_1 and lactate threshold may be because they are sensitive to different physiological signals. The VT_1 is assessed via the ventilatory response to exercise, which is sensitive to various metabolic perturbations, including lactate, pH, CO_2, K^+ and H^+ concentrations (74). These metabolites stimulate peripheral chemoreceptors and skeletal muscle metaboreceptors (164), resulting in a disproportionate increase in minute ventilation relative to $\dot{V}O_2$. The breakpoint in this relationship is assessed as VT_1. In contrast, the lactate threshold is assessed solely via changes in blood lactate concentrations, and thus transient imbalances between lactate production and clearance (65, 69). Plausibly, the VT_1 may be more sensitive to the effect of reduced carbohydrate availability on power output at the moderate-to-heavy intensity transition. In LOW, the reduced muscle glycogen content may have downregulated muscle glycogenolysis (112). Consistent with this, whole-body fat oxidation rates were ~ 0.2-0.3 g/min higher in LOW (Figure 9B). Downregulated muscle glycogenolysis may have led to stimulation of alternative metabolic pathways, such as phosphocreatine metabolism, at lower power outputs. Phosphocreatine metabolism leads to increased H^+ and P_i accumulation (71), which stimulate ventilatory drive via input to skeletal muscle metaboreceptors (164), without increasing blood lactate accumulation. Therefore, the metabolic shifts associated with LOW may have reduced power output at the

moderate-to-heavy intensity transition without impacting the power output vs. blood lactate relationship, and therefore the lactate threshold. This may explain our observation that reduced carbohydrate availability lowered power output at VT_1, but not the lactate threshold.

No effect of lowered carbohydrate availability on the heavy-to-severe intensity transition

Contrary to our hypothesis, reduced carbohydrate availability did not impact power output at the heavy-to-severe transition, assessed using three-minute all-out test end-test power (Figure 7C). These data suggest that factors other than carbohydrate availability govern the heavy-to-severe intensity transition, at least within the range of carbohydrate availabilities studied here (Figure 6A). Consistent with this, a previous study reported no relationship between the magnitude of muscle glycogen depletion during prolonged, heavy-intensity cycling and the magnitude of the reduction in power output at the heavy-to-severe intensity transition, also determined using three-minute all-out tests (102).

It is possible that a negative effect of reduced carbohydrate availability on power output at the heavy-to-severe intensity transition might have been seen with a greater reduction in muscle glycogen levels in LOW. It has been suggested that the negative effects of muscle glycogen depletion on high-intensity performance are only seen when muscle glycogen falls below a threshold value, possibly 250 to 300 $mmol \cdot kg^{-1}$ dry mass (165). In support, researchers previously reported no difference in 75-s exercise performance when muscle glycogen levels were reduced, but still well above this threshold (462 $mmol \cdot kg^{-1}$ dw) (166). In this study four of the nine participants were above this threshold, whilst the mean value is below the proposed threshold (248 ± 97 nmol glycosyl $units \cdot mg^{-1}$ dw). Future studies may consider investigating the effects of more-severe muscle glycogen depletion on power output at the heavy-to-severe intensity transition.

Limitations

When interpretating the results of this study, several limitations should be acknowledged. There was a discrepancy in total energy intake between the glycogen-depleting bout and the experimental trial in HIGH and LOW, whereby participants consumed ~25% more energy in HIGH. Whilst our primary aim was to manipulate carbohydrate availability, the higher total energy intake in HIGH may have introduced a confounding variable. Specifically, the observed greater power output at VT_1 could be partly attributable to greater overall energy availability. To address this in future studies, researchers may consider providing all meals to participants during the dietary manipulation phase to standardise energy and macronutrient intake across conditions, ensuring tighter control over carbohydrate availability and reducing between-condition variability in energy status. This would help isolate the specific effects of carbohydrate availability on physiological outcomes. Another limitation was that we lost some EMG data due to technical issues, which limited the statistical power of our neuromuscular analysis.

Conclusion

In conclusion, our data suggest that lowered carbohydrate availability reduced power output at the moderate-to-heavy transition, as indicated by the reduction in power output at VT_1. We attribute this effect to greater activation of higher-threshold motor units during submaximal cycling, likely in compensation for impaired function of glycogen-depleted fibres, resulting in impaired gross cycling efficiency. In contrast, no effect of reduced

carbohydrate availability was observed at the heavy-to-severe transition, which suggests that reduced carbohydrate availability, at least within the range studied here, does not govern power output at the heavy-to-severe intensity transition. Our data therefore support a potential mechanistic role for the progressive reduction of carbohydrate availability during prolonged exercise in durability of the moderate-to-heavy intensity transition.

Chapter 4: Summary and future directions

The primary aims of this thesis were to investigate the effect of lowered carbohydrate availability on power output at the intensity domain transitions in endurance-trained females, and to explore effects on muscle activation patterns and gross efficiency during submaximal cycling. We hypothesised that lowered carbohydrate availability would reduce power output at the intensity domain transitions, increase recruitment of higher threshold motor units at fixed power outputs and, therefore, increase muscle activation amplitude and median power frequency, resulting in reduced submaximal gross efficiency. The primary findings were that lowered carbohydrate availability: (i) decreased power output at VT_1, with no effect observed at the lactate threshold or critical power; (ii) elevated the median power frequency of the VL and VM during submaximal cycling and (iii) led to a decline in gross cycling efficiency. Throughout this chapter we will discuss the practical applications of the results, and then discuss recommendations for future research directions.

Application to practice

A key finding of this study was that lowered carbohydrate availability impaired power output at the moderate-to-heavy intensity transition (VT_1). This reduction in VT_1 under low-carbohydrate conditions mirrors the deterioration in physiological thresholds commonly observed after prolonged endurance exercise (96, 97, 99, 100, 144), which similarly lowers carbohydrate availability (102, 103), suggesting that carbohydrate availability is a determinant of durability. The elevated median power frequency of the vastus medialis and lateralis observed in the low carbohydrate condition indicates altered neuromuscular recruitment, likely reflecting a compensatory activation of higher-threshold motor units due to glycogen-depleted slow-twitch fibres. This shift, combined with the observed reduction in gross cycling efficiency, supports the mechanistic link between glycogen depletion, impaired muscle contractile function, and reduced metabolic power. Collectively, these results suggest that an athlete's durability is influenced by the maintenance of carbohydrate availability. This has a number of important practical applications.

Firstly, the results from this study highlight the need to consider nutritional status when assessing or prescribing training intensities based on physiological profiling data. For instance, testing an athlete in a low-carbohydrate state may underestimate their actual VT_1 and submaximal efficiency, potentially leading to inappropriate training zone prescription. Therefore, coaches and practitioners should implement strategies that ensure athletes are adequately fueled before key sessions or tests to obtain an accurate representation of their physiological profile. Furthermore, interventions aimed at maintaining carbohydrate availability are likely to enhance durability during prolonged exercise. For example, carbohydrate ingestion during endurance sessions can preserve power output at intensity domain transitions (97, 102, 103). Athletes may benefit from starting sessions with high carbohydrate availability, which can be achieved by consuming a carbohydrate-rich meals the day before and a light pre-session snack to ensure adequate glycogen stores.

Furthermore, the findings of this study contribute new evidence supporting the concept of durability as a individualised performance attribute in endurance-trained athletes. Specifically, this study demonstrated that VT_1

power output was significantly lower under conditions of lowered carbohydrate availability, even during a relatively short submaximal protocol. Therefore, these results suggest that physiological profiling assessments made in fresh conditions may not accurately reflect an athlete's true physiological capacity during real-world training or competition where exercise is prolonged and glycogen stores are progressively depleted. This challenges current practice, where endurance athletes are often profiled under rested conditions and training zones are prescribed accordingly. The observed sensitivity of VT_1 to reduced carbohydrate availability supports the growing research that profiling should extend beyond baseline assessments to include durability-specific evaluations showcasing how an individual's physiological thresholds shift under fatigue or glycogen depleted conditions.

Interestingly, critical power did not differ between low and high carbohydrate conditions. This suggests that critical power may be a more stable marker under diet manipulation compared to VT_1. In practice, this indicates that sessions prescribed relative to critical power are less likely to be affected by acute fluctuations in carbohydrate availability. Further, critical power may serve as a more reliable measure for training intensity prescription even when athletes are in a glycogen-depleted state, for example, following prolonged exercise or if they have back-to-back training days and have not replenished their glycogen stores. Therefore, integrating both VT_1 and CP into physiological profiling is important as VT_1 reflects the athlete's sensitivity to carbohydrate availability, while critical power represents a robust threshold for consistent training prescription.

Future research directions

The present study was conducted in a female-only cohort to address the persistent sex data gap and contribute to a more equitable understanding of endurance performance in females. Sex is now widely acknowledged as a significant biological factor that can mediate physiological responses to exercise (167). However, a significant sex imbalance persists in exercise science research (168–170). Cowley et al. (2021) reported that of 5,261 articles published between 2014 and 2020, 6% featured female-only cohorts. Given what we know about the physiological disparities in response to exercise between the sexes, the persistence of bias in exercise physiology literature is concerning. It should be encouraged for females to receive the same quality and quantity of research to optimise female support. When addressing the demands of elite female athletes, this apparent absence in research presents notable barriers because we cannot adopt and apply an evidence-informed approach to maximise female performance potential (171). A key reason why females are underrepresented within exercise physiology literature is that women are more physiologically variable than men due to fluctuating reproductive hormonal profiles across the menstrual cycle. This introduces potential variability, and the methodological challenges affecting study design are often cited as the basis for excluding female participants in studies. However, several recent studies suggest that these factors may not significantly influence physiological profiling outcomes in trained females (149–151). These findings indicate that, when accounting for training status, hormonal fluctuations across the menstrual cycle or oral contraceptive use may not substantially alter key performance variables. Therefore, future studies should aim to include female participants and use study designs that enable sex-based comparisons. Increasing the representation of females in exercise science research is important to ensure that physiological recommendations are evidence-based and tailored to both sexes.

Furthermore, as our study was conducted on a female-only cohort, future research could investigate the effects of carbohydrate availability on intensity domain transitions in male participants. Males rely more heavily on carbohydrate metabolism during endurance exercise, driven by higher glycogen phosphorylase activity and lower fat oxidation rates (172, 173). These differences in substrate usage suggest that reduced carbohydrate availability may produce a more pronounced effect on power output at the intensity domain transitions in males. Understanding that males have different physiological responses to exercise and substrate metabolism compared to females is important, as it suggests that the effects of altered carbohydrate availability on physiological profiling measures may differ between sexes.

Future studies could explore the link between carbohydrate availability and the power output decline observed during prolonged exercise. One approach would be to examine whether accelerated glycogen depletion, such as through nicotinic acid ingestion, leads to greater reductions in VT_1 during prolonged exercise. By combining a prolonged cycling protocol with pre and post exercise physiological profiling, researchers could determine whether the responses observed under low-carbohydrate conditions in the present study mirror those that occur with progressive glycogen depletion during prolonged endurance exercise. Such studies would provide valuable insight into the role of muscle glycogen as a determinant of durability and guide strategies to optimise carbohydrate availability for sustained performance.

Conclusion

In conclusion, the key findings of this thesis were that reduced carbohydrate availability lowered power output at VT_1, but did not alter the lactate threshold. This may have been attributable to altered neuromuscular recruitment and consequent reductions in gross cycling efficiency. However, reduced carbohydrate availability did not affect the heavy-to-severe transition. Accordingly, the primary recommendations for application of these results are that nutritional status should be considered in physiological profiling, and training prescription and durability should be monitored at an individual level as a part of athlete profiling assessments. The primary recommendation for future research is to examine the link between carbohydrate availability and durability during prolonged exercise, including studies using accelerated glycogen depletion to replicate real-world endurance conditions. Whilst continuing to address the sex data gap by including female-focused studies and enabling sex-based comparisons. These future research findings will provide a more thorough understanding of what makes athletes more durable and enhance the practical application of durability research to improve endurance performance.

Reference List

1. **Maunder E**, **Seiler S**, **Mildenhall MJ**, **Kilding AE**, **Plews DJ**. The Importance of 'Durability' in the Physiological Profiling of Endurance Athletes. *Sports Med* 51: 1619–1628, 2021. doi: 10.1007/s40279-021-01459-0.

2. **Jones AM**, **Poole DC**. Oxygen Uptake Kinetics in Sport, Exercise and Medicine. 0 ed. Routledge.

3. **Bassett DR**. Limiting factors for maximum oxygen uptake and determinants of endurance performance: *Medicine & Science in Sports & Exercise* 32: 70, 2000. doi: 10.1097/00005768-200001000-00012.

4. **Liguzinski P**, **Korzeniewski B**. Oxygen delivery by blood determines the maximal V O_2 and work rate during whole body exercise in humans: in silico studies. *American Journal of Physiology-Heart and Circulatory Physiology* 293: H343–H353, 2007. doi: 10.1152/ajpheart.01371.2006.

5. **Mortensen SP**, **Dawson EA**, **Yoshiga CC**, **Dalsgaard MK**, **Damsgaard R**, **Secher NH**, **González-Alonso J**. Limitations to systemic and locomotor limb muscle oxygen delivery and uptake during maximal exercise in humans. *The Journal of Physiology* 566: 273–285, 2005. doi: 10.1113/jphysiol.2005.086025.

6. **Carlsson M**, **Carlsson T**, **Knutsson M**, **Malm C**, **Tonkonogi M**. Oxygen uptake at different intensities and sub-techniques predicts sprint performance in elite male cross-country skiers. *Eur J Appl Physiol* 114: 2587–2595, 2014. doi: 10.1007/s00421-014-2980-0.

7. **McLaughlin JE**, **Howley ET**, **Bassett DR**, **Thompson DL**, **Fitzhugh EC**. Test of the classic model for predicting endurance running performance. *Med Sci Sports Exerc* 42: 991–997, 2010. doi: 10.1249/MSS.0b013e3181c0669d.

8. **Coyle EF**, **Martin WH**, **Sinacore DR**, **Joyner MJ**, **Hagberg JM**, **Holloszy JO**. Time course of loss of adaptations after stopping prolonged intense endurance training. *J Appl Physiol Respir Environ Exerc Physiol* 57: 1857–1864, 1984. doi: 10.1152/jappl.1984.57.6.1857.

9. **Pelliccia A**, **Maron BJ**, **Spataro A**, **Proschan MA**, **Spirito P**. The Upper Limit of Physiologic Cardiac Hypertrophy in Highly Trained Elite Athletes. *N Engl J Med* 324: 295–301, 1991. doi: 10.1056/NEJM199101313240504.

10. **Roeske WR**, **O'Rourke RA**, **Klein A**, **Leopold G**, **Karliner JS**. Noninvasive evaluation of ventricular hypertrophy in professional athletes. *Circulation* 53: 286–291, 1976. doi: 10.1161/01.CIR.53.2.286.

11. **Hellsten Y**, **Nyberg M**. Cardiovascular Adaptations to Exercise Training. In: *Comprehensive Physiology*. John Wiley & Sons, Ltd, p. 1–32.

12. **Convertino VA**. Blood Volume Response to Physical Activity and Inactivity. *The American Journal of the Medical Sciences* 334: 72–79, 2007. doi: 10.1097/MAJ.0b013e318063c6e4.

13. **Gillen CM**, **Lee R**, **Mack GW**, **Tomaselli CM**, **Nishiyasu T**, **Nadel ER**. Plasma volume expansion in humans after a single intense exercise protocol. *Journal of Applied Physiology* 71: 1914–1920, 1991. doi: 10.1152/jappl.1991.71.5.1914.

14. **Heinicke K**, **Wolfarth B**, **Winchenbach P**, **Biermann B**, **Schmid A**, **Huber G**, **Friedmann B**, **Schmidt W**. Blood Volume and Hemoglobin Mass in Elite Athletes of Different Disciplines. *International Journal of Sports Medicine* 22: 504–512, 2001. doi: 10.1055/s-2001-17613.

15. **Pugh LGCE**. Blood volume changes in outdoor exercise of 8-10 hour duration. *J Physiol* 200: 345–351, 1969. doi: 10.1113/jphysiol.1969.sp008697.

16. **Saltin B**, **Nazar K**, **Costill DL**, **Stein E**, **Jansson E**, **Essén B**, **Gollnick PD**. The Nature of the Training Response; Peripheral and Central Adaptations to One-Legged Exercise. *Acta Physiologica Scandinavica* 96: 289–305, 1976. doi: 10.1111/j.1748-1716.1976.tb10200.x.

17. **Beltz NM**, **Gibson AL**, **Janot JM**, **Kravitz L**, **Mermier CM**, **Dalleck LC**. Graded Exercise Testing Protocols for the Determination of VO_2max: Historical Perspectives, Progress, and Future Considerations. *Journal of Sports Medicine* 2016: e3968393, 2016. doi: 10.1155/2016/3968393.

18. **Burnley M**, **Doust JH**, **Vanhatalo A**. A 3-min All-Out Test to Determine Peak Oxygen Uptake and the Maximal Steady State. *Medicine & Science in Sports & Exercise* 38: 1995–2003, 2006. doi: 10.1249/01.mss.0000232024.06114.a6.

19. **Barnes KR**, **Kilding AE**. Running economy: measurement, norms, and determining factors. *Sports Medicine - Open* 1: 8, 2015. doi: 10.1186/s40798-015-0007-y.

20. **Coyle EF**. Physiological determinants of endurance exercise performance. *Journal of Science and Medicine in Sport* 2: 181–189, 1999. doi: 10.1016/S1440-2440(99)80172-8.

21. **Coyle EF**, **Sidossis LS**, **Horowitz JF**, **Beltz JD**. Cycling efficiency is related to the percentage of Type I muscle fibers. *Medicine & Science in Sports & Exercise* 24: 782, 1992.

22. **Fletcher JR**, **Esau SP**, **Macintosh BR**. Economy of running: beyond the measurement of oxygen uptake. *J Appl Physiol (1985)* 107: 1918–1922, 2009. doi: 10.1152/japplphysiol.00307.2009.

23. **Saunders PU**, **Pyne DB**, **Telford RD**, **Hawley JA**. Factors affecting running economy in trained distance runners. *Sports Med* 34: 465–485, 2004. doi: 10.2165/00007256-200434070-00005.

24. **Hopker J**, **Passfield L**, **Coleman D**, **Jobson S**, **Edwards L**, **Carter H**. The Effects of Training on Gross Efficiency in Cycling: A Review. *Int J Sports Med* 30: 845–850, 2009. doi: 10.1055/s-0029-1237712.

25. **Passfield L**, **Doust JH**. Changes in cycling efficiency and performance after endurance exercise. *Medicine & Science in Sports & Exercise* 32: 1935, 2000.

26. **Horowitz J**, **Sidossis L**, **Coyle E**. High Efficiency of Type I Muscle Fibers Improves Performance. *Int J Sports Med* 15: 152–157, 1994. doi: 10.1055/s-2007-1021038.

27. **Romijn JA**, **Coyle EF**, **Sidossis LS**, **Gastaldelli A**, **Horowitz JF**, **Endert E**, **Wolfe RR**. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology-Endocrinology and Metabolism* 265: E380–E391, 1993. doi: 10.1152/ajpendo.1993.265.3.E380.

28. **Jensen J**, **Rustad PI**, **Kolnes AJ**, **Lai Y-C**. The Role of Skeletal Muscle Glycogen Breakdown for Regulation of Insulin Sensitivity by Exercise. *Front Physio* 2, 2011. doi: 10.3389/fphys.2011.00112.

29. **Maunder E**, **Kilding AE**, **Plews DJ**. Substrate Metabolism During Ironman Triathlon: Different Horses on the Same Courses. *Sports Med* 48: 2219–2226, 2018. doi: 10.1007/s40279-018-0938-9.

30. **Martin WH**, **Klein S**. Use of endogenous carbohydrate and fat as fuels during exercise. *Proc Nutr Soc* 57: 49–54, 1998. doi: 10.1079/pns19980008.

31. **Achten J**, **Gleeson M**, **Jeukendrup AE**. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc* 34: 92–97, 2002. doi: 10.1097/00005768-200201000-00015.

32. **Van Loon LJC**, **Greenhaff PL**, **Constantin-Teodosiu D**, **Saris WHM**, **Wagenmakers AJM**. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology* 536: 295–304, 2001. doi: 10.1111/j.1469-7793.2001.00295.x.

33. **Hargreaves M**, **Spriet LL**. Skeletal muscle energy metabolism during exercise. *Nat Metab* 2: 817–828, 2020. doi: 10.1038/s42255-020-0251-4.

34. **Hickson RC**, **Rennie MJ**, **Conlee RK**, **Winder WW**, **Holloszy JO**. Effects of increased plasma fatty acids on glycogen utilization and endurance. *Journal of Applied Physiology* 43: 829–833, 1977. doi: 10.1152/jappl.1977.43.5.829.

35. **Holloszy JO**, **Coyle EF**. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *Journal of Applied Physiology* 56: 831–838, 1984. doi: 10.1152/jappl.1984.56.4.831.

36. **Maunder E**, **Rothschild JA**, **Fritzen AM**, **Jordy AB**, **Kiens B**, **Brick MJ**, **Leigh WB**, **Chang W-L**, **Kilding AE**. Skeletal muscle proteins involved in fatty acid transport influence fatty acid oxidation rates observed during exercise. *Pflugers Arch - Eur J Physiol* 475: 1061–1072, 2023. doi: 10.1007/s00424-023-02843-7.

37. **Nordby P**, **Saltin B**, **Helge JW**. Whole-body fat oxidation determined by graded exercise and indirect calorimetry: a role for muscle oxidative capacity? *Scandinavian Med Sci Sports* 16: 209–214, 2006. doi: 10.1111/j.1600-0838.2005.00480.x.

38. **Shaw CS**, **Swinton C**, **Morales-Scholz MG**, **McRae N**, **Erftemeyer T**, **Aldous A**, **Murphy RM**, **Howlett KF**. Impact of exercise training status on the fiber type-specific abundance of proteins regulating intramuscular lipid metabolism. *Journal of Applied Physiology* 128: 379–389, 2020. doi: 10.1152/japplphysiol.00797.2019.

39. **Maunder E**, **Plews DJ**, **Wallis GA**, **Brick MJ**, **Leigh WB**, **Chang W-L**, **Stewart T**, **Watkins CM**, **Kilding AE**. Peak fat oxidation is positively associated with vastus lateralis CD36 content, fed-state exercise fat oxidation, and endurance performance in trained males. *Eur J Appl Physiol* 122: 93–102, 2022. doi: 10.1007/s00421-021-04820-3.

40. **Maunder E**, **Plews DJ**, **Kilding AE**. Contextualising Maximal Fat Oxidation During Exercise: Determinants and Normative Values. *Front Physiol* 9: 599, 2018. doi: 10.3389/fphys.2018.00599.

41. **Farinatti P**, **Neto AC**, **Amorim P**. Oxygen consumption and substrate utilization during and after resistance exercises performed with different muscle mass. *International Journal of Exercise Science* 9: 77–88, 2016. doi: 10.70252/AKBM3973.

42. **Ramos-Jiménez A**, **Hernández-Torres RP**, **Torres-Durán PV**, **Romero-Gonzalez J**, **Mascher D**, **Posadas-Romero C**, **Juárez-Oropeza MA**. The Respiratory Exchange Ratio is Associated with Fitness Indicators Both in Trained and Untrained Men: A Possible Application for People with Reduced Exercise Tolerance. *Clin Med Circ Respirat Pulm Med* 2: 1–9, 2008. doi: 10.4137/ccrpm.s449.

43. **Frandsen J**, **Vest S**, **Larsen S**, **Dela F**, **Helge J**. Maximal Fat Oxidation is Related to Performance in an Ironman Triathlon. *Int J Sports Med* 38: 975–982, 2017. doi: 10.1055/s-0043-117178.

44. **Achten J**, **and Jeukendrup AE**. The effect of pre-exercise carbohydrate feedings on the intensity that elicits maximal fat oxidation. *Journal of Sports Sciences* 21: 1017–1025, 2003. doi: 10.1080/02640410310001641403.

45. **Black MI**, **Jones AM**, **Blackwell JR**, **Bailey SJ**, **Wylie LJ**, **McDonagh STJ**, **Thompson C**, **Kelly J**, **Sumners P**, **Mileva KN**, **Bowtell JL**, **Vanhatalo A**. Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. *J Appl Physiol (1985)* 122: 446–459, 2017. doi: 10.1152/japplphysiol.00942.2016.

46. **Jones AM**, **Wilkerson DP**, **DiMenna F**, **Fulford J**, **Poole DC**. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 294: R585–R593, 2008. doi: 10.1152/ajpregu.00731.2007.

47. **Stanley J**, **Peake JM**, **Buchheit M**. Cardiac Parasympathetic Reactivation Following Exercise: Implications for Training Prescription. *Sports Med* 43: 1259–1277, 2013. doi: 10.1007/s40279-013-0083-4.

48. **Coyle EF**, **Coggan AR**, **Hemmert MK**, **Ivy JL**. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *Journal of Applied Physiology* 61: 165–172, 1986. doi: 10.1152/jappl.1986.61.1.165.

49. **Gonzalez JT**, **Fuchs CJ**, **Smith FE**, **Thelwall PE**, **Taylor R**, **Stevenson EJ**, **Trenell MI**, **Cermak NM**, **van Loon LJC**. Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *American Journal of Physiology-Endocrinology and Metabolism* 309: E1032–E1039, 2015. doi: 10.1152/ajpendo.00376.2015.

50. **Coggan AR**, **Coyle EF**. Carbohydrate ingestion during prolonged exercise: effects on metabolism and performance. *Exerc Sport Sci Rev* 19: 1–40, 1991.

51. **Coyle EF**, **Coggan AR**. Effectiveness of Carbohydrate Feeding in Delaying Fatigue during Prolonged Exercise. *Sports Medicine* 1: 446–458, 1984. doi: 10.2165/00007256-198401060-00004.

52. **Elghobashy ME**, **Richards AJ**, **Malekzadeh R**, **Patel D**, **Turner LV**, **Burr JF**, **Power GA**, **Laham R**, **Riddell MC**, **Cheng AJ**. Carbohydrate Ingestion Increases Interstitial Glucose and Mitigates Neuromuscular Fatigue during Single-Leg Knee Extensions. *Medicine & Science in Sports & Exercise* 56: 1495, 2024. doi: 10.1249/MSS.0000000000003440.

53. **Glace BW**, **Kremenic IJ**, **McHugh MP**. Effect of carbohydrate beverage ingestion on central versus peripheral fatigue: a placebo-controlled, randomized trial in cyclists. *Appl Physiol Nutr Metab* 44: 139–147, 2019. doi: 10.1139/apnm-2017-0777.

54. **Noakes TD**. What Is the Evidence That Dietary Macronutrient Composition Influences Exercise Performance? A Narrative Review. *Nutrients* 14: 862, 2022. doi: 10.3390/nu14040862.

55. **Nybo L**. CNS Fatigue and Prolonged Exercise: Effect of Glucose Supplementation. *Medicine & Science in Sports & Exercise* 35: 589, 2003. doi: 10.1249/01.MSS.0000058433.85789.66.

56. **Nielsen J**, **Schrøder HD**, **Rix CG**, **Ørtenblad N**. Distinct effects of subcellular glycogen localization on tetanic relaxation time and endurance in mechanically skinned rat skeletal muscle fibres. *J Physiol* 587: 3679–3690, 2009. doi: 10.1113/jphysiol.2009.174862.

57. **Ørtenblad N**, **Nielsen J**, **Saltin B**, **Holmberg H-C**. Role of glycogen availability in sarcoplasmic reticulum Ca2+ kinetics in human skeletal muscle. *The Journal of Physiology* 589: 711–725, 2011. doi: 10.1113/jphysiol.2010.195982.

58. **Stephenson DG**, **Nguyen LT**, **Stephenson GMM**. Glycogen content and excitation-contraction coupling in mechanically skinned muscle fibres of the cane toad. *The Journal of Physiology* 519: 177–187, 1999. doi: 10.1111/j.1469-7793.1999.0177o.x.

59. **Jones AM**, **Vanhatalo A**. The 'Critical Power' Concept: Applications to Sports Performance with a Focus on Intermittent High-Intensity Exercise. *Sports Med* 47: 65–78, 2017. doi: 10.1007/s40279-017-0688-0.

60. **Poole DC**, **Ward SA**, **Gardner GW**, **Whipp BJ**. Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* 31: 1265–1279, 1988. doi: 10.1080/00140138808966766.

61. **Poole DC**, **Burnley M**, **Vanhatalo A**, **Rossiter HB**, **Jones AM**. Critical Power: An Important Fatigue Threshold in Exercise Physiology. *Medicine & Science in Sports & Exercise* 48: 2320, 2016. doi: 10.1249/MSS.0000000000000939.

62. **Jones AM**, **Grassi B**, **Christensen PM**, **Krustrup P**, **Bangsbo J**, **Poole DC**. Slow Component of V˙O2 Kinetics: Mechanistic Bases and Practical Applications. *Medicine & Science in Sports & Exercise* 43: 2046–2062, 2011. doi: 10.1249/MSS.0b013e31821fcfc1.

63. **Seiler S**, **Haugen O**, **Kuffel E**. Autonomic Recovery after Exercise in Trained Athletes: Intensity and Duration Effects. *Medicine & Science in Sports & Exercise* 39: 1366, 2007. doi: 10.1249/mss.0b013e318060f17d.

64. **Cerezuela-Espejo V**, **Courel-Ibáñez J**, **Morán-Navarro R**, **Martínez-Cava A**, **Pallarés JG**. The Relationship Between Lactate and Ventilatory Thresholds in Runners: Validity and Reliability of Exercise Test Performance Parameters [Online]. *Frontiers in Physiology* 9, 2018. https://www.frontiersin.org/articles/10.3389/fphys.2018.01320 [19 Oct. 2023].

65. **Faude O**, **Kindermann W**, **Meyer T**. Lactate Threshold Concepts. *Sports Med* 39: 469–490, 2009. doi: 10.2165/00007256-200939060-00003.

66. **Jamnick NA**, **Pettitt RW**, **Granata C**, **Pyne DB**, **Bishop DJ**. An Examination and Critique of Current Methods to Determine Exercise Intensity. *Sports Med* 50: 1729–1756, 2020. doi: 10.1007/s40279-020-01322-8.

67. **Pallarés JG**, **Morán-Navarro R**, **Ortega JF**, **Fernández-Elías VE**, **Mora-Rodriguez R**. Validity and Reliability of Ventilatory and Blood Lactate Thresholds in Well-Trained Cyclists. *PLoS One* 11: e0163389, 2016. doi: 10.1371/journal.pone.0163389.

68. **Neves LNS**, **Gasparini Neto VH**, **Araujo IZ**, **Barbieri RA**, **Leite RD**, **Carletti L**. Is There Agreement and Precision between Heart Rate Variability, Ventilatory, and Lactate Thresholds in Healthy Adults? *International Journal of Environmental Research and Public Health* 19: 14676, 2022. doi: 10.3390/ijerph192214676.

69. **Goodwin ML**, **Harris JE**, **Hernández A**, **Gladden LB**. Blood Lactate Measurements and Analysis during Exercise: A Guide for Clinicians. *J Diabetes Sci Technol* 1: 558–569, 2007.

70. **Gladden LB**. Lactate metabolism: a new paradigm for the third millennium. *The Journal of Physiology* 558: 5–30, 2004. doi: 10.1113/jphysiol.2003.058701.

71. **Robergs RA**, **Ghiasvand F**, **Parker D**. Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 287: R502–R516, 2004. doi: 10.1152/ajpregu.00114.2004.

72. **Davis JA**, **Rozenek R**, **DeCicco DM**, **Carizzi MT**, **Pham PH**. Comparison of three methods for detection of the lactate threshold. *Clinical Physiology and Functional Imaging* 27: 381–384, 2007. doi: 10.1111/j.1475-097X.2007.00762.x.

73. **Jamnick NA**, **Botella J**, **Pyne DB**, **Bishop DJ**. Manipulating graded exercise test variables affects the validity of the lactate threshold and V˙O2peak. *PLoS ONE* 13: e0199794, 2018. doi: 10.1371/journal.pone.0199794.

74. **Loat CER**, **Rhodes EC**. Relationship Between the Lactate and Ventilatory Thresholds During Prolonged Exercise: *Sports Medicine* 15: 104–115, 1993. doi: 10.2165/00007256-199315020-00004.

75. **Ramos-Campo DJ**, **Rubio-Arias JA**, **Ávila-Gandía V**, **Marín-Pagán C**, **Luque A**, **Alcaraz PE**. Heart rate variability to assess ventilatory thresholds in professional basketball players. *J Sport Health Sci* 6: 468–473, 2017. doi: 10.1016/j.jshs.2016.01.002.

76. **Jeukendrup AE**, **Wallis GA**. Measurement of Substrate Oxidation During Exercise by Means of Gas Exchange Measurements. *Int J Sports Med* 26: S28–S37, 2005. doi: 10.1055/s-2004-830512.

77. **Stevenson JD**, **Kilding AE**, **Plews DJ**, **Maunder E**. Prolonged cycling reduces power output at the moderate-to-heavy intensity transition. *Eur J Appl Physiol* 122: 2673–2682, 2022. doi: 10.1007/s00421-022-05036-9.

78. **Baron B**, **Noakes TD**, **Dekerle J**, **Moullan F**, **Robin S**, **Matran R**, **Pelayo P**. Why does exercise terminate at the maximal lactate steady state intensity? *Br J Sports Med* 42: 828–833, 2008. doi: 10.1136/bjsm.2007.040444.

79. **Pringle JS**, **Jones AM**. Maximal lactate steady state, critical power and EMG during cycling. *Eur J Appl Physiol* 88: 214–226, 2002. doi: 10.1007/s00421-002-0703-4.

80. **Jones AM**, **Burnley M**, **Black MI**, **Poole DC**, **Vanhatalo A**. The maximal metabolic steady state: redefining the 'gold standard.' *Physiological Reports* 7: e14098, 2019. doi: 10.14814/phy2.14098.

81. **Bellotti C**, **Calabria E**, **Capelli C**, **Pogliaghi S**. Determination of Maximal Lactate Steady State in Healthy Adults: Can NIRS Help? *Medicine & Science in Sports & Exercise* 45: 1208, 2013. doi: 10.1249/MSS.0b013e3182828ab2.

82. **Iannetta D**, **Inglis EC**, **Fullerton C**, **Passfield L**, **Murias JM**. Metabolic and performance-related consequences of exercising at and slightly above MLSS. *Scandinavian Journal of Medicine & Science in Sports* 28: 2481–2493, 2018. doi: 10.1111/sms.13280.

83. **Nixon RJ**, **Kranen SH**, **Vanhatalo A**, **Jones AM**. Steady-state $$\dot{V}{\text{O}}_{2}$$ above MLSS: evidence that critical speed better represents maximal metabolic steady state in well-trained runners. *Eur J Appl Physiol* 121: 3133–3144, 2021. doi: 10.1007/s00421-021-04780-8.

84. **Smith CGM**, **Jones AM**. The relationship between critical velocity, maximal lactate steady-state velocity and lactate turnpoint velocity in runners. *European Journal of Applied Physiology* 85: 19–26, 2001. doi: 10.1007/s004210100384.

85. **Hill AV**. The Physiological Basis of Athletic Records1. *Nature* 116: 544–548, 1925. doi: 10.1038/116544a0.

86. **Monod H**, **Scherrer J**. THE WORK CAPACITY OF A SYNERGIC MUSCULAR GROUP. *Ergonomics* 8: 329–338, 1965. doi: 10.1080/00140136508930810.

87. **Moritani T**, **Nagata A**, **Devries HA**, **Muro M**. Critical power as a measure of physical work capacity and anaerobic threshold. *Ergonomics* 24: 339–350, 1981. doi: 10.1080/00140138108924856.

88. **Vanhatalo A**, **Jones AM**, **Burnley M**. Application of Critical Power in Sport. .

89. **Vanhatalo A**, **Black MI**, **DiMenna FJ**, **Blackwell JR**, **Schmidt JF**, **Thompson C**, **Wylie LJ**, **Mohr M**, **Bangsbo J**, **Krustrup P**, **Jones AM**. The mechanistic bases of the power–time relationship: muscle metabolic responses and relationships to muscle fibre type. *The Journal of Physiology* 594: 4407, 2016. doi: 10.1113/JP271879.

90. **Vanhatalo A**, **Doust JH**, **Burnley M**. Determination of Critical Power Using a 3-min All-out Cycling Test. *Medicine & Science in Sports & Exercise* 39: 548, 2007. doi: 10.1249/mss.0b013e31802dd3e6.

91. **Muniz-Pumares D**, **Karsten B**, **Triska C**, **Glaister M**. Methodological Approaches and Related Challenges Associated With the Determination of Critical Power and Curvature Constant. *The Journal of Strength & Conditioning Research* 33: 584, 2019. doi: 10.1519/JSC.0000000000002977.

92. **Burnley M**, **Jones AM**. Power–duration relationship: Physiology, fatigue, and the limits of human performance. *European Journal of Sport Science* 18: 1–12, 2018. doi: 10.1080/17461391.2016.1249524.

93. **Sylta Ø**, **Tønnessen E**, **Seiler S**. From Heart-Rate Data to Training Quantification: A Comparison of 3 Methods of Training-Intensity Analysis. .

94. **Seiler KS**, **Kjerland GØ**. Quantifying training intensity distribution in elite endurance athletes: is there evidence for an "optimal" distribution? *Scand J Med Sci Sports* 16: 49–56, 2006. doi: 10.1111/j.1600-0838.2004.00418.x.

95. **Halson SL**. Monitoring Training Load to Understand Fatigue in Athletes. *Sports Med* 44: 139–147, 2014. doi: 10.1007/s40279-014-0253-z.

96. **Barrett AMS**, **Maunder E**. Prolonged running reduces speed at the moderate-to-heavy intensity transition without additional reductions due to increased eccentric load. .

97. **Dudley-Rode H**, **Zinn C**, **Plews DJ**, **Charoensap T**, **Maunder E**. Carbohydrate ingestion during prolonged exercise blunts the reduction in power output at the moderate-to-heavy intensity transition. .

98. **Gallo G**, **Faelli EL**, **Ruggeri P**, **Filipas L**, **Codella R**, **Plews DJ**, **Maunder E**. Power output at the moderate-to-heavy intensity transition decreases in a non-linear fashion during prolonged exercise. .

99. **Hamilton K**, **Kilding AE**, **Plews DJ**, **Mildenhall MJ**, **Waldron M**, **Charoensap T**, **Cox TH**, **Brick MJ**, **Leigh WB**, **Maunder E**. Durability of the moderate-to-heavy-intensity transition is related to the effects of prolonged exercise on severe-intensity performance. .

100. **Nuuttila O-P**, **Laatikainen-Raussi V**, **Vohlakari K**, **Laatikainen-Raussi I**, **Ihalainen JK**. Durability in recreational runners: effects of 90-min low-intensity exercise on the running speed at the lactate threshold. *Eur J Appl Physiol* 125: 697–705, 2025. doi: 10.1007/s00421-024-05631-y.

101. **Clark IE**, **Vanhatalo A**, **Bailey SJ**, **Wylie LJ**, **Kirby BS**, **Wilkins BW**, **Jones AM**. Effects of Two Hours of Heavy-Intensity Exercise on the Power–Duration Relationship. .

102. **Clark IE**, **Vanhatalo A**, **Thompson C**, **Joseph C**, **Black MI**, **Blackwell JR**, **Wylie LJ**, **Tan R**, **Bailey SJ**, **Wilkins BW**, **Kirby BS**, **Jones AM**. Dynamics of the power-duration relationship during prolonged endurance exercise and influence of carbohydrate ingestion. *Journal of Applied Physiology* 127: 726–736, 2019. doi: 10.1152/japplphysiol.00207.2019.

103. **Clark IE**, **Vanhatalo A**, **Thompson C**, **Wylie LJ**, **Bailey SJ**, **Kirby BS**, **Wilkins BW**, **Jones AM**. Changes in the power-duration relationship following prolonged exercise: estimation using conventional and all-out protocols and relationship with muscle glycogen. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 317: R59–R67, 2019. doi: 10.1152/ajpregu.00031.2019.

104. **Ørtenblad N**, **Zachariassen M**, **Nielsen J**, **Gejl KD**. Substrate utilization and durability during prolonged intermittent exercise in elite road cyclists. *Eur J Appl Physiol* 124: 2193–2205, 2024. doi: 10.1007/s00421-024-05437-y.

105. **Spragg J**, **Leo P**, **Swart J**. The relationship between training characteristics and durability in professional cyclists across a competitive season. *European Journal of Sport Science* 23: 489–498, 2023. doi: 10.1080/17461391.2022.2049886.

106. **Valenzuela PL**, **Alejo LB**, **Ozcoidi LM**, **Lucia A**, **Santalla A**, **Barranco-Gil D**. Durability in Professional Cyclists: A Field Study. .

107. **Bergström J**, **Hermansen L**, **Hultman E**, **Saltin B**. Diet, Muscle Glycogen and Physical Performance. *Acta Physiologica Scandinavica* 71: 140–150, 1967. doi: 10.1111/j.1748-1716.1967.tb03720.x.

108. **Hermansen L**, **Hultman E**, **Saltin B**. Muscle Glycogen during Prolonged Severe Exercise. *Acta Physiologica Scandinavica* 71: 129–139, 1967. doi: 10.1111/j.1748-1716.1967.tb03719.x.

109. **Alghannam AF**, **Jedrzejewski D**, **Tweddle MG**, **Gribble H**, **Bilzon J**, **Thompson D**, **Tsintzas K**, **Betts JA**. Impact of Muscle Glycogen Availability on the Capacity for Repeated Exercise in Man. *Medicine & Science in Sports & Exercise* 48: 123, 2016. doi: 10.1249/MSS.0000000000000737.

110. **Bangsbo J**, **Graham TE**, **Kiens B**, **Saltin B**. Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J Physiol* 451: 205–227, 1992.

111. **Gollnick PD**, **Piehl K**, **Saubert CW**, **Armstrong RB**, **Saltin B**. Diet, exercise, and glycogen changes in human muscle fibers. *Journal of Applied Physiology* 33: 421–425, 1972. doi: 10.1152/jappl.1972.33.4.421.

112. **Hargreaves M**, **McConell G**, **Proietto J**. Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *Journal of Applied Physiology* 78: 288–292, 1995. doi: 10.1152/jappl.1995.78.1.288.

113. **Pernow B**, **Saltin B**. Availability of substrates and capacity for prolonged heavy exercise in man. *J Appl Physiol* 31: 416–422, 1971. doi: 10.1152/jappl.1971.31.3.416.

114. **Nielsen J**, **Suetta C**, **Hvid LG**, **Schrøder HD**, **Aagaard P**, **Ørtenblad N**. Subcellular localization-dependent decrements in skeletal muscle glycogen and mitochondria content following short-term disuse in young and old men. *American Journal of Physiology-Endocrinology and Metabolism* 299: E1053–E1060, 2010. doi: 10.1152/ajpendo.00324.2010.

115. **Nielsen J**, **Holmberg H-C**, **Schrøder HD**, **Saltin B**, **Ørtenblad N**. Human skeletal muscle glycogen utilization in exhaustive exercise: role of subcellular localization and fibre type. *The Journal of Physiology* 589: 2871–2885, 2011. doi: 10.1113/jphysiol.2010.204487.

116. **Ørtenblad N**, **Westerblad H**, **Nielsen J**. Muscle glycogen stores and fatigue. *The Journal of Physiology* 591: 4405–4413, 2013. doi: 10.1113/jphysiol.2013.251629.

117. **Marchand I**, **Tarnopolsky M**, **Adamo KB**, **Bourgeois JM**, **Chorneyko K**, **Graham TE**. Quantitative assessment of human muscle glycogen granules size and number in subcellular locations during recovery from prolonged exercise. *J Physiol* 580: 617–628, 2007. doi: 10.1113/jphysiol.2006.122457.

118. **Jensen R**, **Ørtenblad N**, **Stausholm M-LH**, **Skjærbæk MC**, **Larsen DN**, **Hansen M**, **Holmberg H-C**, **Plomgaard P**, **Nielsen J**. Heterogeneity in subcellular muscle glycogen utilisation during exercise impacts endurance capacity in men. *The Journal of Physiology* 598: 4271–4292, 2020. doi: 10.1113/JP280247.

119. **Duhamel TA**, **Perco JG**, **Green HJ**. Manipulation of dietary carbohydrates after prolonged effort modifies muscle sarcoplasmic reticulum responses in exercising males. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 291: R1100–R1110, 2006. doi: 10.1152/ajpregu.00858.2005.

120. **Jensen R**, **Nielsen J**, **Ørtenblad N**. Inhibition of glycogenolysis prolongs action potential repriming period and impairs muscle function in rat skeletal muscle. *The Journal of Physiology* 598: 789–803, 2020. doi: 10.1113/JP278543.

121. **Nielsen J**, **Dubillot P**, **Stausholm M-LH**, **Ørtenblad N**. Excitation–Contraction Coupling: Specific ATPases drive compartmentalized glycogen utilization in rat skeletal muscle. *The Journal of General Physiology* 154, 2022. doi: 10.1085/jgp.202113071.

122. **Cairns SP**, **Renaud J-M**. The potassium–glycogen interaction on force and excitability in mouse skeletal muscle: implications for fatigue. *The Journal of Physiology* 601: 5669–5687, 2023. doi: 10.1113/JP285129.

123. **Grisdale RK**, **Jacobs I**, **Cafarelli E**. Relative effects of glycogen depletion and previous exercise on muscle force and endurance capacity. *Journal of Applied Physiology* 69: 1276–1282, 1990. doi: 10.1152/jappl.1990.69.4.1276.

124. **Osborne MA**, **Schneider DA**. Muscle glycogen reduction in man: relationship between surface EMG activity and oxygen uptake kinetics during heavy exercise. *Experimental Physiology* 91: 179–189, 2006. doi: 10.1113/expphysiol.2005.031450.

125. **Felig P**, **Cherif A**, **Minagawa A**, **Wahren J**. Hypoglycemia during Prolonged Exercise in Normal Men. *N Engl J Med* 306: 895–900, 1982. doi: 10.1056/NEJM198204153061503.

126. **Murray B**, **Rosenbloom C**. Fundamentals of glycogen metabolism for coaches and athletes. *Nutrition Reviews* 76: 243–259, 2018. doi: 10.1093/nutrit/nuy001.

127. **Essén B**, **Henriksson J**. Glycogen Content of Individual Muscle Fibres in Man. *Acta Physiologica Scandinavica* 90: 645–647, 1974. doi: 10.1111/j.1748-1716.1974.tb05629.x.

128. **Tsintzas OK**, **Williams C**, **Boobis L**, **Greenhaff P**. Carbohydrate ingestion and single muscle fiber glycogen metabolism during prolonged running in men. *Journal of Applied Physiology* 81: 801–809, 1996. doi: 10.1152/jappl.1996.81.2.801.

129. **Vigh-Larsen JF**, **Ørtenblad N**, **Nielsen J**, **Emil Andersen O**, **Overgaard K**, **Mohr M**. The Role of Muscle Glycogen Content and Localization in High-Intensity Exercise Performance: A Placebo-Controlled Trial. *Medicine & Science in Sports & Exercise* 54: 2073, 2022. doi: 10.1249/MSS.0000000000003002.

130. **Howald H**, **Hoppeler H**, **Claassen H**, **Mathieu O**, **Straub R**. Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. *Pflugers Arch* 403: 369–376, 1985. doi: 10.1007/BF00589248.

131. **Nielsen J**, **Jensen R**, **Ørtenblad N**. Assessments of individual fiber glycogen and mitochondrial volume percentages reveal a graded reduction in muscle oxidative power during prolonged exhaustive exercise. *Scandinavian Med Sci Sports* 34: e14571, 2024. doi: 10.1111/sms.14571.

132. **Gerdle B**, **Henriksson-Larsén K**, **Lorentzon R**, **Wretling M-L**. Dependence of the mean power frequency of the electromyogram on muscle force and fibre type. *Acta Physiologica Scandinavica* 142: 457–465, 1991. doi: 10.1111/j.1748-1716.1991.tb09180.x.

133. **Häkkukinen K**, **Komi PV**, **Alén M**. Effect of explosive type strength training on isometric force- and relaxation-time, electromyographic and muscle fibre characteristics of leg extensor muscles. *Acta Physiologica Scandinavica* 125: 587–600, 1985. doi: 10.1111/j.1748-1716.1985.tb07760.x.

134. **Kupa EJ**, **Roy SH**, **Kandarian SC**, **De Luca CJ**. Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *Journal of Applied Physiology* 79: 23–32, 1995. doi: 10.1152/jappl.1995.79.1.23.

135. **Moritani T**, **Muro M**, **Kijima A**, **Gaffney FA**, **Parsons D**. Electromechanical changes during electrically induced and maximal voluntary contractions: Surface and intramuscular EMG responses during sustained maximal voluntary contraction. *Experimental Neurology* 88: 484–499, 1985. doi: 10.1016/0014-4886(85)90065-2.

136. **Viitasalo JHT**, **Komi PV**. Signal characteristics of EMG during fatigue. *Europ J Appl Physiol* 37: 111–121, 1977. doi: 10.1007/BF00421697.

137. **Goswami A**, **Sadhukhan AK**, **Gupta S**. EMG CHARACTERISTICS AND FIBRE COMPOSITION: STUDY ON RECTUS FEMORIS OF SPRINTERS AND LONG DISTANCE RUNNERS. .

138. **De Luca CJ**. Myoelectrical manifestations of localized muscular fatigue in humans. *Crit Rev Biomed Eng* 11: 251–279, 1984.

139. **Farina D**, **Merletti R**, **Enoka RM**. The extraction of neural strategies from the surface EMG. *J Appl Physiol* 96, 2004.

140. **Krustrup P**, **S□derlund K**, **Mohr M**, **Bangsbo J**. The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. *Pfl□gers Archiv European Journal of Physiology* 447: 855–866, 2004. doi: 10.1007/s00424-003-1203-z.

141. **Tenan MS**, **McMurray RG**, **Troy Blackburn B**, **McGrath M**, **Leppert K**. The relationship between blood potassium, blood lactate, and electromyography signals related to fatigue in a progressive cycling exercise test. *Journal of Electromyography and Kinesiology* 21: 25–32, 2011. doi: 10.1016/j.jelekin.2010.09.002.

142. **Chidnok W**, **Fulford J**, **Bailey SJ**, **DiMenna FJ**, **Skiba PF**, **Vanhatalo A**, **Jones AM**. Muscle metabolic determinants of exercise tolerance following exhaustion: relationship to the “critical power.” *Journal of Applied Physiology* 115: 243–250, 2013. doi: 10.1152/japplphysiol.00334.2013.

143. **Joyner MJ**, **Coyle EF**. Endurance exercise performance: the physiology of champions. *J Physiol* 586: 35–44, 2008. doi: 10.1113/jphysiol.2007.143834.

144. **Stevenson JD**, **Kilding AE**, **Plews DJ**, **Maunder E**. Prolonged exercise shifts ventilatory parameters at the moderate-to-heavy intensity transition. *Eur J Appl Physiol* 124: 309–315, 2024. doi: 10.1007/s00421-023-05285-2.

145. **Seiler S**, **Haugen O**, **Kuffel E**. Autonomic Recovery after Exercise in Trained Athletes: Intensity and Duration Effects. *Medicine & Science in Sports & Exercise* 39: 1366–1373, 2007. doi: 10.1249/mss.0b013e318060f17d.

146. **Bangsbo J**, **Graham TE**, **Kiens B**, **Saltin B**. Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J Physiol* 451: 205–227, 1992.

147. **Jensen R**, **Ørtenblad N**, **Stausholm M-LH**, **Skjaerbaek MC**, **Larsen DN**, **Hansen M**, **Holmberg H-C**, **Plomgaard P**, **Nielsen J**. Heterogeneity in subcellular muscle glycogen utilisation during exercise impacts endurance capacity in men. *J Physiol* 598: 4271–4292, 2020. doi: 10.1113/JP280247.

148. **Tenan MS**, **Blackburn JT**. Exercise-Induced Glycogen Reduction Increases Muscle Activity. .

149. **James JJ**, **Leach OK**, **Young AM**, **Newman AN**, **Mpongo KL**, **Quirante JM**, **Wardell DB**, **Ahmadi M**, **Gifford JR**. The exercise power-duration relationship is equally reproducible in eumenorrheic female and male humans. *Journal of Applied Physiology* 134: 230–241, 2023. doi: 10.1152/japplphysiol.00416.2022.

150. **Mattu AT**, **Iannetta D**, **MacInnis MJ**, **Doyle-Baker PK**, **Murias JM**. Menstrual and oral contraceptive cycle phases do not affect submaximal and maximal exercise responses. *Scandinavian Journal of Medicine & Science in Sports* 30: 472–484, 2020. doi: 10.1111/sms.13590.

151. **Williams JS**, **Stone JC**, **Masood Z**, **Bostad W**, **Gibala MJ**, **MacDonald MJ**. The impact of natural menstrual cycle and oral contraceptive pill phase on substrate oxidation during rest and acute submaximal aerobic exercise. *Journal of Applied Physiology* 135: 642–654, 2023. doi: 10.1152/japplphysiol.00111.2023.

152. **Tarnopolsky MA**, **Zawada C**, **Richmond LB**, **Carter S**, **Shearer J**, **Graham T**, **Phillips SM**. Gender differences in carbohydrate loading are related to energy intake. *J Appl Physiol (1985)* 91: 225–230, 2001. doi: 10.1152/jappl.2001.91.1.225.

153. **Vigh-Larsen JF**, **Ørtenblad N**, **Emil Andersen O**, **Thorsteinsson H**, **Kristiansen TH**, **Bilde S**, **Mikkelsen MS**, **Nielsen J**, **Mohr M**, **Overgaard K**. Fibre type- and localisation-specific muscle glycogen utilisation during repeated high-intensity intermittent exercise. *J Physiol* 600: 4713–4730, 2022. doi: 10.1113/JP283225.

154. **Jones AM**, **Vanhatalo A**, **Burnley M**, **Morton RH**, **Poole DC**. Critical Power: Implications for Determination of V˙O2max and Exercise Tolerance. *Medicine & Science in Sports & Exercise* 42: 1876–1890, 2010. doi: 10.1249/MSS.0b013e3181d9cf7f.

155. **Özgünen KT**, **Çelik U**, **Kurdak SS**. Determination of an optimal threshold value for muscle activity detection in EMG analysis. *Journal of Sports Science and Medicine* 9: 620–628, 2010.

156. **Yoon S**, **Cederbaum LA**, **Côté JN**. Females show less decline in contractile function than males after repeated all-out cycling. .

157. **Del Vecchio A**, **Negro F**, **Felici F**, **Farina D**. Distribution of muscle fibre conduction velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle. *Acta Physiologica* 222: e12930, 2018. doi: 10.1111/apha.12930.

158. **Masuda T**, **De Luca CJ**. Recruitment threshold and muscle fiber conduction velocity of single motor units. *Journal of Electromyography and Kinesiology* 1: 116–123, 1991. doi: 10.1016/1050-6411(91)90005-P.

159. **Houtman CJ**, **Stegeman DF**, **Van Dijk JP**, **Zwarts MJ**. Changes in muscle fiber conduction velocity indicate recruitment of distinct motor unit populations. *Journal of Applied Physiology* 95: 1045–1054, 2003. doi: 10.1152/japplphysiol.00665.2002.

160. **De Luca CJ**, **Hostage EC**. Relationship Between Firing Rate and Recruitment Threshold of Motoneurons in Voluntary Isometric Contractions. *Journal of Neurophysiology* 104: 1034–1046, 2010. doi: 10.1152/jn.01018.2009.

161. **Gollnick PD**, **Piehl K**, **Saltin B**. Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *The Journal of Physiology* 241: 45–57, 1974. doi: 10.1113/jphysiol.1974.sp010639.

162. **Vøllestad NK**, **Blom PCS**. Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiologica Scandinavica* 125: 395–405, 1985. doi: 10.1111/j.1748-1716.1985.tb07735.x.

163. **Schytz CT**, **Ørtenblad N**, **Gejl KD**, **Nielsen J**. Differential utilisation of subcellular skeletal muscle glycogen pools: a comparative analysis between 1 and 15 min of maximal exercise. *The Journal of Physiology* 602: 1681–1702, 2024. doi: 10.1113/JP285762.

164. **Rotto DM**, **Kaufman MP**. Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *Journal of Applied Physiology* 64: 2306–2313, 1988. doi: 10.1152/jappl.1988.64.6.2306.

165. **Vigh-Larsen JF**, **Ørtenblad N**, **Spriet LL**, **Overgaard K**, **Mohr M**. Muscle Glycogen Metabolism and High-Intensity Exercise Performance: A Narrative Review. *Sports Med* 51: 1855–1874, 2021. doi: 10.1007/s40279-021-01475-0.

166. **Hargreaves M**, **Finn JP**, **Withers RT**, **Scroop GC**, **Mackay M**, **Snow RJ**, **Carey MF**, **Halbert J**. Effect of muscle glycogen availability on maximal exercise performance. *Eur J Appl Physiol* 75: 188–192, 1997. doi: 10.1007/s004210050146.

167. **Ansdell P**, **Thomas K**, **Hicks KM**, **Hunter SK**, **Howatson G**, **Goodall S**. Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Experimental Physiology* 105: 2007–2021, 2020. doi: 10.1113/EP088548.

168. **Bruinvels G**, **Burden RJ**, **McGregor AJ**, **Ackerman KE**, **Dooley M**, **Richards T**, **Pedlar C**. Sport, exercise and the menstrual cycle: where is the research? *Br J Sports Med* 51: 487–488, 2017. doi: 10.1136/bjsports-2016-096279.

169. **Costello JT**, **Bieuzen F**, **Bleakley CM**. Where are all the female participants in Sports and Exercise Medicine research? *European Journal of Sport Science* 14: 847–851, 2014. doi: 10.1080/17461391.2014.911354.

170. **Cowley ES**, **Olenick AA**, **McNulty KL**, **Ross EZ**. "Invisible Sportswomen": The Sex Data Gap in Sport and Exercise Science Research. *Women in Sport and Physical Activity Journal* 29: 146–151, 2021. doi: 10.1123/wspaj.2021-0028.

171. **Emmonds S**, **Heyward O**, **Jones B**. The Challenge of Applying and Undertaking Research in Female Sport. *Sports Medicine - Open* 5: 51, 2019. doi: 10.1186/s40798-019-0224-x.

172. **Carter SL**, **Rennie C**, **Tarnopolsky MA**. Substrate utilization during endurance exercise in men and women after endurance training. *American Journal of Physiology-Endocrinology and Metabolism* 280: E898–E907, 2001. doi: 10.1152/ajpendo.2001.280.6.E898.

173. **Tarnopolsky MA**. Sex Differences in Exercise Metabolism and the Role of 17-Beta Estradiol. *Medicine & Science in Sports & Exercise* 40: 648, 2008. doi: 10.1249/MSS.0b013e31816212ff.