INTEGRATION OF FATIGUE AND PERFORMANCE THROUGH THE POWER-DURATION RELATIONSHIP

Submitted by Matthew Ian Black to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Sport and Health Sciences in November 2016

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……M. Black……

ABSTRACT

The hyperbolic power-duration relationship for high-intensity exercise is defined by two parameters: a power asymptote (critical power; CP) and a curvature constant (W′) which have been associated with sustainable, and non-sustainable metabolism, respectively. Conventionally, the power-duration relationship is derived from a series of constant work-rate (CWR) prediction trials. However, it may be advantageous to establish this relationship using the 3-min all-out test, or a series of time-trial (TT) tests. The validity, plasticity and applicability of the power-duration relationship derived using these different protocols has not been experimentally verified. Moreover, although the CP has been shown to represent a threshold in muscle metabolic and neuromuscular responses during single-legged knee-extension exercise, it is unclear whether this is also the case during whole-body exercise. The purpose of this thesis, therefore, was to: 1) evaluate the predictive validity of the laboratory-based 3-min all-out test; 2) investigate the plasticity and applicability of the power-duration relationship; 3) elucidate the mechanistic bases for fatigue during whole-body exercise above- and below-CP. In study 1, the CP (r=-0.83, P<0.001) derived from the 3-min all-out test was more strongly associated with 16.1-km road cycling TT performance than; maximum oxygen uptake ($\dot{V}O_{2max}$) (r=-0.60, P>0.05); gas exchange threshold (GET), (r=-0.60, P>0.05); and, respiratory compensation point (RCP), (r=-0.68, P<0.05). In study 2, the power-duration relationship derived from CWR prediction trials overestimated ramp incremental exercise performance by 2.9 ± 2.4%, and the predictive error was associated with the magnitude of the W′ (r=-0.56; P<0.05). Study 3 demonstrated that the CP derived from a series of self-paced TTs (265 ± 44 W) was greater (P<0.05) than the CP derived from CWR prediction trials

(250 ± 47 W), while W′ was not different between the protocols (TT: 18.1 ± 5.7 kJ, CWR: 20.6 ± 7.4 kJ), and the increase in CP was associated (r=0.88, P<0.05, n=20) with faster mean response time of pulmonary O_2 uptake during the TTs (TT: 34 ± 16 s, CWR: 39 ± 19 s, P<0.05). In study 4, muscle biopsies revealed a similar (P>0.05) muscle metabolic milieu (i.e., low pH, low [PCr] and high [lactate]) at the limit of tolerance (T_{lim}) for all severe-intensity (>CP) work rates irrespective of duration (~2-14 min). The muscle metabolic perturbation was greater at T_{lim} following severe-intensity exercise compared to exercise heavy-intensity exercise (<CP, >GET), and also following severe- and heavy-intensity exercise compared to moderate-intensity exercise (<CP, <GET) (all P<0.05). Moreover, the rates of change in M-wave amplitude and neural drive were significantly correlated with changes in muscle metabolic ([PCr], [lactate]) and blood ionic/acid-base status ([lactate], [K^+]) during severe- and heavy-intensity exercise (all P<0.05), but not during moderate-intensity exercise (P>0.05). Finally, study 5 found no differences in muscle carnosine content or the power-duration relationship following 4-weeks of beta-alanine (BA) supplementation (6.4 $g.d^{-1}$). Therefore, the results of this thesis demonstrate that the CP model is a powerful predictor of exercise performance, but only when the work-rate forcing function of the prediction trials is closely matched to the performance trial. Furthermore, this thesis provides novel insights into the underlying mechanisms that characterise the power-duration relationship during whole-body exercise which explains the plasticity and thus applicability of the power-duration relationship.

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Symbols and abbreviations

[ ]	concentration
Δ	difference
Δ%	percentage (%) difference between GET and $\dot{V}O_2max$
>	above
<	below
µs	micro-second (one-millionth of a second)
^{31}P-MRS	^{31}phosphorous nuclear magnetic resonance spectroscopy
^{1}H-MRS	^{1}hydrogen proton magnetic resonance spectroscopy
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BIF	best individual fit
BLa	blood lactate
Ca^{2+}	calcium
CV%	coefficient of variation (presented as a percentage (%))
CMAP	compound muscle action potential
CP	critical power (i.e., asymptote of the P-T_{lim} relationship)
Cr	creatine
CT	critical torque
CWR	constant work rate
EP	end power of the 3-min all-out test
EMG	electromyogram
F_iO_2	fraction of inspired oxygen
GET	gas exchange threshold
H^+	hydrogen ion/proton
H_2O	water
iEMG	integrated electromyogram
K^+	potassium cation
$[K^+]_e$	concentration of extracellular potassium
kJ	kilojoule

L	litre
LT	lactate threshold
LF	linear factor
m.	muscle
MRT	mean response time (approximated by τ + TD of an exponential)
min	minute
ml	millilitre
mA	milliamp
mM	micromole
M_{max}	maximal M-wave
mV	millivolt
MVC	maximal voluntary contraction
Na^+	sodium cation
O_2	oxygen
P	power output
P_{max}	peak power output
PCr	phosphocreatine
P_i	inorganic phosphate
RCP	respiratory compensation point
rpm	revolutions per minute (cadence)
S	ramp slope
SEE	standard error of the estimate
SR	sarcoplasmic reticulum
τ	time constant (time to reach 63% of an exponential response)
TD	exponential response time delay
T_{lim}	time to the limit of tolerance
TT	time-trial
$\dot{V}CO_2$	carbon dioxide output
$\dot{V}E$	ventilation (expired)
$\dot{V}O_2$	oxygen uptake

$\dot{V}O_{2max}$	maximum oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
W′	curvature constant of the hyperbolic P-T_{lim} relationship
W	work done
WIF	worst individual fit
WR	work rate

Declaration

The material contained within this thesis is original work conducted and written by the author. The following communications and publications are a direct consequence of this work.

Publications

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Black, M.I., Jones, A.M., Bailey, S.J., Vanhatalo, A. (2015). Self-pacing increases critical power and improves performance during severe-intensity exercise. *Applied Physiology, Nutrition, and Metabolism,* **40**, 662-670.

Black, M.I., Jones, A.M., Kelly, J., Bailey, S.J., Vanhatalo, A. The prediction of exercise tolerance during ramp incremental exercise using the power-duration parameters derived from constant work-rate prediction trials. *European Journal of Applied Physiology.*[Epub ahead of print]: DOI 10.1007/s00421-016-3491-y.

Black, M.I., Jones, A.M., Blackwell, J.R., Bailey, S.J., McDonagh, S.T.J., Thompson, C., Kelly, J., Sumners, P., Mileva, K.J., Bowtell, J.L., Vanhatalo, A. (2016). Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. *Journal of Applied Physiology.* [Epub ahead of print]: DOI 10.1152/japplphysiol.00942.2016

Conference communications

Black, M.I., Jones, A.M., Kelly, J., Vanhatalo, A. (2012). The prediction of time-to-exhaustion during ramp incremental exercise using the critical power concept. *BASES Student Conference, London, UK.*

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Black, M.I., Jones, A.M., Bowtell, J.L., Mileva, K.N., Sumners, D.P., Blackwell, J.R., Vanhatalo, A. (2014). Muscle metabolic responses and fatigue mechanisms during moderate-, heavy-, and severe-intensity cycle exercise. *European College of Sport Science, Amsterdam, Netherlands.*

Other Publications

Wylie, L.J., Mohr, M., Krustrup, P., Jackman, S.R., Ermidis, G., Kelly, J., **Black, M.I.,** Bailey, S.J., Vanhatalo, A., Jones, A.M. (2013). Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. *European Journal of Applied Physiology,* **113**, 1673-1684.

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in laboratory testing; and, to attend conferences (oh, and frequent the Raddy). You have exceeded all expectations of a research supervisor; providing an exceptional role-model, whose door was always open for me to ask questions and vent frustrations. Your devotion and commitment to research and my PhD has been truly inspirational and under your tutorship I have achieved more during my PhD than I could have ever hoped. I cannot thank you both enough for your support and guidance throughout my PhD and your valuable contributions to my career. I hope to come close to matching your achievements.

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One of the fundamental characteristics of striated muscle, and the one involving the greatest difficulty in investigation, is the great rapidity with which changes take place in it. There is no doubt that ultimately the muscle is a chemical mechanism…If we were aware of all the chemical events, we should know all that was necessary about the machine which we are studying. Unfortunately, the investigation of chemical events is a slow and laborious process.

A.V. Hill

Chapter 1 INTRODUCTION

In an attempt to understand the limits of human performance, the English physiologist Archibald Vivian Hill produced speed-time curves from the world record times of various athletic events (Figure 1.1). The shape of the curves led Hill to postulate that the causes of fatigue may differ according to exercise intensity and duration. It was evident that very fast muscular contractions, which require fairly large amounts of immediately available energy, could only be maintained for relatively short periods of time. In contrast, exercise associated with slower, less powerful contractions which require a more gradual energy supply could be maintained for much longer. These observations, although formed nearly a century ago, are integral to our understanding of muscle physiology and performance, and provide the foundations for the present body of work.

1.1 Skeletal muscle bioenergetics

Skeletal muscle contraction is dependent on the release of chemical energy from the hydrolysis of intramuscular adenosine triphosphate (ATP), the muscles' sole fuel for force generation. At the onset of exercise, or at the initiation of any muscular contraction, there is an instantaneous increase in energy demand and, thus a concomitant utilisation of intramuscular ATP. However, human skeletal muscle has only a limited store of ATP which can be exhausted within a few seconds. To avert an abrupt and debilitating depletion in intramuscular ATP following the onset of exercise, the immediate and continued resynthesis of this molecule is imperative. In order to prevent a precipitous fall in intramuscular ATP, and to prolong muscular work, ATP can be resynthesised from its products,

adenosine diphosphate (ADP) and inorganic phosphate (P_i), via the energy from endogenous macronutrient catabolism. Importantly, the catabolism of PCr and ATP to P_i and creatine (Cr), and ADP and P_i, respectively, stimulates glycolysis (Kushmerick et al. 1992; Walsh et al. 2001). The pathways for cellular respiration, therefore, occur in tandem and regulate one another.

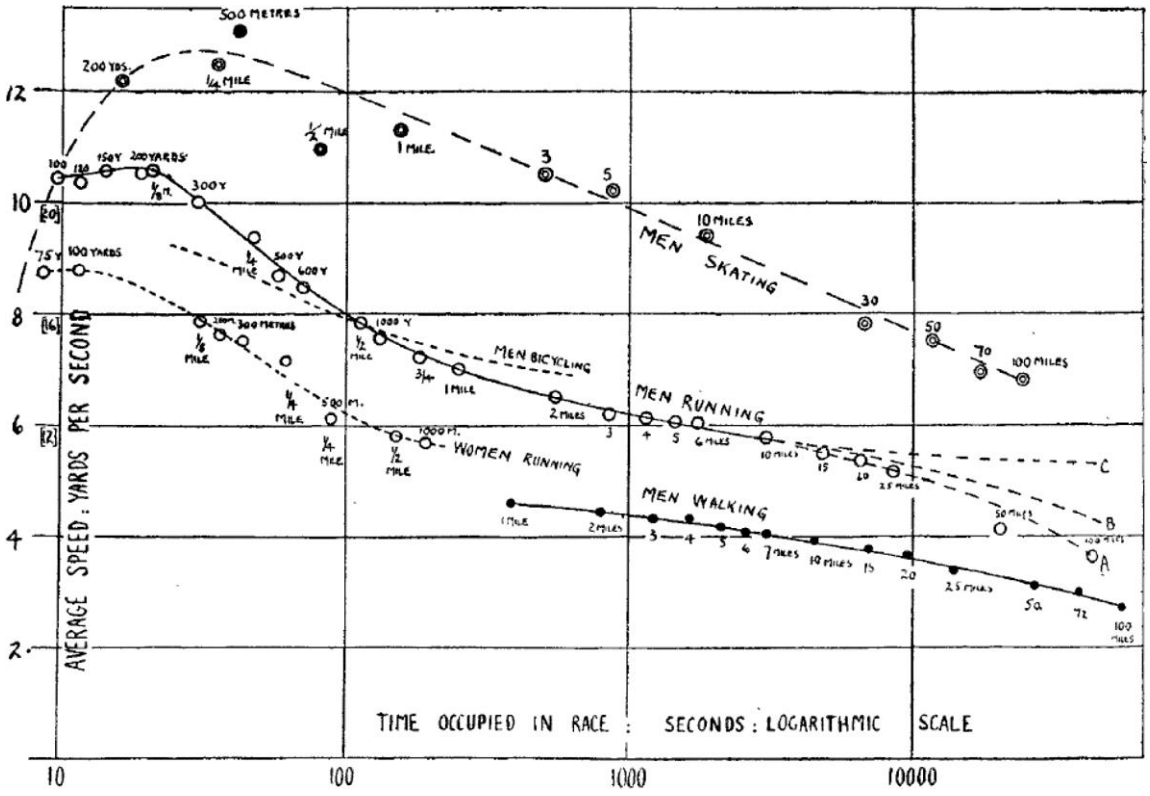

Figure 1.1 A.V. Hill's (Hill, 1925) original plot of world record performances.

Note: time is plotted across the X-axis and speed on the Y-axis. The sporting event is indicated next to each trace. The top dashed trace is for men's speed skating, the solid-lined middle trace represents men's running, the dashed-lined middle trace is illustrative of men's bicycling, the dashed-lined bottom trace demonstrates women's running and the solid-lined trace at the bottom of the figure denotes men's walking.

Glycolysis and hydrolysis of phosphocreatine (PCr), commonly termed 'substrate level phosphorylation', is stimulated by the increase in intramuscular ADP concentration (Kushmerick et al. 1992; Walsh et al. 2001). Following the onset of exercise, muscle PCr is rapidly hydrolysed in a process catalysed by the enzyme creatine kinase. The chemical energy liberated during the hydrolysis of each PCr molecule is sufficient to resynthesise 1 molecule of ATP. This process activates the anaerobic catabolism of glucose and glycogen (glycolysis), which releases sufficient chemical energy to resynthesise 2 or 3 molecules of ATP, respectively. However, whilst substrate level phosphorylation permits the rapid resynthesis of ATP, these metabolic pathways are reliant on finite metabolic substrates (PCr and glycogen). Furthermore, the breakdown of PCr and anaerobic glycolysis results in the accumulation of inorganic phosphate (P_i) and hydrogen ions (H^+), metabolites that have been reported to interfere with the process of muscle contraction (Allen, 2009; Allen et al. 2008; Fowles et al. 2002; Green, 1998; 2004; McKenna et al. 2008). Therefore, the resynthesis of ATP via substrate level-phosphorylation is not sustainable.

Although a slower means of ATP resynthesis, oxidative phosphorylation has a greater ATP yield compared to substrate level phosphorylation, and can utilise carbohydrate and fat (and when carbohydrate and fat stores are depleted, protein) as metabolic substrates. For example, in contrast to anaerobic glycolysis, which has a net gain of 2 ATP molecules, glucose oxidation yields 38 molecules of ATP. Fat sources are even more energy rich, with the oxidation of palymitate, a free fatty acid, liberating sufficient energy for the resynthesis of 129 ATP molecules. Furthermore, oxidative phosphorylation is not associated with the accumulation of fatigue-related metabolites (i.e. P_i and H^+), but instead results in the production of

carbon dioxide (CO_2) and water (H_2O) which are easily regulated through ventilation and osmosis, respectively. However, in comparison to substrate level phosphorylation, the rate of resynthesis of ATP via oxidative phosphorylation is much slower.

The constraints placed on the capacity and rate of ATP resynthesis via substrate level phosphorylation and oxidative phosphorylation influence and shape the muscles' ability to generate and sustain muscular force. Substrate metabolism, therefore, underpins athletic performance and dictates the relationship between speed (or power) and time to the limit of tolerance (T_{lim}).

1.2 The critical power model

Since the pioneering work of A.V. Hill, there has been much interest in the relationship between speed/power and time, and the changes within the muscle cell that may be implicated in this relationship. In 1965, Monod and Scherrer investigated the maximal work capacity for synergistic small muscle mass exercise and discovered that there was a linear relationship between work done (W) and time to the limit of tolerance (T_{lim}). The slope of this relationship, termed the CP, was thought to be indicative of the 'fatigueless task', whereas the *y*-intercept (W′) was reflective of a 'fixed energetic reserve' that can be utilised during exercise above the CP (Figure 2; Monod and Scherrer, 1965). Accordingly, the CP was considered to represent the maximal rate of oxidative phosphorylation and, thus the W′ was believed to reflect substrate level phosphorylation (Moritani et al. 1981). However, the premise that the W′ is a fixed energetic reserve has more recently been challenged by its association with the pulmonary oxygen uptake

($\dot{V}O_2$) response profile, specifically $\dot{V}O_2$ slow component which represents an increased oxygen cost to the exercise (described further in section 2.1.2.2) (Murgatroyd et al. 2011; Vanhatalo et al. 2011b), and also by studies that have demonstrated a decrease, or an increase in the size of the W′ following inspiration of a hyperoxic (Vanhatalo et al. 2010), or hypoxic (Dekerle et al. 2012; Simpson et al. 2015) gas mixture, respectively. These findings suggest that the W′ is not reflective of a fixed energetic reserve *per se* but is instead a finite amount of work that can be performed above the CP which is associated with the $\dot{V}O_2$ slow component, depletion of PCr, and the accumulation of fatigue-related metabolites.

The intensity and duration of muscular contraction and the appearance of fatigue-related metabolites is therefore dictated by the relative contribution of each metabolic pathway to the overall ATP turnover, which itself is dependent on the position of the work rate relative to the CP. During exercise below the CP, ATP turnover can be maintained via oxidative phosphorylation, such that a steady state can be achieved in $\dot{V}O_2$ (Poole et al. 1988), muscle pH, and muscle PCr and P_i concentrations (Jones et al. 2008a). As such, the CP can be considered to represent the highest sustainable rate of oxidative metabolism (Jones et al. 2008a; 2010; Monod and Scherrer, 1965; Moritani et al. 1981; Poole et al. 1988; 2016). At exercise intensities above the CP, and by definition represented by the W′, there is a continued increase in the reliance on substrate level phosphorylation and thus the accumulation of fatigue-related metabolites to a "critical value" (Vanhatalo et al. 2010). $\dot{V}O_2$ steady state cannot be attained >CP and instead $\dot{V}O_2$ continues to rise until VO_{2max} is achieved (Burnley and Jones, 2007; Jones et al. 2010; Poole et al. 1988). Accordingly, interventions that reduce the utilisation of the W′, by increasing the rate of energy supplied by oxidative

phosphorylation, would be expected to improve exercise tolerance and performance (Burnley and Jones, 2007; Jones et al. 2010).

The CP model describes the interaction of two endogenous energy supply components (CP and W′), which characterise the relationship between power and time, and defines the amount of work that can be performed above the CP in a given amount of time (Jenkins and Quigley, 1990; Moritani et al. 1981; Poole et al. 1988; Smith and Hill, 1993; Smith and Jones, 2001). The CP model, therefore, provides a framework to investigate the physiological responses to exercise, and their relationship to fatigue and performance. This thesis was designed to explore the predictive validity, applicability, plasticity and mechanistic underpinnings of the power-duration relationship.

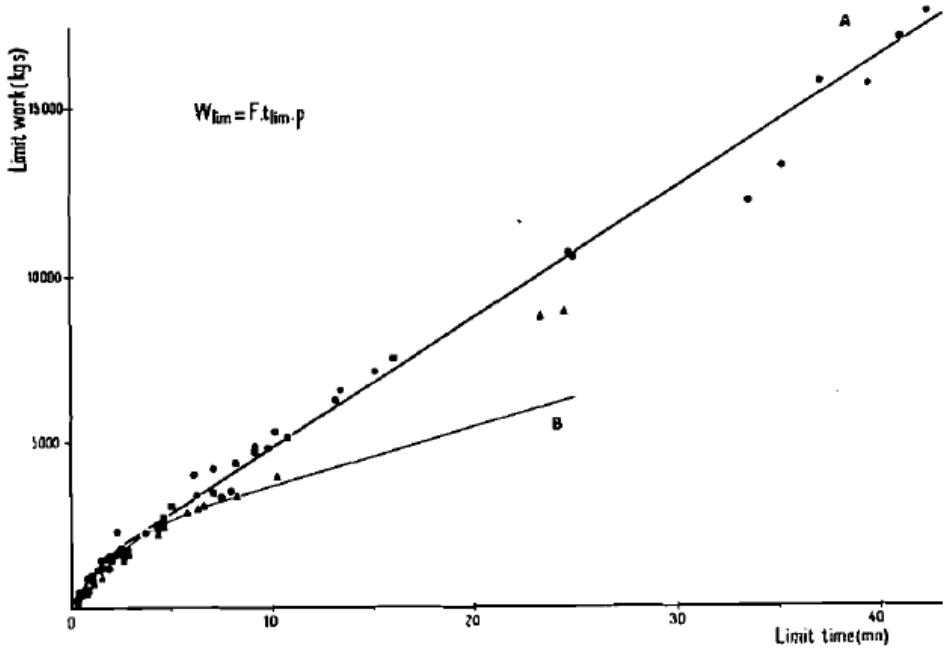

Figure 1.2 The original plot of the two-parameter linear W-T_{lim} model (Monod and Scherrer, 1965).T_{lim} is plotted on the X-axis and W on the Y-axis. Lines A and B represent the linear transformations for the relationship between T_{lim} and isometric contraction force, equivalent to the hyperbolic P-T_{lim} relationship, measured during intermittent and continuous isometric exercise, respectively.

Chapter 2 LITERATURE REVIEW

2.1 Fatigue

Fatigue is defined as, and is evidenced by, the progressive decline in mechanical performance of the muscle(s) (i.e., the force generating capacity and the rate of contraction), which is reversible with a period of rest (Allen et al. 2008; Enoka and Duchateau, 2008; Enoka and Stuart, 1992; Fitts, 1994; Gandevia, 2001; Sejersted and Sjogaard, 2000). Whilst the muscle(s) can be considered as the mechanical machinery responsible for the generation of force and movement, their ability to contract is dependent on the receipt of an action potential from a neural stimulus initiated within the motor cortex region of the brain. Inhibition or disturbance at any site along the brain-muscle pathway, or within the muscle itself, may impair force or power generation. Fatigue that occurs through processes at or distal to the neuromuscular junction are commonly referred to as being peripheral in origin (Bigland and Ritchie, 1978; Gandevia, 2001). Conversely, an inhibition or disruption in the neural drive to the muscle, which occurs proximal to the neuromuscular junction, is defined as central fatigue (Gandevia, 2001).

The relative contribution from central and peripheral fatigue mechanisms likely varies in an exercise-intensity dependent manner. Although fatigue is one of the most researched areas in exercise physiology, the mechanisms that govern exercise tolerance/performance, and ultimately fatigue, and the methods used to determine the origin(s) of fatigue are hotly debated (e.g., see the Point:Counterpoint series started by the Point of Amann and Secher, 2009, and Taylor et al. 2009). A definitive understanding/explanation of fatigue remains elusive. The following literature review will provide a summary of our current understanding of the mechanistic bases for muscle fatigue, introduce a

two-parameter CP model, and suggest interventions that may potentially offset the fatigue process(es) and, thus improve exercise tolerance.

2.1.1 Skeletal muscle contraction

Skeletal muscle contraction is the result of a process in which an electrical potential (action potential) signals a sequence of reactions that causes mechanical activation of the contractile myofibrils. Briefly, upon the arrival of a neural stimulus at the neuromuscular junction from the motor cortex, the neurotransmitter acetylcholine is produced and subsequently released into the space between the presynaptic terminal and the muscle fibre membrane (sarcolemma). Acetylcholine results in the opening of channels positioned along the sarcolemma resulting in an influx of sodium cations (Na^+) and a concomitant efflux of potassium cations (K^+) from the muscle cell, resulting in its depolarisation. Provided there is sufficient depolarisation (i.e., the voltage of the cell attains threshold; -55 mV), voltage gated channels open, resulting in passing of the action potential along the transverse tubules (t-tubules) to the sarcoplasmic reticulum (SR), initiating the release of calcium ions (Ca^{2+}) into the sarcoplasm surrounding the myofibril. Ca^{2+} binds with troponin C which firstly displaces tropomyosin, exposing the myosin binding sites on the actin filament, and provided that ATP is present, the myosin and actin filament will repeatedly attach and detach resulting in the sliding of filaments and the generation of muscular force (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). When the muscle is no longer stimulated, ATP activated pumps along the SR membrane reuptake Ca^{2+} resulting in a concomitant relaxation and repolarisation of the muscle cell (Ebashi et al. 1976; Fitts, 2008; Fuchs et al. 1974; MacIntosh et al. 2012).

2.1.2 Skeletal muscle fatigue

Reductions in the substrates used to resynthesize ATP (i.e., PCr, glycogen) may impair skeletal muscle function. Moreover, the products of substrate-level phosphorylation (i.e., P_i and H^+), and increased extracellular K^+, a result of repeated muscle activation, have also been implicated in the fatigue process. The cellular bases for skeletal muscle fatigue has been the subject of numerous reviews (e.g.'s., Allen and Westerblad, 2001; Allen et al. 2008; Allen and Trajanovska, 2012; Fitts, 1994; 2008; Green et al. 1998; 2004; MacIntosh et al. 2012; Westerblad and Allen, 2002; Westerblad et al. 2002). The mechanisms associated with the fatigue-inducing effects for the: depletion of PCr and the concomitant accumulation of intramuscular P_i; accumulation of H^+; increased extracellular K^+; and, the depletion of muscle glycogen will be described below.

2.1.2.1 The depletion of PCr and the role of increased intramuscular P_i

The accumulation of intramuscular P_i is linked to the breakdown of muscle PCr which is essential for the resynthesis of ATP at exercise onset, or to sustain muscular force generation during high-intensity exercise. Indeed, severe-intensity exercise has been shown to result in an inexorable decrease in muscle PCr and increase in P_i until exhaustion (Jones et al. 2008a; Vanhatalo et al. 2010). An increase in P_i has been associated with: i) a reduction in the force output from the binding of myosin and actin filaments; ii) a reduction in myofibrillar Ca^{2+} sensitivity; and iii) inhibition of Ca^{2+} release from the SR, due to the formation of a calcium-phosphate precipitate (Allen et al. 2008; Allen and Westerblad, 2001; Fitts, 2008). It is noteworthy that the other product of PCr hydrolysis, creatine (Cr),

has little effect on muscle contractility (Murphy et al. 2004), but has been found to increase the sensitivity of mitochondrial respiration to ADP (Walsh et al. 2001).

2.1.2.2 The role of intramuscular pH

The rapid breakdown of glucose and glycogen via glycolysis necessitates the conversion of pyruvate to lactate, resulting in large increases in lactate during high-intensity exercise. The reduced ability to perform high-intensity exercise, and the concomitant increases observed in muscle and blood [lactate], has implicated lactate in the fatigue process. However, it is now widely accepted that lactate *per se* does not cause muscle fatigue (Brooks, 2001; Cairns, 2006; Hall et al. 2016; Nielsen, 2001; Robergs et al. 2004). Rather, H^+ accumulation and the resultant decrease in muscle pH ($-\log_{10}[H^+]$) is more likely to contribute to the fatigue process (Fitts, 2008). Decreased muscle pH has been found to inhibit cross-bridge cycling (Fabbiato and Fabbiato, 1978; Metzger and Moss, 1987) and depress the maximal shortening velocity (Edman and Mattiazi, 1981; Metzger and Moss, 1987; Thompson et al. 1992), specifically in fast-twitch muscle fibres (Metzger and Moss, 1987; 1990a; 1990b) resulting in a reduced force generating capacity (Knuth et al. 2006; Pate et al. 1995; Westerblad et al. 1997). Furthermore, decreased muscle pH has been shown to inhibit glycolysis (Gevers and Dowdle, 1963; Spriet et al. 1989; Trivedi and Danforth, 1966) and reduce the rate of oxidative phosphorylation (Jubrias et al. 2003). Therefore, decreased muscle pH may induce fatigue via direct inhibition of cross-bridge cycling and by reducing the capacity/rate for ATP resynthesis via substrate-level phosphorylation and oxidative phosphorylation during high-intensity exercise.

2.1.2.3 The role of increased extracellular K^+ concentration

The influx of Na^+ and efflux of K^+ from the muscle cell are essential for muscle contraction. However, during high-intensity exercise, extracellular $[K^+]$ ($[K^+]_e$) has been shown to increase 4-fold to attain values ranging from 10-14 mM (Juel et al., 2000; Mohr et al., 2004; Nielsen et al., 2004). Due to the regulatory role of K^+ in the maintenance of resting membrane potential and excitability, thus its important role in translating the action potential across the sarcolemma and t-tubules to the SR, increased $[K^+]_e$ has been identified as a likely factor contributing to muscle fatigue (Fitts, 1994; McKenna et al. 2008). Indeed, several in-vitro studies have shown that increasing $[K^+]_e$ above 8 mM impairs muscle contractility due to decreased SR Ca^{2+} release (Renaud and Light, 1992; Cairns et al. 1995; MacIntosh et al. 2012). Interestingly, a greater $[K^+]_e$ is evident in a more acidic environment owing to a greater opening of the K^+-ATP channel (Davies et al. 1990; Davies et al. 1991; Standen et al. 1992). Although $[K^+]$ has been shown to be considerably greater in the interstitium than the venous plasma following exercise (Juel et al. 2000), both interstitial and plasma $[K^+]$ increase proportionally to an increase in work rate (Juel et al. 2000; Medbo et al. 1990). Therefore, due to its ability to discriminate between exercise intensity, its purported role in muscle membrane excitability, and the relative ease and expense of assessment, plasma $[K^+]$ has typically been determined as a proxy for $[K^+]_e$.

2.1.2.4 The role of muscle glycogen

Muscle glycogen is a major source of chemical energy for ATP resynthesis. The importance of muscle glycogen as an energy substrate is emphasised in its

well-established relationship with exercise capacity, which describes a concomitant decrease in muscle glycogen content and fatigue-resistance (Ahlborg et al. 1967; Bangsbo et al. 1992; Bergstrom et al. 1967; Gollnick et al. 1972; Hargreaves et al. 1995; Hermansen et al. 1967; Sjogaard 1983; 1986). In addition to being an essential substrate for the regeneration of ATP, it has also been demonstrated that under conditions where [ATP] were held high and constant, that low muscle [glycogen] can impair muscle function (Nielsen et al. 2009; Stephenson et al. 1999). The association between low muscle [glycogen] and impaired muscle function can be attributed to glycogen's modulatory role in the release of Ca^{2+} from the sarcoplasmic reticulum (Chin and Allen, 1997; Duhamel et al. 2006a; 2006b; Nielsen et al. 2009; Ortenblad et al. 2011; Gejl et al. 2014). In keeping with glycogen's role in excitation-contraction coupling, individuals deficient in glycogen phosphorylase (McArdle's disease) do not experience a considerable fall in pH but demonstrate an earlier decline in muscle membrane excitability (M-wave amplitude) during exercise (Cooper et al. 1989). Furthermore, glucose administration has been shown to partially restore both the M-wave amplitude and muscle contractility (Karelis et al. 2002; Marcil et al. 2005; Stewart et al. 2007), supporting the notion that glycogen modulates muscle function. It is therefore likely that glycogen depletion is the predominant cause of peripheral fatigue during prolonged moderate- and heavy-, relative to severe-intensity exercise. However, there is a paucity of human in-vivo studies exploring: the mechanistic bases for fatigue during whole-body exercise within each intensity domain; and, the association between glycogen and muscle membrane excitability.

2.1.2.5 Summary

It should be recognised that numerous metabolites and ions change in combination during exercise of an intact muscle. Thus, it is important to consider the combination of these changes on the fatigue process, rather than the changes in any metabolite or ion in isolation. This is emphasised by a recent study which infused exogenous metabolites into intact human muscle interstitium (Pollak et al. 2014). Pollak and colleagues (2014), found that a combination of metabolites typically associated with moderate endurance exercise or vigorous exercise was associated with fatigue and pain sensations, but this was not reported when the same concentration of metabolites were infused in isolation. These findings emphasise the importance of considering the combined effect of changes that occur during human exercise (i.e., increased muscle temperature, metabolic and ionic changes etc.) on muscle fatigue, and these changes should be considered in relation to muscle excitability and exercise intolerance during whole-body exercise performed within each distinct submaximal exercise intensity domain.

2.1.3 The exercise intensity domains: the muscle metabolic and pulmonary $\dot{V}O_2$ responses

The muscle metabolic response to exercise can be determined non-invasively, and with a high temporal resolution, using ^{31}phosphorous magnetic resonance spectroscopy (^{31}P-MRS). However, ^{31}P-MRS requires considerable expertise, is relatively expensive, and is limited to the assessment of small muscle mass exercise. Alternatively, the percutaneous muscle biopsy technique can be used to gain insight into muscle metabolism during whole-body exercise. However, this

technique is not without its limitations: it is highly invasive, and lacks temporal resolution, providing a “snapshot” into muscle metabolism which is dependent on factors such as the site and depth of biopsy and the fibre type population of the sample. Importantly, muscle O_2 uptake, indicative of the rate of muscle oxidative phosphorylation, is accurately reflected in the pulmonary $\dot{V}O_2$ response (Barstow et al. 1990; Grassi et al., 1996; Koga et al., 2005; Krustrup et al. 2004; 2009). Therefore, with a few caveats, the breath-by-breath measurement of $\dot{V}O_2$ provides a non-invasive and relatively inexpensive means to estimate the muscle metabolic rate during exercise.

The $\dot{V}O_2$ response differs considerably depending on the intensity of exercise, and can be used to ascertain which exercise intensity domain an individual is working within (Barstow, 1994; Whipp, 1994). The submaximal exercise intensity continuum comprises three domains: the moderate, heavy and severe, each of which is associated with distinct physiological responses (Hill et al. 2002; Jones and Doust, 2001; Jones and Poole, 2005). In addition to these submaximal exercise intensity domains, an additional ‘supra maximal’ domain exists, that has been termed the extreme intensity domain (Hill et al. 2002). The submaximal exercise intensity domains and their inherent muscle metabolic and $\dot{V}O_2$ responses will be described below.

2.1.3.1 Moderate exercise intensity domain

During moderate-intensity exercise, which comprises all work rates below the gas exchange threshold (GET), $\dot{V}O_2$ increases in an exponential fashion with a functional ‘gain’ (increase in $\dot{V}O_2$ per unit increase in work rate) that approximates

10 ml·min^{-1}·W^{-1} (Mallory et al. 2002; Whipp and Wasserman, 1972). Due to the exponential nature of the $\dot{V}O_2$ response, and the immediate increase in muscle ATP turnover during the transition to a higher work rate, there is a temporary mismatch between the energy requirement of the contracting muscle(s) and the energy that is provided by oxidative phosphorylation, termed the 'O_2 deficit' (Krogh and Lindhard, 1913). Therefore, the immediate increase in ATP turnover and the exponential increase in oxidative metabolism necessitate elevated rates of ATP resynthesis from substrate-level phosphorylation at exercise onset. Consequently, a $\dot{V}O_2$ 'steady-state' is typically achieved within 2-3 minutes (Whipp and Wasserman, 1972; Whipp and Mahler, 1980). Upon the attainment of the $\dot{V}O_2$ steady-state, ATP resynthesis can be fully sustained by oxidative phosphorylation, and there is no significant elevation in blood [lactate] (square brackets denotes concentration), and little disturbance to the intramuscular metabolic milieu (i.e., H^+, P_i, pH, ADP) relative to resting values (Jones et al. 2008a; Poole et al. 1988). Consequently, exercise can be continued for several hours, with fatigue likely mediated by muscle glycogen depletion, muscle damage, increased core temperature and central fatigue (Gonzàlez-Alonso et al. 1997; St Clair Gibson et al. 2001).

2.1.3.2 Heavy exercise intensity domain

During exercise above the GET, the $\dot{V}O_2$ steady state is supplemented by an additional $\dot{V}O_2$ slow component. This so-called $\dot{V}O_2$ slow component emerges ~100-180 s into the exercise bout and elevates $\dot{V}O_2$ to a greater level than that predicted from the extrapolation from work rates below the GET (Linnarsson et al. 1974; Barstow and Molé, 1991; Paterson and Whipp, 1991; Burnley and Jones,

2007; Billat et al. 1998; Gaesser and Poole, 1996). Importantly, during exercise above the GET but below the CP (i.e., the heavy-exercise intensity domain), $\dot{V}O_2$, blood [lactate], muscle metabolites (i.e., ADP and P_i) and muscle pH will eventually stabilise and remain submaximal irrespective of exercise duration (Poole et al. 1988; Whipp and Ward, 1990). The mechanistic bases for the $\dot{V}O_2$ slow component have been linked to the progressive recruitment of less efficient, more glycolytic type II muscle fibre types (Garland et al. 2004; Krustrup et al. 2004) and the high O_2 cost of fatigued muscle fibres that either no longer contribute to force generation or do so at a far greater O_2 cost per unit of external work done (Vanhatalo et al. 2011b; Zoladz et al. 2008). Exercise within the heavy intensity domain may be sustained for a few hours, with fatigue likely to be related to hyperthermia and the depletion of muscle glycogen (Hughes et al. 1982).

2.1.3.3 Severe exercise intensity domain

The severe intensity domain encompasses all work rates above the CP for which the $\dot{V}O_{2max}$ can be attained (Jones and Poole, 2005; Hill et al. 2002; Poole et al. 1988). However, unlike the moderate and heavy exercise intensity domains, within the severe domain a metabolic steady-state is unattainable. Instead, the $\dot{V}O_2$ slow component drives $\dot{V}O_2$ towards its maximum and, provided that exercise is continued until exhaustion, $\dot{V}O_{2max}$ is achieved (Jones and Poole, 2005; Hill et al. 2002; Poole et al. 1988). The inability to maintain the requisite power output during exercise within this domain is likely due to the depletion of high-energy substrate (PCr), the accumulation of P_i, ADP and H^+, and a reduction in intramuscular K^+ (Allen, 2009; Allen et al. 2008; Dutka et al. 2005; Green, 1998; Jones et al. 2008a; Vanhatalo et al. 2010; McKenna et al. 2008; Westerblad and

Allen, 1996). Unlike the other exercise intensity domains, the tolerable duration of exercise can be predicted from the hyperbolic relationship between power output and time to exhaustion (Monod and Scherrer, 1965; Poole et al. 1988; Hill et al. 2002).

2.2 The power-duration relationship: a two component model of fatigue

The proximity of the exercise power output to the CP defines the muscle metabolic response and, thus plays an important role in exercise tolerance. The mathematical functions that characterise severe-intensity exercise tolerance/performance, the level of error associated with the parameters produced by these functions, and the physiological underpinnings of these models will be described below.

2.2.1 The CWR protocol and the two-parameter CP model

Conventionally, the power-time ($P\text{-}T_{lim}$) relationship is constructed using data from a series of severe-intensity constant work rate (CWR) 'prediction trials' for which the tolerable duration should range from 2-15 min (Jones et al. 2010; Poole et al. 1988). This approach requires the completion of 3-5 CWR trials performed to exhaustion on separate days, with $\dot{V}O_{2max}$ being achieved at T_{lim} for each trial. The two-parameter model entails three mathematically equivalent equations:

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

$$W = CP \cdot T_{lim} + W' \quad \text{[Equation 2]}$$

$$P = W'/T_{lim} + CP \quad \text{[Equation 3]}$$

Where P represents power output, T is equal to time, and W is the amount of work done. Equation 1 describes the hyperbolic P-T_{lim} relationship (Figure 3A). The power-asymptote of this relationship represents the CP (discussed further in section 2.3), and the curvature constant is representative of a "fixed energetic reserve" (Monod and Scherrer, 1965), which is referred to as the W′ (Fukuba et al. 2003; Gaesser et al. 1995; Poole et al. 1988; Smith and Hill, 1993). The amount of work (W) that can be performed, is a function of P and T_{lim} ($W = P \cdot T_{lim}$), and therefore the hyperbolic P-T_{lim} relationship can be linearised by plotting W against T_{lim} (Equation 2, Figure 3B), the y-intercept representative of the W′ and the slope of the relationship equivalent to CP. In addition, the P-T_{lim} relationship can also be linearised by plotting P against the inverse of T ($1/T_{lim}$; Equation 3, Figure 3C), such that the CP is provided by the y-intercept and the slope of the relationship provides the W′.

2.2.2 The 3-parameter CP model

The 2-parameter CP model assumes that is possible to utilise the W′ instantaneously thus produce power outputs well in excess of peak power output. The 3-parameter model attempts to constrain peak power output by setting the peak power output (P_{max}) as the y-axis intercept of the P-T_{lim} hyperbola (Equation 4; Gaesser et al., 1995; Morton, 1986).

$$P = (W'/(T_{lim} - (W'/(CP - P_{max}))) + CP \qquad \text{[Equation 4]}$$

Whilst the introduction of the 3rd parameter constrains P_{max} to within physiological limits, this model consistently underestimates CP and overestimates W′ relative to the 2-parameter models (Bull et al. 2000; Gaesser et al. 1995). Moreover, the

3-parameter model increased the standard error of the estimate (SEE) for the CP estimates from a worst-case scenario of 15 W (equivalent to a coefficient of variation % (CV%) of 7.2%) for the 2-parameter models to a worst-case scenario of 39 W (CV%, 22%) for the 3-parameter model (Bull et al. 2000). The SEE associated with the W′ estimate from the 3-parameter model (worst-case, SEE: 68.0 kJ) was also 2-fold greater than that derived from the 2-parameter model (worst-case, SEE: 34.9 kJ) (Bull et al. 2000), although this should be interpreted with caution given the greater W′ associated with the 3-parameter model. Despite presenting an upper-limit to maximal instantaneous power output, there is greater error associated with the parameter estimates. Given the close agreement in the parameter estimates derived from the 2-parameter models, and its relative widespread application, this thesis will focus on the original 2-parameter CP model.

2.2.3 Estimation of the power-duration relationship using the 3-min all-out test

The advent of the 3-min all-out test has expedited the estimation of the power-duration parameters. The 3-min all-out test is a single visit protocol, based on the premise that following the depletion of the W′, the highest sustainable power output is equal to the CP (Burnley et al. 2006). The parameter estimates derived from the 3-min all-out test have been shown to be reliable and closely match the CP derived from a series of CWR tests (Burnley et al. 2006; Vanhatalo et al. 2007; 2008a). Moreover, the all-out test profile is sufficiently sensitive to detect a change in the CP following high-intensity interval training (Vanhatalo et al. 2008a), and can be used to predict exercise tolerance in the laboratory (Bailey et

al. 2011; Chidnok et al. 2012; Vanhatalo et al. 2008a). However, the predictive validity of the all-out protocol has not been tested such that it is presently unknown whether the CP estimated from the laboratory based 3-min all-out test can be used to predict TT performance in the field.

2.2.4 The sources of error associated with the estimation of the power-duration parameters

As described above, the parameter estimates of the power-duration relationship may be derived via the application of linear (Equations 2 and 3), or non-linear (Equation 1) regression analysis to a series of CWR or TT prediction trials, or alternatively by using a single 3-min all-out test. The conventional method has two sources of error that may influence the power-duration parameters: 1) the reliability of T_{lim} (i.e., CWR) or time to completion (i.e., TT), which have been shown to have a coefficient of variation (CV%) ranging from 2-17% (Poole et al. 1988; Hopkins et al. 2001), and 0.6-4.6% (Hopkins et al. 2001), respectively; and, 2) the error associated with fitting the experimental data to the mathematical model. It has been proposed that an acceptable level of accuracy between the experimental data and the mathematical model is attained when the standard error is less than 5% for the CP, and less than 10% for the W' (Hill and Smith 1994; 1999). However, these guidelines for acceptable error in the parameter estimates have been set arbitrarily and have not been experimentally verified. In the few studies that have determined the test-retest reliability of the conventionally derived CP and W', where the experimental trials are performed under identical conditions (i.e., not confounded by a prior bout of exercise, administration of a supplement, or a training intervention), the CV% has been shown to range from

3-6% and 8-17% for the CP and W', respectively (Gaesser and Wilson, 1988; Nebelsick-Gullett et al. 1988). An advantage of the 3-min all-out test over the conventional method is that determination of the CP and W' does not require linear or non-linear regression analysis. Consequently, errors attributable to extrapolation of a relationship to an asymptote or intercept cannot occur. The power-profile from the 3-min all-out test is reproducible with the CV% for the CP and W' approximating 3% and 9%, respectively (Burnley et al. 2006; Vanhatalo, 2008). Therefore, both the conventional and 3-min all-out test protocols provide valid and reliable estimates for the CP and W' when rigorous testing and analysis methods are used.

2.3 Physiological underpinnings of the CP and W′

The physiological relevance of the CP has been the subject of numerous investigations (e.g. Barker et al. 2006; Bosquet et al. 2011; Brickley et al. 2002; 2007; Burnley et al. 2012; Carter et al. 2005; Chidnok et al. 2013a; b; Copp et al. 2010; Jones et al. 2008a; Miura et al. 1999; 2000; Poole et al. 1988; Vanhatalo et al. 2010; 2016). Our current understanding of the physiological underpinnings of the CP and W' will be described below.

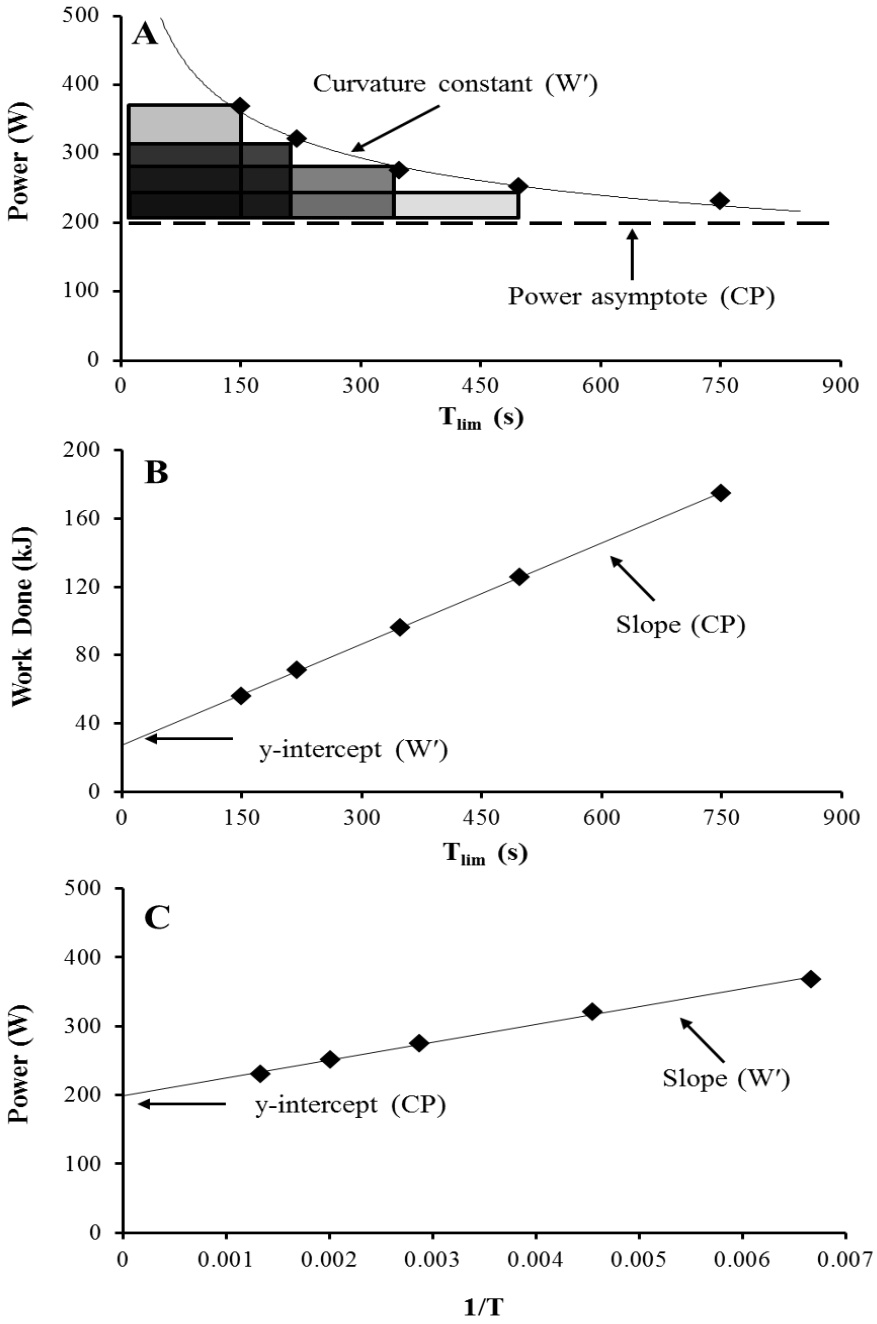

Figure 2.1 The two-parameter hyperbolic P-T_{lim} model (panel A), W-T_{lim} model (panel B) and 1/T_{lim} model (panel C) with data derived from 5 CWR prediction trials (black diamonds) from cycling exercise. In panel A, the dashed line indicates the CP; the shaded rectangles represent the curvature constant of the hyperbola (W′). Note that the W′ is always of the same fixed magnitude, regardless of its rate of expenditure. The axes of the P-T_{lim} model are reversed to improve clarity.

2.3.1 Muscle metabolic responses during exercise above and below CP

The muscle metabolic responses to single-legged knee extension exercise has been shown to depend on the position of the work rate in relation to the CP. Specifically, exercise performed 10% above the CP (Jones et al. 2008a), or any severe-intensity work rate performed in normoxia (Fraction of inspired O_2; F_iO_2, 0.21) or hyperoxia (F_iO_2, 0.70; Vanhatalo et al. 2010), is associated with the progressive depletion of muscle high-energy phosphates and a progressive accumulation of fatigue related metabolites (i.e. H^+ and P_i). Moreover, exercise needs to be reduced to a work rate below the CP for any restoration of muscle [PCr], [P_i], [ADP] or pH to occur (Chidnok et al. 2013a). In contrast, during exercise below (-10%) the CP, a steady-state can be achieved in muscle PCr, P_i and pH (Jones et al. 2008a). A steady-state in muscle metabolism during exercise below (-5%) the CP, and a progressive disturbance to the muscular metabolic milieu during exercise above (+5%) the CP has recently been demonstrated during cycling exercise (Vanhatalo et al. 2016). Furthermore, Vanhatalo et al. (2016) demonstrated that the CP was associated with the percentage of highly oxidative type I muscle fibres, whereas the W' was not correlated with muscle fibre type distribution. Collectively, these studies demonstrate that the CP represents a threshold above which a steady-state cannot be achieved in muscle metabolism, and is linked to the percentage of oxidative type I muscle fibres. In contrast, exercise above the CP (i.e., where the W' is utilised) is associated with the progressive depletion of a finite energy reserve, which is inherently linked with the accumulation of fatigue-related metabolites and depletion of energy substrates.

2.3.2 Reconstitution of W′

The CP model explains that the W′ is accessed during exercise >CP and at its exhaustion the highest work rate that can be sustained is equal to the CP (Jones et al. 2010). Moreover, the CP model explains that reconstitution of W′ can only occur during exercise <CP. Consistent with this notion, Coats et al. (2003) found that exercise duration was extended to a greater extent when the intensity of the recovery exercise was progressively lowered, consistent with a greater W′ reconstitution. These findings were later supported by Chidnok et al. (2015) who demonstrated greater [PCr], pH, [ADP] and [P_i] recovery (i.e., a return towards baseline) when recovery exercise was performed at a "greater distance" <CP. Furthermore, the reestablishment of muscle homeostasis is dependent on the recovery duration, with a greater [PCr], pH, [ADP] and [P_i] recovery following a longer relative to a shorter time period for recovery (Chidnok et al. 2013a). Given the relationship between muscle metabolic and ionic perturbations, W′ and its recovery, $\dot{V}O_2$ kinetics, and ultimately exercise performance (Jones et al. 2010), interventions designed to expedite the return of muscular homeostasis via manipulation of one or several muscle metabolites or ions may also improve subsequent exercise performance

2.3.3 Neuromuscular responses during exercise above and below CP

The CP may also represent the threshold for neuromuscular fatigue development (Moritani et al. 1993; Burnley et al. 2012). In 1993, Moritani and colleagues found that the integrated electromyographic (iEMG) signal derived from *m. vastus lateralis* during cycling could be stabilised at a given work rate, but continued to

gradually increase once this threshold was exceeded. Furthermore, Burnley et al. (2012) found that the rate at which fatigue developed, and the site of the fatigue (i.e. central or peripheral fatigue) was different above and below the critical torque during single-leg, knee-extension exercise. The rate of change in the maximal voluntary contraction (MVC) torque, and the potentiated doublet torque were disproportionately larger during exercise above, compared to below, the CT. These findings suggest that exercise above the critical torque for small muscle mass exercise is predominantly limited by the development of peripheral fatigue, whereas central fatigue may play a greater role during exercise below the CT.

2.3.4 Summary

Collectively, the muscle metabolic and neuromuscular data indicate that the CP represents an important physiological threshold, that once exceeded, results in a: i) precipitous change in fatigue related substrates/metabolites until exercise cessation (Jones et al. 2008a; Vanhatalo et al. 2010; Chidnok et al. 2013a); and, ii) a disproportionate increase in the rate of peripheral fatigue development (Burnley et al. (2012). Moreover, reconstitution of W′ cannot occur until exercise is reduced to a work rate <CP and its recovery is dependent on: i) the proximity of the recovery work rate to the CP, with a greater recovery evident at work rates a "greater distance" <CP; and, ii) the duration of the recovery period, a positive association between recovery duration and W′ reconstitution. Much of our current understanding of the intensity dependent in-vivo fatigue process is based on single-legged knee extension exercise. However, whole-body exercise is associated with: a greater ventilatory load (Wuthrich et al. 2014), increasing the likelihood of respiratory muscle fatigue (Smith et al. 2014); increased feedback

from type III/IV muscle afferent fibres, which may modulate central drive (Rossman et al. 2012; 2014); and restricted muscle perfusion and reduced vascular conductance (Calbet et al. 2004; Mortensen et al. 2008; Smith et al. 2014). These key differences may influence the aetiology and/or time course of fatigue. It is presently unknown whether the CP represents a neuromuscular threshold for fatigue development during whole-body exercise. Furthermore, it is unclear whether a similar level of muscle metabolic perturbation and a similar reduction in muscle excitability are observed at T_{lim} during severe-intensity (i.e., >CP) whole-body exercise of different durations. It is also unclear whether manipulation of the intramuscular milieu, specifically by increasing muscle buffering capacity, may be effective in expediting the recovery in muscle pH. By elevating muscle pH, improved muscle buffering may reduce the extent of muscle metabolic perturbation and thus improve exercise performance and alter the power-duration relationship.

2.4 The influence of different work-rate forcing functions and pacing on the power-duration relationship

Similar parameter estimates can be derived from CWR prediction trials and a 3-min all-out test (Burnley et al. 2006; Simpson et al. 2015; Vanhatalo et al. 2007; 2008). Moreover, Chidnok et al. (2013b) reported that the work done above the CP derived from the 3-min all-out test is fixed during ramp incremental, 3-min all-out, CWR, and a work-matched self-paced time trial, suggesting that the power-duration relationship is unaltered by the work-rate forcing function and/or pacing strategy utilised. However, it should be noted that defining the work done above the 3-min all-out test CP for each of the discrete work-rate forcing functions

(i.e., ramp incremental test, CWR and/or self-paced time trial) assumes that the CP is fixed. This assumption is challenged by the well-established ergogenic effect(s) of an appropriate fast-start pacing strategy, which have been shown to accelerate the $\dot{V}O_2$ response at exercise onset and thus reduce the magnitude of the O_2 deficit, permitting an improved exercise tolerance/performance compared to an even- or slow-start strategy (Bailey et al. 2011; 2015; Bishop et al. 2002; Hettinga et al. 2009; Jones et al. 2008b). Theoretically, any alteration in exercise tolerance/performance should be reflected by a change(s) in the parameter estimates of the power-duration relationship. Accordingly, the rate of adjustment in $\dot{V}O_2$ to the required oxidative energy demand (i.e., the phase II time constant (τ)) has been associated with the CP (Murgatroyd et al. 2011), suggesting that an intervention that could speed τ may increase CP. Moreover, a faster $\dot{V}O_2$ response is linked to a reduced reliance on substrate level phosphorylation and, therefore would be expected to attenuate the utilisation of high-energy phosphates and the accumulation of fatigue-related metabolites associated with the W'.

The fixed nature of the parameter estimates of the power-duration relationship irrespective of the work-rate forcing function is further challenged by Morton et al. (1997) whom reported a trend for a ~18% reduction in the W′ (P=0.07) when CP and W′ were estimated from a series of ramp incremental prediction trials at different ramp rates relative to CWR prediction trials. Notably, during a fast-ramp incremental protocol the $\dot{V}O_2$ conforms to quasi-linear first order kinetics (Whipp et al. 1981; Wilcox et al. 2016), whereas during severe-intensity CWR exercise the $\dot{V}O_2$ kinetics manifests an initial fast (or primary) component followed by a delayed, progressive increase in $\dot{V}O_2$ (i.e., $\dot{V}O_2$ slow component) (Burnley and Jones, 2007). Given that the slow component appears to be almost entirely

eradicated (or hidden) during fast-ramp incremental exercise (Wilcox et al. 2016), it is possible that the fixed work capacity indicated by the W′ may not be accessible to the same extent as during severe-intensity CWR exercise, consistent with the tendency for lower W′ (Morton et al. 1997).

Collectively, these studies demonstrate that manipulation of the work-rate forcing function and/or the pacing strategy utilised may alter the $\dot{V}O_2$ kinetics and thus influence the parameter estimates of the power-duration relationship. However, the influence of the work-rate imposed or the pacing strategy utilised on the CP and W′ has yet to be determined.

2.5 The influence of muscle pH on the power-duration relationship: the role of extracellular and intracellular buffering capacity

The resynthesis of ATP during severe-intensity exercise performance (i.e., >CP) requires a large contribution from substrate-level phosphorylation, resulting in the production of lactate and the accumulation of H^+. The large increases in $[H^+]$ during severe-intensity exercise reduces muscle pH (i.e., $pH = -\log_{10} [H^+]$) from ~7.1 at rest to ~6.4 at T_{lim} (Hermansen and Osnes, 1972; Sahlin et al. 1976). As discussed in section 2.1, H^+ accumulation thus the resultant decrease in muscle pH occurs in tandem with increased ionic and metabolic perturbations and, notwithstanding the deleterious effects of decreased muscle pH on muscle contractile function and muscle energetics, muscle fatigue may indeed be caused by the attainment of a combination of metabolites and ions. Therefore, attenuating the decrease in muscle pH may delay the attainment of this “critical” muscle metabolic milieu and thus improve exercise tolerance and/or performance. Accordingly, exercise-based interventions that have enhanced skeletal muscle

buffering capacity have been associated with improved exercise tolerance/performance (Harmer et al. 2000; Weston et al. 1997).

Further to the efficacious influence of exercise, dietary supplementation has been shown to be an effective means to improve buffering capacity. Acute oral ingestion of sodium bicarbonate has been shown to increase the extracellular bicarbonate buffering pool and increase H^+ efflux (Juel, 1996), thus improve exercise tolerance/performance (Bishop et al. 2004; Goldfinch et al. 1988; McNaughton et al. 2008; Price et al. 2003; Sale et al. 2011). Furthermore, and consistent with the associations between the W′ and $\dot{V}O_2$ kinetics (Murgatroyd et al. 2011; Vanhatalo et al. 2011), sodium bicarbonate supplementation has been shown to reduce the development of the $\dot{V}O_2$ slow component (Berger et al. 2006). The improved exercise performance capabilities and the mechanistic underpinnings associated with the ingestion of sodium bicarbonate should be reflected by an alteration in the power-duration relationship. However, despite notably enhancing blood-buffering capacity, Vanhatalo et al. (2011a) reported no significant alterations in the power-duration relationship following sodium bicarbonate ingestion. It may be postulated that the enhanced blood-buffering capacity could not be utilised due to the rapid accumulation of H^+ during the 3-min all-out test used by Vanhatalo et al. (2010a) to characterise the power-duration relationship, and delay in H^+ efflux. It is plausible that an intramuscular buffer which acts within the myocyte may provide a more effective means to buffer H^+ during the 3-min all-out test than an extracellular buffer which is reliant on H^+ efflux.

Beta-alanine (β-alanine) is considered to be the rate-limiting precursor to carnosine synthesis, a potent intramuscular pH buffer (Harris et al. 2006). Indeed, β-alanine supplementation has been shown to significantly increase the

intramuscular carnosine content (see Figure 2.2 for a summary of the dose-response relationship), and to improve exercise tolerance (del Favero et al., 2012; Hill et al. 2007; Sale et al. 2011) and performance (Baguet et al. 2010; Donovan et al. 2014; Hobson et al. 2013; Hoffman et al. 2015; Saunders et al. 2012; Suzuki et al. 2002; Van Thienen et al. 2009). β-alanine supplementation, via an increase in muscle carnosine content, may therefore attenuate the rate of muscle H^+ accumulation and reduce the decrease in muscle pH during exercise. Improved muscle buffering capacity may reduce the extent of exercise-induced disruption to excitation-contraction coupling, glycolytic flux and PCr recovery and therefore may permit a higher sustainable steady-state to be achieved (i.e., increase the CP). Alternatively, increased muscle buffering capacity may delay the attainment of a "critical" muscle metabolic milieu (Vanhatalo et al. 2010) and thereby increase the W'. Although improved buffering capacity has been shown to improve exercise tolerance/performance, it is presently unclear whether these improvements are due to alterations in the CP, W′ or both. Furthermore, despite the ergogenic effects of β-alanine supplementation being attributed to enhanced muscle buffering capacity, no study has yet assessed its influence on muscle pH during exercise in humans. Therefore, the extent to which muscle pH may be altered following β-alanine supplementation, and how this may change the parameters of the power-duration relationship is unclear and warrants further investigation.

2.6 Summary

The ability to perform high-intensity exercise is described by the hyperbolic relationship between power and time, which is constrained by the rate of ATP resynthesis, the depletion of finite energy substrates, and the accumulation of fatigue related metabolites. The power-asymptote (CP) and the curvature constant (W') of the power-duration relationship can be used to predict severe-intensity exercise tolerance/performance and have been associated with sustainable, and non-sustainable metabolism, respectively. Conventionally, the power-duration relationship was derived from a series of CWR trials. However, it may be advantageous to establish this relationship using the 3-min all-out test, or a series of TT tests. Whilst the construct validity and the reliability of the 3-min all-out test have been determined, its ability to predict performance in the field has not been experimentally verified. Furthermore, the work-rate forcing function and pacing strategy utilised may alter the $\dot{V}O_2$ response and influence the utilisation of the muscles' finite energy stores and the accumulation of fatigue-related metabolites, and subsequently impact on exercise tolerance/performance. The work-rate forcing function may therefore alter the power-duration relationship, having implications for its predictive accuracy. Although, during knee-extension exercise, the CP has been found to represent a threshold in muscle metabolic (^{31}P-MRS; Jones et al. 2008) and neuromuscular responses (Burnley et al. 2012), it is important to establish whether this is also the case during larger muscle mass exercise, such as cycling.

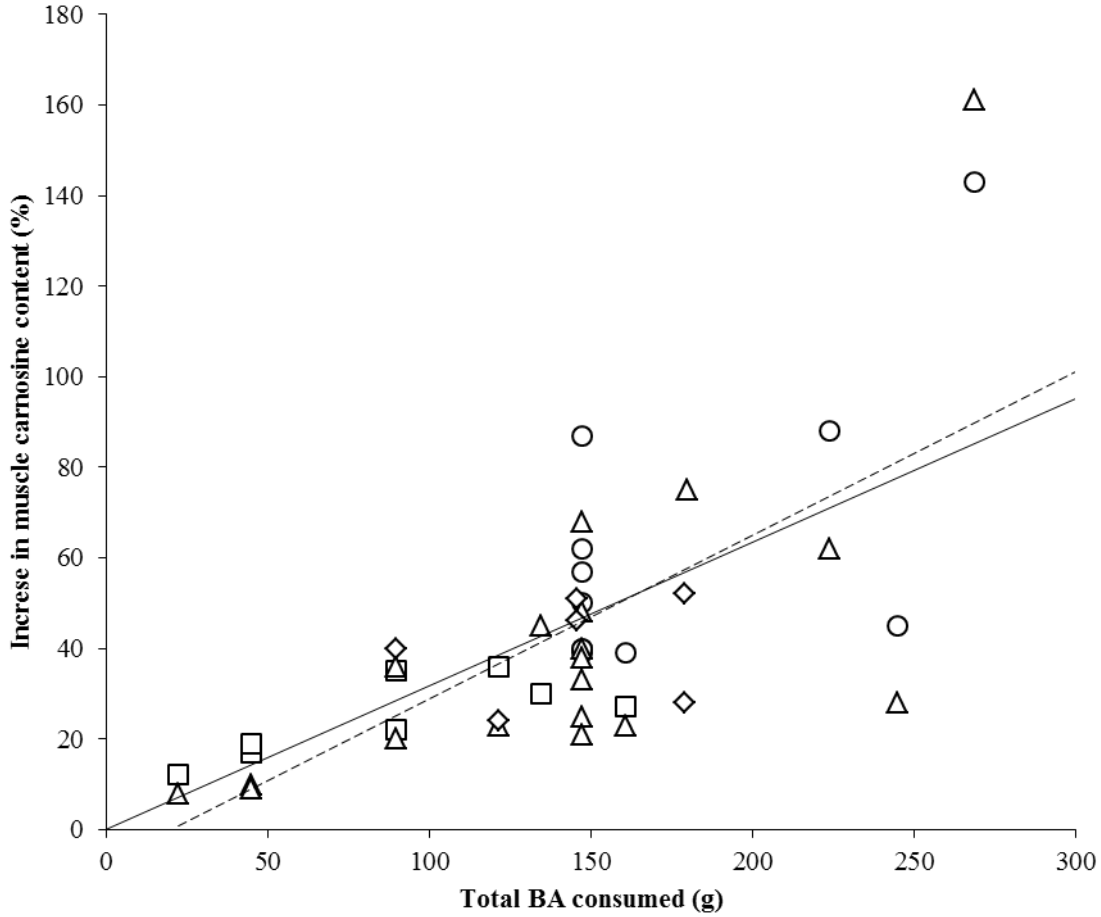

Figure 2.2 The total amount of carnosine ingested and the corresponding group mean increase in muscle carnosine content (%) reported in the literature* for the soleus (circles), tibialis anterior (squares), gastrocnemius (triangles), and the vastus lateralis (diamonds) muscles. For clarity, the line of best fit (dashed line; r^2=0.496, y=0.3617x-7.3642) and the line of origin (solid line; y=0.317x) have also be included.

*based on the findings reported by Baguet et al. (2009); (2010); Bex et al. (2014); Chung et al. (2014); Danaher et al. 2015; Gross et al. 2014; Harris et al. (2006); Hill et al. (2007); Hoffman et al. (2015); Kendrick et al. (2009); Stegen et al. (2013); Stellingwerf et al. (2013).

2.7 Aims and hypotheses

2.7.1 Aims

The overall aim of this thesis was to advance understanding of the methodological rigour and practical application of the critical power concept and to elucidate its key mechanistic underpinnings during whole-body exercise. The specific aims of the five experimental chapters are as follows:

1) Evaluate the efficacy of the parameter estimates derived from the 3-min all-out test in predicting 16.1-km road cycling time-trial performance.

2) Determine the accuracy with which ramp incremental exercise may be predicted by parameter estimates derived from a series of CWR prediction trials.

3) Determine the effect of the pacing strategy utilised during a series of severe-intensity prediction trials on the power-duration relationship and the $\dot{V}O_2$ response. A secondary aim of this experimental chapter was to evaluate the accuracy with which TT's may be predicted by parameter estimates derived from a series of CWR prediction trials.

4) Elucidate the mechanistic bases of fatigue development during severe- (>CP), heavy- (<CP and >GET), and moderate-intensity (<CP and <GET) cycling exercise.

5) Investigate the influence of muscle pH manipulation on the power-duration relationship.

2.7.2 Hypotheses

This thesis will address the following hypotheses:

1) The 3-min all-out test CP will be significantly correlated with 16.1-km road TT performance. Furthermore, the 3-min all-out test CP will be more strongly correlated with performance than other well-known aerobic fitness parameters: the gas exchange threshold (GET); the respiratory compensation point (RCP); and $\dot{V}O_2$max.

2) The CP and W′ derived from CWR prediction trials will overestimate ramp incremental test performance.

3) The CP derived from a series of self-paced TT's will be greater than the CP derived from a series of CWR prediction trials, and the $\dot{V}O_2$ response will be faster during TT compared to CWR prediction trials. Also, the accuracy of TT performance prediction will be compromised when predicted on the basis of the power-duration parameters derived from a series of CWR prediction trials.

4) A consistent muscle metabolic milieu (i.e., low [PCr], low muscle pH) will be attained at T_{lim} during cycling exercise performed at work rates above CP. Exercise below the CP will be associated with progressively less perturbation in muscle pH, [PCr], [lactate] and [ADP], but with progressively greater reductions in muscle glycogen. Also, the rate of neuromuscular fatigue development will be greater above, compared to below CP due to greater muscle metabolic and ionic perturbation.

5) β-alanine supplementation will increase muscle carnosine content and thus improve the intramuscular buffering capacity. Improved buffering capacity

will result in an upward and rightward shift in the power-duration relationship and enhance recovery from a previous bout(s) of high-intensity exercise.

Chapter 3 GENERAL METHODS

3.1 General Experimental Procedures

All of the exercise tests, with exception of the 16.1-km road time trial (Chapter 4), were conducted at sea level, in an air conditioned exercise physiology laboratory with an ambient temperature of 18-22ºC. Prior to data collection, the procedures employed in each of the experimental chapters were approved by the University of Exeter Research Ethics Committee.

3.2 Subjects

The subjects who volunteered to participate in these investigations were recruited from the staff and student community at the University, and local cycling clubs. Prior to testing, subjects were provided with written and verbal explanation of all risks and benefits associated with taking part in the experiment(s). Verbal explanation was provided by a person(s) trained in obtaining valid informed consent (see appendix). Subjects were informed that while their anonymity would be preserved and their data safely stored, the data may be published in academic journals and/or presented at conferences. Subjects were also informed of their right to withdraw from the investigation at any time with no disadvantage to themselves. Any additional questions and/or concerns of the subjects were answered and, provided they understood and were happy with all aspects of the study, they provided their written informed consent. All subjects were non-smokers who were free from known disease. Subjects were instructed to report to the laboratory in a well hydrated state having completed no strenuous exercise for at least 24 hours, and to avoid alcohol for 24 hours, and food or

caffeine for 3 hours prior to testing. Each subject underwent testing at the same time of day (± 2 hours) and all subjects were familiarised with the exercise mode(s) and experimental procedures prior to testing.

3.3 Health and Safety

Great care was taken during all testing to ensure a safe environment for the subjects. All laboratory procedures adhered to the health and safety guidelines established by the Sport and Health Sciences Department at the University of Exeter. Trained experimenters were used to collect muscle tissue and venous blood samples. All samples were collected, stored and disposed of in accordance with the Human Tissue Act (2004). During all laboratory tests, experimenters wore a disposable laboratory coat and disposable latex gloves. Experimenters wore sterile powder free vinyl gloves during tests involving muscle tissue sampling (Chapter 7). Ergometers, trolleys and work surfaces were cleaned using dilute Virkon disinfectant, respiratory apparatus were disinfected according to the manufacturer's recommendations and the percutaneous biopsy needles were sterilised prior to use. All sharps and biohazard materials were disposed of appropriately.

3.4 Measurement Procedures

3.4.1 Descriptive Data

For all experimental chapters, each subject's age, height and body mass were recorded prior to and at the completion of all testing for each experiment. Height

was measured using a Seca stadiometer (SEC-225, Seca, Hamburg, Germany) and recorded to within 0.1 cm, and body mass was recorded using regularly calibrated standard laboratory scales (SEC-780, Seca, Hamburg, Germany) and recorded to within 0.1 kg. Both height and body mass were recorded with the subjects unshod, but otherwise wearing the clothing as during the experimentation.

3.4.2 Cycle Ergometry

All experimental chapters included exercise tests conducted on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), which can be used to administer various work rate forcing functions. The ergometer functions that were used in the series of experiments which comprise this thesis include the hyperbolic, step and linear modes. The hyperbolic function allows work rate to increase as a function of time and this ergometer mode was employed during the ramp incremental exercise tests. The step function allows work rate to be increased, or decreased, rapidly (1000 $W \cdot s^{-1}$), for a predetermined duration. This mode was employed during all constant work rate tests. Both the hyperbolic and step modes control the external power output independent of pedal cadence by instantaneously adjusting the flywheel resistance via electrical braking. In contrast, the linear ergometer mode imposed work rate in a cadence dependent manner given by the following equation:

$$\text{Linear factor} = \text{Power Output} / \text{Cadence}^2 \qquad \text{[Equation 5]}$$

In this mode the ergometer imposes a fixed work rate such that the attainment of a particular cadence will elicit a known power output. The linear mode was

employed during the all-out sprint exercise tests described in chapters 4, and 8, and during the self-paced exercise trials in chapter 6. The ergometer was regularly calibrated by a qualified technician according to the manufacturer's guidelines.

3.4.3 Exercise Tolerance

In all experimental chapters, time to the limit of tolerance (T_{lim}) was defined as the point at which the subject was unable to maintain the required work rate despite strong verbal encouragement. Specifically, during cycling exercise T_{lim} was defined as the point at which the subject's cadence dropped by more than 10 rpm from their preferred cadence (a value between 80-90 rpm that was to be held constant throughout the exercise). During the single-legged knee-extension exercise performed in the prone position (Chapter 8), T_{lim} was taken as the time at which the subject was unable to keep pace with the required contraction frequency (40 repetitions·min^{-1}).

3.4.4 Pulmonary Gas Exchange

Pulmonary gas exchange was measured breath-by-breath during all cycle ergometer tests. During these tests, subjects wore a nose clip and breathed through a low-dead-space (90 mL), low-resistance (0.75 mmHg.L^{-1}.s^{-1}) mouthpiece and impeller turbine transducer assembly (Jaeger Triple V). The inspired and expired gas volume and concentration signals were continuously sampled at a frequency of 100 Hz, the latter using differential paramagnetic (O_2) and infrared absorption (CO_2) analysers (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. These analysers were calibrated before each test with gases of known concentration, and the turbine

volume transducer was calibrated using a 3 L syringe (Hans Rudolph, Kansas City, MO, USA). The volume and concentration signals were time aligned by accounting for the delay in capillary gas transit and analyser rise time relative to the volume signal. Following the completion of each test, raw breath-by-breath gas exchange and ventilation data were exported for later analysis. Oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$) were calculated using standard formulae (Beaver et al. 1973).

3.4.5 Venous blood sampling

In Chapters 6 and 7 venous blood samples were obtained to determine whole blood [lactate] and [glucose], and plasma [K^+] and [Na^+]. For these measurements, the antecubital fossa was initially cleaned with an alcohol swab and a cannula (Insyte-W™, Becton-Dickinson, Madrid, Spain) was inserted into the subject's vein. All samples were drawn into a 5-mL heparin tube (Terumo Corporation, Leuven, Belgium). Whole blood [lactate] and [glucose] were analysed using an automated blood lactate analyser (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The remaining whole blood was then centrifuged at 4,000 rpm for 7 min (Hettich EBA 20, Germany) before plasma was extracted and analysed for [K^+] and [Na^+] (9180 Electrolyte Analyser, F. Hoffman-La Roche, Basel, Switzerland). All analysers were calibrated regularly in accordance with the manufacturer's guidelines.

3.4.6 Ramp Incremental Tests

Ramp incremental tests were conducted in each experimental chapter for the determination of $\dot{V}O_{2peak}$ and gas exchange threshold (GET). Subjects performed 3

min of baseline cycling at 20 W and 80 rpm, after which the work rate was increased at a rate of 30 $W \cdot min^{-1}$ in a linear fashion until volitional exhaustion was achieved or until the subject was unable to maintain the 80 rpm pedal rate. $\dot{V}O_{2peak}$ was determined as the highest mean $\dot{V}O_2$ during any 30-s period. To estimate the GET, data were firstly reduced to 10 s averages and the GET was then determined as: 1) the first disproportionate increase in $\dot{V}CO_2$ versus $\dot{V}O_2$; 2) an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E$/ $\dot{V}CO_2$; and 3) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The GET was estimated independently by three experienced assessors using code labelled data files such that the assessors were blind to the subject being assessed. In all cases at least two assessors identified the same value. All subsequent work rates were calculated with account taken of the mean response time for $\dot{V}O_2$ during ramp incremental exercise (i.e., two-thirds of the ramp rate was deducted from the work rate at the GET).

3.4.7 Determination of the Power-Duration Relationship

During all experimental chapters, the power-duration relationship was assessed for each subject using the 3-min all-out test and/or a series of severe intensity prediction trials performed at different work rates (approximately 60%Δ, 70% Δ, 80% Δ and 90% Δ; where Δ refers to the difference between the work rates at GET and $\dot{V}O_{2peak}$), in a randomised order. The work rates for each prediction trial were selected to obtain a range of T_{lim} between approximately 2 and 15 min. Each trial began with a period of 3 or 4 min of unloaded baseline pedalling at the subject's preferred cadence (the duration of unloaded baseline pedalling remained constant for each prediction trial in any given experimental chapter), followed by a

step increase to the required work rate. Subjects were instructed to remain seated and to maintain their preferred cadence for as long as possible. The CP and W′ parameters were estimated using equations 1-3. The SEE associated with the CP and W′ were expressed as coefficients of variation (CV%, i.e., relative to the parameter estimate). The 'total error' associated with the modelling of the power-duration parameters was calculated as the sum of the CV% associated with the CP and the CV% associated with the W′. The sum of the CV% was optimised for each individual by selecting the model with the smallest total error (Equation 1, 2 or 3) to produce the 'best individual fit' parameter estimates. If the standard errors associated with the CP and W′ exceeded 5 and 10 %, respectively, after three prediction trials had been performed, a fourth prediction trial was completed. Any prediction trials where the end-exercise $\dot{V}O_2$ was <95% of the individual's ramp test determined $\dot{V}O_{2peak}$ were excluded from the modelling of the power-duration relationship.

The 3-min all-out test was preceded by 3 or 4 min of unloaded baseline pedalling at the subject's preferred cadence, immediately followed by the application of a fixed resistance. The fixed resistance for the 3-min all-out test was set using the linear mode of the ergometer such that on reaching their preferred cadence the subject would achieve a power output equivalent to 50%Δ. Subjects were given a 5 s countdown to the start of test, during which they were instructed to increase their cadence to approximately 110-120 rpm. To ensure an all-out effort, subjects were instructed and strongly encouraged to attain their peak power as quickly as possible, and to maintain the cadence as high as possible throughout the test. No time-based feedback was provided in order to prevent pacing. Power output was recorded second-by-second and the peak power output was determined as the

highest 1 s value. The power and $\dot{V}O_2$ profiles of the 3-min all-out tests were visually inspected and the test was repeated if there was any indication that the effort was submaximal. Figure 3.1 provides two examples of failed tests, with clear indication of voluntary pacing of effort and/or an inability to achieve and maintain the $\dot{V}O_{2peak}$. If the test was approved, the CP was calculated by averaging the power output over the final 30 s of the test, and the W′ was estimated as the power-time integral above CP (Burnley et al. 2006, Vanhatalo et al. 2007; 2008a; 2008b).

3.4.8 Statistical Methods

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS). Specific information regarding the statistical tests implemented is given within each experimental chapter. Statistical significance was accepted at $P<0.05$.

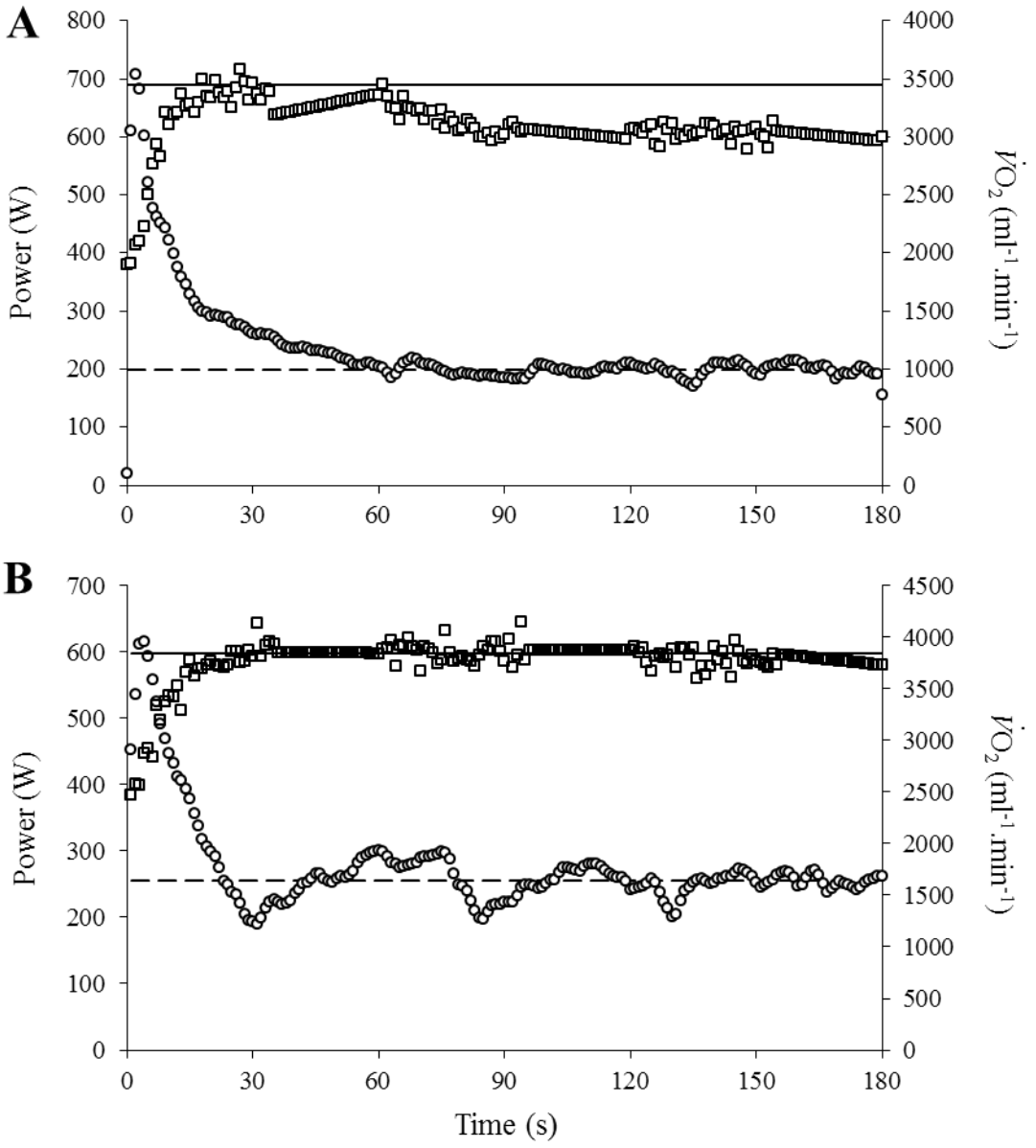

Figure 3.1 Two examples of failed 3-min all-out tests. In panel A the $\dot{V}O_2$ profile (square markers) shows that the subject achieved, but did not sustain their $\dot{V}O_2$peak (solid line). In panel B the $\dot{V}O_2$ profile (square markers) shows that the subject achieved and sustained their $\dot{V}O_{2peak}$ (solid line), but the power profile (circle markers) shows intermittent bursts of increased effort. The dashed line indicates the average power over the final 30 s of the trial. These data sets were recorded during work presented in Chapter 4 and were discarded from further analysis.

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ORIGINAL ARTICLE

Critical power derived from a 3-min all-out test predicts 16.1-km road time-trial performance

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Abstract
It has been shown that the critical power (CP) in cycling estimated using a novel 3-min all-out protocol is reliable and closely matches the CP derived from conventional procedures. The purpose of this study was to assess the predictive validity of the all-out test CP estimate. We hypothesised that the all-out test CP would be significantly correlated with 16.1-km road time-trial (TT) performance and more strongly correlated with performance than the gas exchange threshold (GET), respiratory compensation point (RCP) and $\dot{V}O_2$ max. Ten club-level male cyclists (mean ± SD: age 33.8 ± 8.2 y, body mass 73.8 ± 4.3 kg, $\dot{V}O_2$ max 60 ± 4 ml·kg^{-1}·min^{-1}) performed a 10-mile road TT, a ramp incremental test to exhaustion, and two 3-min all-out tests, the first of which served as familiarisation. The 16.1-km TT performance (27.1 ± 1.2 min) was significantly correlated with the CP (309 ± 34 W; $r = -0.83$, $P < 0.01$) and total work done during the all-out test (70.9 ± 6.5 kJ; $r = -0.86$, $P < 0.01$), the ramp incremental test peak power (433 ± 30 W; $r = -0.75$, $P < 0.05$) and the RCP (315 ± 29 W; $r = -0.68$, $P < 0.05$), but not with GET (151 ± 32 W; $r = -0.21$) or the $\dot{V}O_2$ max (4.41 ± 0.25 L·min^{-1}; $r = -0.60$). These data provide evidence for the predictive validity and practical performance relevance of the 3-min all-out test. The 3-min all-out test CP may represent a useful addition to the battery of tests employed by applied sport physiologists or coaches to track fitness and predict performance in atheletes.

Keywords: *Power–duration relationship, W′, $\dot{V}O_2$ max, gas exchange threshold, respiratory compensation point*

Introduction

The sub-maximal exercise intensity continuum comprises three domains – moderate, heavy and severe, each of which is associated with distinct physiological responses to exercise (Jones & Poole, 2005). These domains are demarcated by well-known aerobic fitness parameters: the upper limit of the moderate domain is indicated by the lactate threshold (LT) or gas exchange threshold (GET); the boundary between the heavy and severe domains is given by the critical power (CP); and the upper limit of the severe domain is determined by the highest work rate that results in the attainment of maximal oxygen uptake ($\dot{V}O_2$ max) prior to exhaustion (Hill, Poole, & Smith, 2002; Jones & Poole, 2005; Poole, Ward, Gardner, & Whipp, 1988). The GET, CP and $\dot{V}O_2$ max all tend to be correlated with endurance exercise performance, with the relative importance of each being determined by the event duration (Bentley, McNaughton, Thompson, Vleck, & Batterham, 2001; Coyle, Coggan, Hopper, & Walters, 1988; Ingham, Whyte, Jones, & Nevill, 2002; Jones & Carter, 2000).

Although the $\dot{V}O_2$ max is able to differentiate endurance performance capability in heterogeneous groups, the threshold parameters (such as the GET and CP) represent stronger performance predictors than the $\dot{V}O_2$ max when training status is more homogeneous (Bassett & Howley, 2000; Nicholson & Sleivert, 2001). The LT (or GET) is a strong predictor of performance in endurance events lasting >2 h and the exercise intensity that can be sustained over a marathon distance closely corresponds with the LT (Bassett & Howley, 2000; Jones, 2006). The predictive capability of the LT is inversely related to race distance, such that in events lasting ≤30 min

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the demarcation point between the heavy and severe exercise intensity domains may become a superior predictor of performance (Jones & Doust, 1998). The respiratory compensation point (RCP), which can also be derived from the incremental exercise test, indicates the onset of ventilatory compensation for metabolic acidosis (Wasserman, Hansen, Sue, Whipp, & Casaburi, 1994). The RCP typically occurs at a power output close to the CP (Dekerle, Baron, Dupont, Vanvelcenaher, & Pelayo, 2003). A 10-km run or a 16.1-km cycle time-trial (TT) is performed at an intensity that is close to the CP (Brickley et al., 2007; Kolbe, Dennis, Selley, Noakes, & Lambert, 1995; Smith, Dangelmaier, & Hill, 1999).

The GET, RCP and $\dot{V}O_2$ max can be assessed in a single incremental exercise test. However, the CP is rarely used to predict performance because its assessment is so arduous and time consuming – typically requiring several (~3–5) laboratory visits (Hill et al., 2002; Poole et al., 1988; Vanhatalo, Doust, & Burnley, 2007). This is unfortunate because the CP is a performance parameter based on the measurement of external power output and time and it may, therefore, be a more powerful predictor of performance than traditional parameters of aerobic fitness which are based on the behaviour of blood lactate or pulmonary gas exchange alone (LT/GET and $\dot{V}O_2$ max). In this light, it is interesting that a single-visit protocol for the determination of CP has been developed (Burnley, Doust, & Vanhatalo, 2006; Vanhatalo, Doust, & Burnley, 2008b; Vanhatalo et al., 2007). The estimated CP from this 3-min all-out test has been shown to be reliable and to closely match the CP derived from conventional procedures (Burnley et al., 2006; Vanhatalo et al., 2007). Moreover, the test is sufficiently sensitive to detect a change in CP following training (Vanhatalo et al., 2008b) and can be used to predict exercise tolerance in the laboratory (Bailey, Vanhatalo, DiMenna, Wilkerson, & Jones, 2011; Chidnok et al., 2012; Vanhatalo et al., 2008b). However, it is presently not known whether the CP estimated from the laboratory-based all-out test can be used to predict TT performance in the field.

Therefore, the purpose of this study was to evaluate the efficacy of the all-out CP test in predicting cycle TT performance. We hypothesised that the all-out test CP would be significantly correlated with 16.1-km road TT performance. It was expected that the all-out test CP would be more strongly correlated with performance than the GET, RCP and $\dot{V}O_2$ max.

Methods

Subjects

Ten club-level cyclists (mean ± SD: age 33.8 ± 8.2 y, height 1.79 ± 0.06 m, body mass 73.8 ± 4.3 kg) volunteered to participate in this study. Written informed consent and a physical activity readiness questionnaire (PAR-Q) were obtained prior to data collection. Subjects were instructed to report to all testing sessions in a similar state, following their usual pre-competition routine. The study was approved by the local Research Ethics Committee.

Experimental design

Subjects reported for testing on four occasions, including three laboratory tests and a in atheletes. TT. All laboratory tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). The seat and handlebar positions were adjusted for comfort and the same settings were replicated for all tests. Subjects performed a ramp incremental test to volitional exhaustion for the determination of the GET, RCP and $\dot{V}O_2$ max. During the second and third laboratory visits, subjects completed a 3-min all-out test with the first serving as a familiarisation trial, which was not included in the subsequent data analysis. The 3-min all-out tests and the 16.1-km TT were performed in a randomised order. The trials were separated by at least 24 h.

Determination of the GET, RCP and $\dot{V}O_2$ max

The ramp incremental protocol included 4 min of unloaded pedalling, followed by a ramp increase in power output of 30 W min^{-1} until volitional exhaustion. Subjects were instructed to maintain their preferred cadence, i.e. the cadence they would select during a 10-mile TT, throughout the test (80 rpm, $n = 3$; 90 rpm, $n = 7$). The test was terminated when the cadence fell by more than 10 rpm below their preferred cadence for more than 5 s despite strong verbal encouragement. The GET was determined as: 1. the first disproportionate increase in $\dot{V}CO_2$ versus $\dot{V}O_2$; 2. an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$; and 3. the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The RCP was determined as: 1. the first disproportionate increase in $\dot{V}_E$ versus $\dot{V}CO_2$ and 2. an increase in $\dot{V}_E/\dot{V}CO_2$. $\dot{V}O_2$ max was determined as the highest mean $\dot{V}O_2$ during a 30-s period. The GET and RCP were estimated independently by three experienced assessors using code labelled data files such that the assessors were blind to the subject being assessed. The GET and RCP were determined as the majority agreement; in all cases at least two assessors identified the same value.

All-out test

Subjects performed 4 min of unloaded pedalling at their preferred cadence, followed by a 3-min all-out sprint. The fixed resistance for the all-out sprint was set using the linear mode of the ergometer such that on reaching their preferred cadence the subjects would achieve a power output equivalent to 50% of the difference between GET and $\dot{V}O_2$ max (linear factor = power/preferred cadence^2). Subjects were given a 5 s countdown to the start of the test. To ensure an all-out effort, subjects were instructed and strongly encouraged to attain their peak power output as quickly as possible, and to maintain their cadence as high as possible until instructed to stop. No time-based feedback was provided in order to prevent pacing. Power output was recorded second-by-second, and the peak power was determined as the highest 1-s value. CP was estimated as the mean power output over the final 30 s of the test, and the W′ was estimated as the power-time integral above CP (Vanhatalo et al., 2007, 2008b).

16.1-km road TT

The TT was performed during December in Exeter (Devon, UK), on a dry day, with little wind, and an ambient air temperature of ~10.1°C. Subjects performed the TT on their standard road racing bike, without any aerodynamic equipment. All subjects were familiar with the 16.1-km road TT route which they used regularly as part of club training sessions. Subjects were instructed to follow their normal pre-competition warm-up, to give a maximal 16.1-km TT effort, and not to draft. All subjects performed the TT on the same day, within the same hour. To reduce the possibility of drafting, start times were separated by a 1-min interval and assigned based on previous TT performance, i.e. the fastest cyclist started first, followed by the second fastest and so on. Time to completion was recorded to the nearest second. To ensure safety during the event, all subjects were sent, and required to sign a document detailing, the course route, and the technical rules and regulations of the Cycling Time Trials governing body for England and Wales.

Measurements

Throughout all laboratory tests, subjects wore a nose clip and breathed through a low-dead space (90 mL), low resistance (0.75 mmHg L^{-1} s^{-1}) mouthpiece and impeller turbine transducer assembly (Jaeger Triple V, Jaeger GmbH, Hoechberg, Germany). Inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz. The gases were drawn continuously from the mouthpiece through a 1.5 m sampling line (0.5 mm internal diameter) to paramagnetic (O_2) and infrared (CO_2) analysers (Jaeger Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). These analysers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, KS). The volume and concentration signals were time aligned, accounting for the transit delay in capillary gas and analyser rise time relative to the volume signal. $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were calculated for each breath using standard formulae.

Statistical analysis

Relationships between the TT performance and laboratory measures (GET, RCP, $\dot{V}O_2$ max and the all-out test parameters) were determined using Pearson's correlation coefficients. Paired samples *t*-tests were used to evaluate differences between the $\dot{V}O_2$ peak values achieved during the all-out test and the $\dot{V}O_2$ max measured in the ramp test. TT performance was predicted using linear regression. 'Cook's distance' test was used to determine the overall influence of each individual data point on the overall model fit. According to this criterion, the Cook's distance for any single data point must not exceed 1 (Cook & Weisberg, 1982). The standard error of estimate between the actual and predicted TT performance were determined. Statistical significance was accepted at $P<0.05$ and data are presented as mean ± SD.

Results

The 10 subjects completed the 16.1-km TT in 27.1 ± 1.2 min. The mean $\dot{V}O_2$ max measured in the ramp incremental test was 4.41 ± 0.25 L·min^{-1} (60 ± 4 ml·kg^{-1}·min^{-1}) and the peak power (peak P) was 433 ± 30 W. The GET occurred at 2.17 ± 0.34 L·min^{-1} and 151 ± 32 W, which represented approximately 35% peak power. The RCP occurred at 315 ± 29 W (3.70 ± 0.27 L·min^{-1}), representative of approximately 75% peak power and approximately 57% of the difference between the GET and $\dot{V}O_2$ max (57% Δ).

The $\dot{V}O_2$ peak achieved during the all-out test (4.36 ± 0.30 L·min^{-1}, 59 ± 4 ml·kg^{-1}·min^{-1}) was not significantly different to the values achieved during the ramp incremental test ($P>0.05$). The all-out test peak power output was 883 ± 107 W (~204% ramp test peak power) which was attained 5–10 s after the start of the test. The CP was 309 ± 34 W (4.20 ± 0.41 W·kg^{-1}), which represented ~71% ramp test peak power and approximately 56% Δ. The work done above CP (W′) was 15.0 ± 2.1 kJ. The total work done during the test was 70.9 ± 6.5 kJ.

There was no significant difference ($P>0.05$) between the CP and RCP; however, the two were not significantly correlated ($r=0.59$, $P>0.05$). The CP was significantly correlated with $\dot{V}O_2$ max ($r=0.79$, $P<0.01$) and ramp incremental test peak power ($r=0.84$, $P<0.01$). The CP and the GET were not correlated ($r=0.59$, $P>0.05$).

The 16.1-km TT performance was significantly correlated with the CP ($r=-0.83$, $P<0.01$), total work done during the all-out test ($r=-0.86$, $P<0.01$), ramp incremental test peak power ($r=-0.75$, $P<0.05$) and the RCP ($r=-0.68$, $P<0.05$), but not with the GET ($r=-0.21$) or the $\dot{V}O_2$ max ($r=-0.60$). The regression equations for time-trial completion (TTC; min) time against each predictive variable were as follows:

CP (W): $TTC = -0.031 \cdot CP + 36.55$
All-out test total work done (kJ): $TTC = -0.16 \cdot TWD + 38.68$
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$): $TTC = -2.14 \cdot \dot{V}O_{2max} + 36.28$
Ramp test peak power (W): $TTC = -0.032 \cdot peak\ P + 40.75$
RCP (W): $TTC = -0.029 \cdot RCP + 36.28$
GET (W): $TTC = -0.0082 \cdot GET + 28.30$

The relationships between the predicted TTC based on the above regression equations and the actual TTC are shown in Figure 1. The effect of each data point on the model's predictive ability was determined using Cook's distance. The maximum Cook's distance values for each regression were: CP 0.566; all-out test total work done 0.446; $\dot{V}O_2$ max 0.315; ramp test peak power 0.721; RCP 0.602; and GET 1.168. The predictive capability of the model was found to be acceptable (i.e. Cook's distance <1) in all cases, with the exception of the GET (Figure 1, panel F).

Discussion

The main finding of the present study was that the all-out test CP was significantly correlated with 16.1-km TT performance, with the correlation being stronger than those between GET, RCP or $\dot{V}O_2$ max and the 16.1-km TT performance. This is the first study to demonstrate that the CP derived from the 3-min all-out test is related to field-based athletic performance, and, therefore, supports the validity and practical utility of the test.

The $\dot{V}O_2$ max values (60 ± 4 $ml \cdot kg^{-1} \cdot min^{-1}$) indicate that the subjects in the present study were well trained. Within homogeneous groups of aerobically well-trained individuals (as indicated by $\dot{V}O_2$ max), threshold parameters (LT/GET and CP) have been reported to be stronger predictors of performance than $\dot{V}O_2$ max per se (Bassett & Howley, 2000; Nicholson & Sleivert, 2001). In the present study, there was no correlation between either $\dot{V}O_2$ max or GET and TT performance whereas the CP and RCP were significantly correlated with TT performance. The GET demarcates the boundary between the moderate and heavy exercise intensity domains, and is strongly related to the speed that can be sustained during a marathon (Bassett & Howley, 2000; Jones, 2006). However, the predictive ability of the LT/GET is less strong during shorter duration aerobic events (Jones & Doust, 1998). The 16.1-km TT was completed in ~27 min, requiring a greater rate of adenosine triphosphate (ATP) turnover than would be the case during marathon running. Indeed, the exercise intensity during 16.1-km cycling TT would be expected to approximate the CP (Brickley et al., 2007).

It has previously been reported that the CP, derived using the conventional prediction trial protocol, is significantly correlated with both 17-km and 40-km TT cycling events (Smith et al., 1999). This conventional method for CP determination requires the completion of 3–5 exhaustive severe-intensity constant power output trials on separate days (Hill et al., 2002; Poole et al., 1988; Vanhatalo et al., 2007, 2008b). This limitation has hampered application of the CP concept to athlete performance diagnosis and prognosis. The advent of the 3-min all-out test circumvents this limitation by providing an accurate and expeditious estimation of CP in a single laboratory visit. Vanhatalo et al. (2007, 2008b) reported a close agreement between the CP derived from the all-out test and the CP determined by the conventional method, thereby providing evidence for the criterion validity of the novel all-out test. The findings of the present study are consistent with previous research regarding the relationship between CP and performance (Smith et al., 1999), and demonstrate for the first time that the all-out CP test has strong ecological and predictive validity.

The total work done during the all-out test was the variable most strongly correlated to 16.1-km TT performance. The all-out test is predicated on the notion that there is a fixed amount of work that can be performed above the CP (W′) and, once this has been depleted, the highest sustainable power output is the CP (Burnley et al., 2006; Vanhatalo et al., 2007). Subjects produce maximal effort throughout the all-out test, necessitating the recruitment of all available (task-specific) motor units and the rapid depletion of the W′, so that during the final 30 s of the test the power output closely approximates the CP (Burnley et al., 2006; Vanhatalo et al., 2007, 2008b). The total work done during the test is, therefore, a function of both the CP and the W′. Given that performance during high-intensity endurance exercise will be related to the maximal sustainable oxidative metabolic rate (i.e. CP) and the 'anaerobic capacity' (which may be reflected in

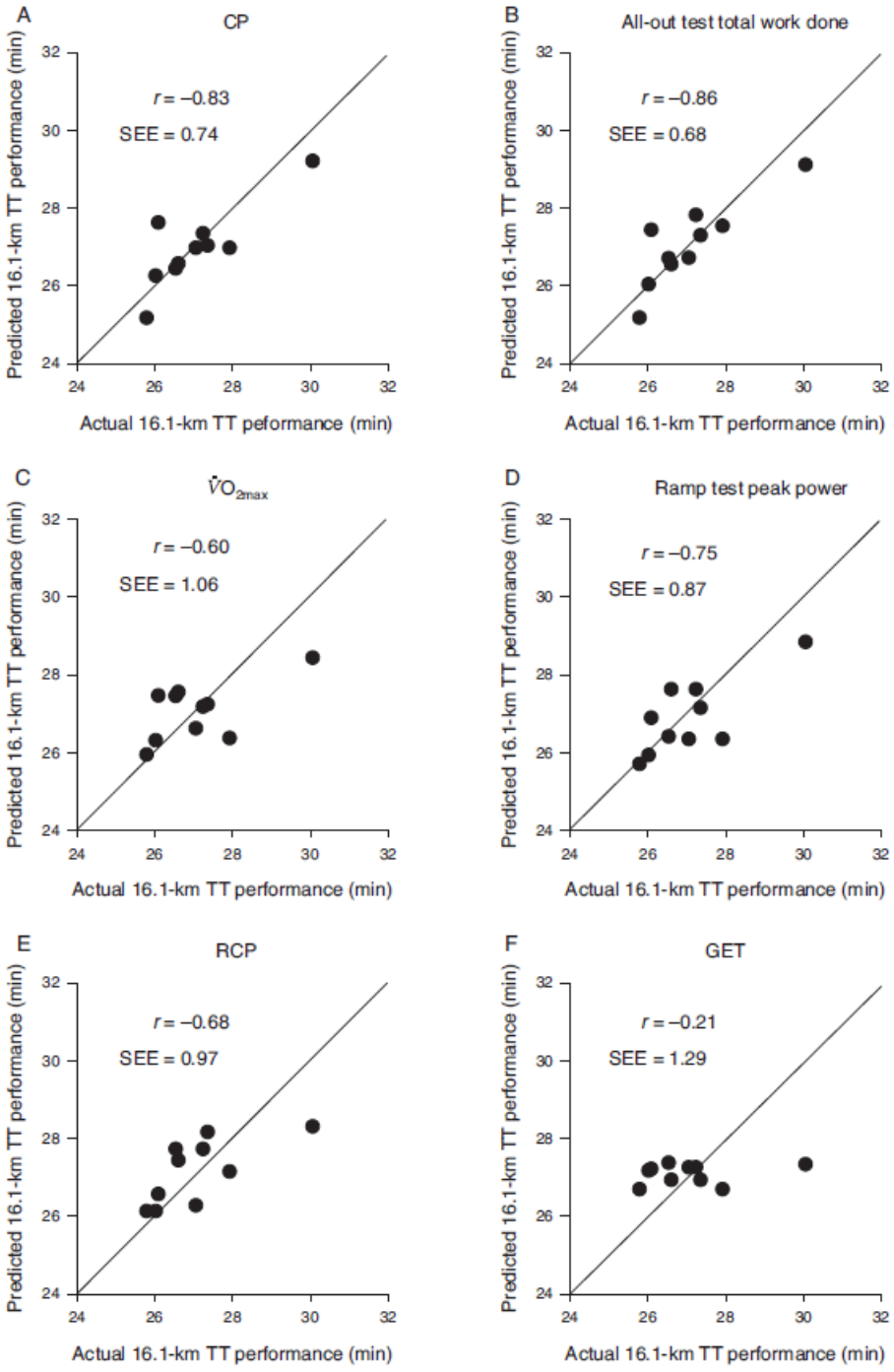

Figure 1. Illustration of the relationships between the actual 16.1-km TT performance and predicted TT performance based on CP (panel A), total work done during the all-out test (panel B), the $\dot{V}O_2$ max (panel C), the ramp test peak power (panel D), the RCP (panel E) and the GET (panel F). The 16.1-km TT performance was most closely correlated with the CP, the total work done in the all-out test, and the ramp test peak power. The solid line in each panel indicates the line of identity.

the W′), the strong correlation between the total work done in the all-out test and 16.1-km cycle TT performance might not be considered surprising. The ramp incremental test peak power, which also reflects both aerobic and anaerobic contributions to energy metabolism, was also significantly correlated with TT performance, consistent with previous studies (Bentley et al., 2001). However, this relationship

was not as strong as for the either the total work done or the CP derived from the all-out test.

It is worth noting that ramp incremental test peak power, GET (when expressed as a power output) and RCP all display some degree of protocol-dependency, with faster ramp rates resulting in higher power outputs for a given $\dot{V}O_2$ (Bentley & McNaughton, 2003; Davis et al., 1982). This has the potential to confound, or at least complicate, interpretation of any relationship between these parameters of aerobic fitness and exercise performance. In contrast, the determination of CP (either via conventional constant power output trials or the all-out test) is less sensitive to the vagaries of test protocol (Vanhatalo, Doust, & Burnley, 2008a), and reflects the power output at a specific highest sustainable $\dot{V}O_2$ (Barker, Poole, Noble, & Barstow, 2006). In the present study, there was no significant difference between the mean CP and RCP, although the two were not significantly correlated. Others have suggested that the RCP might be used to provide an estimate of the CP (Barker et al., 2006). Along with Cross and Sabapathy (2012), however, we would emphasise that although the RCP and the CP may occur at a similar fraction of $\dot{V}O_2$ max, there is no reason to suspect a mechanistic link between the two. Moreover, the RCP does not occur at a consistent $\dot{V}O_2$ and is strongly protocol-dependent (disappearing altogether at slow ramp rates; Wasserman et al., 1994), such that the lack of significant difference between CP and RCP is likely to be coincidental.

One of the main strengths of the 3-min all-out test is that it provides an expeditious estimate of the CP (Vanhatalo et al., 2007, 2008b). However, the all-out test may have other advantages, including a reduced error associated with the determination of the CP compared to the conventional protocol. The test–retest coefficient of variation for the all-out test CP, which is determined as the mean power output during the final 30 s of the test, is $<3\%$ (Burnley et al., 2006). In contrast, the conventional prediction trial protocol for the assessment of CP has two sources of error: the error associated with the mathematical fit of a linear or non-linear regression equation and the errors associated with the test–retest reliability of time-to-exhaustion in each prediction trial. There is no established consensus on the number and duration of trials needed and the optimal mathematical modelling procedure, and although it has been suggested that the coefficient of variation for the mathematical fit of CP should be less than 5% (Hill, 1993) this is not routinely reported in the literature. The total error associated with the conventional CP protocol is exacerbated by the error associated with the time-to-exhaustion in each prediction trial, which has been criticised for lacking reproducibility and ecological validity (Jeukendrup, Saris, Brouns, & Kester, 1996; Taylor & Batterham, 2002). Compared to the conventional method, the 3-min all-out test may, therefore, represent a more objective, reproducible and time-efficient method for CP assessment (Burnley et al., 2006; Vanhatalo et al., 2007, 2008b).

In conclusion, the results of the present study demonstrate for the first time that the laboratory-based all-out test CP is significantly correlated with 16.1-km TT road performance. The correlation between all-out test CP and TT performance was higher than the correlations between the ramp test peak power output, $\dot{V}O_2$ max, RCP and GET and TT performance. These results provide evidence for the ecological validity and practical performance relevance of the all-out test. In addition to its ability to predict performance, the all-out test CP can be established objectively, with high test–retest reliability, in a single laboratory visit. The 3-min all-out test CP may, therefore, represent a useful addition to the battery of tests employed by applied sport physiologists or coaches to track fitness and predict athletic performance.

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ORIGINAL ARTICLE

The constant work rate critical power protocol overestimates ramp incremental exercise performance

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Abstract
Purpose The parameters of the power-duration relationship (i.e., the critical power, CP, and the curvature constant, W′) may theoretically predict maximal performance capability for exercise above the CP. The CP and W′ are associated with the parameters of oxygen uptake ($\dot{V}O_2$) kinetics, which can be altered by manipulation of the work-rate forcing function. We tested the hypothesis that the CP and W′ derived from constant work-rate (CWR) prediction trials would overestimate ramp incremental exercise performance.
Methods Thirty subjects (males, $n = 28$; females, $n = 2$) performed a ramp incremental test, and 3–5 CWR prediction trials for the determination of the CP and W′. Multiple ramp incremental tests and corresponding CP and W′ estimates were available for some subjects such that in total 51 ramp test performances were predicted.
Results The ramp incremental test performance (729 ± 113 s) was overestimated by the CP and W′ estimates derived from the best (751 ± 114 s, $P < 0.05$) and worst (749 ± 111 s, $P < 0.05$) individual fits of CWR prediction trial data. The error in the prediction was inversely correlated with the magnitude of the W′ for the best ($r = -0.56$, $P < 0.05$) and worst individual fits ($r = -0.36$, $P < 0.05$).
Conclusions The overestimation of ramp incremental performance suggests that the CP and W′ derived from different work-rate forcing functions, thus resulting in different $\dot{V}O_2$ kinetics, cannot be used interchangeably. The present findings highlight a potential source of error in performance prediction that is of importance to both researchers and applied practitioners.

Keywords Power-duration relationship · Critical power · W′ · Performance prediction

Abbreviations

Δ	Work rate difference between GET and the $\dot{V}O_{2peak}$
CP	Critical power
CV %	Coefficient of variation
CWR	Constant work rate
GET	Gas exchange threshold
iEMG	Integrated electromyography
P	Power
S	Ramp slope
SEE	Standard error of estimate
τ	Time constant
T_{lim}	Limit of tolerance
W	Work
$\dot{V}_E$	Minute ventilation
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
W′	Curvature constant of the power-duration relationship

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Introduction

High-intensity exercise performance is well described by the hyperbolic relationship between power (P) and time, which can be derived from a series of constant work rate (CWR) trials performed until the limit of tolerance (T_{lim}) (Hill 1993; Jones et al. 2008; Monod and Scherrer 1965; Moritani et al. 1981; Poole et al. 1988). This hyperbolic relationship is constrained by the capacity and rate at which adenosine triphosphate can be resynthesised via aerobic and anaerobic pathways. The power-asymptote of this hyperbola, termed the critical power (CP), denotes the highest work rate at which a physiological steady-state can be attained and is therefore considered to represent the highest work rate that can be sustained without a significant contribution from anaerobic metabolism (Jones et al. 2008; Poole et al. 1988). The curvature constant (W′) of the power-duration relationship is indicative of a fixed amount of work that can be performed above the CP and is associated with the progressive rise in pulmonary oxygen uptake ($\dot{V}O_2$), and the accumulation of fatigue-related metabolites (i.e., inorganic phosphate, hydrogen ions, interstitial potassium; Allen et al. 2008) until the attainment of maximal O_2 uptake ($\dot{V}O_{2max}$) (Poole et al. 1988) and the concomitant achievement of a critical level of intramuscular metabolic perturbation (Vanhatalo et al. 2010). Resolving the parameters of the power-duration relationship, therefore, permits the prediction of exercise performance or tolerance at work rates above the CP according to the equation:

$$T_{lim} = W'/(P - CP) \quad (1)$$

Although the power-duration relationship is conventionally derived from a series of CWR prediction trials, equivalent parameter estimates can also be obtained in a single 3 min all-out test (Burnley et al. 2006; Vanhatalo et al. 2007, 2008). In contrast to CWR exercise, the 3 min all-out test requires subjects to produce their maximal instantaneous power output throughout the test (Burnley et al. 2006; Vanhatalo et al. 2007, 2008). Despite the considerable differences in the work-rate forcing functions between these two testing protocols, similar CP estimates are derived (Simpson et al. 2015; Vanhatalo et al. 2007, 2008). Furthermore, the size of the W′, determined as the work done above CP, has been shown to be similar between ramp incremental, 3 min all-out, and work-matched self-paced time-trial and CWR exercise (Chidnok et al. 2013a). However, Morton et al. (1997) reported a trend for a ~18% lower W′ ($P = 0.07$) when power-duration parameters were estimated from a series of ramp incremental prediction trials at different ramp rates relative to CWR prediction trials. The tendency for a smaller W′ in ramp compared to CWR protocol indicates that the conventional CWR prediction trial protocol may not accurately predict T_{lim} during ramp incremental exercise.

Performance in ramp incremental exercise, where work rate is increased as a linear function of time (e.g., 1 W every 2 s) can be predicted using a modified version of Eq. 1:

$$T_{lim} = CP/S + \sqrt{(2W'/S)} \quad (2)$$

where S represents the ramp slope (e.g., 0.5 W s^{-1}) (Morton 1994). The ramp incremental test represents a distinct work-rate forcing function to test the applicability of the CP and W′ estimates derived from CWR prediction trials. During a fast-ramp protocol, the $\dot{V}O_2$ conforms to quasi-linear first-order kinetics (Whipp et al. 1981; Wilcox et al. 2016), whereas during severe CWR exercise the $\dot{V}O_2$ kinetics manifests an initial fast (or primary) component followed by delayed, progressive increase in $\dot{V}O_2$ termed the 'slow component' (Burnley and Jones 2007; Poole et al. 1988). The time constant (τ) of the primary component has been inversely correlated with CP and endurance performance (Murgatroyd et al. 2011), while the amplitude of the slow component has been positively correlated with the W′ (Murgatroyd et al. 2011; Vanhatalo et al. 2011). Given that the slow component appears to be almost entirely eradicated (or hidden) during fast-ramp incremental exercise (Wilcox et al. 2016), it is possible that the fixed work capacity indicated by the W′ may not be accessible to the same extent as during severe CWR exercise, consistent with the tendency for lower W′ (Morton et al. 1997).

The purpose of this study, therefore, was to evaluate the accuracy with which ramp incremental exercise performance may be predicted by the power-duration parameters derived from a series of CWR prediction trials. We hypothesized that, due to the differences in $\dot{V}O_2$ kinetics, the CP and W′ derived from CWR prediction trials would overestimate the ramp incremental test performance using Eq. 2, and that the prediction error would be related to the W′ but not CP.

Methods

Overview

This work was a retrospective analysis of data collected during previous research studies for which subjects had performed a ramp incremental test and a series of CWR prediction trials (Black et al. 2015; Kelly et al. 2013; Vanhatalo et al. 2007, 2008). Data were collected in two laboratories (University of Wales Aberystwyth and University of Exeter) and tests were performed after informed consent was provided and following the completion of a health screen questionnaire. Experimental procedures were approved by the local ethics committees. Where available, multiple ramp incremental tests and multiple corresponding

parameter estimates (CP and W′) were assessed per subject: 19 males had performed two ramp incremental tests and two sets of prediction trials within the same experimental study, and one male had completed two experimental studies including four ramp incremental tests and four sets of prediction trials. Subjects performed 3–5 prediction trials in all cases (3 trials, 9 cases; 4 trials, 32 cases; 5 trials, 10 cases). In total, 51 data sets, obtained from 30 subjects (males $n = 28$, age, 27 ± 8 years, body mass 75.8 ± 9.8 kg, height 1.79 ± 0.07 m; females $n = 2$, age, 27 ± 4 years, body mass 57.5 ± 0.7 kg, height 1.72 ± 0.03 m) were included in this analysis. Where data had been collected following a supplementation regimen (Kelly et al. 2013), only data from the placebo trials were included in the analysis. The ramp test performance was predicted using parameter estimates derived from CWR prediction trials, where all tests for a given individual were performed within 4 weeks. Subjects were instructed to report to all testing sessions well-hydrated, having avoided strenuous physical activity and caffeine ingestion for 24 and 3 h prior to testing, respectively. Within each study, testing was performed at the same time of day for each subject and laboratory visits were separated by at least 24 h.

Protocol

Determination of peak oxygen uptake and GET

All exercise tests were performed using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The ergometer seat and handlebars were adjusted for comfort, with the cyclists' own pedals fitted if required, and with the same settings replicated for subsequent tests. The ramp protocol consisted of a period of unloaded pedaling (3 or 4 min), followed by a ramp increase in work rate of 30 W min^{-1} (1 W every 2 s) until volitional exhaustion. Subjects were instructed to maintain their preferred cadence (70–90 rpm) for as long as possible. The test was terminated when the pedal rate fell by more than 10 rpm below their preferred cadence for more than 10 s despite strong verbal encouragement. Power output was recorded to the nearest Watt. The ramp rate (30 W min^{-1}) and the end-test power output permitted the determination of T_{lim} to the nearest second. During this and all subsequent tests, breath-by-breath pulmonary gas exchange and ventilation were measured. Subjects wore a nose clip and breathed through a mouthpiece and impeller turbine assembly (Jaeger Triple V, Hoechburg, Germany). The inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analysers (Jaeger Oxycon Pro, Hoechburg, Germany) via a capillary line connected to the mouthpiece. These analysers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, KS). The volume and concentration signals were time-aligned, accounting for the transit delay in capillary gas and analyser rise time relative to the volume signal. Oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$) were calculated using standard formulae (Beaver et al. 1973) and displayed breath-by-breath. Subsequently, the breath-by-breath data were converted to second-by-second data using linear interpolation. The peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) was determined as the highest $\dot{V}O_2$ over a 30 s period. The data were reduced to 10 s mean values for the estimation of the GET, which was determined as: (1) the first disproportionate increase in $\dot{V}CO_2$ versus $\dot{V}O_2$; (2) an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$, and; (3) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension.

Determination of the power-duration relationship

The CP and W′ were estimated from a series of CWR prediction trials performed at different work rates (approximately 60, 70, 80, and 100% $\dot{V}O_{2peak}$; where Δ refers to the work rate difference between the GET and the $\dot{V}O_{2peak}$). Each prediction trial began with a period of unloaded cycling (3 or 4 min) followed by an abrupt transition to the appropriate work rate. Subjects were instructed to maintain their preferred cadence, which was the same as that chosen during the ramp incremental test, for as long as possible. Trials were terminated when cadence fell by more than 10 rpm below their preferred cadence for more than 5 s or 10 s (for details see; Black et al. 2015; Kelly et al. 2013; Vanhatalo et al. 2007, 2008) despite strong verbal encouragement. Subjects were not informed of the work rate or the performance of any trial until all experimental trials had been completed.

Data analyses

The CP and W′ were estimated using three models: the hyperbolic ($P–T_{lim}$) model, where the work rate is plotted against time (Eq. 1); the linear work-time ($W–T_{lim}$) model, where the work done (W) is plotted against time (Eq. 3); and the linear inverse-of-time ($1/T_{lim}$) model (Eq. 4), where work rate is plotted against the inverse of time.

$$W = CP\ T_{lim} + W' \quad (3)$$

$$P = W'\ (1/T_{lim}) + CP \quad (4)$$

The standard error of the estimate (SEE) associated with the CP and W′ were expressed as coefficients of variation (CV %, i.e., relative to the parameter estimate).

Table 1 The parameter estimates derived from Eqs. 1, 3 and 4, and the best (BIF) and worst individual fits (WIF). Total error indicates the sum of the coefficients of variation (CV %) associated with critical power (CP) and the curvature constant (W′) of the power-duration relationship

	R^2	CP (W)	SEE (W)	CV %	W′ (kJ)	SEE (kJ)	CV %	Total error (CV %)
W–T_{lim} model	0.995–1.000	241 ± 48	3 ± 2	1.53 ± 1.22	18.6 ± 5.5	1.3 ± 0.8	7.6 ± 5.8	9.1 ± 6.9
1–T_{lim} model	0.931–1.000	242 ± 50	5 ± 3	2.10 ± 1.73	17.9 ± 4.4	1.2 ± 0.8	6.9 ± 4.9	8.9 ± 6.5
P–T_{lim} model	0.917–1.000	240 ± 48	3 ± 2	1.46 ± 1.29	18.5 ± 4.9	1.6 ± 1.3	9.2 ± 7.0	10.7 ± 8.1
BIF	0.969–1.000	242 ± 48	3 ± 2	1.33 ± 1.07	18.4 ± 5.7	1.0 ± 0.6	5.7 ± 4.2	7.3 ± 5.1
WIF	0.931–1.000	240 ± 50	5 ± 3	2.14 ± 1.84	18.5 ± 4.6	1.8 ± 1.3	9.8 ± 7.1	12.0 ± 8.5

SEE standard error of estimate, T_{lim} time to the limit of tolerance, $1/T_{lim}$ linear inverse-of-time model, P–T_{lim} hyperbolic power-time model, W–T_{lim} linear work-time model

The total error associated with the modelling of the power-duration parameters was calculated as the sum of the CV % associated with the CP and the W′. The sum of the CV % was optimised for each individual by selecting the model (Eqs. 1, 3 or 4) with the smallest total error to produce the "best individual fit" parameter estimates. Similarly, the parameter estimates from a model associated with the largest total error were grouped together to produce the "worst individual fit" parameter estimates. The best fit and worst fit CP and W′ derived from the CWR prediction trials were then used to retrospectively calculate T_{lim} during the ramp incremental exercise test using Eq. 2 (Morton 1994).

Statistical analyses

One-way analysis of variance was used to assess differences in power-duration parameters between models (Eqs. 1, 3, 4, and the best and worst individual fits), and for differences between the $\dot{V}O_{2peak}$ achieved in the ramp incremental test and CWR prediction trials. Paired samples t tests and Bland–Altman analysis were used to evaluate differences between the actual and predicted T_{lim} for the ramp incremental tests. Pearson's product moment correlation coefficient was used to assess relationships between the actual and predicted T_{lim} for the ramp incremental test, and the relationships between the error in estimation for the ramp incremental test T_{lim} and the CP, and W′, respectively. Statistical significance was accepted at $P < 0.05$ and data are presented as mean ± SD.

Results

The $\dot{V}O_{2peak}$ measured during the ramp incremental test was 4.06 ± 0.60 L min^{-1} (54.7 ± 7.5 mL kg^{-1} min^{-1}) and the peak work rate was 365 ± 57 W. The GET occurred at 2.19 ± 0.44 L min^{-1} and 141 ± 38 W. The $\dot{V}O_{2peak}$ measured during the ramp incremental test was not different from the mean $\dot{V}O_{2peak}$ in CWR prediction trials (4.05 ± 0.59 L min^{-1}) measured at T_{lim} ($P > 0.05$).

There were no differences in CP or W′ estimates between the three models (i.e., Eqs. 1, 3, 4), or the best fit and the worst fit parameter estimates ($P > 0.05$; Table 1). The CP estimate from the best fit model corresponded to 66 ± 4% of the ramp incremental test peak power and 45 ± 6% Δ.

The actual ramp incremental test T_{lim} (729 ± 113 s) was significantly correlated with the predicted T_{lim} calculated using the CP and W′ from the best fit model (751 ± 114 s, $r = 0.96$, $P < 0.001$) and the worst fit model (749 ± 111 s, $r = 0.97$, $P < 0.001$) (Fig. 1). However, both the best fit and worst fit models significantly overestimated T_{lim} with a mean bias of 22 s (CV 2.9 ± 2.4%) and 20 s (CV 2.6 ± 2.0%), respectively (Fig. 1). The error in the prediction was negatively correlated with the W′ from the best fit model ($r = -0.56$, $P < 0.001$) and the worst fit model ($r = -0.36$, $P = 0.01$), but was not significantly related to the CP ($P > 0.05$ for best and worst fit models) (Fig. 2).

Discussion

The principal and novel findings of this study were that the CP and W′ derived from a series of CWR prediction trials significantly overestimated ramp incremental test performance. The overestimation in ramp incremental test performance was associated with the magnitude of the W′, but not the CP. These findings may have important implications for normalisation of work rate in research settings, as well as for applied performance prediction, using the power-duration parameters derived from the conventional CWR prediction trial protocol.

In theory, when the CP and W′ are known, the power-duration relationship (Eqs. 1, 3, 4) should be applicable to predict performance in any severe intensity exercise bout irrespective of the work rate forcing function (Fukuba et al. 2003; Hill 1993; Jones et al. 2010; Morton 2006). To test this assumption, we performed a retrospective analysis of data sets for which the power-duration relationship had been estimated from a series of CWR prediction trials and used these parameter estimates to predict each subject's

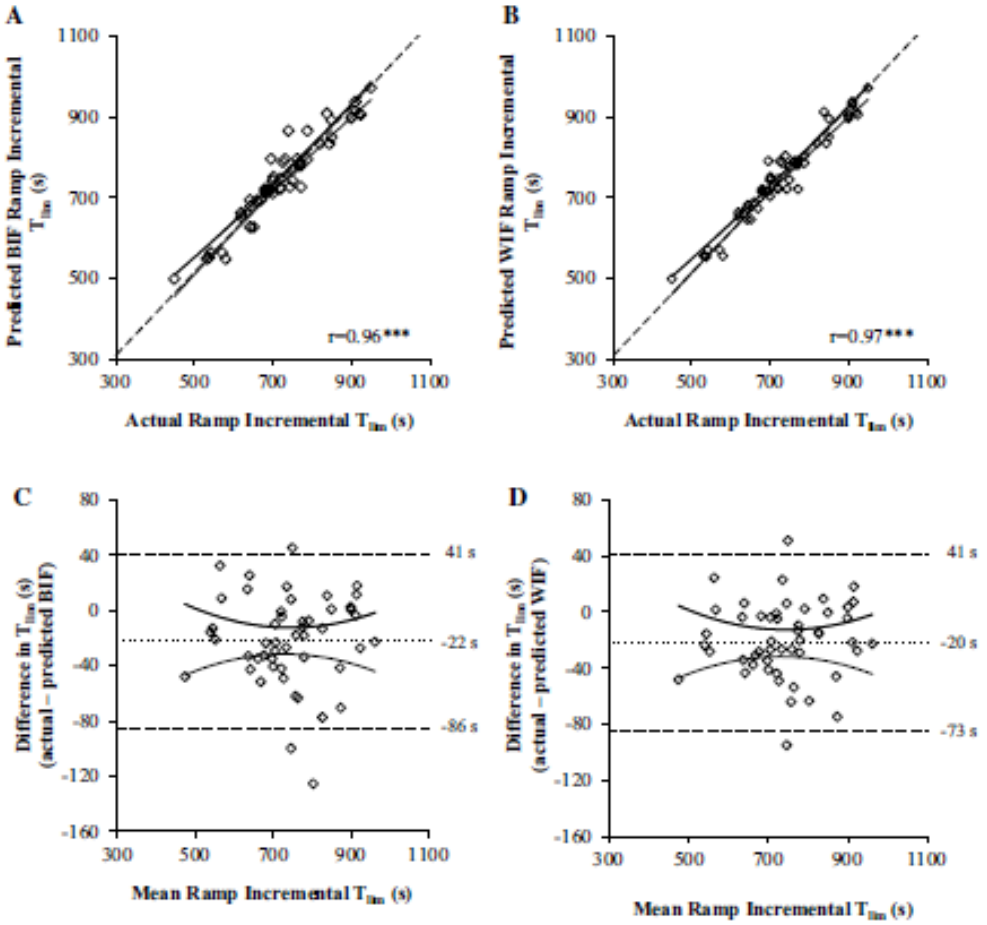

Fig. 1 Bland-Altman plots of the relationship (**a** and **b**) and the limits of agreement (**c** and **d**) between the actual and predicted ramp incremental T_{lim} using the 'best individual fit' (BIF; **a** and **c**) and the 'worst individual fit' (WIF; **b** and **d**). **a** and **b** the *line* of origin (*dashed line*) and 95% confidence intervals (*solid lines*) are presented. **c** and **d**, the mean difference (*dotted line*), the 95% confidence intervals (*solid line*) and the limits of agreement (*dashed line*) are provided. ***$P < 0.001$

ramp incremental exercise performance (Eq. 2; Morton 1994). The actual ramp incremental performance was overestimated by ~3% irrespective of whether the best or worst individual fits were used (Fig. 1). It should be noted that the coefficient of variation between the actual and predicted T_{lim} (~3%, or ~11 W) in the present study, consistent with previous data (CV %, 3 ± 3%, $n = 7$; Chidnok et al. 2013a), is fivefold greater than the typical test–retest reliability of a 30 W min^{-1} ramp incremental test performance (CV 0.53%; Weston and Gabbett 2001). This small, but consistent, overestimation in the performance prediction highlights the need for caution when using CP and W′ estimates derived from CWR protocols to predict exercise tolerance during ramp incremental exercise and potentially also during other work-rate forcing functions.

It has been previously shown that similar power-duration parameter estimates can be derived from two protocols employing contrasting work-rate forcing functions, that is: (1) a series of CWR trials, where the subject maintains a specified work rate for as long as possible; and (2) a 3 min all-out test, in which the subject exerts their maximal instantaneous power output throughout (Burnley et al. 2006; Simpson et al. 2015; Vanhatalo et al. 2007, 2008). Similarly, it has also been shown that the magnitude of the W′ is similar irrespective of its rate of utilisation (Fukuba et al. 2003; Chidnok et al. 2013a). It is important to note that in these experiments (Fukuba et al. 2003; Chidnok et al. 2013a) the W′ was estimated as the 'work done >CP', assuming that the CP itself was unaffected by different work rate forcing functions. Although the power-duration relationship was not

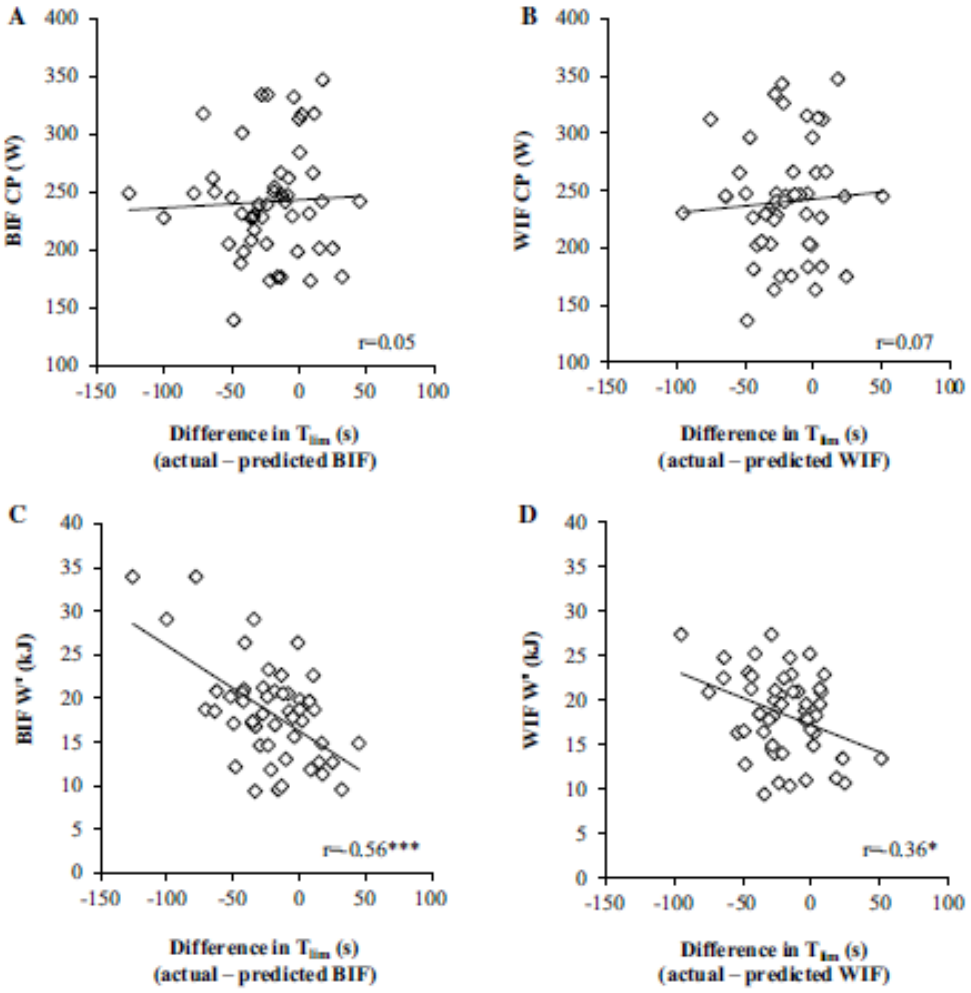

Fig. 2 Relationship between the difference in actual and predicted T_{lim} derived from the 'best individual fit' (BIF; **a** and **c**) and the 'worst individual fit' (WIF; panels B and D) and the CP (**a** and **b**), and W′ (**c** and **d**). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

established for the ramp incremental exercise in the present study, our findings suggest that there was a reduction in the CP and/or W′ during ramp incremental exercise relative to the CWR prediction trials. The only study to date that has directly compared the CP and W′ estimates derived from a series of CWR and ramp incremental prediction trials reported no difference in the CP but a tendency for a lower W′ during ramp incremental exercise (Morton et al. 1997). It is therefore likely that the overestimation of ramp incremental performance was due to a reduction in the W′ in ramp incremental exercise relative to the CWR protocol.

The mechanisms underlying a smaller W′ during ramp incremental exercise relative to CWR exercise may relate to differences in the motor unit recruitment patterns and $\dot{V}O_2$ kinetics in response to different work rate forcing functions. The severe intensity CWR exercise trials necessitate a progressive increase in motor unit recruitment and/or firing frequency, which is consistent with an increase in integrated electromyography (iEMG) until T_{lim} (Vanhatalo et al. 2011). A similar increase in iEMG response is evident during ramp incremental exercise (Chidnok et al. 2013a; Scheuermann et al. 2002), but unlike CWR exercise, performance is dependent on the subjects' ability to increase their work rate to meet the continually increasing, externally imposed work rate (e.g., 0.5 W s^{-1}). There is some evidence to suggest that the accessible portion of the W′ may be partly determined

by the rate of its utilisation and not merely by the capacity of W′ remaining (Chidnok et al. 2013b) The inability to achieve the higher imposed work rate, rather than task failure of motor units at a given constant work rate, may limit the accessible portion of the W′, thus reducing ramp incremental exercise performance relative to that predicted from CWR prediction trials. In contrast, the power profile during the 3 min all-out test is not externally imposed but rather reflects the subject's ability to generate maximal force which declines with time. Therefore, despite a reversal in the iEMG profile in the 3 min all-out test relative to CWR and ramp incremental exercise (i.e., a progressive decline in iEMG throughout the test) (Vanhatalo et al. 2011), it appears possible to access the W′ to the same extent during all-out and CWR severe intensity exercise (Simpson et al. 2015; Vanhatalo et al. 2007, 2008).

Although each subject attained a consistent $\dot{V}O_{2peak}$ at T_{lim} following all experimental trials, the $\dot{V}O_2$ kinetics differed significantly between protocols. During ramp incremental exercise, the $\dot{V}O_2$ increases in proportion to the increase in work rate, displaying a quasi-linear response which persists even at work rates above the GET, at least during fast-ramp incremental protocols (Rossiter 2011; Whipp et al. 1981; Wilcox et al. 2016). In contrast, following an abrupt step increase to a constant work rate within the severe intensity domain (>CP), the $\dot{V}O_2$ increases exponentially and is supplemented by an additional $\dot{V}O_2$ slow component which elevates $\dot{V}O_2$ to a greater value than that predicted from the extrapolation of $\dot{V}O_2$ from work rates below the GET (Burnley and Jones 2007; Rossiter 2011; Poole et al. 1988). Since the amplitude of the $\dot{V}O_2$ slow component is positively correlated with the size of the W′ (Murgatroyd et al. 2011; Vanhatalo et al. 2011), it is possible that the overestimation of ramp incremental exercise performance by the CWR prediction trial protocol may be related to the limited scope for the development of the $\dot{V}O_2$ slow component (and thus, incomplete access to W′) during ramp incremental compared to CWR exercise. It may be speculated that accuracy of the ramp test performance prediction by the CWR prediction trial protocol may be improved by reducing the ramp rate considerably, thus revealing an upwardly curvilinear $\dot{V}O_2$ response (Scheuermann et al. 2002).

An important observation in the present study was that the error in the ramp test performance prediction by Eq. 2 was correlated with the W′, such that the greatest overestimation was evident in subjects with the largest W′ (Fig. 2). There was no relationship between the prediction error and the CP. These relationships provide further support for the interpretation that the accuracy of the ramp test performance prediction might have been adversely influenced by a discrepancy between the size of the W′ determined in a CWR protocol and the accessible portion of this W′ during ramp incremental exercise.

The close agreement between the parameter estimates derived from Eqs. 1, 3 and 4; the goodness of fit of each model to the experimental data; and the similarity of the CP estimates derived from the best (242 ± 48 W) and the worst (240 ± 50 W) individual fits (Table 1) manifest low incidence of random and systematic errors in the prediction trial data (Hill and Smith 1994). The CP and W′ estimates derived from the best and worst individual fits, therefore, predicted ramp test performance to a similar degree of (in) accuracy (Fig. 1). It should be noted, however, that the range of errors associated with the mathematical modelling of the W′ was considerably broader within the worst (CV % 0.11–34.4%) compared to the best individual fit (CV % 0.08–15.5%) (Table 1). Further research is warranted to identify whether the selection of the 'best individual model fit' for each subject is superior to conventional 'one model fits all' approach when predicting self-paced, maximal exercise performance that better reflects competitive sport.

In conclusion, ramp incremental exercise performance was not accurately predicted by the power-duration parameters derived from a series of CWR prediction trials. The parameter estimates overestimated actual performance. This overestimation was likely due to a reduction in the accessible portion of the W′ in the ramp test due to differences between the work-rate forcing functions and $\dot{V}O_2$ kinetics in the two protocols (i.e., CWR vs. ramp incremental). This is consistent with the association between the predictive error and the magnitude of the W′. Whilst it is recognised that ramp incremental exercise represents an extreme work-rate forcing function atypical of any sport, the inaccuracy in the prediction of ramp incremental performance highlights a potentially important consideration for the matching of prediction trials to the performance test. The present findings are consistent with the notion that the power-duration parameters are sensitive to interventions that alter $\dot{V}O_2$ kinetics. Further investigation is warranted into effects of different work-rate forcing functions on the power-duration relationship when predicting exercise tolerance and performance in both research and applied settings.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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ARTICLE

Self-pacing increases critical power and improves performance during severe-intensity exercise

Matthew I. Black, Andrew M. Jones, Stephen J. Bailey, and Anni Vanhatalo

Abstract: The parameters of the power-duration relationship for severe-intensity exercise (i.e., the critical power (CP) and the curvature constant (W')) are related to the kinetics of pulmonary O_2 uptake, which may be altered by pacing strategy. We tested the hypothesis that the CP would be higher when derived from a series of self-paced time-trials (TT) than when derived from the conventional series of constant work-rate (CWR) exercise tests. Ten male subjects (age, 21.5 ± 1.9 years; mass, 75.2 ± 11.5 kg) completed 3–4 CWR and 3–4 TT prediction trial protocols on a cycle ergometer for the determination of the CP and W'. The CP derived from the TT protocol (265 ± 44 W) was greater ($P < 0.05$) than the CP derived from the CWR protocol (250 ± 47 W), while the W' was not different between protocols (TT: 18.1 ± 5.7 kJ, CWR: 20.6 ± 7.4 kJ, $P > 0.05$). The mean response time of pulmonary O_2 uptake was shorter during the TTs than the CWR trials (TT: 34 ± 16, CWR: 39 ± 19 s, $P < 0.05$). The CP was correlated with the total O_2 consumed in the first 60 s across both protocols ($r = 0.88$, $P < 0.05$, $n = 20$). These results suggest that in comparison with the conventional CWR exercise protocol, a self-selected pacing strategy enhances CP and improves severe-intensity exercise performance. The greater CP during TT compared with CWR exercise has important implications for performance prediction, suggesting that TT completion times may be overestimated by CP and W' parameters derived from CWR protocols.

Key words: power-duration relationship, critical power, W', $\dot{V}O_2$ mean response time, time trial, severe-intensity exercise.

Résumé : Les paramètres de la relation puissance-durée au cours d'un exercice très intense (c.-à-d. la puissance critique (« CP ») et la constante de courbure (« W' »)) s'appliquent à la cinétique de la captation pulmonaire d'oxygène qui peut être modifiée par la vélocité adoptée. Nous vérifions l'hypothèse selon laquelle CP est plus élevée lorsqu'issue d'une série de courses autodéterminées contre-la-montre (« TT ») comparativement à une série courante d'efforts en régime stable (« CWR »). Dix sujets masculins (âgé, 21,5 ± 1,9 ans; masse, 75,2 ± 11,5 kg) participent à des protocoles de prédiction comportant 3-4 CWR et 3-4 TT sur cycloergomètre pour la détermination de CP et de W'. La CP obtenue lors du protocole TT (265 ± 44 W) est plus élevée ($P < 0,05$) que la CP du protocole CWR (250 ± 47 W); en contrepartie, W' ne varie pas d'un protocole à l'autre (TT: 18,1 ± 5,7 kJ, CWR: 20,6 ± 7,4 kJ, $P > 0,05$). Le temps de réponse moyen de la consommation d'oxygène est plus bref en TT qu'en CWR (TT: 34 ± 16, CWR: 39 ± 19 s, $P < 0,05$). La CP est corrélée à la consommation totale d'O_2 durant les 60 secondes initiales dans les deux protocoles ($r = 0,88$, $P < 0,05$, $n = 20$). D'après ces résultats, le protocole d'effort autodéterminé élève plus la CP comparativement au protocole CWR courant d'où une meilleure performance au cours d'un exercice très intense. La CP plus élevée en TT qu'en CWR a des implications importantes pour la prédiction de performance, car le temps de performance au TT pourrait être surestimé par les paramètres de CP et de W' obtenus lors des protocoles CWR. [Traduit par la Rédaction]

Mots-clés : relation puissance-durée, puissance critique, W', temps de réponse moyen du $\dot{V}O_2$, contre-la-montre, exercice d'intensité très élevée.

Introduction

The tolerable duration of exercise decreases in a hyperbolic fashion as power output increases within the severe-intensity exercise domain (Jones et al. 2010; Morton 2006). This hyperbolic power-duration relationship is characterised by a power-asymptote, termed critical power (CP), and a curvature constant, termed W'. The CP denotes the highest work rate that can be sustained without a progressive loss of intramuscular and systemic homeostasis (Jones et al. 2008*a*; Poole et al. 1988) and is strongly associated with endurance exercise performance (Black et al. 2014; Smith et al. 1999). The W' represents a fixed amount of work that can be performed above CP before exhaustion (Moritani et al. 1981; Poole et al. 1988). During exercise above CP, where pulmonary O_2 uptake ($\dot{V}O_2$) continues to increase until its maximum ($\dot{V}O_{2max}$) is achieved (Poole et al. 1988), exercise tolerance is predictable according to the power-duration relationship, based on the premise that the size of the W' remains constant irrespective of its rate of expenditure (Chidnok et al. 2013; Morton 2006). Knowledge of the CP and W' is therefore of considerable importance for understanding the limitations to high-intensity exercise tolerance and for predicting athletic performance (Jones et al. 2010; Morton 2006).

The parameters of the power-duration relationship are conventionally determined by modelling the relationship between work rate and time to exhaustion (T_{lim}) as derived from a series of constant work rate (CWR) exercise tests (Hill 1993; Housh et al. 1989; Jones et al. 2008*a*; Moritani et al. 1981; Poole et al. 1988). It has been suggested, however, that time trial (TT) tests, where subjects manipulate their own pace to complete a set distance or amount of work as quickly as possible, are more reliable than CWR tests and more closely replicate competitive sport (Hopkins et al. 2001; Jeukendrup et al. 1996). While TT-type performance tests have been used in the field to determine the power-duration relationship (Karsten et al. 2014; Quod et al. 2010), it is presently not

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known whether CWR and TT protocols performed under identical experimental conditions yield equivalent CP and W' parameter estimates.

It has been suggested that a self-selected "parabolic" pacing strategy, typically involving a fast start, may enable the individual to maintain a higher mean power output for the duration of a TT than would be possible with an externally imposed CWR test (Thomas et al. 2013). A fast start pacing strategy has been shown to augment the $\dot{V}O_2$ response early in exercise and lead to a greater tolerable duration of severe-intensity exercise than would be predicted based on the CP and W' derived from CWR prediction trials (Jones et al. 2008*b*). These findings suggest that a fast start pacing strategy may improve exercise performance by increasing CP, W', or both. If the power-duration relationship is significantly impacted by variations in pacing strategy, this may have considerable implications for the accuracy with which competitive TT performance can be predicted based on the CP and W' as traditionally measured. There is a strong body of evidence to suggest that adopting a fast start pacing strategy increases the rate at which $\dot{V}O_2$ increases towards its maximum and improves severe-intensity exercise performance compared with a CWR pacing strategy (Aisbett et al. 2009; Bailey et al. 2011; Hettinga et al. 2009; Jones et al. 2008*b*). As the CP has been shown to be inversely correlated with the phase II $\dot{V}O_2$ time constant (τ) (Murgatroyd et al. 2011), it is conceivable that a parabolic pacing strategy, which is typically self-selected during a TT, may augment the $\dot{V}O_2$ response at exercise onset and increase the CP.

The purpose of this study, therefore, was to determine the effect of pacing strategy during severe-intensity prediction trials on the CP and the $\dot{V}O_2$ mean response time (MRT). We tested the hypotheses that the CP derived from a series of self-paced TTs would be greater than the CP derived from CWR prediction trials, and that the $\dot{V}O_2$ MRT would be lower during TT compared with CWR prediction trials. A secondary purpose of this study was to evaluate the accuracy of TT performance prediction on the basis of power-duration parameters derived from a CWR prediction trial protocol.

Materials and methods

Subjects

Ten healthy, physically active male subjects (mean ± SD: age, 21.5 ± 1.9 years; height, 1.79 ± 0.07 m; mass, 75.2 ± 11.5 kg) volunteered to participate in this study. The study was approved by the University of Exeter Research Ethics Committee. Written informed consent was obtained and a physical activity readiness questionnaire (PAR-Q) was completed prior to data collection. Subjects were instructed to report to all testing sessions well-hydrated, having avoided strenuous physical activity and caffeine ingestion for 24 h and 3 h prior to testing, respectively. Testing was completed at the same time of day (±2 h) for each subject and laboratory visits were separated by at least 24 h.

Experimental design

Subjects reported to the laboratory on 11–18 occasions over a 6-week period, such that subjects typically completed 2–3 exercise tests per week. All tests were performed on an electronically braked cycle ergometer (Lode, Excalibur, Groningen, the Netherlands). The ergometer seat and handlebar height were adjusted for comfort and the same settings were replicated for subsequent tests. Subjects performed a ramp incremental test to volitional exhaustion for the determination of the gas exchange threshold (GET) and the peak O_2 uptake ($\dot{V}O_{2peak}$). Subjects then performed 3–4 CWR prediction trials and 3–4 TT prediction trials in a semi-randomised order for the determination of the power-duration relationship. The CWR trial always preceded the corresponding TT to enable the calculation of an appropriate fixed resistance (i.e., linear factor) for the TT and to ensure similar durations and cadences of CWR and TT prediction trials. Trials were randomised such that the comparative TT test was not always administered immediately after the comparative CWR test (by way of example, test 1: CWR3, test 2: CWR2, test 3: TT3, test 4: TT2, test 5: CWR1, test 6: TT1 etc.). Each TT prediction trial was preceded by at least 1 familiarisation session. Following the completion of all CWR and TT prediction trials, the ramp incremental test was repeated to assess potential training effects that might have occurred during the study period.

Determination of the GET and $\dot{V}O_{2peak}$

The ramp incremental test included 4 min of "unloaded" (20 W) pedalling, followed by a ramp increase in power of 30 W·min^{-1}. Subjects were instructed to maintain their preferred cadence (80 revolutions per minute (rpm), $n = 7$; 90 rpm, $n = 3$) throughout the test. The test was terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement. The GET was determined as (i) the first disproportionate increase in carbon dioxide output ($\dot{V}CO_2$) versus $\dot{V}O_2$; (ii) an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$; and (iii) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The GET was estimated independently by 3 experienced assessors using code-labelled data files. In all cases at least 2 assessors identified the same value. The $\dot{V}O_{2peak}$ was determined as the highest 15-s rolling mean $\dot{V}O_2$ recorded during the test.

Determination of the power-duration relationship using CWR trials

CP and W' were estimated from 3–4 CWR prediction trials (3 trials, $n = 5$; 4 trials, $n = 5$), performed at different work rates (approximately 60% Δ, 70% Δ, 80% Δ, and 100% $\dot{V}O_{2peak}$; where Δ refers to the work rate difference between the GET and the $\dot{V}O_{2peak}$). As a quality control measure of the mathematical modelling of power-duration parameters, a priori criteria were set for the standard errors associated with the CP and W', such that if the standard errors associated with the CP and W' exceeded 5% and 10%, respectively, after 3 prediction trials had been performed, a fourth prediction trial was completed. Any prediction trials where the end-exercise $\dot{V}O_2$ was <95% of the individual's ramp test determined $\dot{V}O_{2peak}$ were excluded from the modelling of the power-duration relationship. The work rates for each trial were selected to obtain a range of T_{lim} between approximately 2 and 15 min, with a minimum of 5 min between the shortest and longest trials. Each trial started with 4 min of unloaded pedalling at the subject's preferred cadence, followed by a step increase to the required work rate. Subjects were instructed to remain seated and to maintain their preferred cadence for as long as possible. Strong verbal encouragement was provided throughout, but subjects were not informed of the work rate or the elapsed time. The test was terminated when cadence fell by more than 10 rpm below the preferred cadence for more than 5 s. The T_{lim} was recorded to the nearest second.

Determination of the power-duration relationship using TTs

The total work done during each CWR prediction trial for each subject was used as a "target total work done" for the corresponding self-paced, maximal TT. The Lode Excalibur Sport is an electromagnetically braked ergometer, which enables TTs to be performed against a fixed resistance. For the TTs, the ergometer was set on the so-called "linear mode", where the fixed resistance (i.e., linear factor) is set according to the equation Linear factor = CWR/rpm^2, where CWR represents the work rate in the given CWR prediction trial and rpm represents the cadence self-selected by the subject during the initial ramp test. The same a priori criteria for the standard errors were applied as for the CWR protocol, such that if the standard errors associated with the CP and W' exceeded 5% and 10%, respectively, after 3 prediction trials

had been performed, a fourth prediction trial was completed. Had a subject performed only 3 CWR prediction trials, the amount of work to be completed during the fourth TT was estimated using eq. 2, and the power output for the determination of the linear factor was estimated using eq. 3. Therefore, the CP and W' were estimated from 3-4 TT prediction trials (3 trials, $n = 3$; 4 trials, $n = 7$), and the work rates for each trial were selected to obtain a range of T_{lim} between approximately 2 and 15 min, with a minimum of 5 min between the shortest and longest trials. Each test started with 4 min of unloaded pedalling at each subject's preferred cadence, followed immediately by the TT.

The linear mode does not allow the subject to change the linear factor (i.e., the "gear") during the protocol. In other words, the conditions during the TT simulated a situation where the subject pedals a bicycle along a level road, and while it is not possible to change the gear, speed can be varied by varying cadence. For maximal TT performance, subjects were instructed to complete the given target work done as quickly as possible. Subjects were familiarized to each self-paced target work done, such that they performed a minimum of 2 trials at each target work done where the criteria for a maximal TT performance were as follows: (*i*) attainment of >95% of the $\dot{V}O_{2peak}$ measured in the ramp incremental test; and (*ii*) the performance times between the 2 consecutive TTs varying by less than 5%. Upon meeting these criteria, the shortest time taken to achieve the designated amount of work for each TT was used for analysis. Visual and verbal feedback regarding the amount of work completed was deliberately provided throughout the trials to mimic real-world TT conditions. Subjects were instructed to remain seated and were provided with strong verbal encouragement.

In all tests, subjects wore a nose clip and breathed through a low-dead space (90 mL), low-resistance (0.75 mm $Hg \cdot L^{-1} \cdot s^{-1}$) mouthpiece and impeller turbine transducer assembly (Jaeger Triple V, Jaeger GmbH, Hoechburg, Germany). Inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz. The gases were drawn continuously from the mouthpiece through a 1.5-m sampling line (0.5 mm internal diameter) to paramagnetic (O_2) and infrared (CO_2) analysers (Jaeger Oxycon Pro, Hoechburg, Germany). These analysers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, KS). The volume and concentration signals were time-aligned, accounting for the transit delay in capillary gas and analyser rise time relative to volume signal. The $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ were calculated for each breath using standard formulae. The $\dot{V}O_{2peak}$ during the CWR and TT prediction trials was calculated as the highest 15-s rolling mean value.

Data analyses

The CP and W' parameters were estimated using 3 models: the hyperbolic power-time ($P\text{-}T_{lim}$) model (eq. 1); the linear work-time ($W\text{-}T_{lim}$) model, where the total work done (W) is plotted against time (eq. 2); and the linear inverse-of-time ($1/T_{lim}$) model, where power output is plotted against the inverse of time (eq. 3):

$$T_{lim} = W'/(P - CP) \quad (1)$$

$$W = CP \cdot T_{lim} + W' \quad (2)$$

$$P = W' \cdot (1/T_{lim}) + CP \quad (3)$$

The power (P) during the TTs was defined as the mean power output measured across the duration of each trial. The standard errors of estimate (SEEs) associated with the CP and W' were expressed as coefficients of variation (CV%; i.e., relative to the parameter estimate). The "total error" associated with the modelling of the power-duration parameters was calculated as the sum of the CV% associated with the CP and the CV% associated with the W'. The sum of the CV% was optimised for each individual by selecting the model with the smallest total error (eq. 1, 2, or 3) to produce the "best individual fit" parameter estimates. Similarly, the parameter estimates from a model associated with the largest total error were grouped together to produce the "worst individual fit" parameter estimates. The "best fit" and "worst fit" CP and W' derived from the CWR prediction trials were then used to calculate the predicted TT completion time (T_{lim}) for each target work done (W) by rearranging eq. 2:

$$T_{lim} = (W - W')/CP \quad (4)$$

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, etc., and those values lying more than 4 SD from the local mean were removed. Subsequently, the breath-by-breath data were converted to second-by-second data using linear interpolation and time aligned to the start of the exercise. A nonlinear least squares algorithm was used to fit the data. Given that the subjects only completed 1 trial at each work rate, it was not justified to use a bi-exponential model to characterise the $\dot{V}O_2$ kinetics as the statistical confidence in the derived parameter estimates would be low. Therefore, a single exponential model without time delay, with the fitting window commencing at $t = 0$ s (equivalent to the MRT), was used to characterise the overall $\dot{V}O_2$ response as described in the following equation:

$$\dot{V}O_2(t) = \dot{V}O_2\text{baseline} + A \cdot (1 - e^{-t/\tau}) \quad (5)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time (t), $\dot{V}O_2$ baseline is the average of the $\dot{V}O_2$ measured over the final 120 s of baseline pedalling, A and τ represent the amplitude and time constant, respectively, describing the overall increase in $\dot{V}O_2$ above baseline. The total O_2 consumed (in litres) was also calculated for the first 60 s of each exercise bout as well as for the entire exercise bout.

Statistical analyses

Two-way ANOVAs with repeated measures were conducted to assess differences in power-duration parameters between models (eqs. 1–3 and the best and worst individual fits) and protocols (CWR vs. TT). Independent samples 1-way ANOVA was used to assess differences in the $\dot{V}O_{2peak}$ achieved in the TT ($n = 37$) and CWR ($n = 35$) tests and the ramp test $\dot{V}O_{2peak}$ ($n = 10$ pre- and poststudy). Paired samples t tests were used to evaluate differences in the $\dot{V}O_2$ MRT, total O_2 consumed, mean power output, the mean power output over the first 30 s of the TT, and trial completion time between the corresponding work-matched CWR and TT prediction trials ($n = 33$). Paired samples t tests were also used to evaluate differences in the actual versus predicted TT completion times ($n = 37$) between the CWR best and worst individual fits. Pearson's product moment correlation coefficient was used to assess the relationships between the $\dot{V}O_2$ MRT and the CP ($n = 20$), the total O_2 consumed and the CP ($n = 20$), the relationship between the relative change in CP and the relative change in W' between the CWR and TT protocols ($n = 10$), and the relationship between actual and predicted TT performance ($n = 37$). Statistical significance was accepted at $P < 0.05$ and data are presented as means ± SD.

Results

The $\dot{V}O_{2peak}$ measured in the initial ramp incremental test was 4.14 ± 0.56 $L \cdot min^{-1}$ (56 ± 6 $mL \cdot kg^{-1} \cdot min^{-1}$) and the peak power output was 377 ± 59 W. The GET occurred at 2.06 ± 0.33 $L \cdot min^{-1}$ and 122 ± 30 W. There was no significant difference between the

Table 1. The parameter estimates derived from eqs. 1–3 and the best and worst individual fits for the time trial (TT) and constant work-rate (CWR) protocols. Total error indicates the sum of coefficients of variation (CV%) associated with critical power (CP) and the curvature constant (*W'*).

	R^2	CP (W)	SEE (W)	CV%	*W'* (kJ)	SEE (kJ)	CV%	Total error (CV%)
CWR protocol								
W-T_{lim} model	0.996–1.000	248±44	4.6±5.6	1.7±1.7	21.4±7.0	1.8±2.0	9.5±9.8	11.2±11.5
1/T_{lim} model	0.964–0.998	251±47	4.9±3.1	1.9±1.0	19.1±5.1	1.3±0.7	7.3±4.7	9.3±5.6[c]
P-T_{lim} model	0.965–0.999	247±43	4.8±4.7	1.9±1.6	20.6±6.0	2.5±2.1[c]	12.4±9.3[c,e]	14.3±10.8[c]
Best individual fit	0.974–1.000	250±47	3.6±3.3	1.4±0.9	20.6±7.4	1.2±0.7[d]	6.7±4.9[d]	8.0±5.7[d]
Worst individual fit	0.987–0.998	247±44	10.0±6.6	4.1±2.7	20.3±5.2	3.6±2.2[c]	18.5±10.7[c]	22.6±12.4[c]
TT protocol								
W-T_{lim} model	0.996–1.000	265±45[a]	5.3±2.3	2.1±1.2	18.5±6.8	2.1±0.9[c,e]	12.3±5.1[c,e]	14.4±5.7[c,e]
1/T_{lim} model	0.935–0.999	265±44[a]	5.9±3.0	2.3±1.3	18.0±5.7	1.4±0.8[d]	8.9±5.2[d]	11.2±6.2[d]
P-T_{lim} model	0.804–0.994	264±45[b]	7.1±6.2	2.6±2.0	18.4±8.4	3.4±2.5[c,e]	20.7±13.1[c,e]	23.3±14.5[c,e]
Best individual fit	0.983–1.000	265±44[a]	5.5±2.8	2.2±1.3	18.1±5.7	1.4±0.8[d]	8.5±4.9[d]	10.7±6.0[d]
Worst individual fit	0.804–0.998	265±44[b]	7.5±6.0	2.8±1.9	17.8±7.3	3.4±2.5[c]	20.8±13.0[c]	23.6±14.3[c]

Note: SEE, standard error of estimate; T_{lim}, time to exhaustion; 1/T_{lim}, linear inverse-of-time; P-T_{lim}, hyperbolic power-time model; *W*-T_{lim}, linear work-time.

[a]Significantly different from the respective CWR protocol ($P < 0.05$).

[b]Statistical trend towards difference from the respective CWR protocol ($P < 0.06$).

[c]Significantly different from the best individual fit ($P < 0.05$).

[d]Significantly different from the worst individual fit ($P < 0.05$).

[e]Significantly different from the 1/T_{lim} model within the same protocol ($P < 0.05$).

pre- and poststudy $\dot{V}O_{2peak}$ determined during ramp incremental exercise ($P > 0.05$).

The power-duration parameters

The T_{lim} in the CWR prediction trials was 384 ± 189 s (range: 135 to 896 s). The $\dot{V}O_{2peak}$ values in the CWR prediction trials (4.14 ± 0.47 L·min^{-1}) were not significantly different from the $\dot{V}O_{2peak}$ achieved during the ramp incremental test ($P > 0.05$). The mean cadence during the CWR prediction trials was 83 ± 4 rpm. There were no differences in CP or *W'* estimates between the 3 models (eqs. 1–3), or the best individual fit and the worst individual fit within the CWR protocol ($P > 0.05$; Table 1). The CP estimate from the best individual fit corresponded to 66% ± 4% of ramp incremental test peak power and 46% ± 8%Δ.

The completion times of the TT prediction trials was 371 ± 185 s (range: 135 to 899 s). The TT completion times were significantly shorter than the T_{lim} in the respective CWR prediction trials ($P < 0.05$). The $\dot{V}O_{2peak}$ values in the TT prediction trials (4.21 ± 0.45 L·min^{-1}) were not significantly different from the values attained in the CWR prediction trials, or the values achieved during the ramp incremental test ($P > 0.05$). The mean cadence during the TT prediction trials was 84 ± 5 rpm. There were no differences in CP or *W'* estimates between the 3 models (eqs. 1–3), or the best individual fit and the worst individual fit within the TT protocol ($P > 0.05$; Table 1). The CP estimate from the best individual fit corresponded to 71% ± 8% ramp incremental test peak power and 53% ± 12%Δ.

The mean power outputs maintained during corresponding work-matched prediction trials ($n = 33$) were significantly greater during the TT prediction trials (330 ± 56 W) compared with the CWR prediction trials (318 ± 59 W) ($P < 0.05$). The mean power output over the first 30 s of the TT tests (357 ± 78 W) was significantly greater than the mean power output during the CWR prediction trials ($P < 0.05$; $n = 33$), representing 107% ± 8% of the TT mean power. The pacing strategy of a representative subject is displayed in Fig. 1.

The best individual fit for the CWR protocol was derived from the P-T_{lim} model in 1 subject, *W*-T_{lim} model in 4 subjects and 1/T_{lim} model in 5 subjects, whereas the best individual fit for the TT protocol was derived from the W-T_{lim} model in 1 subject and 1/T_{lim} model in 9 subjects. There were no differences ($P > 0.05$) in the SEEs associated with the CP or *W'* modelling between the CWR and TT protocols (Table 1). However, there were significant differences in the SEEs associated with the *W'* parameter and the total error between models *within* the CWR and TT protocols (specific differences indicated in Table 1).

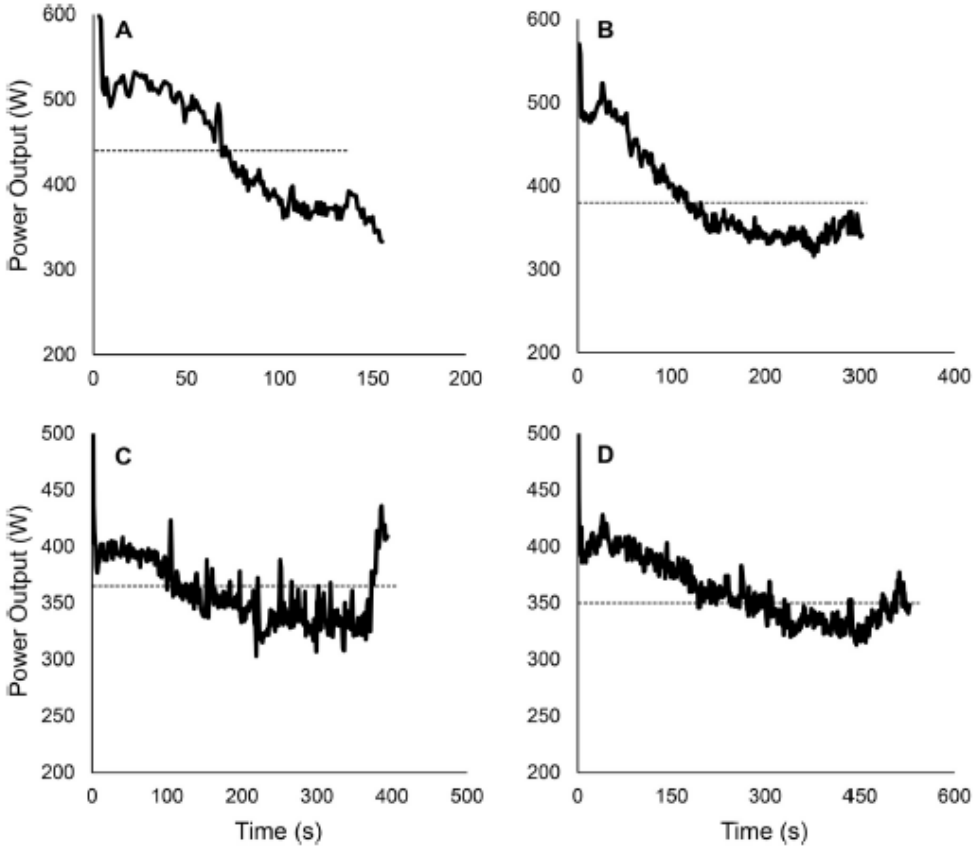
As there were no differences in the power-duration parameters derived from different models within the CWR and TT protocols (Table 1), the estimates from the best individual fit, which were associated with the lowest errors, were chosen for further analyses. The CP derived from the TT protocol was ~7% greater than that derived from the CWR protocol ($P < 0.05$; Fig. 2A). The CP estimates from the TT and CWR protocols were positively correlated ($r = 0.91$, $P < 0.01$). The *W'* estimates were not significantly different between the CWR and TT protocols ($P > 0.05$; Fig. 2B). The *W'* estimates from the 2 protocols were correlated ($r = 0.67$, $P < 0.05$). There was a significant inverse correlation between the relative difference in CP (ΔCP) and the relative difference in *W'* (Δ*W'*) between the CWR and TT protocols ($r = -0.74$, $P < 0.01$).

$\dot{V}O_2$ response

The overall rate of increase in $\dot{V}O_2$ during the prediction trials was characterised using the MRT. The MRT during the corresponding work-matched CWR and TT prediction trials ($n = 33$) was significantly shorter during the TT prediction trials (34 ± 16 s) compared with the CWR prediction trials (39 ± 19 s, $P < 0.05$; Fig. 2C). The mean MRT for all CWR prediction trials for each individual, and the mean MRT for all TT prediction trials for each individual, were calculated. When combined, these CWR and TT protocol mean MRTs ($n = 20$) were inversely correlated with the CP derived from the respective protocols ($r = -0.66$, $P < 0.05$).

The total O_2 consumed during the first 60 s of exercise was significantly greater during the corresponding work-matched TT (2.87 ± 0.43 L) compared with the CWR (2.75 ± 0.45 L) prediction trials ($P < 0.05$). The total work done during the first 60 s was also significantly greater during the corresponding work-matched TT (21.1 ± 4.5 kJ) compared with the CWR (19.3 ± 3.6 kJ) prediction trials ($P < 0.05$), such that the total work done per unit O_2 consumed over the first 60 s was slightly but significantly greater during the TTs (7.3 ± 0.7 kJ·L^{-1}·min^{-1}) than the CWR trials (7.0 ± 0.6 kJ·L^{-1}·min^{-1}, $P < 0.05$). The mean of total O_2 consumed in the first 60 s for all CWR trials for each individual, and the mean of total O_2 consumed in the first 60 s for all TT trials for each individual were calculated. When combined, these CWR and TT protocol means for O_2 consumed in the first 60 s ($n = 20$) were positively correlated with the CP derived from the respective protocols ($r = 0.88$, $P < 0.05$). The total O_2 consumed during the entire

Fig. 1. The pacing strategy of a representative subject during short (A), short-intermediate (B), long-intermediate (C), and long (D) time trials. The power output of the corresponding constant work-rate prediction trial is shown as a dashed line in each panel.



exercise bout was not different between the work-matched CWR (21.44 ± 10.33 L) and TT (21.36 ± 10.04 L) prediction trials ($P > 0.05$; $n = 33$).

There was no significant difference between the actual TT completion time (356 ± 185 s) and the predicted completion time calculated according to eq. 4 and the best individual fit CP and W' estimates from the CWR protocol (362 ± 189 s) ($P = 0.11$; $n = 37$). However, the predicted TT completion time based on the worst individual fit from the CWR protocol (451 ± 188 s) was significantly greater than the actual completion time ($P < 0.05$; $n = 37$).

Discussion

The principal novel findings of this study were that (i) the CP derived from a series of TT prediction trials was significantly greater compared with that derived from a series of CWR prediction trials; (ii) the W' was not significantly affected by differences in pacing strategy (TT vs. CWR); and, (iii) the $\dot{V}O_2$ MRT was significantly greater during the CWR compared with the TT tests, with a significant inverse relationship between the MRT and the CP. These results indicate that performance was improved in the TT compared with CWR tests, concomitant with a greater CP and augmented $\dot{V}O_2$ response, whereas W' was not significantly influenced by differences in pacing between the CWR and TT prediction trials. Direct quantification of the effects of pacing on the power-duration parameters is a key novel contribution of this study and, collectively, the present findings offer important novel insights into the potential mechanisms by which a fast starting strategy may be ergogenic. Our findings also highlight a potential source of error, stemming from a significant difference in CP between protocols, when predicting TT performance on the basis of CWR prediction trials.

The CP estimate was ~7% greater when derived from the TT protocol compared with the CWR protocol. The magnitude of this increase is similar to the influence of cadence on CP (~6% difference between 60 rpm vs. 100 rpm; Barker et al. 2006; Hill et al. 1995) but smaller than the increase in CP observed following high-intensity interval training (~10%–15%; Gaesser and Wilson 1988; Vanhatalo et al. 2008). The power profiles of the self-paced TT prediction trials were indicative of a "parabolic" pacing strategy where the power outputs over the early stages of the trial were higher than the mean power output, followed by a gradual decline in power and (with the exception of the shortest trial) a subsequent increase in power during a "sprint finish". The shorter $\dot{V}O_2$ MRT and greater total O_2 consumed during the first 60 s in the TT protocol were likely to result from higher power outputs at the onset of the TT compared with the CWR trials, which would generate a greater "error signal" between the muscle ATP requirement and the rate of oxidative phosphorylation in the early phase of exercise (Bailey et al. 2011; Jones et al. 2008*b*). It is possible that the greater rate of increase in oxidative phosphorylation during

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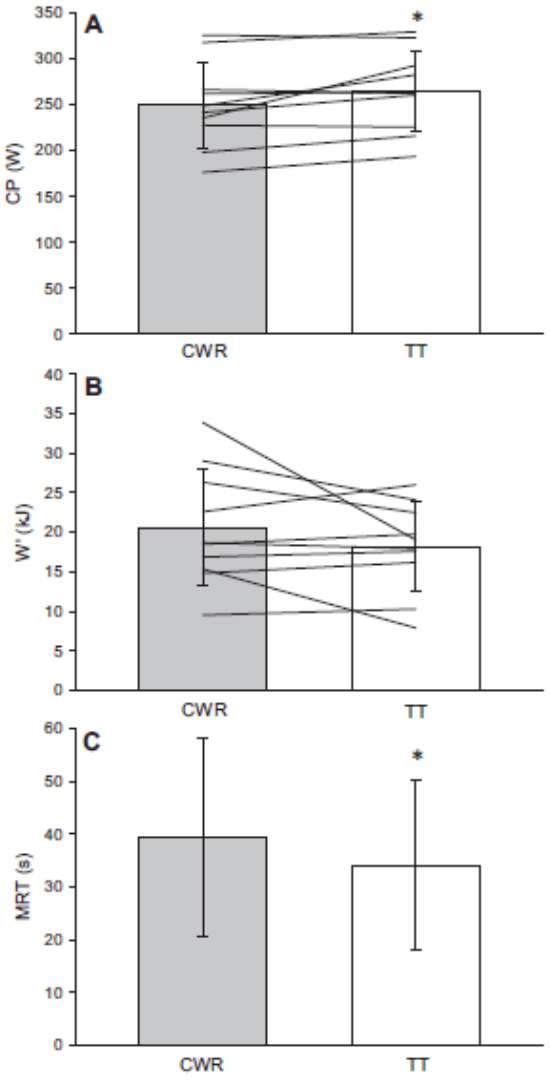
Fig. 2. The group mean (±SD) critical power (CP) derived from the time-trial (TT) protocol was 6% greater than the CP derived from the constant work-rate (CWR) protocol (A). The individual differences in CP between TT and CWR protocols ranged from −2 to +57 W. The group mean curvature constant (W') was 12% lower in TT compared with CWR protocol (B) and the individual differences ranged from −14.8 to +3.4 kJ. In panels A and B, the solid lines indicate individual responses. The group mean response time (MRT) derived from all TT tests was 14% shorter compared with the CWR protocol (C). Individual responses (n = 33) are not shown in panel C for clarity. *, $P < 0.05$.



the self-paced TT relative to the CWR trials reduced the reliance on nonoxidative metabolism (i.e., the O_2 deficit) across the exercise transient (but see caveats discussed later), thereby reducing the extent of metabolic perturbation early on in exercise and enabling a greater power output to be maintained throughout the trial (Bailey et al. 2011; Jones et al. 2008*b*). The improved exercise performance associated with the fast-start pacing strategy during the self-paced TTs compared with CWR tests is compatible with this interpretation and was reflected in the power-duration relationship as an elevated CP with no significant change in the W'.

The present study showed no statistically significant change in the size of the available W' despite the different rates of W' utilisation between CWR and self-paced TT protocols (Chidnok et al. 2013). However, the change in W' was inversely correlated with the increase in CP, such that the individuals with the greatest increases in CP had the greatest reductions in the W'. The interrelated nature of the W' and CP has been previously illustrated following interventions such as training and inspiration of hyperoxic gas (Jenkins and Quigley 1992; Vanhatalo et al. 2010), which are known to reduce the amplitude of the $\dot{V}O_2$ slow component (Jones et al. 2011). It has been proposed that the inter-individual variability in the changes in W' following interventions that influence the $\dot{V}O_2$ kinetics may depend on the relative changes in the CP and the $\dot{V}O_2$peak (Bailey et al. 2011; Jones et al. 2010; Vanhatalo et al. 2010). In the present study, the $\dot{V}O_{2peak}$ remained unaltered but the CP increased in the TT compared with the CWR protocol, indicating that the "range" of the severe domain (defined as the relative difference between CP and $\dot{V}O_{2peak}$), and therefore the scope for the $\dot{V}O_2$ slow component to develop, was smaller in the TT protocol. It may be speculated that the significant inverse correlation between the changes in W' and CP may reflect a smaller $\dot{V}O_2$ slow component, consequent to fast-start pacing strategy, during TT compared with CWR trials.

The fast-start pacing strategy adopted by the subjects during the self-paced TTs significantly augmented the $\dot{V}O_2$ response following exercise onset compared with the CWR tests (Fig. 2C). This finding is consistent with previous studies that have reported a greater rate of $\dot{V}O_2$ increase when a fast-start strategy is adopted (Aisbett et al. 2009; Bailey et al. 2011; Bishop et al. 2002; Hanon et al. 2008; Jones et al. 2008*b*). However, not all reports support the performance enhancing effect of fast-start pacing strategy (Hanon et al. 2008; Mattern et al. 2001). Bailey et al. (2011) showed that an externally imposed fast-start pacing strategy increased the rate at which $\dot{V}O_2$ increased towards its maximum during both 3- and 6-min trial durations, but exercise performance was only improved in the 3-min performance trial. This difference was attributed, in part, to the observation that the subjects were only able to attain $\dot{V}O_{2peak}$ in the 3-min trial when using a fast-start, but not an even-pace or slow-start, pacing strategy (Bailey et al. 2011). In the present study, the $\dot{V}O_{2peak}$ was consistently attained in all TT and CWR prediction trials, suggesting that the underlying mechanism for the inferior performance during the CWR compared with TT tests was not associated with the inability to attain $\dot{V}O_{2peak}$ and/or the incomplete utilisation of the W' (indeed, the W' was not significantly influenced by self-pacing). It is possible that the different durations of the fast-start phase in the study by Bailey et al. (2011) resulted in a greater demand on the anaerobic metabolic pathways during the 6-min than the 3-min trials, with a concomitant increase in the O_2 deficit. In the present study, subjects were allowed to self-select their pacing strategy as opposed to having the fast-start imposed externally (Bailey et al. 2011). This may have enabled subjects to preserve a sufficient proportion of the W', thus permitting a greater mean power output to be achieved throughout the TT compared with the CWR tests. In this regard, the potential ergogenic effect of a fast-start strategy on performance appears to be dependent on the dynamic relationship between event duration, and the duration and intensity of the fast-start phase (Bailey et al. 2011; Foster et al. 2004; Wood et al. 2014).

The tolerable duration of severe-intensity exercise has been proposed to be determined by the characteristics of the $\dot{V}O_2$ kinetic response, the $\dot{V}O_{2peak}$, and the "anaerobic capacity" (Burnley and Jones 2007). These variables are associated with the parameters that define the power-duration relationship: the CP has been shown to be related to the rate at which the $\dot{V}O_2$ increases at exercise onset

(i.e., the phase II τ; Murgatroyd et al. 2011) and the W' is related to the amplitude of the $\dot{V}O_2$ slow component (Murgatroyd et al. 2011; Vanhatalo et al. 2011). Given the breath-by-breath variability inherent in pulmonary $\dot{V}O_2$ measurement, accurate characterization of the $\dot{V}O_2$ phase II τ requires the averaging of $\dot{V}O_2$ responses from several step transitions in work rate. Since only a single baseline-to-exercise transition was completed for each prediction trial in the present study, we determined the MRT of the $\dot{V}O_2$ response to provide a broad assessment of the rate at which $\dot{V}O_2$ projected towards its maximum.

It is important to note that ATP turnover rate is not constant during TT exercise and the shorter MRT in the TT compared with CWR tests should not be considered indicative of faster $\dot{V}O_2$ kinetics per se. Nevertheless, the MRT assessment in this study enabled comparison of the rate at which $\dot{V}O_2$ projected towards its maximum value between the TT and CWR protocols. The significant relationships between CP and the MRT ($r = -0.66$), and the CP and the total O_2 consumed during the first 60 s of exercise ($r = 0.88$), may suggest that the rate of $\dot{V}O_2$ increase during the exercise bout influences the power-duration relationship and severe-intensity exercise performance. Our finding of an inverse correlation between the CP and the $\dot{V}O_2$ MRT is consistent with the inverse relationship between CP and the $\dot{V}O_2$ phase II τ reported previously (Murgatroyd et al. 2011). The findings of the current study, therefore, support the notion that $\dot{V}O_2$ kinetics and the parameters of the power-duration relationship may share similar physiological determinants (Burnley and Jones 2007; Jones et al. 2010; Murgatroyd et al. 2011; Vanhatalo et al. 2011).

The augmented $\dot{V}O_2$ early in the TTs compared with the CWR trials is a direct reflection of the fast-start strategy employed in the former, and therefore the higher power outputs selected. The fast-start pacing strategy observed during the TT led to enhanced CP (with no significant changes in $\dot{V}O_{2peak}$ and W') and improved severe-intensity exercise performance. It appears that the fast-start pacing strategy selected by subjects does not accelerate substrate-level phosphorylation to the extent that it invokes a sufficient muscle metabolic perturbation to precipitate fatigue (and result in premature depletion of the W'). While ATP turnover is not constant over time during TT (or even CWR) exercise (Bangsbo et al. 2001), there is evidence that muscle efficiency may be higher in the early compared with the later stages of an exercise transient (Krustrup et al. 2003; Wüst et al. 2011). It may be speculated that subjects can take advantage of this metabolic "window of opportunity" wherein the selection of relatively high initial power outputs enables a more rapid acceleration of $\dot{V}O_2$ without invoking a substantial, and deleterious, metabolic cost.

It is assumed that when the CP and W' are known, the power-duration relationship (eqs. 1–3) can be used to predict performance in any continuous exercise bout provided that the work rate remains within the severe-intensity exercise domain (Hill 1993; Jones et al. 2010; Morton 2006). We used eq. 4 and the CP and W' estimates derived from the CWR prediction trials to predict TT performance. The predicted TT duration was overestimated (on average by ~27%) when worst individual fit parameters were used and tended to be overestimated (by ~2%) even when the best individual fit parameters were used because of the significantly greater CP during TT compared with CWR exercise (Fig. 3). It should be noted that the CV between actual and predicted TT performance was ~6%, which is slightly greater than the typical test–retest reliability of TT performance of similar durations (0.6%–4.6%; Hopkins et al. 2001). For comparison, the accuracy of TT prediction in the present study was similar to the accuracy of predicting T_{lim} (~2%–13%) during CWR exercise on the basis of the power-duration relationship derived from CWR prediction trials (Housh et al. 1989). Although it may be argued that the magnitude of error in TT performance prediction on the basis of CWR prediction trials is only marginally greater than the reliability of TT performance, the significant difference in CP between CWR and TT exercise nevertheless results in a consistent under-prediction of TT performance, which is likely to be meaningful when attempting to predict marginal alterations in athletic performance. It is recommended that the best individual fit CP and W' estimates are established using eqs. 1–3 to minimize the potential prediction error arising from differences in pacing.

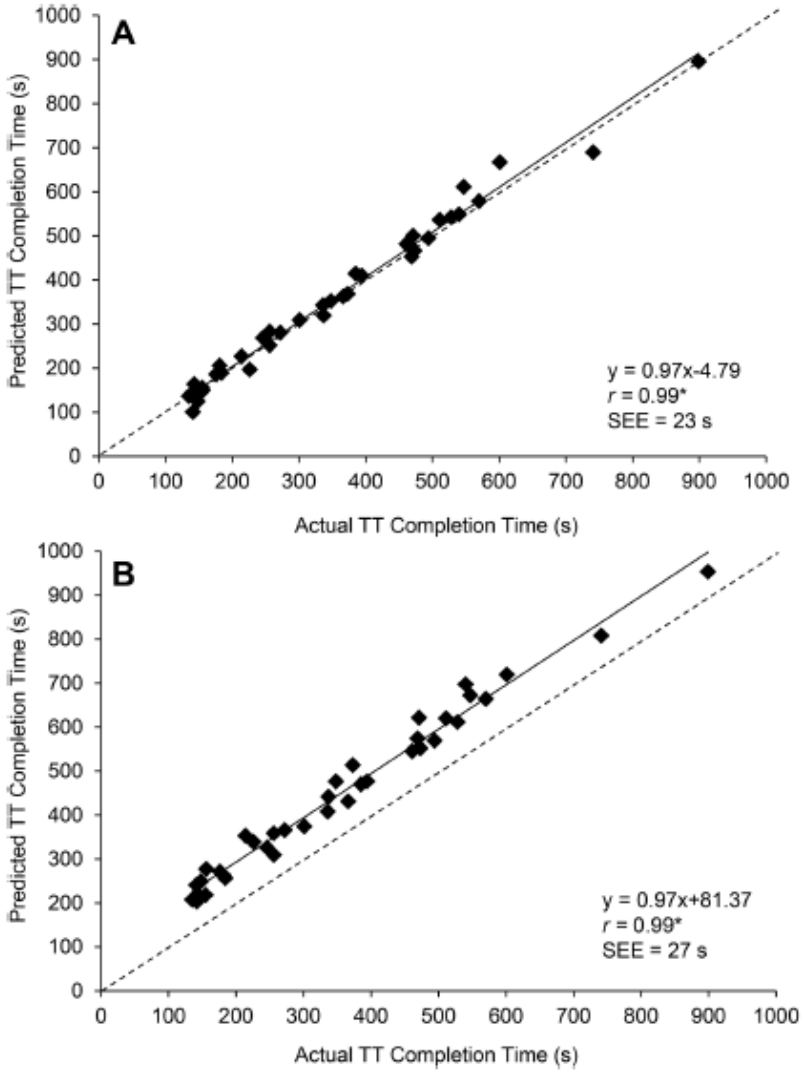
The close agreement between the parameter estimates derived from eqs. 1–3 within the CWR and TT protocols indicates that the incidence of random and systematic errors in the prediction trial data was low (Hill and Smith 1994). The goodness of fit was further demonstrated by the finding that the error associated with the modeled CP was not significantly different between the best (~1%–2%) and worst (~3%–4%) individual fits (Table 1). However, the error associated with the W' was significantly greater for the worst (~19%–21%) compared with the best (~7%–9%) individual fit, which likely negatively influenced the accuracy of performance prediction (Fig. 3). It is important to note that if inconsistencies in the attainment of $\dot{V}O_{2peak}$ in the prediction trials occur, this may impact on the modelled power-duration parameters. Inclusion of data from prediction trials where the $\dot{V}O_{2peak}$ has not been attained has been associated with an underestimation of CP (Sawyer et al. 2012). The criteria of (*i*) no significant differences in parameter estimates between models (eqs. 1–3); and (*ii*) attainment of >95% of $\dot{V}O_{2peak}$ in all CWR prediction trials are therefore advisable to ensure reasonably accurate TT performance prediction (CV ~5%–9%) when the best individual fit parameter estimates are used.

In addition to the physiological determinants of performance, which were the main focus of the present study, it should be noted that subject motivation also plays a role in determining maximal high-intensity exercise performance. The participants in this study were instructed and strongly encouraged to provide a maximal effort in all tests (CWR and TT) and were challenged to achieve their $\dot{V}O_{2peak}$ every time. Indeed, attainment of $\dot{V}O_{2peak}$ was used as a criterion of maximal effort, indicating consistent metabolic strain across all trials. Although verbal encouragement was kept consistent between conditions, it is possible that subject motivation might have been influenced by the feedback given on work completed during the TTs but not during the CWR tests. Potential differences in subject motivation between time-to-exhaustion, open-ended CWR tests and the fixed-endpoint TTs might have contributed to differences in performance to some extent. The definitive assessment of the potential influence of motivation on the power-duration relationship is beyond the scope of the present study and warrants further investigation.

Recent development of user-friendly on-bike power meters now affords an opportunity to assess maximal performance within the power-duration framework in an actual competitive situation on the road. An additional advantage of on-bike power meters is that the cyclist is able to control the gear as well as the cadence during performance, as opposed to the "fixed resistance – variable cadence" model applied in the present study. The aim of the current study was to investigate in a controlled laboratory environment whether prediction trial protocols with distinct power profiles (Fig. 1) result in similar CP and W' estimates. Our finding that the CP was significantly greater in a TT than CWR protocol opens up interesting avenues for field assessment of power output distribution during competitive TTs.

In conclusion, the CP was greater and the $\dot{V}O_2$ MRT was shorter during self-paced TT exercise compared with CWR exercise, while the W' was not significantly affected by differences in pacing strategy. The significant difference in CP between the TT and CWR protocols was reflected in a small but consistent under-prediction of TT performance when the CP and W' were derived from CWR prediction trials. It is therefore recommended that the best individual fit CP and W' estimates are established using eqs. 1–3, and where possible, TT prediction protocols, in which subjects are

Fig. 3. The relationships between actual time-trial (TT) performance and TT performance predicted on the basis of the "best" and "worst" individual fits derived from the constant work-rate trials (panels A and B, respectively). The predicted TT durations derived from the best individual fit were not significantly different from the actual TT durations, whereas the TT durations predicted from the worst individual fit were significantly different from the actual TT durations. CP, critical power; SEE, standard error of estimate; W', curvature constant. *, $P < 0.05$.



permitted to self-pace, are used for determination of the CP and W' when accurate predictions of performance are desired. This approach may represent an important step forward in the application of the power-duration relationship to modelling athletic performance.

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Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains

Original Investigation

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Running head: Metabolic and neuromuscular correlates of fatigue

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ABSTRACT

The lactate or gas exchange threshold (GET) and the critical power (CP) are closely associated with human exercise performance. We tested the hypothesis that the limit of tolerance (T_{lim}) during cycle exercise performed within the exercise intensity domains demarcated by GET and CP is linked to discrete muscle metabolic and neuromuscular responses. Eleven males performed a ramp incremental exercise test, 4-5 severe-intensity (SEV; >CP) constant-work-rate (CWR) tests until T_{lim}, a heavy-intensity (HVY; <CP but >GET) CWR test until $T_{lim,}$ and a moderate-intensity (MOD; <GET) CWR test until T_{lim}. Muscle biopsies revealed that a similar (P>0.05) muscle metabolic milieu (i.e., low pH and [PCr] and high [lactate]) was attained at T_{lim} (~2-14 min) for all SEV exercise bouts. The muscle metabolic perturbation was greater at T_{lim} following SEV compared to HVY, and also following SEV and HVY compared to MOD (all P<0.05). The normalised M-wave amplitude for the m. vastus lateralis (VL) decreased to a similar extent following SEV (-38±15%), HVY (-68 ± 24%), and MOD (-53±29%), (P>0.05). Neural drive to the VL increased during SEV (4±4%; P<0.05) but did not change during HVY or MOD (P>0.05). During SEV and HVY, but not MOD, the rates of change in M-wave amplitude and neural drive were correlated with changes in muscle metabolic ([PCr], [lactate]) and blood ionic/acid-base status ([lactate], $[K^+]$) (P<0.05). The results of this study indicate that the metabolic and neuromuscular determinants of fatigue development differ according to the intensity domain in which the exercise is performed.

NEW AND NOTEWORTHY (75 words)

This is the first study to investigate both the putative metabolic and neuromuscular determinants of fatigue during whole-body exercise performed within discrete exercise intensity domains demarcated by the gas exchange threshold (GET) and the critical power (CP). Our findings build-on those of previous studies, and demonstrate for the first time, during whole-body exercise, that the GET and CP demarcate exercise intensity domains within which fatigue development is determined by discrete mechanisms.

KEYWORDS:

Critical power; neuromuscular fatigue; muscle metabolism; cycling-exercise

INTRODUCTION

Intense and/or prolonged excitation of muscle leads to a reversible decline in its force generating capacity and rate of contraction, commonly known as fatigue (22-24, 28, 58). This temporary reduction in muscle performance may be attributed to central factors that limit the neural drive for muscle contraction, and to peripheral factors, which occur at, or distal to, the neuromuscular junction and that often involve metabolic and ionic perturbations that reduce the muscle's ability to respond to neural stimulation (2, 3, 26, 31, 44).

The extent of the muscle metabolic and ionic, and blood acid-base and respiratory perturbations experienced during exercise is dependent on the exercise intensity, which can be categorised into three distinct domains demarcated by physiological thresholds (34 ,75). The upper limit of the 'moderate' exercise intensity domain is indicated by the lactate threshold (LT; which is often estimated using the gas exchange threshold (GET)), and the boundary between the 'heavy' and 'severe' exercise intensity domains is given by the critical power (CP). Using ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS), it has been demonstrated that severe-intensity, single-leg knee-extension exercise is associated with a progressive loss of muscle homeostasis with time (i.e. progressive reductions in muscle phosphocreatine concentration ([PCr]) and pH and an increase in inorganic phosphate concentration ($[P_i]$)) (9, 33, 36, 72). In contrast, heavy- and moderate-intensity, small muscle mass exercise is associated with much more limited muscle metabolic perturbation with new 'steady-state' values of [PCr], pH and $[P_i]$ being achieved within a few minutes of the initiation of exercise (36, 51, 70). These intensity-related differences in muscle metabolic, as well as related blood

acid-base and respiratory gas exchange, responses to exercise (36, 54, 71, 76) likely underpin the close relationships reported between these threshold phenomena (LT/GET and CP) and human exercise performance (8).

The role of exercise intensity in defining the extent and dynamics of muscle metabolic perturbation implies that exercise intensity may also influence the nature of neuromuscular fatigue development (3, 23, 25, 42, 44, 55, 56). The peripheral component to fatigue, as estimated non-invasively using surface electromyography (EMG), electrical muscle stimulation and/or transcranial magnetic stimulation, appears to be especially important during high-intensity exercise (48, 67, 68), whereas central fatigue may be more prominent during prolonged, low-intensity exercise (41, 48, 62, 64, 68). The intensity-dependent interaction between peripheral and central components of fatigue is thought to be modulated by changes in afferent feedback arising from the muscle metabolic milieu. Consistent with this, the critical torque (CT; analogous with the CP) for small muscle mass exercise has been shown to represent a threshold in the development of neuromuscular fatigue (10), such that severe-intensity knee-extensor contractions (>CT) were associated with elevated motor unit recruitment and a disproportionate increase in the rate of neuromuscular fatigue development relative to heavy-intensity contractions (<CT).

It is presently unclear whether the determinants of neuromuscular fatigue development during whole-body exercise, such as cycling, differ according to the intensity domain in which exercise is performed. Previous studies have assessed neuromuscular fatigue before and after self-paced maximal time trial cycle

exercise (68) and during constant-work-rate (CWR) cycling performed ostensibly within the severe-intensity domain (69). These studies suggested that, in contrast to knee extension exercise (10), the level of peripheral fatigue at exhaustion for cycling may also be intensity-dependent above CP (69). Compared to small muscle mass exercise, whole-body exercise is associated with greater rates of pulmonary ventilation and gas exchange (63, 77), differences in cardiac output and muscle perfusion (13, 49, 63), and greater activity of type III/IV muscle afferents that may modulate central drive (55, 56). It is possible that these factors impact the relationship between muscle metabolic changes and neuromuscular fatigue development during exercise.

To date, the physiological and neuromuscular responses to whole-body exercise, and their possible inter-relationship, has not been assessed within distinct exercise intensity domains. The purpose of this study was therefore to evaluate possible differences in the muscle metabolic and systemic responses to different, well-defined, intensities of exercise, with the aim of elucidating whether the exercise intensity domain influences the determinants of neuromuscular fatigue. Based on earlier studies investigating small muscle mass exercise (36, 72), we tested the hypotheses that: 1) a consistent muscle metabolic milieu ([ATP], [PCr], [lactate], pH) and neuromuscular responses (muscle excitability and neural drive) will be attained at the limit of tolerance (T_{lim}) during severe-intensity exercise (>CP); 2) severe-intensity exercise will be associated with greater muscle metabolic perturbation compared to heavy- and moderate-intensity exercise; and 3) the rate of neuromuscular fatigue development will be greater during severe-

compared to heavy- and moderate-intensity exercise due to greater muscle metabolic and ionic perturbations.

METHODS

Ethical approval

The protocols were approved by the host institution's Research Ethics Committee and conducted in accordance with the code of the ethical principles of the World Medical Association (Declaration of Helsinki). Subjects gave written informed consent to participate after the experimental procedures, associated risks, and potential benefits of participation had been explained.

Subjects

Eleven healthy recreationally active males (mean ± SD: age 21.8 ± 1.9 years, height 1.79 ± 0.05 m, body mass 78.2 ± 8.1 kg) volunteered to participate in this study, 8 of whom volunteered to provide muscle tissue samples. One of the subjects whom volunteered for the biopsy procedure withdrew from the study having completed only the severe-intensity exercise trials. This subject's data were excluded from statistical difference tests, but was included in the correlational analysis. All subjects were in good health and had no known history of neurological or motor disorder. Subjects were instructed to report to all testing sessions in a rested and fully hydrated state, ≥3 h post-prandial, and to avoid strenuous exercise and refrain from caffeine and alcohol in the 24 h prior to testing. Each subject started each experimental trial at the same time of day (±2 h). All trials were performed on the same electronically-braked cycle ergometer (Lode, Excalibur, Groningen, The Netherlands).

Experimental design

Each subject visited the laboratory on ~7 occasions over a 6-wk period with each visit separated by a minimum of 24 h. A minimum of 7 days recovery was provided following the heavy- and moderate-intensity exercise tests. After the completion of a ramp incremental test (visit 1), subjects performed 4-5 CWR severe-intensity exercise tests to define the power-duration relationship, a heavy-intensity CWR test and a moderate-intensity CWR test, completed in a randomised order (Figure 1) except that the severe-intensity tests always preceded the heavy-intensity test. Pulmonary gas exchange was measured continuously during all tests, with the exception of the moderate-intensity test in which it was measured periodically for 10 min intervals, with the mid-point of collection coinciding with blood sample collection and femoral nerve stimulation (see below). We encouraged the subjects to continue exercising during the moderate-intensity test to enable 10 min of gas exchange data to be collected immediately prior to exercise cessation. EMG data were obtained continuously from m. vastus lateralis (VL) and m. vastus medialis (VM) throughout the exercise period with stimulation of the femoral nerve delivered at regular intervals (Figure 1) to quantify the neuromuscular changes occurring during the exercise protocols. Venous blood samples were obtained before and during exercise for the moderate-, heavy-, and for three of the severe-intensity exercise tests. In addition, muscle tissue was obtained at rest, and immediately following the moderate-, heavy-, and three of the severe-intensity exercise tests (Figure 1). The severe-intensity tests were performed at different work-rates (spanning 60%Δ to $\dot{V}O_2$peak; (where Δ refers to the work-rate difference between the GET and the $\dot{V}O_2$peak). Subsequently, the shorter (85 ± 5%Δ), intermediate (75 ± 5%Δ), and longer (65 ± 5%Δ) duration

exercise tests were grouped and compared to test for differences in muscle, neuromuscular, and blood responses within the severe-intensity domain.

Incremental test

On the first laboratory visit, subjects completed a ramp incremental test for the determination of the $\dot{V}O_2$peak and gas exchange threshold (GET). The ergometer seat height and handlebars were adjusted for comfort and the same settings were replicated for each subsequent test. Initially, subjects completed 3 min of baseline cycling at 20 W, after which the work-rate was increased by 30 $W \cdot min^{-1}$ until volitional exhaustion. The subjects cycled at a constant self-selected pedal rate (80 rpm, n = 9, 90 rpm, n = 2), which was recorded and reproduced in subsequent tests. The test was terminated when the pedal rate fell by more than 10 rpm below the preferred value for more than 5 s despite strong verbal encouragement. Breath-by-breath pulmonary gas exchange data were collected continuously throughout the test and recorded as 10-s moving average for data analysis. $\dot{V}O_{2peak}$ was determined as the highest mean $\dot{V}O_2$ during any 30-s period. The GET was determined as: 1) the first disproportionate increase in $\dot{V}CO_2$ versus $\dot{V}O_2$; 2) an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E$/ $\dot{V}CO_2$; and 3) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension (5).

CWR tests

All CWR tests started with 3 min of cycling at 20 W, followed by a step increase to the required work-rate. Subjects were instructed to remain seated and to maintain their preferred pedal rate for as long as possible. Strong verbal encouragement

was provided, but subjects were not informed of either the work-rate or the elapsed time. The tests were terminated when pedal rate fell by more than 10 rpm below the preferred value for more than 5 s. The T_{lim} was recorded to the nearest second.

The parameters of the power-duration relationship (CP and W′) were estimated by completion of 4-5 severe-intensity exercise tests (4 trials, *n* = 9; 5 trials *n* = 2) at different work-rates (approximately 60%Δ, 70%Δ, 80%Δ and 100% $\dot{V}O_{2peak}$) resulting in T_{lim} ranging between approximately 2 and 14 min (32). If the standard errors associated with the CP and W′ exceeded 5 and 10 %, respectively, after four exercise tests had been completed, a fifth test was performed. Any tests in which the end-exercise $\dot{V}O_2$ was <95% of the individual's ramp test determined $\dot{V}O_2$peak were excluded from the modelling of the power-duration relationship.

The CP and W′ (the amount of work done above the CP) parameters were estimated using three models: the hyperbolic P-T_{lim} model (Equation 1); the linear work-time (W-T_{lim}) model, where the total work done (W) is plotted against time (Equation 2); and the linear inverse-of-time ($1/T_{lim}$) model, where power output is plotted against the inverse of time (Equation 3):

$$T_{lim} = W' / (P - CP) \quad [1]$$

$$W = CP \cdot T_{lim} + W' \quad [2]$$

$$P = W' (1/ T_{lim}) + CP \quad [3]$$

The standard errors of the estimate associated with the CP and W′ were expressed as coefficients of variation (CV%, i.e. relative to the parameter estimate). For each individual, the 'best fit' model associated with the lowest CV% for CP and W' was used for further analyses (7).

The work-rate for the heavy-intensity CWR trial was equal to the lower bound of the 95% confidence limit in the CP parameter (36). The moderate-intensity CWR trial was performed at a work-rate corresponding to 90% of the GET. Subjects were permitted to ingest water *ad libitum* during the heavy- and moderate-intensity tests.

Pulmonary gas exchange

Breath-by-breath pulmonary gas exchange and ventilation were measured continuously during all exercise tests, with the exception of the moderate-intensity test, where it was measured at discrete time points (Figure 1). Subjects wore a nose clip and breathed through a mouthpiece and impeller turbine assembly (Jaeger Triple V, Jaeger, Hoechberg, Germany). The inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz (Oxycon Pro, Jaeger, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analysers were calibrated before each trial with gases of known concentration and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by accounting for the delay in capillary gas transit and the analyser rise time relative to the volume signal.

Blood analyses

Venous blood samples were drawn into 5-mL heparinised syringes (Terumo Corporation, Leuven, Belgium) from a cannula (Insyte-W™, Becton-Dickinson, Madrid, Spain) inserted into the subject's antecubital vein. The blood was analysed for [lactate] and [glucose] within ~5 min of collection (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The remaining whole blood was then centrifuged at 4,000 rpm for 7 min (Hettich EBA 20, Germany) before plasma was extracted and analysed for [K^+] (9180 Electrolyte Analyser, F. Hoffman-La Roche, Basel, Switzerland).

Neuromuscular Function

EMG was used to continuously record the VL and VM activity during exercise, using active bipolar bar electrodes with single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA), positioned over the muscle belly (SENIAM guidelines). The ground electrode was positioned on the patella. Double-sided adhesive interfaces and hypoallergenic medical tape were used to keep the EMG sensors in place and to reduce skin impedance. The leads connected to the electrodes were secured using hypoallergenic medical tape to minimise artefacts due to movement of the leads. The skin area underneath each electrode was shaved, abraded, and cleaned with alcohol swabs prior to electrode placement to minimise skin impedance. The EMG signal was considered of good quality when the average rectified EMG baseline level for each muscle was below 2 μV (19). The EMG signals were pre-amplified (1,000x), band-pass filtered (20-450 Hz, Bagnioli-8, DelSys Inc, Boston, MA), and digitised at a sampling rate of 2,000 Hz and resolution of 16 bits using a Power 1401 mk-II analog-to-digital converter and

Spike 2 data collection software run by custom written sampling configuration (CED, Cambridge Electronic Design, UK).

The location of the optimal site for transcutaneous femoral nerve stimulation was determined whilst the subject was positioned on the cycle ergometer. Using a cathode (Boots UK Ltd, Nottingham, England) placed approximately 2 cm medial of the femoral pulse, and an anode (Boots UK Ltd, Nottingham, England) placed at the anterior aspect of the iliac crest, single electrical pulses generated by a constant current stimulator (DS7 A, Digitimer Ltd, UK) were delivered. The cathode was systematically moved vertically and horizontally and the amplitude of the compound muscle action potential (CMAP, M-wave) was monitored to identify the optimal position of the cathode for attaining maximal peak-to-peak M-wave amplitude during the cycling trials.

Following the attachment of the EMG and the stimulation electrodes, the crank angle at which stimulation was to be delivered during the trials was determined for each subject. The subject was positioned on the cycle ergometer and cycled at a moderate work-rate (20 W below GET) for 1 min. The EMG activity obtained during this period was rectified and averaged for 20 complete crank revolutions. The duration of each revolution was determined by a custom-made magnetic switch that generated an event marker signal on each occasion that the crank passed top dead centre (i.e. 0º). For each subject, the crank angle at which the rectified VL EMG activity was maximal was determined, and the magnet was moved to this position for all trials (65 ± 5° relative to the top dead centre). A

sequencer script triggered 3 stimulations, with at least 1 and up to 10 pedal revolutions between stimuli, when an event marker was produced.

A standard M-wave recruitment curve protocol was completed during each laboratory visit. The subject cycled at 20 W below GET throughout the recruitment curve protocol. A single-pulse electrical stimulation (200 μs) was delivered at the individually identified crank angle as described above. The current was increased in 20 mA increments until the M-wave amplitude plateaued at the maximal M-wave amplitude (M_{max}). Three stimulations were delivered at each current intensity in a pseudo-random fashion. A pulse of 130% M_{max} current was applied during the exercise tests (mean stimulation intensity: 350 ± 50 mA).

Muscle biopsy

The biopsy site was prepared on the alternate thigh to the EMG and peripheral nerve stimulation setup. Local anaesthesia was applied (2-3 ml of 20 $mg.ml^{-1}$ lidocaine) and an incision was made in the medial region of the VL. Muscle samples were obtained using needle biopsy with suction (6). Resting muscle samples were obtained prior to any exercise on the first laboratory visit and post-exercise biopsies were taken within ~10 s of the cessation of each exercise test with the subject supported on the ergometer. The muscle tissue was rapidly frozen in liquid nitrogen.

Muscle tissue analysis

The frozen muscle samples from each biopsy were weighed before and after freeze-drying to determine water content. After freeze-drying, the muscle samples

were dissected free from blood, fat and connective tissue. Prior to muscle metabolite analysis, 200 µl of 3 M perchloric acid was added to approximately 2.5 mg d.w. muscle. The solution was then centrifuged and placed on ice for 30 min. It was subsequently neutralised to pH 7.0 with 255 µl of cooled potassium bicarbonate ($KHCO_3$) and centrifuged (10,000 *g*). The supernatant was analysed for PCr, ATP and lactate by fluorometric assays (38). An aliquot containing 1-2 mg d.w. muscle was extracted in 1 M hydrochloric acid (HCl) and hydrolysed at 100ºC for 3 h before glycogen content was determined using the hexokinase method (38). Muscle pH was measured using a glass electrode following the homogenisation of 1-2 mg d.w. of muscle in a non-buffering solution containing 145 mM KCl, 10 mM NaCl and 5 mM iodoacetic acid.

EMG analysis

The EMG signal from the VL and VM was filtered and rectified (described above), and processed using a custom written script to measure peak-to-peak M-wave amplitude and M-wave area. The root-mean-square (RMS) of the EMG signal (an index of the power of the signal) was calculated as the mean over a 25 ms pre-stimulation period at each stimulation time point. The EMG RMS amplitudes and the M-wave parameters were normalised to the corresponding values attained after 1 min of exercise during each trial to evaluate temporal changes in the voluntary muscle activation level (i.e. the EMG RMS amplitude) and the peripheral neuromuscular excitability (i.e. the M-wave amplitude and area). In addition, the voluntary EMG RMS amplitude was normalised to the nearest M-wave amplitude to assess changes in neural drive (RMS/M; 45). The rates of change in M-wave and EMG parameters from baseline cycling to T_{lim} were calculated for each

exercise to quantify the rate of neuromuscular fatigue development in each intensity domain.

Statistical analyses

One-way ANOVAs with repeated measures were used to assess differences between severe-intensity exercise tests in $\dot{V}O_2$peak, muscle [ATP], [PCr], [pH], [lactate], and [glycogen], M-wave amplitude, M-wave area, voluntary EMG amplitude and RMS/M, and blood and plasma variables at T_{lim}. The data from the severe-intensity tests were subsequently averaged for each individual for comparison with the heavy- and moderate-intensity tests. Differences in $\dot{V}O_2$peak, muscle [ATP], [PCr], [pH], [lactate], and [glycogen] between the severe-, heavy- and moderate-intensity tests were assessed using one-way ANOVAs. Two-way repeated measures ANOVAs (condition x time) were used to analyse differences in M-wave amplitude and area, and voluntary EMG amplitude for the VL and VM, and blood and plasma variables at common time-points (baseline, 1 min, 3 min and T_{lim}) among the severe-, heavy-, and moderate-intensity tests. Significant interaction and main effects were followed up with Bonferroni post-hocs. Relationships between the rates of change of metabolic and neuromuscular variables were assessed using Pearson's product-moment correlation coefficients. Statistical significance was set at $P<0.05$ and data are presented as mean ± SD.

RESULTS

The $\dot{V}O_{2peak}$ measured in the ramp incremental test was 4.32 ± 0.46 $L \cdot min^{-1}$ (56 ± 8 $mL \cdot kg^{-1} \cdot min^{-1}$) and the peak work-rate was 385 ± 50 W. The GET occurred at 2.33 ± 0.34 $L \cdot min^{-1}$ and 137 ± 24 W.

Physiological responses within the severe-intensity domain

The T_{lim} in the severe-intensity CWR exercise tests ranged from 2.2 to 13.9 min. There were no differences between the three models (Equations 1-3) in the CP or W′ estimates (*P*>0.05; Table 1). The CP from the best fit model corresponded to 64 ± 7% of ramp test peak work-rate and 45 ± 11%Δ.

The $\dot{V}O_2$peak during the shorter (~85%Δ: 4.43 ± 0.50 L·min^{-1}), intermediate (~75%Δ: 4.49 ± 0.47 L·min^{-1}) and longer (~65%Δ: 4.41 ± 0.47 L·min^{-1}) severe-intensity tests were not different from the $\dot{V}O_2$peak achieved during the ramp incremental test (all *P*>0.05). Moreover, no significant differences were observed at T_{lim} among the three severe-intensity tests for any of the muscle tissue variables or for blood [lactate] (all *P*>0.05; Figure 2). There were also no differences in plasma [K^+] at T_{lim} among the shorter (5.6 ± 0.6 mM), intermediate (5.8 ± 1.1 mM,) and longer (5.7 ± 0.6 mM) severe-intensity tests (*P*>0.05).

Physiological responses during severe-, heavy- and moderate-intensity exercise

Pulmonary $\dot{V}O_2$, blood [lactate] and plasma [K^+] during moderate-, heavy- and severe-intensity exercise are illustrated in Figure 3**.** The T_{lim} for heavy-intensity exercise (231 ± 56 W) was 43.5 ± 16.2 min (range: 20.5 to 67.4 min) and the $\dot{V}O_2$ at T_{lim} (3.78 ± 0.53 L·min^{-1} ; 87 ± 4% of $\dot{V}O_{2peak}$) was lower than the ramp test $\dot{V}O_{2peak}$ (*P*<0.05). The T_{lim} for the moderate-intensity exercise (113 ± 19 W) was 211.1 ± 57.0 min (range: 180 to 360 min) and the $\dot{V}O_2$ at T_{lim} (2.22 ± 0.38 L·min^{-1}, 52 ± 8% of $\dot{V}O_2$peak) remained below the GET (*P*<0.05) throughout the exercise.

During severe-intensity exercise, blood [lactate] increased rapidly until T_{lim} and was significantly greater than baseline after 3 min (P<0.05). During heavy-intensity exercise, the rate of blood [lactate] increase was slower than during severe-intensity exercise such that blood [lactate] did not differ from baseline until after 10 min (P<0.05), and no further increase was observed between 10 min and T_{lim} (P>0.05) (Figure 3B). Plasma [K^+] was elevated above baseline at all measurement time points during heavy- and severe-intensity exercise (all P<0.05). The [K^+] continued to rise throughout severe-intensity exercise, whereas it stabilised during heavy-intensity exercise beyond 6 min (Figure 3C). During moderate-intensity exercise, blood [lactate] did not change from baseline (P>0.05) while plasma [K^+] was elevated above resting baseline at 1 min (P<0.05), with no further increase thereafter (all time points P>0.05).

Muscle metabolic variables at rest and at T_{lim} following moderate-, heavy- and severe-intensity exercise are illustrated in Figure 4. For severe- and heavy-intensity exercise, muscle [ATP], [PCr] and pH were lower and muscle [lactate] was greater at T_{lim} relative to rest (all P<0.05), with no significant changes in muscle [glycogen] (P>0.05). In contrast, for moderate-intensity exercise, muscle [PCr] at T_{lim} was greater than for severe- and heavy-intensity exercise (all P<0.05), and muscle [glycogen] was both lower than at rest and lower than at T_{lim} for heavy- and severe-intensity exercise (all P<0.05). Muscle [pH] and [lactate] did not change significantly from rest during moderate-intensity exercise (P>0.05).

Neuromuscular responses during severe-, heavy- and moderate-intensity exercise

The coefficients of variation (CV%) between trials during unloaded cycling were 25% (VL) and 35% (VM) for the peak-to-peak M-wave amplitude, and 32% (VL) and 32% (VM) for the M-wave total area. The CV% between stimulations during unloaded cycling was 11% (VL) and 9% (VM) for the peak-to-peak M-wave amplitude and 10% (VL) and 9% (VM) for the M-wave total area. The mean M_{max} amplitudes measured during cycling at 20 W below GET (VL 2.77 ± 1.43 and VM 0.99±1.18 mV) were not different between visits (all P>0.05). No significant differences were observed between trials in the neural drive to VL and VM during cycling at 20 W below GET.

Neuromuscular excitability: M-wave amplitude and M-wave area

The M-wave characteristics at T_{lim} for the three severe-intensity exercise tests, and for moderate-intensity, heavy-intensity and the mean of the severe-intensity exercise tests are shown in Figure 5A-D. Peripheral neuromuscular excitability at T_{lim}, indicated by the M-wave amplitude and M-wave area, did not differ among the severe-intensity tests (all P<0.05) (Figure 5B, D). The M-wave amplitude and M-wave area at T_{lim} were greater for severe-intensity exercise compared to both heavy- and moderate-intensity exercise in the VM (P<0.05), and the M-wave area at T_{lim} was also greater in severe- than in heavy-intensity exercise in VL (P<0.05) (Figure 5A, C). Differences in M-wave characteristics between severe-, heavy- and moderate-intensity exercise at each measurement time point are shown in Figure 6A-D.

Voluntary activation and neural drive

Voluntary muscle activation level, measured as EMG RMS amplitude, and neural drive, as indicated by RMS/M-wave amplitude, did not differ at T_{lim} among the severe-intensity exercise tests (all $P<0.05$) (Figure 5F, H). Both EMG RMS and RMS/M were greater at T_{lim} for severe-intensity compared to heavy- and moderate-intensity exercise in the VM ($P<0.05$) (Figure 5E). In the VL, the EMG RMS at T_{lim} was also greater for severe- than for heavy-intensity exercise and the RMS/M was greater for severe- than for moderate-intensity exercise (both $P<0.05$) (Figure 5E, G). The only difference in neuromuscular variables observed at T_{lim} between moderate- and heavy-intensity exercise was a significantly greater EMG RMS in the VL (Figure 5E). Differences in EMG RMS and RMS/M severe-, heavy- and moderate-intensity exercise at each measurement time point are shown in Figure 6E-H.

Relationships between physiological and neuromuscular variables

During severe-intensity exercise, the M-wave amplitude decreased in parallel with [PCr] depletion and plasma K^+ accumulation (Table 2). Moreover, increased neural drive (RMS/M) was related to high blood [lactate] and plasma [K^+], and to low muscle [PCr], and high muscle [lactate] and [glycogen] (Table 2). During heavy-intensity exercise, the reduction in M-wave amplitude was related to low muscle [PCr] and high plasma [K^+], and increased neural drive was related to high plasma [K^+] and low muscle [PCr], and high muscle [lactate] and [glycogen] (Table 2). During moderate-intensity exercise, the M-wave amplitude was inversely correlated with the reduction in [PCr] (Table 2).

DISCUSSION

To our knowledge, the present study is the first to combine muscle biopsy, blood analyses and measurements of neuromuscular excitability and neural drive (via electrical stimulation of the femoral nerve during exercise) to assess the muscle metabolic, acid-base and neuromuscular responses to cycling performed within discrete exercise intensity domains (34). The data presented herein provide novel insight into the *in vivo* relationships between exercise intensity, muscle metabolic perturbation and neuromuscular function and support the notion that LT/GET and CP separate exercise intensity domains within which exercise tolerance is limited by discrete fatigue mechanisms. Specifically, the results demonstrate that a similar muscle metabolic milieu (i.e., [ATP], [PCr], [lactate] and pH) was attained at T_{lim} irrespective of work-rate within the severe-intensity domain. The muscle metabolic perturbation was greater (i.e., lower [ATP] and pH, and higher [lactate]) at T_{lim} following severe- compared to heavy-intensity exercise, and also following severe- and heavy- compared to moderate-intensity exercise. In contrast, more extensive muscle glycogen depletion occurred during moderate- compared to both severe- and heavy-intensity exercise. However, while the results indicate that CP represents a critical threshold for both muscle metabolic control and neuromuscular fatigue development, the importance of the GET in separating exercise intensity domains was less obvious; unlike some muscle metabolic, pulmonary gas exchange and blood [lactate] responses, neuromuscular indices of fatigue development were not strikingly different between moderate-intensity and heavy-intensity exercise.

Fatigue during severe-intensity exercise

The T_{lim} during the severe-intensity exercise tests ranged from 2.2 min to 13.9 min and in all cases, subjects achieved $\dot{V}O_2$peak. Historically, the amount of work that can be done above CP (i.e., the curvature constant of the power-duration relationship, W′), and therefore the cause(s) of exercise intolerance within the severe-intensity domain, has been linked to the depletion of the high-energy phosphates and a source related to anaerobic glycolysis, along with a finite amount of stored O_2 (46, 47). Consistent with this, recent studies have demonstrated that, at least for small muscle mass exercise, the utilisation of this finite energy store (W′) coincides with the depletion of muscle PCr and the accumulation of fatigue-related metabolites (i.e. P_i, H^+) until a consistent, presumably 'limiting' value is attained (36, 72). The findings of the current study indicate that, irrespective of work-rate or exercise duration (~2-14 min), T_{lim} during severe-intensity exercise is associated with the attainment of consistently low values of muscle [PCr] (~23% of resting value), [ATP] (~76% of resting value) and pH (~6.56), and consistently high values of muscle [lactate] (~1382% of resting value), as well as blood [lactate] (~838% of resting value). It should be noted that the observed muscle metabolite and substrate changes are reflective of the homogenate muscle sample and therefore reflect the mean values for that particular muscle portion. It is known that the depletion of muscle [PCr] during exercise displays significant regional heterogeneity (14, 57). It is therefore possible that the subjects' eventual failure to maintain the requisite power output was caused by the attainment of sufficiently low values of [PCr] and, perhaps, [ATP], and/or sufficiently high values of muscle metabolites ([P_i], [ADP], [H^+] and their sequelae) within some of the recruited muscle fibres (54 see also 3, 12, 24,

25). Clearly, subjects either could not, or would not, tolerate this 'critical combination' of substrate and metabolite concentrations, but it is not possible to ascertain whether this was related to direct effects of the muscle metabolic milieu on contractile function (18) or to the attainment of some individual sensory 'critical fatigue threshold' which might constrain central motor drive and muscle activation via feedback from type III/IV neural afferents (4). The appreciable metabolic perturbation we observed during severe-intensity exercise was associated with a concomitant decrease in M-wave amplitude in both the VL and VM. A strong inverse correlation was observed between both the voluntary EMG RMS amplitude and neural drive, and the changes in [ATP] and [PCr] (Table 2). This is consistent with there being greater engagement of central neural mechanisms (e.g. muscle fibre recruitment and firing frequency modulation) in order to compensate for peripheral fatigue development.

We have proposed that the changes in muscle metabolic status that occur concomitantly with the expenditure of the W' are driving the continued development of the $\dot{V}O_2$ slow component during severe-intensity exercise (8, 36, 73). Thus, exercise intolerance in this intensity domain is associated with the complete utilisation of W′, the attainment of some 'critical' combination of muscle substrate and/or metabolite concentrations, and the achievement of $\dot{V}O_2$peak (8, 15, 35, 50, 73). In the present study, we observed a reduction in muscle excitability in parallel with the increased metabolic stress. The reduction in muscle membrane excitability is likely mediated, at least in part, by changes in plasma [K^+] (Table 2). Increased extracellular [K^+] impairs force generation due to a [K^+]-induced depolarisation of the cell membrane, resulting in a reduced amplitude

of the action potential (11, 43). This process attenuates Ca^{2+} release from the sarcoplasmic reticulum, reducing cross-bridge formation and the force generating capacity of the myocyte (39). In our study, the increased plasma [K^{+}] was accompanied by a transient increase in neural drive which was brought about via a preservation of the EMG amplitude with reduced M-wave amplitude. It was notable that the reductions in M-wave amplitude and M-wave area in the VM during exhaustive severe exercise were less pronounced compared to moderate and heavy exercise (Figure 5 A and C), suggesting that the muscle excitability was preserved to a greater extent than at lower exercise intensities. It is important, however, to consider this finding in the context of increasing neural drive during severe exercise (Figure 6 G and H) which implies that exercise cessation was not due to central fatigue. Low muscle pH attained during severe exercise may attenuate the reduction in muscle membrane excitability (3, 25). Furthermore, the muscle glycogen content, a key regulator of sarcoplasmic Ca^{2+} release rate and thus muscle excitability (16, 53), did not fall significantly during severe exercise. Precisely how the utilisation of the W', the associated alterations in muscle substrate and metabolite concentrations, and ionic changes influence muscle excitability warrants further investigation.

Fatigue during heavy-intensity exercise

Heavy-intensity exercise was maintained for an average of 43.5 min (T_{lim} ranged from 20.5 to 67.4 min) and, in contrast to severe-intensity exercise, no subject achieved $\dot{V}O_{2peak}$ at T_{lim} (~87% $\dot{V}O_{2peak}$). Consistent with our second hypothesis, the muscle metabolic perturbation experienced following heavy-intensity exercise was less than that observed following severe-intensity exercise, but was greater

than that observed following moderate-intensity exercise. At T_{lim}, significant reductions were observed in muscle [PCr] (~66%), [ATP] (~12%), [pH] (~97%]) and [glycogen] (~59%), and there was a significant increase in muscle [lactate] (~447%) relative to resting values. Similarly, blood [lactate] and plasma [K^+] displayed greater perturbation relative to moderate-intensity exercise, but less perturbation relative to severe-intensity exercise (Figure 3). It is of interest that the decrease in muscle excitability from rest to T_{lim} was greater during heavy-intensity than during severe-intensity exercise (Figure 5). Following the onset of exercise, plasma [K^+] increased rapidly to attain a peak value at 10 min which was sustained until T_{lim}; the reduction in M-wave amplitude followed a similar temporal profile. It is therefore likely that the initial reduction in M-wave amplitude was a result of plasma [K^+] accumulation which reduced the release of Ca^{2+} from the sarcoplasmic reticulum, impairing excitation-contraction coupling (39, 74). As heavy-intensity exercise continued, it is possible that the combined metabolic and ionic perturbation, coupled with the ~60% decrease in muscle [glycogen], may have further impaired Ca^{2+} release and cross-bridge formation (2, 3, 24, 25, 39, 43, 44) and/or the sensitivity of the myofilaments to Ca^{2+} (18). Although more complicated than for severe-intensity exercise, fatigue development during heavy-intensity exercise appears to be related to the combined influence of ionic changes on muscle membrane excitability, muscle metabolite accumulation, and the decrease in energy substrate, which act collectively to impair excitation-contraction coupling.

Fatigue during moderate-intensity exercise

Moderate-intensity exercise, performed at a work-rate of 20 W below the GET, was continued for an average of 211 min with subjects working at ~52% $\dot{V}O_2$peak at T_{lim}. Muscle metabolic perturbation was relatively slight in this domain (Figure 3). For example, at the end of exercise, muscle [PCr] had fallen to ~76% of the baseline value and pH had fallen by 0.1 unit from the resting value, while blood [lactate] and plasma [K^+] were also largely unchanged (Figure 2). There was, however, a large reduction (–83%) in muscle [glycogen] (1, 30, 60, 61). It is therefore likely that the development of peripheral fatigue within the moderate-intensity domain is related to the depletion of muscle glycogen and impairment in neuromuscular excitability and transmission (16, 29, 52, 53, 65). In addition to being an essential substrate for the regeneration of ATP, it has been demonstrated that under conditions where [ATP] is held high, that low muscle [glycogen] can impair muscle function (52, 65). The association between low muscle [glycogen] and impaired muscle function can be attributed to glycogen's modulatory role in the release of Ca^{2+} from the sarcoplasmic reticulum (16, 20, 21, 29, 52, 53). In keeping with glycogen's role in excitation-contraction coupling, individuals deficient in glycogen phosphorylase (McArdle's disease) do not experience a considerable fall in pH but demonstrate an earlier decline in the M-wave amplitude during exercise (17). Furthermore, glucose administration during exercise has been shown to partially restore both the M-wave amplitude and muscle contractility (37, 40, 66), supporting the notion that carbohydrate availability modulates muscle excitability and contractile function. The findings of the present study show that moderate-intensity exercise (<GET) can be sustained for a long duration with little change in muscle metabolites and indicate that

muscle glycogen depletion is the likely mechanism responsible for the decline in neuromuscular function and exercise intolerance.

The majority of research investigating neuromuscular fatigue development during exercise has focused on small muscle groups and has been limited to the assessment of neuromuscular function pre-exercise and as soon as possible (usually within 2-3 minutes) post-exercise. Considering the task-specific nature of neuromuscular fatigue development, and the rapid recovery in muscle function (within 2 min) after high-intensity cycle exercise (27), it is possible that the previously reported changes in neuromuscular function pre- to post-exercise underestimate fatigue development *during* exercise. Recently, Sidhu et al. (59) adopted an approach that uses the motor compound action potential (M-wave) for the assessment of changes in neuromuscular function during cycle exercise. Adopting a similar approach to Sidhu et al. (59), we found large reductions in the M-wave amplitude and M-wave area in both the VL and VM during exercise to T_{lim} in each discrete exercise intensity domain. This suggests that changes in muscle excitability linked to the fatigue process can occur consequent to a wide range of perturbations in muscle and blood chemistry, with limited differentiation between exercise intensity domains. The consistency of indices of neuromuscular fatigue during severe-intensity cycling exercise in our study contrasts with a recent report of Thomas et al. (69) in which peripheral fatigue, assessed post-exercise using electrical stimulation during isometric contractions, was greater at higher work-rates within the severe-intensity domain. It is possible that this reflects differences in the experimental techniques employed, and underlines the

importance of accounting for the task-specificity of fatigue and the dynamics of muscle recovery post-exercise (10).

Conclusion

This study employed a novel and rather comprehensive combination of invasive and non-invasive techniques that enabled simultaneous assessment of metabolic, ionic, systemic and neuromuscular factors that define muscular performance. This approach permitted elucidation of the relative importance of these factors in neuromuscular fatigue development during exhaustive cycle exercise performed within each of the well-defined exercise intensity domains. This study is consistent with the notion that the GET and the CP demarcate exercise intensity domains within which fatigue is mediated by distinct mechanisms. Exercise intolerance within the severe-intensity domain (>CP) was associated with the attainment of a consistent critical muscle metabolic milieu (i.e., low [PCr] and pH and high [P_i]). In contrast, moderate-intensity exercise (<GET) was associated with more significant depletion of muscle [glycogen]. The cause(s) of fatigue during heavy-intensity exercise (>GET, <CP) was/were more obscure with intermediate changes in muscle metabolic perturbation and glycogen depletion being apparent. These results are consistent with the notion that both the GET and CP demarcate exercise intensity domains characterised by distinct respiratory and metabolic profiles. Strikingly, CP represents a boundary above which both metabolic and neuromuscular responses conform to a consistent ceiling or nadir irrespective of work-rate and exercise duration.

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Figure Legends

Table 1 The CP and W' parameter estimates derived from Equations 1-3 and the 'best fit' model.

Table 2 The correlation coefficients between the rate of change in blood and muscle tissue variables and the rate of change in neuromuscular variables measured in *m. vastus lateralis*. * P<0.05.

Figure 1. Schematic of the exercise protocol. Group mean work-rates are shown for the severe- (solid line), heavy- (dotted line) and moderate- (dashed line) intensity trials. All trials were started with a 3-min "warm-up" phase at 20 W, followed by an immediate "step" increase to the required work-rate. Subjects were encouraged to continue exercising for as long as possible. The dashed arrows indicate the collection of venous blood, and femoral nerve stimulation. The solid arrows indicate the collection of muscle tissue. N.B., for clarity, the resting muscle sample obtained prior to the first trial is not shown.

Figure 2. Muscle metabolic responses ([ATP] panel A, [PCr] panel B, pH panel C, [lactate] panel D, [glycogen] panel E) and blood [lactate] (panel F) at T_{lim} were not different following exhaustive exercise at three different severe-intensity work-rates. R = rest; S1 = short trials at ~85%Δ (T_{lim} = 224 ± 41 s); S2 = intermediate trials at ~75%Δ (T_{lim} = 333 ± 131 s); and S3 = long trials at ~65%Δ (T_{lim} = 475 ± 145 s). * Different from S1, S2 and S3 (P<0.05).

Figure 3. Pulmonary $\dot{V}O_2$ (panel A), blood [lactate], (panel B) and plasma [K^+] (panel C) response to severe- (solid circle), heavy- (clear circle) and moderate- (solid triangle) intensity exercise. To aid clarity error bars have been omitted from

all but the final data point. a = different from moderate-intensity $P<0.05$; b = different from heavy-intensity $P<0.05$.

Figure 4. Muscle [ATP] (panel A), [PCr] (panel B), [pH] (panel C), [lactate] (panel D), and [glycogen] (panel E) at rest (white triangle), and following severe- (black circle), heavy- (white circle), and moderate-intensity exercise (black triangle). * = different from rest $P<0.05$; a = different from moderate-intensity $P<0.05$; b = different from heavy-intensity $P<0.05$; c = different from severe-intensity $P<0.05$.

Figure 5. The group mean ± SD M-wave amplitude and M-wave area (normalised to maximum M-wave during baseline pedalling) indicating peripheral neuromuscular excitability (panels A-D); voluntary EMG RMS amplitude (normalised to M-wave amplitude at 1 min of exercise) indicating muscle activation level (panels E and F); and RMS/M-wave (normalised to corresponding M-wave amplitude at each measurement time point) indicating central fatigue (panels G and H) at the limit of tolerance (T_{lim}) for moderate-, heavy- and severe-intensity exercise (panels A, C, E, G) and for three work-rates (severe 1 ~85%Δ, severe 2 ~75%Δ and severe 3 ~65%Δ) within the severe-intensity domain (panels B, D, F, H). There were no significant differences among the severe-intensity work-rates in muscle excitability (BD) or in indices of central fatigue (FH). VL = *m. vastus lateralis*; VM = *m. vastus medialis*; EMG = electromyogram; RMS = root mean square; a = different from moderate-intensity $P<0.05$; b = different from heavy-intensity $P<0.05$; c = different from severe-intensity $P<0.05$.

Figure 6. The normalised M-wave amplitude (panels A and B), M-wave area (panels C and D), voluntary EMG RMS amplitude (panels E and F), and RMS/M-wave amplitude (panels G and H) during severe- (solid circle), heavy- (clear

circle), and moderate-intensity (solid triangle) exercise in *m. vastus lateralis* (VL) and *vastus medialis* (VM). M-wave amplitude and area were normalised to maximum M-wave during baseline pedalling, EMG RMS was normalised to M-wave amplitude at 1 min of exercise, and RMS/M-wave was normalised to corresponding M-wave amplitude at each measurement time point. Error bars have been omitted from all but the final data point to aid clarity. [a] Different from rest; [b] different from severe-intensity (P<0.05); [c] different from heavy-intensity (P<0.05); [d] different from moderate-intensity (P<0.05); and [e] trend for difference from heavy-intensity (P=0.055).

Table 1

	R^2	CP (W)	SEE (W)	CV%	W' (kJ)	SEE (kJ)	CV%
W-Tlim model	0.993 – 1.000	253 ± 54	6 ± 3	2.6 ± 1.4	22.5 ± 5.3	2.3 ± 1.0	11.0 ± 6.2
1/Tlim model	0.939 – 0.999	252 ± 52	7 ± 4	3.0 ± 2.3	20.7 ± 5.2	1.9 ± 1.1	9.5 ± 5.6
P-Tlim model	0.919 – 1.000	248 ± 52	5 ± 3	2.2 ± 1.4	22.4 ± 3.8	2.5 ± 1.8	11.3 ± 9.4
Optimised fit model	0.944 – 1.000	250 ± 53	5 ± 2	2.0 ± 1.2	22.5 ± 6.1	1.8 ± 0.8	8.3 ± 4.5

Table 2

			M-wave Amplitude	M-wave Area	Voluntary EMG	Neural Drive
Severe	n = 33	BLa	-0.30	-0.11	0.57*	0.47*
		Plasma [Na^+]	-0.23	0.08	0.62*	0.55*
		Plasma [K^+]	-0.39*	0.04	0.68*	0.64*
	n = 24	[PCr]	0.59*	0.34	-0.80*	-0.80*
		[lactate]	-0.40	-0.20	0.44*	0.55*
		[glycogen]	-0.22	0.09	0.46*	0.56*
		[pH]	-0.13	0.01	0.36	0.37
		[ATP]	0.21	-0.25	-0.60*	-0.59*
Heavy	n = 10	BLa	-0.42	-0.53	0.13	0.49
		Plasma [Na^+]	-0.80*	-0.83*	-0.23	0.80*
		Plasma [K^+]	-0.88*	-0.93*	-0.29	0.86*
	n = 7	[PCr]	0.93*	0.94*	-0.28	-0.72*
		[lactate]	-0.25	-0.28	0.63	0.66
		[glycogen]	-0.15	-0.27	0.53	0.77*
		[pH]	0.13	-0.07	0.78*	0.27
		[ATP]	-0.26	-0.26	0.32	0.63
Moderate	n = 10	BLa	0.08	0.13	0.05	0.10
		Plasma [Na^+]	-0.09	-0.13	0.14	0.42
		Plasma [K^+]	0.12	0.05	0.18	0.49
	n = 7	[PCr]	-0.67*	-0.75*	-0.36	0.58
		[lactate]	-0.44	-0.52	-0.34	0.04
		[glycogen]	-0.10	-0.25	0.43	0.23
		[pH]	0.19	0.14	0.06	-0.30
		[ATP]	0.09	-0.07	0.59	0.24

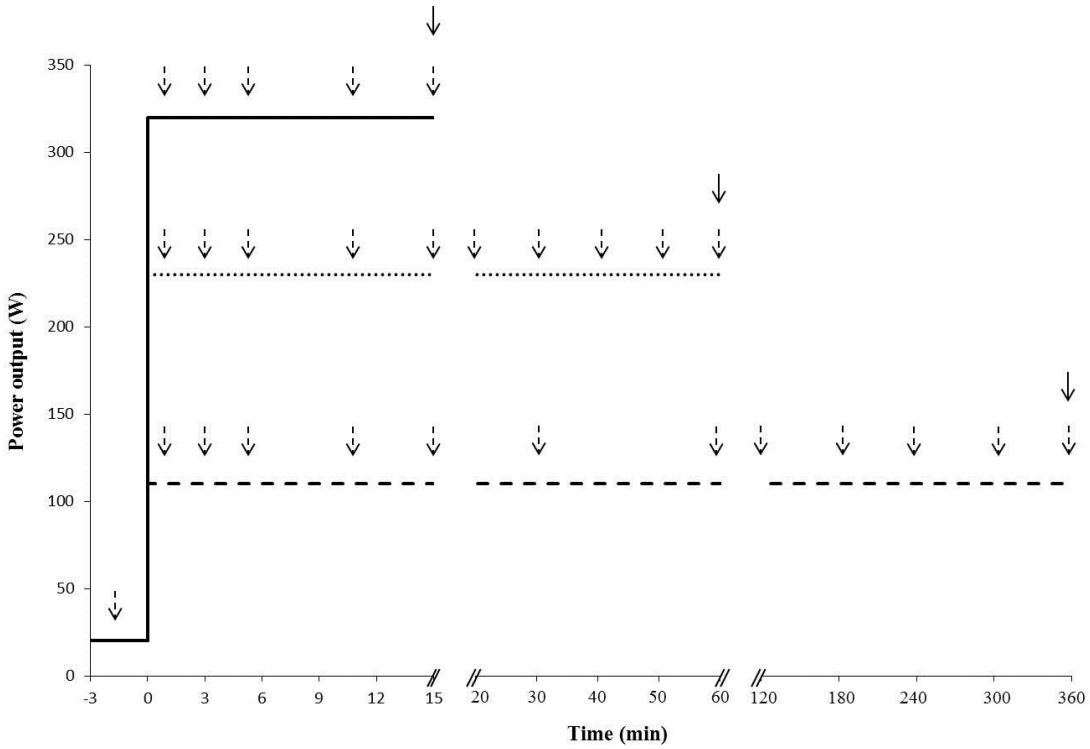

Power output (W)
350
300
250
200
150
100
50
0
-3
0
3
6
9
12
15
20
30
40
50
60
120
180
240
300
360
Time (min)

Figure 1

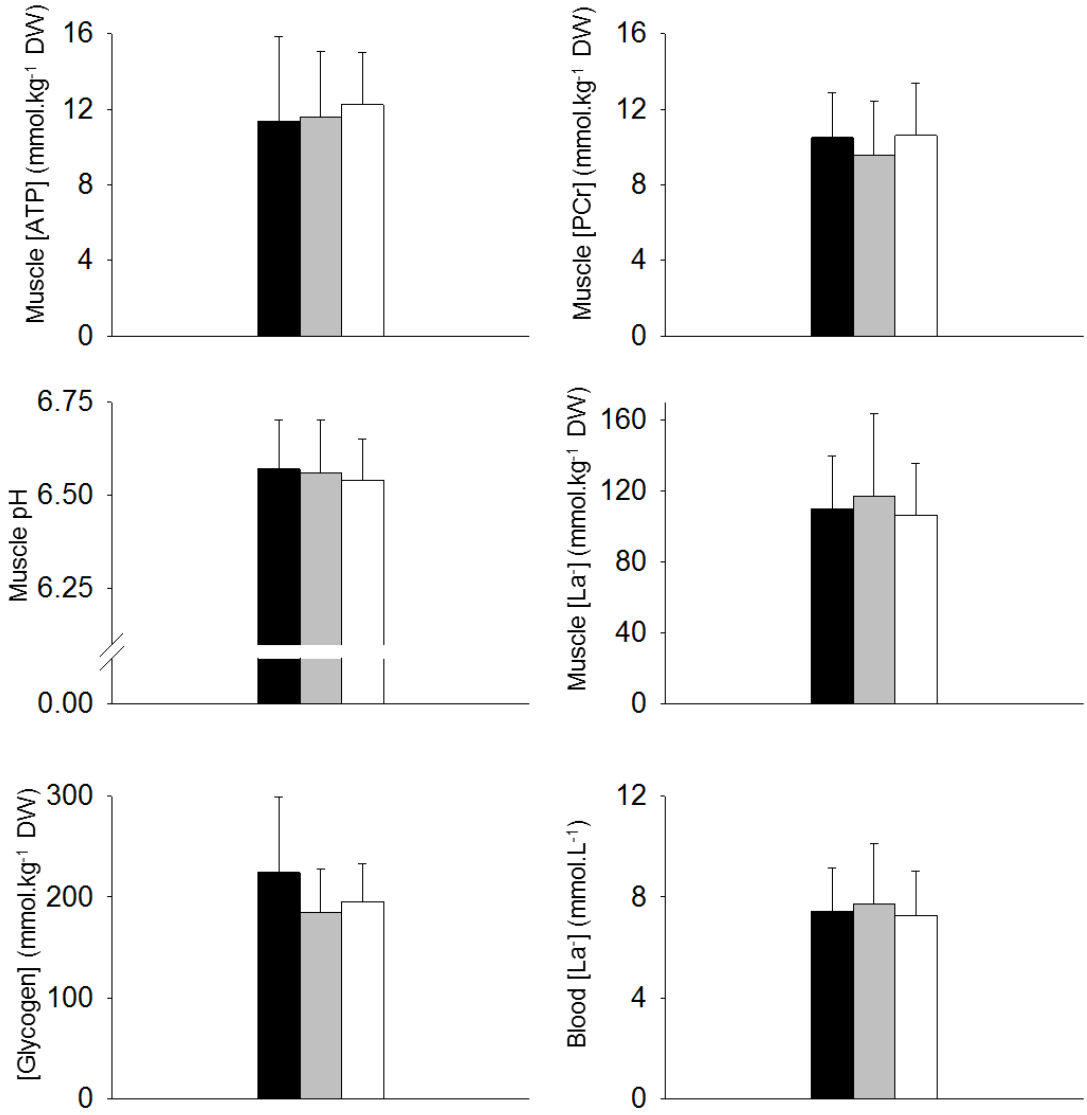

Muscle [ATP] (mmol.kg-1 DW)
16
12
8
4
0
Muscle [PCr] (mmol.kg-1 DW)
16
12
8
4
0
Muscle pH
6.75
6.50
6.25
0.00
Muscle [La-] (mmol.kg-1 DW)
160
120
80
40
0
[Glycogen] (mmol.kg-1 DW)
300
200
100
0
Blood [La-] (mmol.L-1)
12
8
4
0

Figure 2

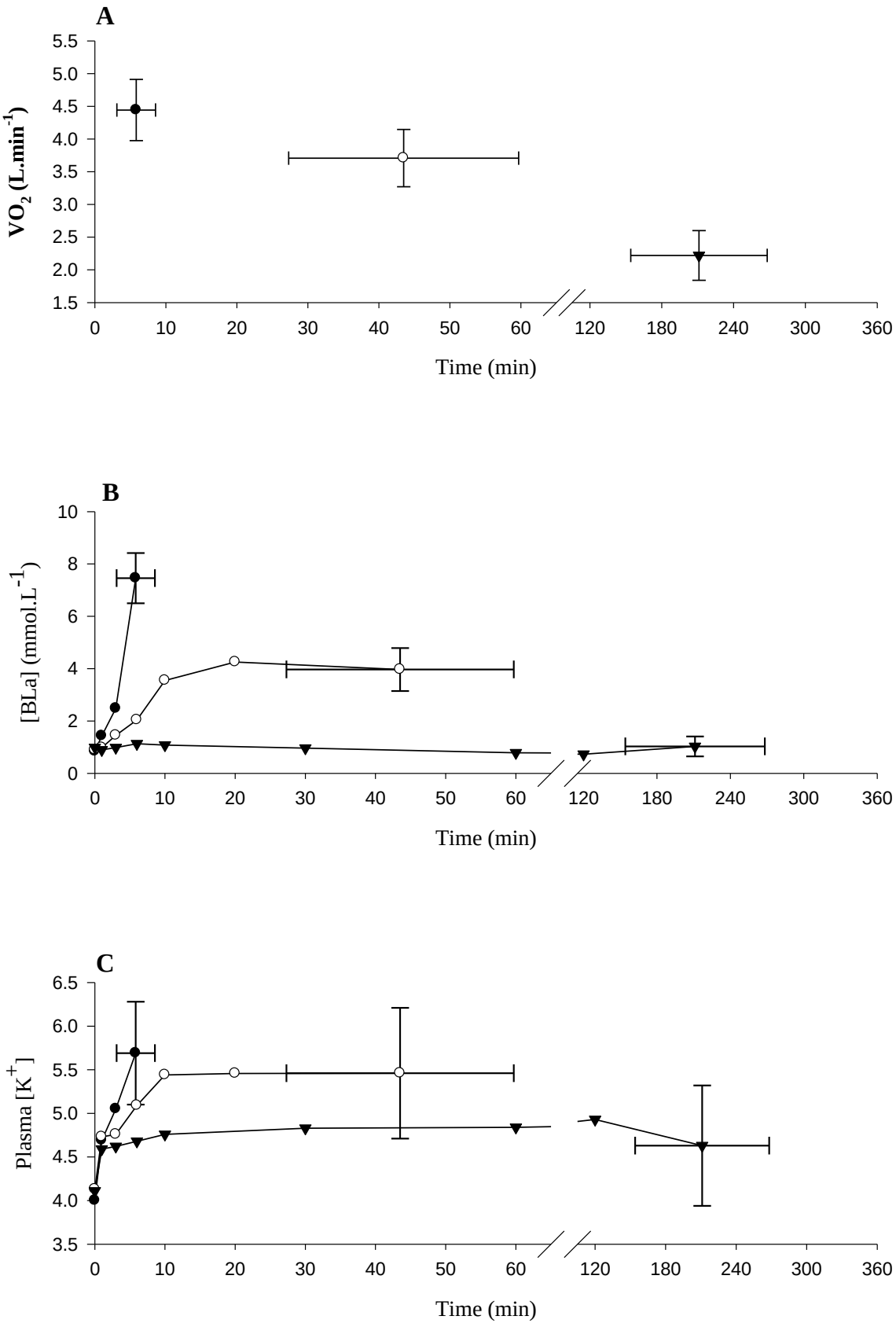

A
VO_2 (L.min^{-1})
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0
10
20
30
40
50
60
120
180
240
300
360
Time (min)
B
[BLa] (mmol.L^{-1})
10
8
6
4
2
0
Time (min)
C
Plasma [K$^+$]
6.5
6.0
5.5
5.0
4.5
4.0
3.5
Time (min)

Figure 3

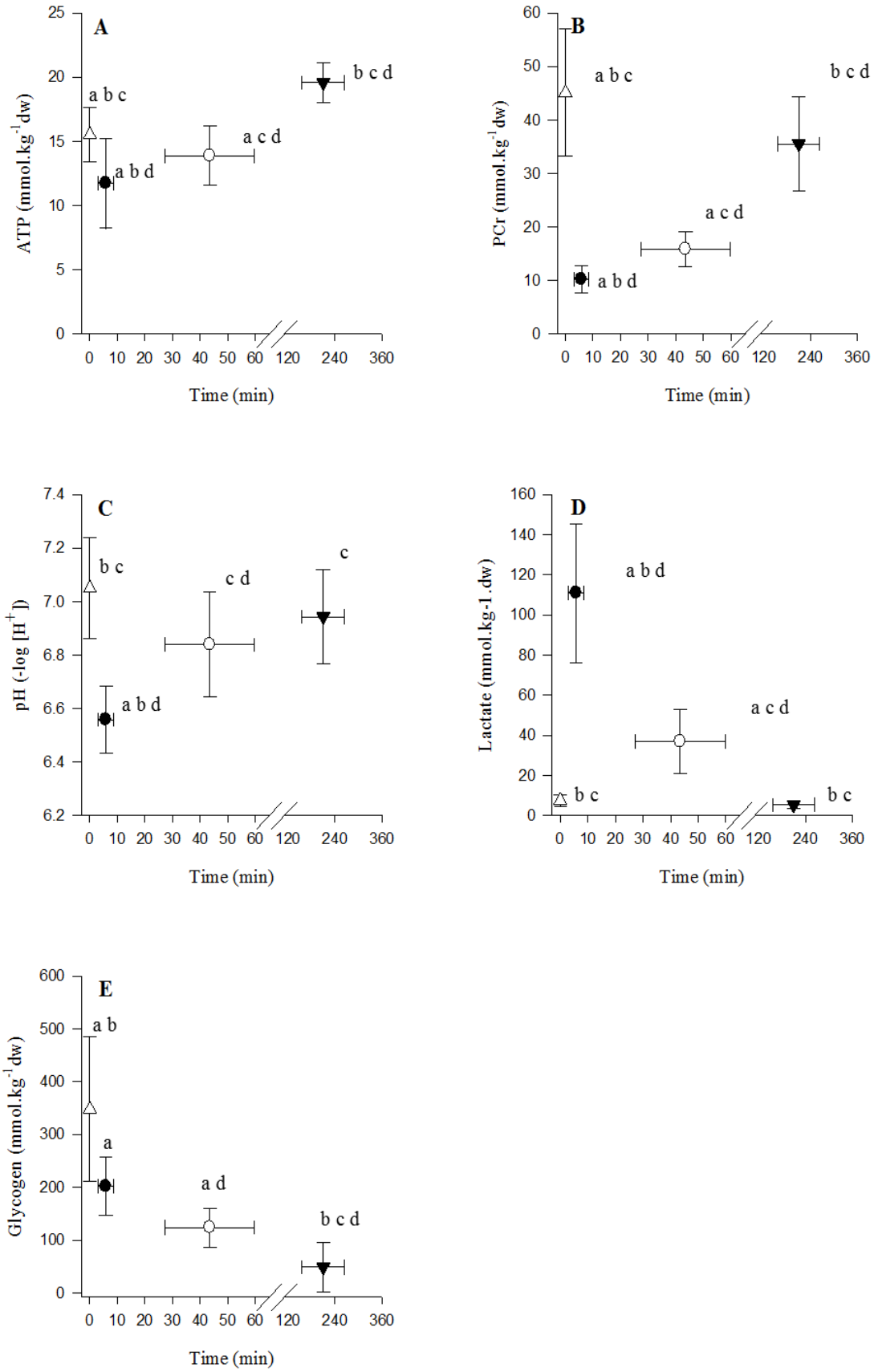

A
ATP (mmol.kg-1dw)
a b c
a b d
a c d
b c d
B
PCr (mmol.kg-1dw)
a b c
a b d
a c d
b c d
C
pH (-log [H+])
b c
a b d
c d
c
D
Lactate (mmol.kg-1.dw)
a b d
a c d
b c
b c
E
Glycogen (mmol.kg-1dw)
a b
a
a d
b c d
Time (min)
0 10 20 30 40 50 60 120 240 360

Figure 4

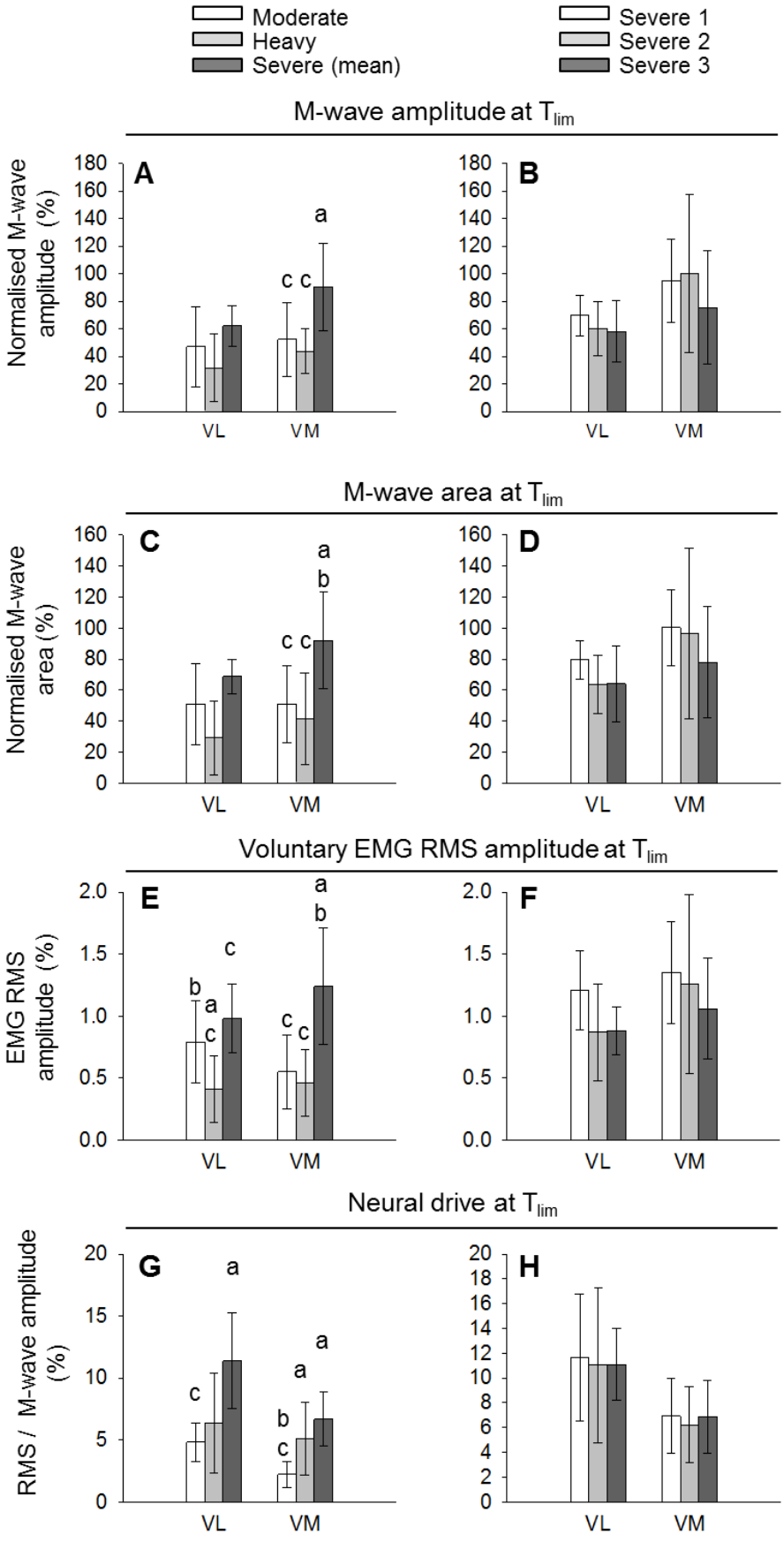
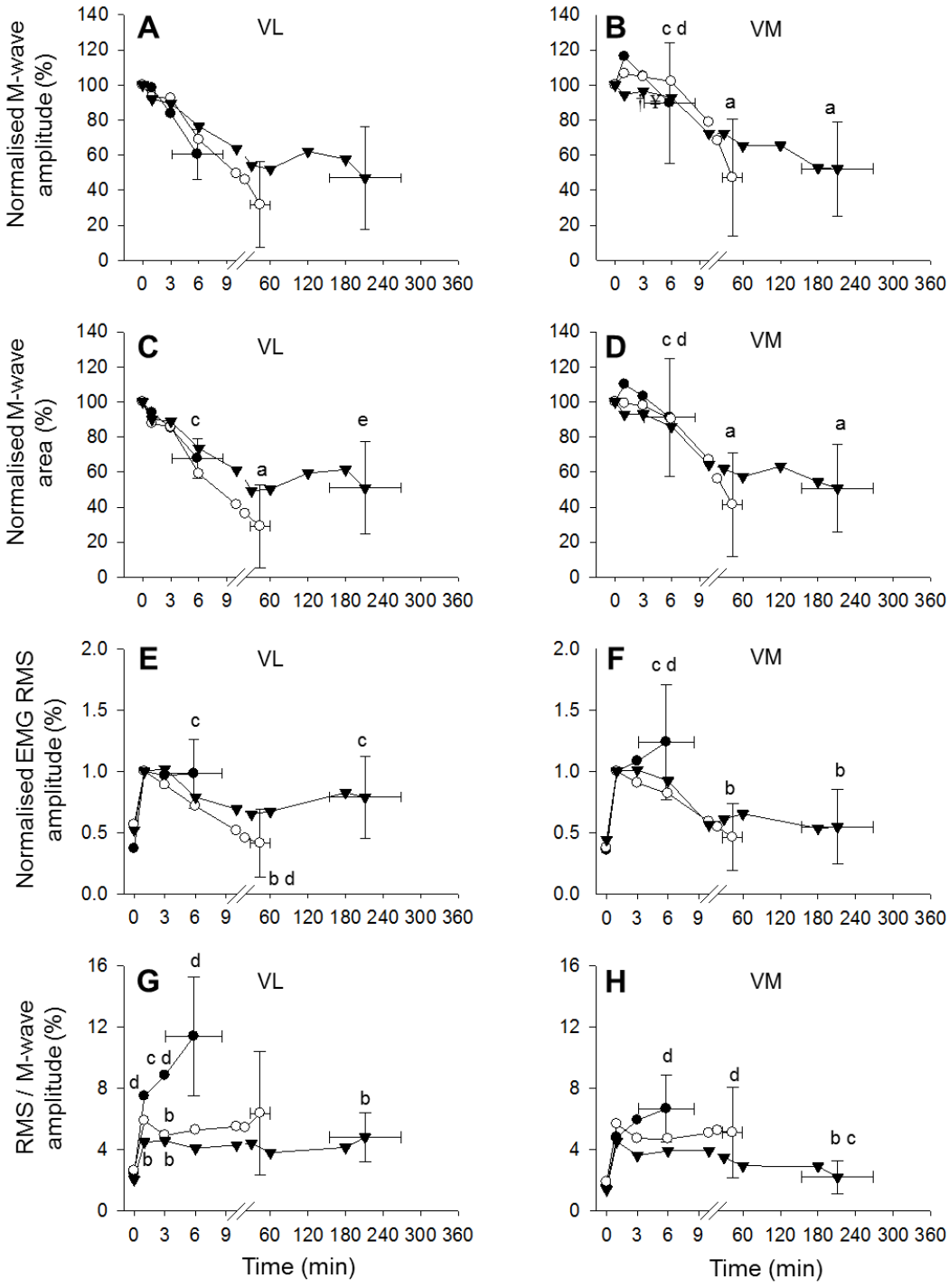

Moderate
Heavy
Severe (mean)
Severe 1
Severe 2
Severe 3
M-wave amplitude at T_{lim}
Normalised M-wave amplitude (%)
A
B
M-wave area at T_{lim}
Normalised M-wave area (%)
C
D
Voluntary EMG RMS amplitude at T_{lim}
EMG RMS amplitude (%)
E
F
Neural drive at T_{lim}
RMS / M-wave amplitude (%)
G
H
VL
VM

Figure 5


A
VL
B
c d
VM
a
a
C
VL
c
e
a
D
c d
VM
a
a
E
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c
c
b d
F
c d
VM
b
b
G
VL
d
c d
d
b
b
b
b
H
VM
d
d
b c
Normalised M-wave amplitude (%)
Normalised M-wave area (%)
Normalised EMG RMS amplitude (%)
RMS / M-wave amplitude (%)
0 3 6 9 60 120 180 240 300 360
Time (min)

Figure 6

The effects of β-alanine supplementation on muscle pH and the power-duration relationship during high-intensity exercise

Original Investigation

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Abstract

Purpose: To investigate the influence of β-alanine (BA) supplementation on muscle carnosine content, muscle pH and the power-duration relationship (i.e., the CP, W′, and the W′ recovery). **Methods:** In a double-blind, randomised, placebo-controlled study, 20 recreationally-active males ingested either BA (6.4 $g.d^{-1}$ for 28 d) or placebo (PL) (6.4 $g.d^{-1}$ for 28 d). Subjects completed a ramp incremental test and two 3-min all-out tests separated by 1-min active recovery on a cycle ergometer pre- and post-supplementation for the determination of CP and W′ (bout 1), and for determination of the work performed above CP during bout 2 (W>CP) as an index of W′ recovery. Muscle pH was assessed using ^{31}P-MRS during incremental and intermittent knee-extension exercise (INT KEE), and muscle carnosine content was determined using ^{1}H-MRS. **Results:** There were no differences in the change in muscle carnosine content from pre- to post-intervention (PL: 2 ± 25% vs. BA: 15 ± 25%) or in muscle pH during INC KEE (PL: 0.04 ± 0.15 vs. BA: -0.04 ± 0.12) or INT KEE (PL: 0.08 ± 0.22 vs. BA: -0.05 ± 0.13) (P>0.05) between PL and BA, but blood pH (PL: -0.06 ± 0.10 vs. BA: 0.09 ± 0.13) during the ramp incremental cycling test was elevated post-supplementation in the BA group only (P<0.05). The changes from pre- to post-supplementation in the CP (PL: -8 ± 18 W vs. BA: -6 ± 17 W), W′ (PL: 1.8 ± 3.3 kJ vs. BA: 1.5 ± 1.7 kJ) and W' recovery (PL: 1.6 ± 3.5 kJ vs. BA: 0.7 ± 2.7 kJ) were not different between groups. No relationships were detected between muscle carnosine content and indices of exercise performance. **Conclusions:** BA supplementation had a variable effect on muscle carnosine content and had no influence on intramuscular pH during high-intensity intermittent knee-extension exercise. The small increase in blood pH during incremental cycling exercise following BA supplementation was

not sufficient to significantly alter the power-duration relationship or exercise performance.

Introduction

The critical power (CP) model of high-intensity exercise performance is defined by two parameters: the CP, representing the highest sustainable rate of oxidative phosphorylation, and the W′, notionally derived from substrate level phosphorylation (Jones et al. 2008; 2010; Monod and Scherrer, 1965; Moritani et al. 1981; Poole et al. 1988). Metabolic acidosis, a consequence of exercise >CP, has been implicated in the fatigue process (Chin and Allen, 1998; Fitts, 1994), attaining a consistent and presumably critically low value at task failure (Poole et al. 1988; Vanhatalo et al. 2010). In keeping with the notion that metabolic acidosis contributes to muscular fatigue, improved muscle H^+ efflux is a good predictor of intense exercise performance (Hostrup and Bangsbo, 2016). Intramuscular H^+ accumulation has been shown to interfere with the release of Ca^{2+} from the sarcoplasmic reticulum and thus impair excitation-contraction coupling (Debold et al. 2008; Knuth et al. 2006), inhibit glycolysis (Gevers and Dowdle, 1963; Trivedi and Danforth, 1966; Spriet et al. 1987) and slow PCr recovery following exercise (Harris et al. 1976). The ability to attenuate the rate of muscle H^+ accumulation during exercise and enhance its removal from the muscle during recovery may therefore reduce the extent of exercise-induced disruption to excitation-contraction coupling, glycolytic flux, and PCr recovery and permit increased performance during continuous and intermittent high-intensity exercise.

Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide found in high concentrations within skeletal muscle (Harris et al. 2006), which, due to the pKa of its imidazole side-chain (6.83), is a potent buffer for intramuscular H^+ accumulation during contractions (Bate-Smith, 1938). β-alanine (BA) has been identified as the rate-limiting factor in carnosine synthesis (Dunnett et al. 1999; Harris et al. 2006) and its ingestion has been shown to increase intramuscular carnosine content (Baguet et al. 2009; Bex et al. 2014; Harris et al. 2006; Stegen et al. 2013, 2014; Stellingwerf et al. 2012) and thus presumably improve intramuscular buffering capacity. Indeed, some investigations have reported improved high-intensity exercise tolerance and performance following BA supplementation (Quesnele et al. 2014; Hobson et al. 2012; Saunders et al. 2016). However, other studies have shown no significant effect of BA supplementation on performance (Ducker et al. 2013; Jagim et al. 2013; Saunders et al. 2012a; Sweeney et al. 2010), despite subjects following an appropriate supplementation strategy (Hobson et al. 2012) and completing a test ostensibly >CP that would be expected to result in a large decline in muscle pH. Furthermore, despite possible ergogenic effects of BA supplementation being attributed to enhanced intramuscular buffering capacity, no study has yet assessed differences in muscle pH during exercise in humans following BA supplementation.

Given that the W′ has been proposed to represent a work capacity comprising the energy available from the “anaerobic” energy pathways (Miura et al., 1999; 2000; Moritani et al. 1981), it would be expected that an increased glycolytic flux during exercise >CP would increase W′. Attenuating the decline in muscle pH may delay the attainment of a “critical” intramuscular milieu which is associated with the complete utilisation of the W′ (Poole et al. 2008; Vanhatalo et al. 2010), and thus

should be reflected by an increased W′. Enhanced muscle buffering may also expedite the restoration of muscular homeostasis following exercise >CP by permitting enhanced PCr recovery and improving glycolytic flux during a subsequent >CP exercise bout. Improved intramuscular buffering capacity may therefore be ergogenic during exercise >CP through enhanced W′ and improved W′ recovery kinetics.

The purpose of this study was to evaluate the physiological and performance effects of 4 weeks of BA supplementation (6.4 $g.d^{-1}$) on exercise performance and the power-duration relationship. We hypothesised that BA supplementation would: 1) increase the muscle carnosine content at rest and intramuscular pH during high-intensity, intermittent knee-extension exercise; 2) enhance muscle [PCr] recovery and thus improve performance during high-intensity, intermittent knee-extension exercise; and 3) increase the size of the W′ estimated in a 3-min all-out cycling test and improve all-out and incremental cycling performance.

Methods

Subjects

Twenty healthy male subjects (mean ± SD: age 22.3 ± 3.2 y, height 1.77 ± 0.07 m, mass 79.0 ± 14.4 kg) volunteered to participate in this study. Prior to testing, subjects were informed of the protocol and possible risks of participation and, subsequently, provided written consent to participate. All procedures were approved by the local Research Ethics Committee and conformed to the code of ethics of the Declaration of Helsinki.

Experimental design

Subjects visited the laboratory on 5 occasions over a 2-wk period pre-supplementation, and 6 occasions over a 2-wk period post-supplementation. Prior to supplementation, baseline carnosine content was determined using ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) (visit 1). Additionally, during visit 1, subjects performed incremental knee extension exercise (INC KEE) with muscle metabolic changes assessed via ^{31}P magnetic resonance spectroscopy (^{31}P-MRS). During visit 2, subjects performed an incremental cycling test for the determination of the gas exchange threshold (GET) and $\dot{V}O_{2max}$. Subjects were familiarised to a 'repeated 3-min all-out' protocol (visit 3), which involved the performance of two 3-min all-out tests (described in Burnley et al. 2006; Vanhatalo et al. 2007) separated by 1 min of passive recovery. Subjects returned to the laboratory on a separate occasion to complete an experimental 3-min all-out test (visit 4). During visit 5, participants performed an intermittent knee extension exercise (INT KEE) with metabolic changes assessed via ^{31}P-MRS. The order of tests was randomised with the exception of the: incremental cycling test, which was necessary to determine the linear factor for the repeated 3-min all-out test; the familiarisation to the repeated all-out test, which always preceded the experimental test; and the INC KEE was performed prior to the INT to set the appropriate load.

In a double-blind, placebo controlled design subjects were assigned to BA (Carnosyn®, Natural Alternative International, San Marcos CA) or a cornflower placebo (PL) group. Groups were matched for physical characteristics (age, body mass, height), baseline carnosine content, and physiological fitness measures

($\dot{V}O_{2max}$, GET, CP, W'). Subjects consumed 8 capsules per day of PL or BA, including two with every meal (breakfast, lunch, dinner) and 2 before bed such that the BA dose was 6.4 $g \cdot d^{-1}$. Following 4 weeks of supplementation, each subject reported to the laboratory to begin post-supplementation tests. Subjects were instructed to continue their supplementation regime during the *post*-supplementation visits, and therefore supplemented their diet for a total of 6 weeks. The post-supplementation visits comprised the same exercise tests and were performed in the same order as the pre-supplementation visits, with the addition of a carnosine scan following the completion of all visits 6 weeks into supplementation. Subjects were instructed to follow their normal dietary and exercise habits throughout the study.

Experimental visits were scheduled at the same time of day (±3 h) and subjects were instructed to report to all testing sessions in a rested and well-hydrated state, having avoided strenuous exercise for 24 h and caffeine for 3 h prior to each test. Subjects were asked to maintain their normal dietary and exercise behaviour throughout the study.

Exercise tests

All cycling tests were performed on the same electronically-braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The ergometer seat and handlebars were adjusted for comfort, and settings were recorded and replicated for subsequent visits. The ramp incremental protocol consisted of 3 min of unloaded baseline pedalling followed by a ramp increase in power output of 30 $W.min^{-1}$ until task failure. Subjects were instructed to maintain their self-selected

cadence (80 rpm, *n* = 18; 85 rpm, *n* = 1; 90 rpm, *n* = 1) for as long as possible. The test was terminated when the pedal rate fell >10 rpm below the chosen cadence for >5 s despite strong verbal encouragement. $\dot{V}O_{2peak}$ was determined as the highest 30-s mean value. The GET was established from the gas exchange data averaged in 10 s time bins using the following criteria: 1) the first disproportionate increase in $\dot{V}CO_2$ versus $\dot{V}O_2$; 2) an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E$/ $\dot{V}CO_2$; and 3) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension.

The repeated 3-min all-out test began with 3 min of baseline pedalling (20 W), at the same self-selected cadence chosen during the incremental ramp test, followed by two 3-min all-out efforts separated by 1 min of active recovery (20 W cycling). Subjects were asked to accelerate to 110-120 rpm over the final 5 s of the baseline period, and the final 5 s of the active recovery. The resistance on the pedals during the 3-min all-out test was set using the linear mode of the ergometer such that on reaching their preferred cadence the power output would be equivalent to 50% of the difference between the power output at GET and $\dot{V}O_{2max}$ (linear factor = power/cadence^2). To ensure an all-out effort, subjects were instructed and strongly encouraged to attain their peak power output as quickly as possible, and to maintain their cadence as high as possible until instructed to stop. CP was determined as the average power output during the final 30 s during bout 1. End test power (EP) was determined as the average 30 s power output during the final 30 s of bout 2. Bout 1 W′ was defined as the amount of work done above bout 1 CP. During bout 2, the amount of work performed above bout 1 CP (W>CP), was determined to provide a measure of recovery. The $\dot{V}O_{2peak}$ during

each bout of the repeated 3-min all-out test was calculated as the highest 15 s rolling mean value.

To assess muscle metabolism during exercise subjects performed single-legged knee-extension exercise in a prone position within a magnetic resonance scanner, as described by Vanhatalo et al. (2010). The incremental exercise consisted of 30 s of exercise lifting 1 kg, followed by a 0.5 kg increase in mass every 30 s until task failure. For the intermittent exercise protocol the weight within the load basket was 120% of the peak work rate recorded in the incremental test with the protocol consisting of 60 s of high-intensity work, followed by 18 s of passive rest, with the sequence repeated until task failure.

Pulmonary gas exchange

Breath-by-breath pulmonary gas exchange data were collected continuously during all cycling tests, with subjects wearing a nose clip and breathing through a low-dead space, low resistance mouthpiece and impeller turbine assembly (Triple V, Jaeger, Hoechberg, Germany). The inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analysers (Oxycon Pro, Jaeger) via a capillary line connected to the mouthpiece. These analysers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, KS). The volume and concentration signals were time-aligned, accounting for the transit delay in capillary gas and analyser rise time relative to the volume signal. The $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were calculated for each breath using standard formulae.

Blood analyses

Venous blood samples were drawn into 5-mL lithium heparin tubes (Terumo Corporation, Leuven, Belgium) at baseline and at discrete time-points during the 3-min all-out test (bout 1 and 2: 30 s, 60 s, 90 s 170 s, and 30 s post-test) and the ramp incremental test (120 s, 240 s, 360 s, 480 s, 600 s, T_{lim}, T_{lim} +60 s, T_{lim} +120 s) from a cannula (Insyte-W™, Becton-Dickinson, Madrid, Spain) inserted into the subject's antecubital vein. The blood was analysed for lactate (La) (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH) and 1.5 mL of whole blood was extracted and stored at -80°C for subsequent determination of pH.

MRS measurements

Muscle carnosine content was measured in the vastus medialis (VM), vastus lateralis (VL), and rectus femoris (RF) muscles using ^{1}H-MRS. Subjects were secured to the scanner bed in the supine position via Velcro straps which were fastened across the thigh to minimise movement during the scans. Following the acquisition of a localiser series, a high-resolution imaging series of the thigh (Fast spin echo, echo train 19, repetition time of 2,660 ms, echo time of 13 ms, slice 4 mm, pixel 0.625 x 0.625 mm) was acquired for the definitive placement of the MRS voxel. A 4-element flexible surface coil was used to obtain high resolution images of the thigh and subsequent single-voxel point resolved spectroscopy was used with the following parameters: repetition time of 2,000 ms, echo time of 30 ms, 128 excitations, 1,024 data points, spectral bandwidth of 1,200 Hz, and a total acquisition time of 4.24 min.. The integral of the carnosine-H2 peak (at ~8 parts per million (ppm)) was quantified relative to the water peak integral

(x1000) using the AMARES fitting algorithm in the jMRUI (version 3) software package (http://www.mrui.uab.cat/mrui). Muscle [carnosine] was expressed as a ratio relative to the water peak. To determine the reliability of this assessment, a separate cohort of 6 subjects visited the laboratory on consecutive days for the determination of baseline muscle carnosine content.

Concentrations of phosphorous-containing muscle metabolites and pH during exercise were determined as previously described (Vanhatalo et al. 2010). Intracellular pH was calculated using the chemical shift of the P_i spectra relative to the PCr peak (Taylor et al. 1983). The [PCr] and [P_i] were expressed as percentage change relative to resting baseline, which was assumed to represent 100%. Resting and end-exercise values of [PCr], [P_i] and pH were calculated over the last 30 s of the rest or exercise period. The ADP concentration was calculated as described by Kemp et al. (2001).

Statistical analyses

Independent samples t-tests were used to assess for differences between the groups prior to supplementation. Two-way repeated measures (RM) split-plot ANOVAs (supplement x time) were used to test for differences during the ramp incremental test ($\dot{V}O_2$max, GET, peak power output, blood pH and [La]) the repeated 3-min all-out test (CP, EP, W′, W>CP, peak power output and total work done (TWD), blood pH and [La]), the muscle metabolite concentrations during INC KEE and INT KEE, and muscle carnosine content. Two-way RM split-plot ANOVAs were used to test for differences in the blood pH and [La] across each time point during the ramp incremental test, and repeated 3-min all-out test. An

independent samples two-way ANOVA was used to explore the absolute change in CP or EP, W′ or W>CP, TWD and peak power output between bout 1 and bout 2 of the repeated 3-min all-out test. Post hoc analysis with Fisher's LSD was used to identify the origin of significant interaction effects. The coefficient of variation (CV %) was used to assess the day-to-day variability in determination of muscle carnosine content. Pearson's product-moment correlation coefficients were used to assess the relationships between muscle carnosine content and performance. All data are presented as mean ± SD. Statistical analysis was performed using SPSS version 22 (SPSS Inc., Chicago, Illinois, USA) with significance set as $P<0.05$.

Results

There were no significant differences in physical characteristics or physiological fitness measures at baseline between the PL and BA supplementation groups (Table 1).

Muscle carnosine content

No significant differences in muscle carnosine content were observed following 4 and 6 weeks of supplementation (Figure 1). The data from the two post supplementation carnosine scans were therefore averaged. BA supplementation did not significantly increase muscle carnosine content in the VM, VL, RF, or across the whole thigh muscle (Figure 1). The day-to-day variability (CV %) in the

assessment of baseline muscle carnosine content by ^{1}H-MRS was 15 ± 8% (range: 3% to 26%).

Indices of buffering capacity

No differences were observed in muscle [pH] between the supplementation groups (BA vs. PL), or within the supplementation groups (Pre vs. Post) at rest, at T_{lim}, or at any time-point during INC KEE or INT KEE (Figure 2). Metabolite changes during the INT KEE and INC KEE are displayed in Table 2. No differences were observed in muscle PCr responses during INT KEE following either supplementation strategy (P>0.05). There was a significant group x time interaction effect on mean blood pH during the ramp test (P<0.01) but no interaction effect on blood [La] (P>0.05) (Figure 3). The BA group had a greater blood pH post- (7.44 ± 0.12) relative to pre-supplementation (7.34 ± 0.05) (P<0.05), while the PL group had a lower blood pH post-supplementation (7.33 ± 0.08) compared to pre-supplementation (7.38 ± 0.09) (P<0.05) (Figure 3). There was no significant group x time interaction effect for blood pH (P>0.05) or blood [La] (P>0.05) when explored across each individual time-point during ramp incremental exercise. There were no significant interaction effects on blood pH or [La] during the repeated 3-min all-out test.

Power-duration relationship

The group mean power profiles for both bouts of the repeated 3-min all-out test are displayed in Figure 4. The $\dot{V}O_{2peak}$ values attained during bout 1 (Pre: 3.90

± 0.52 L.min^{-1}; Post: 3.75 ± 0.48 L.min^{-1}) and bout 2 (Pre: 3.97 ± 0.40 L.min^{-1}; Post: 3.79 ± 0.47 L.min^{-1}) of the repeated 3-min all test were not significantly different from $\dot{V}O_{2peak}$ (Pre: 3.82 ± 0.41 L.min^{-1}; Post: 3.68 ± 0.41 L.min^{-1}) achieved during the ramp incremental test (P>0.05). There were no significant interaction effects on CP or EP, W′ or W>CP, TWD and peak power output (P<0.05) (Table 3; Figure 5). No significant relationships were observed between the absolute or percentage change in whole muscle carnosine content or the blood [La] or blood [pH], and the absolute or percentage change in CP, W', TWD, or peak power output (all P>0.05).

Ramp incremental cycling test performance

There was a significant interaction effect on peak power output (P<0.05), which tended to increase (P=0.08) in the BA group from pre- to post-supplementation (Δ: 7 ± 10 W), with no change observed in the PL group (Δ: -5 ± 13 W) (Figure 6). PL and BA supplementation had no effect on the GET estimated in the ramp incremental test (P<0.05) (Figure 6).

Incremental knee-extension exercise performance

There was no significant interaction effect on INC KEE performance (P>0.05) (Figure 6). The T_{lim} during INC KEE was similar in the BA (594 ± 53 s) and PL (600 ± 62 s) groups at baseline and post-supplementation (BA: 594 ± 102 s vs. PL 625 ± 116 s) (P>0.05).

Intermittent knee-extension exercise performance

There was no significant interaction effect on INT KEE performance (P>0.05) (Figure 6). No baseline differences were observed for T_{lim} between the BA (401 ± 92 s) and PL (464 ± 155 s) groups (P>0.05). No significant *between* or *within* group differences were observed in T_{lim} for the BA (476 ± 172 s) or PL (482 ± 120 s) groups following supplementation (P>0.05).

Table 1 Group mean (± SD) baseline physical characteristics and physiological responses to the ramp incremental cycling test, bout 1 of the repeated 3-min all-out cycling test, and whole thigh muscle carnosine content for the placebo (PL) and β-alanine (BA) groups. The post supplementation whole thigh muscle carnosine values are presented as the mean of the two post supplementation scans. No significant differences were observed between the groups (P>0.05).

	PL	BA
Physical characteristics		
Mass (kg)	81.0 ± 18.1	77.1 ± 10.0
Age (y)	21 ± 3	23 ± 4
Physiological characteristics		
Ramp test $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	3.81 ± 0.52	3.64 ± 0.28
Ramp test peak power (W)	350 ± 49	344 ± 30
GET (W)	125 ± 15	115 ± 16
CP (W)	236 ± 47	232 ± 32
W′ (kJ)	17.8 ± 5.6	18.5 ± 1.8
Muscle carnosine (whole thigh) (A.U.)	55.15 ± 25.97	56.09 ± 19.63

Table 2 Muscle phosphocreatine ([PCr]) and inorganic phosphate ([Pi]) concentrations and pH at T_{lim} during incremental (INC KEE) and intermittent (INT KEE) knee extension exercise pre- and post-supplementation for the placebo (PL) and β-alanine (BA) groups. [PCr] and [Pi] are expressed relative to resting baseline.

	INC KEE				**INT KEE**			
	PL		**BA**		**PL**		**BA**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
End-exercise [PCr] (%)	53 ± 13	52 ± 9	52 ± 9	51 ± 10	49 ± 9	47 ± 12	40 ± 8	44 ± 10
End-exercise [Pi] (%)	442 ± 132	412 ± 73	418 ± 105	431 ± 91	422 ± 84	414 ± 110	510 ± 134	469 ± 58
Baseline pH	7.03 ± 0.02	7.04 ± 0.04	7.05 ± 0.03	7.05 ± 0.01	7.04 ± 0.02	7.04 ± 0.02	7.04 ± 0.01	7.05 ± 0.02
End-exercise pH	6.77 ± 0.20	6.82 ± 0.14	6.88 ± 0.07	6.84 ± 0.12	6.65 ± 0.23	6.57 ± 0.25	6.29 ± 0.15	6.33 ± 0.23
ΔpH	0.26 ± 0.20	0.22 ± 0.15	0.14 ± 0.03	0.19 ± 0.12	0.38 ± 0.24	0.46 ± 0.24	0.75 ± 0.16	0.72 ± 0.24

Table 3 Group mean (± SD) critical power (CP), W′, total work done (TWD), end test power (EP) and work done above CP (W>CP) determined during bout 1 and 2 of the 3-min all-out test pre- and post-supplementation. No significant interaction effects (group x time) were observed.

		PL		BA	
		Pre	**Post**	**Pre**	**Post**
Bout 1	**CP (W)**	236 ± 47	229 ± 52	232 ± 32	226 ± 38
	W′ (kJ)	17.7 ± 5.5	19.5 ± 3.6	18.5 ± 1.8	20.0 ± 1.6
	TWD (kJ)	60.3 ± 8.6	60.7 ± 9.1	60.2 ± 6.4	60.6 ± 7.4
	Peak power output (W)	858 ± 184	930 ± 162	960 ± 137	946 ± 100
Bout 2	**EP (W)**	226 ± 52	210 ± 51	222 ± 25	211 ± 31
	W>CP (kJ)	5.6 ± 2.7	4.0 ± 4.0	5.0 ± 2.4	4.3 ± 3.2
	TWD (kJ)	48.1 ± 9.6	45.2 ± 9.2	46.7 ± 5.3	44.9 ± 6.2
	Peak power output (W)	741 ± 190	732 ± 163	700 ± 74	741 ± 125

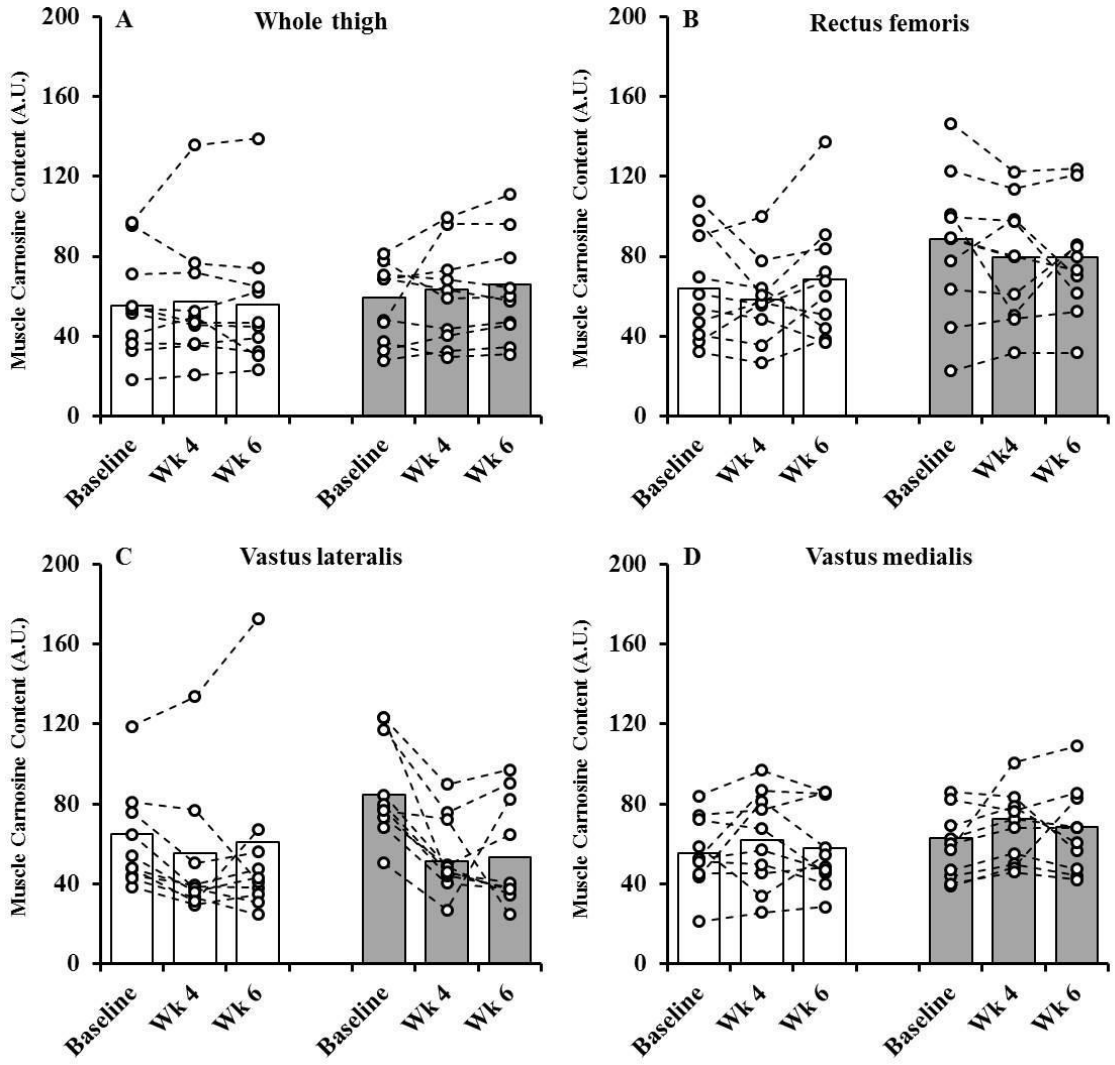

Figure 1 The muscle carnosine content for the placebo (white) and β-alanine (grey) groups for the whole quadriceps (panel A), rectus femoris (panel B), vastus lateralis (panel C) and vastus medialis (panel D). Dashed lines indicate individual responses in muscle carnosine content. For clarity, error bars were omitted.

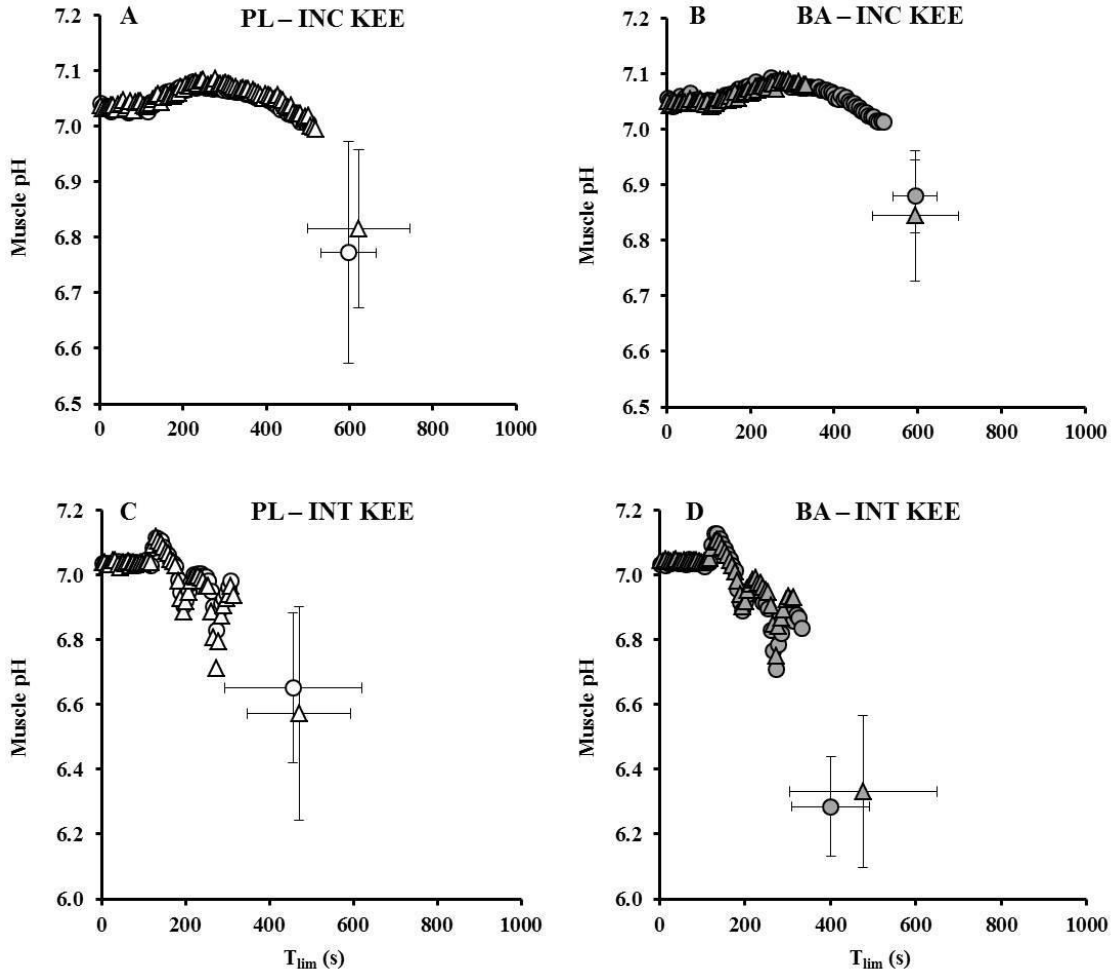

Figure 2 The placebo (PL; white) and β-alanine (BA; grey) group mean muscle pH response during incremental (INC KEE) (panels A and B) and intermittent (INT KEE) (panels C and D) knee-extension exercise pre- (circles) and post- (triangles) supplementation. Error bars represent SD. For clarity, error bars are omitted for all data points except T_{lim}.

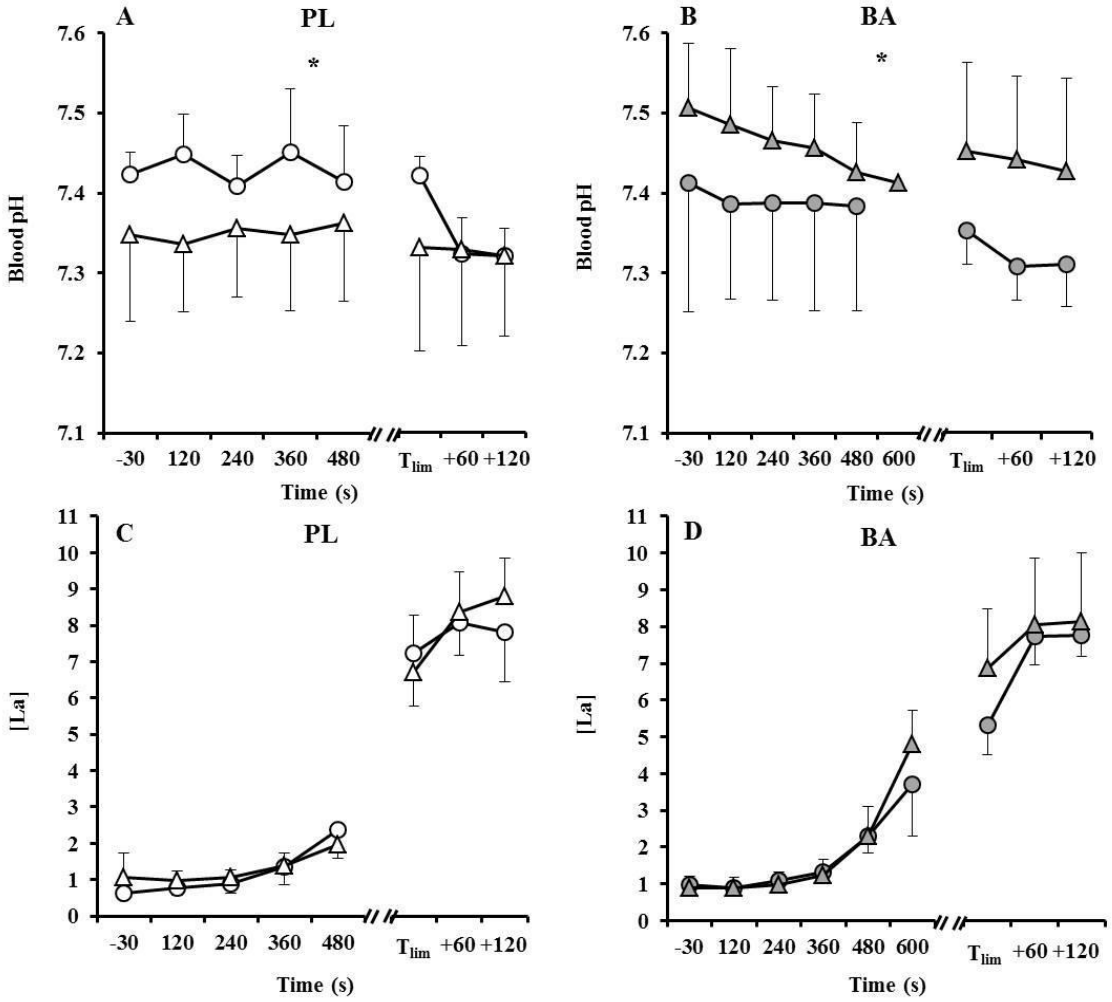

Figure 3 The placebo (white) and β-alanine (grey) group mean blood pH (panels A and B) and blood lactate ([La]) (panels C and D), during the pre- (circles) and post- (triangles) supplementation ramp incremental test. Significant interaction effects for the mean across all time-points are denoted by * (P<0.05). Error bars represent SD.

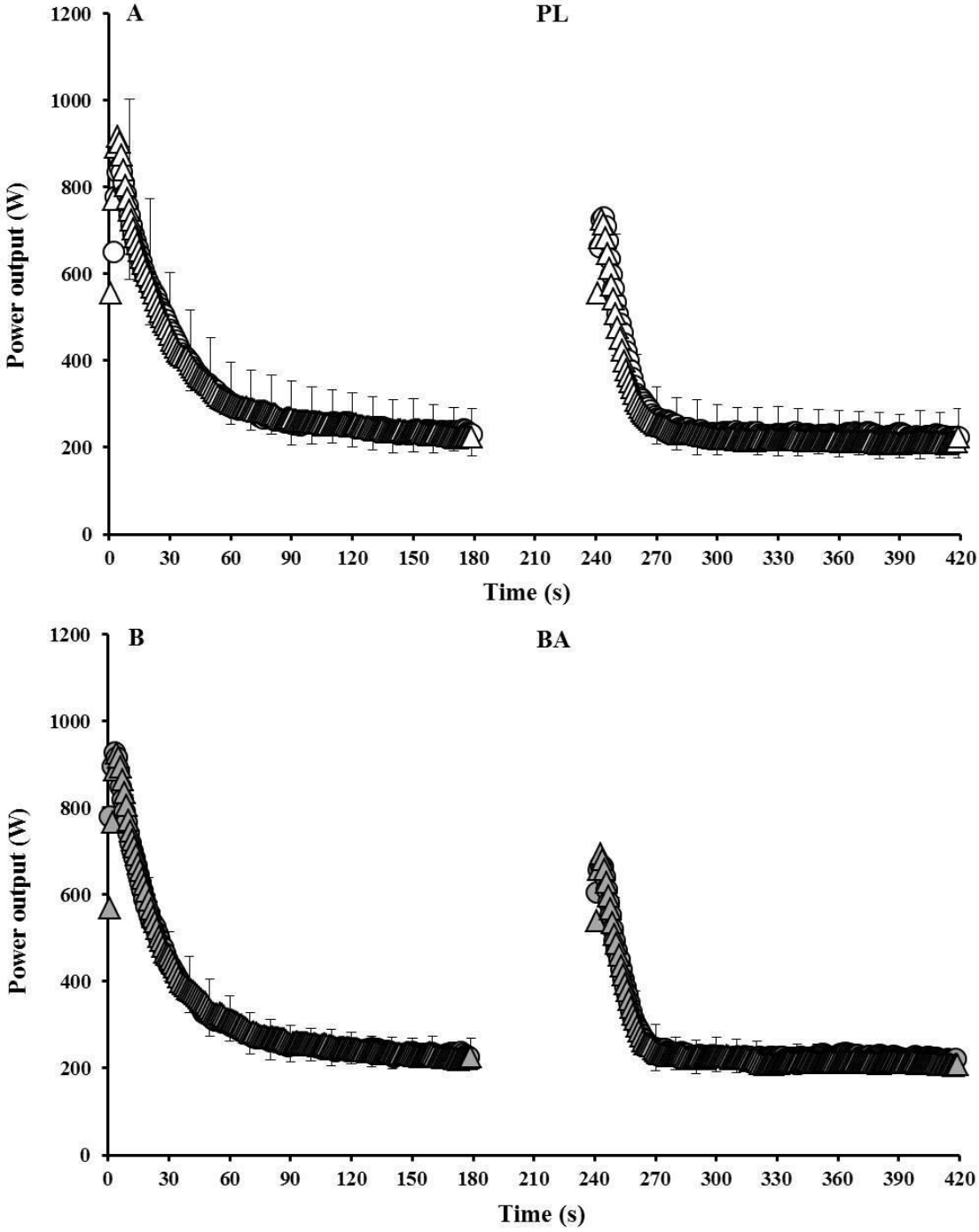

Figure 4 The group mean power profiles during the repeated 3-min all-out test for placebo (PL; panel A) and β-alanine (BA; panel B) groups pre- (circles) and post- (triangles) supplementation. The data are shown as second-by-second values. SD is presented in 10 s intervals and displayed by negative error bars for the pre-, and positive error bars for the post-supplementation trials.

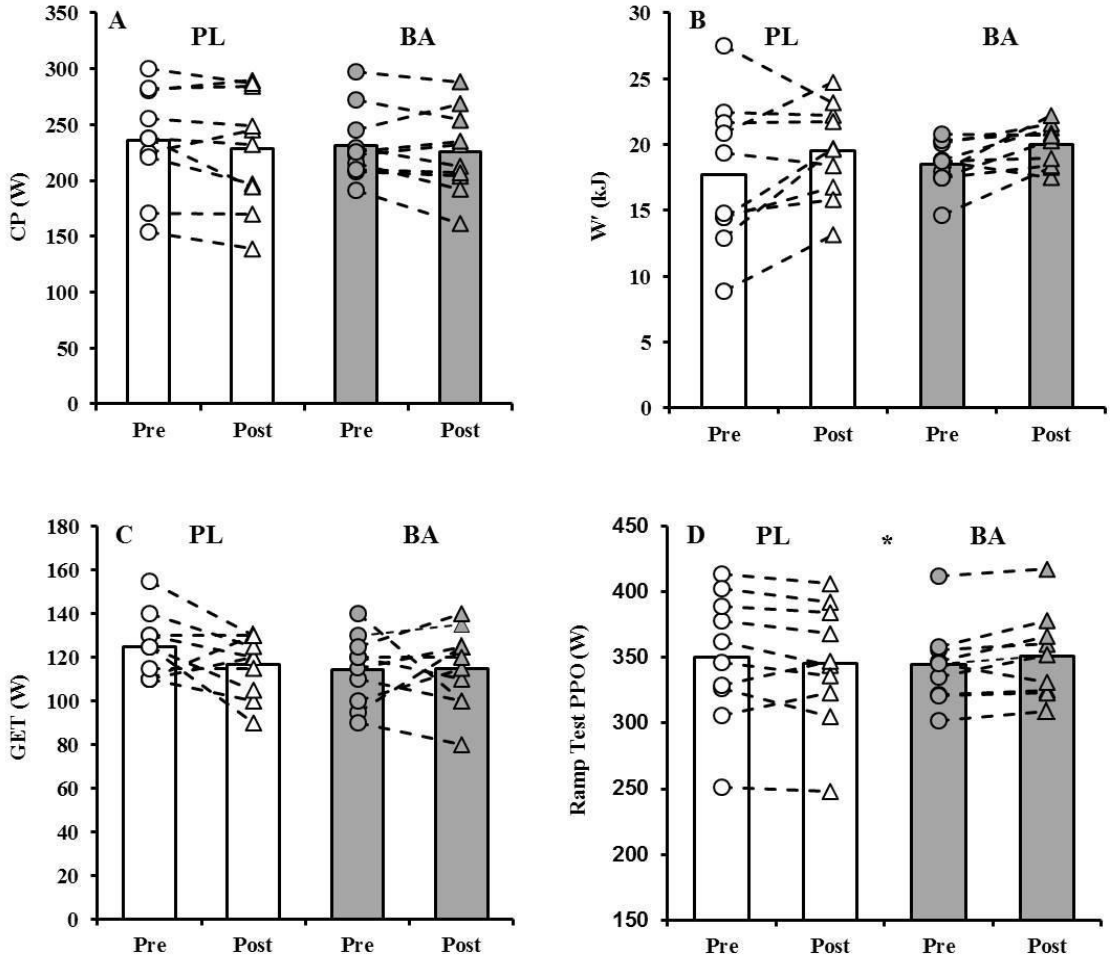

Figure 5 The placebo (PL; white) and β-alanine (BA; grey) group mean and individual critical power (CP; panel A) and W′ (panel B) determined during the 3-min all-out test, and power output at GET (panel C) and peak power output (PPO) (panel D) determined during the ramp incremental test. Pre- and post-supplementation data are presented as circles and triangles, respectively. Significant interaction effects are denoted by * (P<0.05).

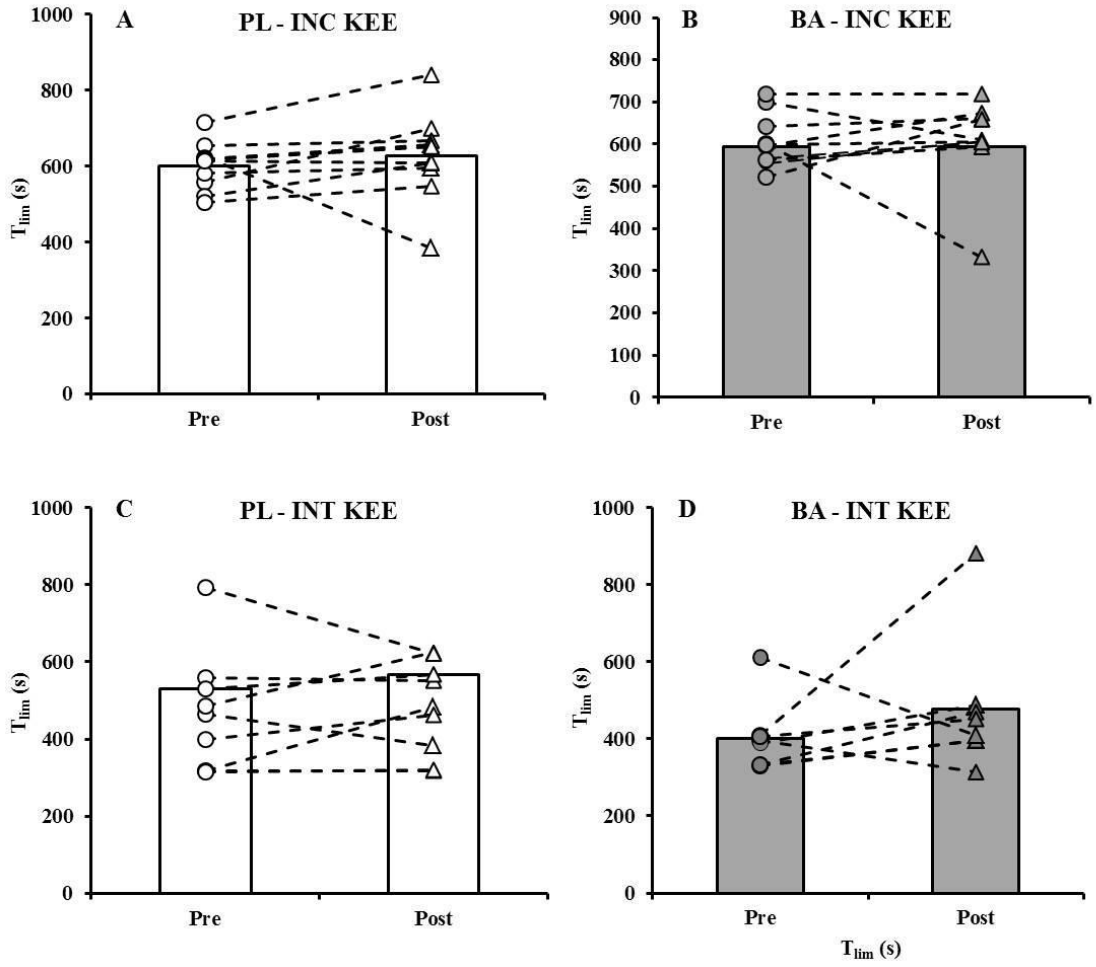

Figure 6 The placebo (PL; white) and β-alanine (BA; grey) group mean and individual T_{lim} during incremental (INC KEE) (panels A and B) and intermittent (INT KEE) (panels C and D) knee-extension exercise pre- (circles) and post- (triangles) supplementation.

Discussion

We employed a comprehensive exercise testing regimen, which included whole-body and single-legged exercise modalities and the use of ^{1}H- and ^{31}P-magnetic resonance spectroscopy to determine muscle carnosine content and muscle metabolic changes during exercise, respectively, to investigate the influence of BA supplementation on exercise performance. The principal findings of this study were that BA supplementation did not significantly increase muscle carnosine content or alter intramuscular pH or performance during incremental and intermittent knee-extension exercise, or alter the power-duration relationship. However, BA supplementation attenuated the fall in blood pH and improved performance by 2 ± 3% during ramp incremental cycle exercise. Although there was great inter-individual variability in muscle carnosine responses to BA supplementation, no relationships were observed between muscle carnosine content and blood pH or exercise performance.

The findings of the current study indicate that muscle carnosine content was not increased following 4 and 6 weeks of BA ingestion (6.4 g.d^{-1}). This is in contrast to previous studies that have assessed muscle carnosine content using ^{1}H-MRS and have shown that BA supplementation results in increased carnosine content in muscles of the calf (Baguet et al. 2009; 2010; Bex et al. 2014; Danaher et al. 2014; del Favero et al. 2012; Hoffman et al. 2015; Stegen et al. 2013; 2014), thigh (Harris et al. 2006), upper arm and shoulder (Bex et al. 2014). Whilst these studies have employed a variety of different supplementation strategies, and although baseline muscle carnosine content and loading rates appear to be muscle specific (Baguet et al. 2009; 2010; Bex et al. 2014; Danaher et al. 2014; Stegen et al.

2013; 2014), they have consistently reported ~45% increase in muscle carnosine following a total BA dose of ~181 g during the supplementation period. In the present study subjects had ingested a total of 179 g BA after 4 weeks and 269 g BA after 6 weeks. Given the large increases in muscle carnosine content typically reported, our method for determination of muscle carnosine content should have been sufficiently sensitive (CV ~15%) to detect changes between PL and BA groups.

A large inter-individual variability was observed in the relative change in muscle carnosine content in response to BA supplementation (-17% to 103%). This finding is consistent with Hill et al. (2007) and Baguet et al. (2009) who reported a range of -1% to 161%, and 2% to 69%, respectively. Whilst a greater baseline carnosine content has been observed in human type II muscle fibres (Suzuki et al. 2002; Hill et al. 2007), both type I and type II muscle phenotypes have been shown to respond equally well to BA supplementation (Baguet et al. 2009; Hill et al. 2007). Therefore, individual differences in muscle fibre type composition are unlikely to explain inter-individual variation in muscle carnosine response to BA supplementation. The training status of the muscle may influence the magnitude of increase in carnosine, with a greater relative increase reported in more highly exercised muscle groups following BA supplementation, possibly due to a greater delivery of BA to the muscle mediated by an increased blood flow and increased capillary density, and/or a contraction induced stimulation of TauT and PAT1 transporters which facilitate BA uptake into the myocyte (Bex et al. 2014). Given that the subjects in the current study were matched at baseline, were not trained in any particular sport, and that there was no association between muscle carnosine increase and parameters of fitness (i.e., $\dot{V}O_{2peak}$, GET, CP, W′), it is unlikely that

these factors can explain the inter-individual differences in muscle carnosine responses. It has been shown that L-histidine concentrations decrease by ~27% and ~31% relative to baseline following 12 and 23 days of 6 $g.d^{-1}$ BA supplementation, respectively (Blancquaert et al. in press). It is possible that reduced L-histidine bioavailability at baseline and as a consequence of BA supplementation may, in part, explain differences in muscle carnosine responses between subjects in the current study and previous research (Baguet et al. 2009; Bex et al. 2014; Harris et al. 2006; Stegen et al. 2013, 2014; Stellingwerf et al. 2012). Our results indicate that 4-6 wks of BA supplementation may not always result in a measurable increase in muscle carnosine content. The factors regulating muscle carnosine content require further research.

The ergogenic effect of BA supplementation is primarily attributed to its role in the synthesis of muscle carnosine, a potent intramuscular pH buffer (Bate-Smith et al. 1938). However, to our knowledge, no previous study has assessed muscle pH during exercise in humans following BA supplementation. In the current study, therefore, we used ^{31}P-MRS to assess muscle pH during single-legged knee-extension exercise. It was shown that BA supplementation did not result in changes in muscle pH during incremental or intermittent single-legged knee-extension exercise and no performance improvement was observed. In addition to intermittent, single-legged, knee-extension exercise, we used a repeated 3-min all-out cycling test to determine whether BA supplementation may improve recovery from intense whole-body exercise. The second bout of the 3-min all out test was used to determine the amount of work that could be performed above CP (W>CP) and thus served as an index of W′ recovery. In agreement with Saunders et al. (2012a), but in contrast with Saunders et al. (2012b), W′ recovery was not

improved, consistent with no change in pH or performance during the INT KEE and no effect on muscle carnosine content following BA supplementation.

There was a small but potentially meaningful change in blood pH and performance during the ramp incremental cycle exercise test following BA supplementation, despite no significant change in muscle carnosine content. The mean increase of ~2% in ramp test peak power in the BA group is similar in magnitude to coefficients of variation shown for ramp test peak power across 4-6 weeks of placebo supplementation (2.5 %, present study) and 4-5 weeks with no dietary or exercise intervention (1.1%; Lindsay et al. 1996). The increased blood pH and ramp test performance are in contrast with previous studies that have shown no significant improvements in incremental test performance following BA supplementation (Van Thienen et al. 2009; Zoeller et al. 2007), and the present finding that BA supplementation did not alter the power-duration relationship or blood pH during the 3-min all-out test. The CP and W′, estimated using a conventional protocol of multiple prediction trials, have been shown to be similarly unaffected by BA supplementation (Smith-Ryan et al. 2012). It can be estimated using prediction equation by Morton (1994), and assuming no change in CP, that a performance improvement of ~2% in the ramp incremental cycling test following BA would necessitate a ~12% increase in W', which is greater than the test-retest reliability CV% of W'(~9%; Vanhatalo, 2008a). The 3-min all-out test has been shown to be as sensitive as the conventional prediction trial protocol to detect changes in the power-duration relationship following training (Vanhatalo et al. 2008b) and acute normobaric hypoxia (Simpson et al. 2015), but no effects on CP or W' were shown following sodium bicarbonate ingestion, despite an elevated blood pH (Vanhatalo et al. 2010). Therefore, small ergogenic effects by dietary

interventions that may transiently enhance muscle (present study; Smith-Ryan et al. 2012) or blood (Vanhatalo et al. 2010) buffering capacity in some individuals do not result in significant shifts in the power-duration relationship.

The ergogenic effects of BA supplementation on high-intensity exercise performance are equivocal (meta-analysis see Hobson et al. 2012; Saunders et al. 2016), with improved exercise performance having been reported by some (examples, Hoffman et al. 2015; Saunders et al. 2012b;) but not all (examples, Ducker et al. 2013; Jagim et al. 2013; Saunders et al. 2012a; Sweeney et al. 2010) previous studies. The discrepancy between findings does not appear consistently linked to differences in supplementation regimes or criterion exercise test protocols. Ducker et al. (2013) and Jagim et al. (2013) observed no effect of BA supplementation despite following the same supplementation regime (6.4 $g.d^{-1}$ for 4 wks) as Hoffman et al. (2015). The repeated performance of short, sprint-intervals (Ducker et al. 2013; Saunders et al. 2012a; Sweeney et al. 2010) or the high-intensity constant work-rate protocols (Jagim et al. 2013), which resulted in task failure in ~1 min to ~2.5 min, would be expected to require substantial contributions from glycolysis and thus decrease muscle pH (Bogdanis et al. 1995; 1998; Vanhatalo et al. 2016). Test duration and supplementation strategy is therefore unlikely to explain the lack of differences observed during the knee-extension protocols, the power-duration relationship, and W>CP in the current study. Although interpretation of the studies reporting no significant improvements following BA supplementation is limited due to the omission of a carnosine assessment, it seems reasonable to suggest that BA supplementation may not have sufficiently increased muscle carnosine content in most individuals within these studies.

Limitations

It was not possible to assess muscle carnosine content on every laboratory visit due to a large number of tests. A temporal lag of 3-4 days between some performance test visits and muscle carnosine scans may have influenced the accuracy of correlations between muscle carnosine and exercise performance indices. Previous research has shown, however, that muscle carnosine content is relative stable and has a slow wash-out rate following BA supplementation (Stellingwerf et al. 2012). There was great variability in muscle carnosine in some individuals between 4 and 6 weeks of supplementation (Figure 1). It may, therefore, be speculated that muscle carnosine content may have been elevated in the majority of the subjects in the BA group at the time of the ramp incremental test but not the 3-min all-out tests. It should also be considered that there are substantial differences in the time courses of excitation-contraction coupling, glycolytic rate, muscle H^+ efflux, hyperaemia response and the rate of W' utilisation between 3-min all-out sprint and ramp incremental exercise protocols. To what extent these factors may have influenced the efficacy of BA supplementation was beyond the scope of the present investigation.

Conclusions

A variety of high-intensity exercise tests comprising different work-rate forcing functions and exercise modalities were used to assess ergogenic effects of BA supplementation. The findings of this study demonstrate that BA supplementation had a variable effect on muscle carnosine content and had no influence on

intramuscular pH during high-intensity incremental or intermittent knee-exercise exercise. The small increase in blood pH during whole-body ramp incremental exercise following BA supplementation resulted in a significantly greater increase in performance relative to the PL group, but this ergogenic effect was not sufficient to alter the power-duration relationship.

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Chapter 9 GENERAL DISCUSSION

This thesis has addressed important questions regarding the predictive validity, applicability, plasticity, and the mechanistic underpinnings of the power-duration relationship. The predictive validity of the CP derived from the 3-min all-out test had not been tested in the field, nor had its predictive capability been compared to other more traditional predictors of performance (i.e., $\dot{V}O_{2max}$, GET, RCP, ramp test peak power output). The omission of empirical evidence to support the predictive validity of the CP derived from the 3-min all-out test may have limited its use in the applied setting. Prior to the work conducted in this thesis, the CP and W′ were considered to remain constant irrespective of the work-rate forcing function applied or the pacing strategy utilised, although the notion of *constancy* is in conflict with a body of literature demonstrating an improved exercise performance following the implementation of an appropriate fast-start pacing strategy (Aisbett et al. 2009; Bailey et al. 2011; Bishop et al. 2002; Hettinga et al. 2009; Jones et al. 2008b; Wood et al. 2014). Furthermore, investigation into the mechanistic bases for fatigue during whole-body exercise above and below the CP was necessary to inform the design of interventions aiming to offset the fatigue process(es) and improve exercise tolerance and performance. The empirical studies that form this thesis were designed to address the gaps in knowledge relating to the CP model and to further our understanding of mechanisms that underpin exercise (in)tolerance. The new knowledge arising from this thesis extends our understanding of the factors that define the limits of human performance.

9.1 Summary of the main findings

9.1.1 The predictive validity of the CP derived from the 3-min all-out test

Chapter 4 sought to determine whether the CP derived from the laboratory based 3-min all-out test can be used to accurately predict performance in the field. Furthermore, this chapter compared the predictive validity of parameters derived from the 3-min all-out test (i.e., CP, W′ and total work done) and the ramp incremental test (i.e., $\dot{V}O_{2max}$, peak power output, power output at GET and RCP). It was demonstrated that 16.1-km road time-trial (TT) performance time was significantly correlated with CP (r = -0.83), total work done during the 3-min all-out test (r = -0.86), the ramp incremental test peak power (r = -0.75), and the RCP (r = -0.68).

In addition to the analyses presented in the experimental chapter, a stepwise multiple linear regression was performed to explore whether the parameters derived from the 3-min all-out test and the ramp incremental test could in combination provide a better prediction for 16.1-km TT performance. However, no variables combined to provide a better prediction of performance than the total work done during the 3-min all-out test. Given that the total work done is an index of both the CP and W′, the total work done was excluded and a subsequent stepwise multiple linear regression was performed, though no variables combined with CP to offer a stronger performance prediction.

These data provide evidence for the predictive validity and practical relevance of the laboratory based 3-min all-out test for performance in the field and complements previous research that has established the criterion validity (Vanhatalo et al. 2007), face validity (Burnley et al. 2006) and sensitivity

(Vanhatalo et al. 2008) of the 3-min all-out test parameters. The 3-min all-out test is therefore a useful addition to the tests employed by applied sport physiologists and/or coaches to rank athletes, monitor fitness and predict performance. Furthermore, these findings suggest that strategies that successfully increase the CP have the potential to improve exercise performance.

9.1.2 The work-rate forcing function influences the accuracy with which the CP and CP and W' can predict performance

Chapter 5 was designed to investigate the accuracy with which the CP and W' derived from the conventional CWR protocol can predict performance of a disparate work-rate forcing function i.e., ramp incremental exercise. It was demonstrated that the parameter estimates of the power-duration relationship derived from the CWR protocol significantly overestimated ramp incremental exercise performance. This overestimation was positively associated with the size of the W', suggesting that subjects could not access the W' during ramp incremental exercise to the same extent as was possible during CWR exercise. These data provided empirical evidence to demonstrate that the parameters of the power-duration relationship are not constants and the work-rate forcing function, and potentially the pacing strategy utilized, alter the shape of the power-duration relationship.

9.1.3 Speeding of overall $\dot{V}O_2$ kinetics during self-paced versus constant work-rate exercise is associated with increased critical power

Based on the findings of Chapter 5, we postulated that the inability to fully access the CWR W′ during the ramp incremental exercise was due to differences in $\dot{V}O_2$ kinetics between the CWR and ramp incremental protocols. Chapter 6, therefore, assessed the power-duration relationship and $\dot{V}O_2$ kinetics derived from CWR prediction trials and corresponding work-matched self-paced TT prediction trials. The CP estimate was ~7% greater and the W′ was unchanged when derived from self-paced relative to CWR prediction trials. The increase in CP was significantly related to a faster $\dot{V}O_2$ mean response time (MRT). These data provide further empirical evidence which demonstrates that the CP and W′ are not constants and their ability to change is linked to $\dot{V}O_2$ kinetics. Furthermore, these data highlight the importance for "matching" the distribution of power output, thus "matching" $\dot{V}O_2$ kinetics, between the prediction trials and the criterion performance task to ensure accurate determination of the power-duration relationship. These data provide insight into the mechanism(s) by which a fast-start pacing strategy may be ergogenic to performance (Burnley and Jones, 2007), and further support the mechanistic link between parameters of $\dot{V}O_2$ kinetics and the parameter estimates of the power-duration relationship (Murgatroyd et al. 2011; Vanhatalo et al. 2011b).

9.1.4 The mechanistic bases for fatigue during exercise above and below CP during whole-body exercise

Having determined the predictive validity (Chapter 4), the applicability and plasticity (Chapter 5 and 6) of the power-duration relationship it was important to further understand the mechanistic bases for fatigue during whole-body exercise above- and below-CP. Chapter 7 was the first study to explore the muscle metabolic (as assessed by the percutaneous needle biopsy technique), neuromuscular (femoral nerve electrical stimulation), $\dot{V}O_2$, and venous blood profiles simultaneously during whole-body exercise within each intensity domain (i.e., severe, heavy, moderate) and therefore represents a thorough investigation of the mechanisms implicated in whole-body fatigue. Severe-intensity (>CP) exercise was associated with the attainment of the same "critical" muscle metabolic milieu at T_{lim} (i.e., high [lactate] and low pH, and low [PCr] [ATP]), and despite an increased neural drive and unchanged muscle membrane excitability, could only be maintained for a relatively brief period of time (<15 min). Furthermore, the same $\dot{V}O_{2max}$, [BLa], and plasma [K^+] were achieved at T_{lim}. In contrast, during exercise <CP the "critical" muscle metabolic milieu or $\dot{V}O_{2max}$ was not attained, although exercise was sustained for a much longer duration and continued until T_{lim}. However, exercise <CP was associated with large reductions in glycogen and large decreases in muscle membrane excitability. The results of this study indicate that the muscle metabolic and neuromuscular responses can be understood in terms of the CP concept. This provides further evidence to support the importance of the CP concept in determining the degree of muscle metabolic perturbation, the development of neuromuscular fatigue and the capacity to perform exercise.

9.1.5 The influence of pH manipulation on the power-duration relationship

It was demonstrated in Chapter 7 that a consistent “critical” muscle pH was attained at T_{lim} during severe-intensity exercise. It was therefore hypothesized that enhanced muscle buffering capacity would attenuate the decrease in muscle pH thus delay the attainment of a “critical” muscle metabolic milieu associated with task failure during severe intensity exercise (Chapter 7). An improved exercise tolerance was expected to be reflected by an increased W′. To investigate the influence of pH manipulation on the power-duration relationship subjects consumed β-alanine (BA), which is considered to be the rate-limiting precursor to the synthesis of muscle carnosine, a potent intramuscular buffer. The muscle carnosine content did not increase significantly and showed great inter-individual variability in responses to BA supplementation. BA did not alter intramuscular pH or performance during intermittent or incremental knee-extension exercise. Although BA attenuated the fall in blood pH and improved performance during ramp incremental cycle exercise, no changes were observed in the power-duration relationship. The comprehensive battery of tests used in this investigation suggests that small ergogenic effects achieved through BA supplementation are not sufficient to significantly alter the power-duration relationship.

9.2 Integration of findings

9.2.1 Novel insights into the validity, applicability and plasticity of the power-duration relationship

Knowledge of the power-duration relationship permits accurate estimation of the amount of work that can be performed above CP prior to exhaustion (Chidnok et

al. 2013b; Murgatroyd et al. 2011; Vanhatalo et al. 2011). The predictive validity and applicability of the laboratory-based 3-min all-out test to determine 16.1-km cycling TT performance in the field was tested in Chapter 4. The 3-min all-out test CP was shown to be a powerful predictor of field performance, capable of discriminating between riders finishing position during a 16.1-km road TT. These data are consistent with earlier findings that the CP or critical speed derived from modelling of multiple, exhaustive prediction trials is correlated with 17-km and 40-km TT cycling events (Smith et al. 1999) and 10-km running performance (Kolbe et al. 1995). The 3-min all-out test can be recommended to coaches, athletes and sports science practitioners as a time efficient means to monitor and predict athletic performance.

One of the tenets of the CP model is that the amount of work completed above CP before task failure remains constant irrespective of the rate of W' expenditure (Monod & Scherrer 1965; Morton, 2006; Poole et al. 1988), suggesting that the power-duration relationship can be used to predict performance during exercise of different work-rate forcing functions as long as work-rate remains above CP. Work completed above CP before task failure during CWR severe-intensity exercise has been shown to equal the W′ derived from a CWR prediction trial protocol by some (Fukuba et al. 2003; Murgatroyd et al. 2011) but not all previous studies (Dekerle et al. 2015). Furthermore, the W' estimated in a 3-min all-out test has been shown to provide a close estimation of work done >CP during work-matched CWR exercise resulting in task failure in ~3 min (Chidnok et al. 2013; Vanhatalo et al. 2011b). It should be noted, however, that defining the W' as the work done >CP during exercise of a different work-rate forcing function assumes that the CP itself remains unchanged.

The findings of Chapters 5 and 6 provide evidence to refute that the CP and W' are constants instead demonstrating their plasticity and sensitivity to changes in work-rate. The main findings of Chapter 5 show that the power-duration relationship derived from a series of CWR prediction trials overestimates actual ramp incremental performance and this overestimation is inversely associated with the magnitude of W′; likely due to the absence of the $\dot{V}O_2$ slow component during ramp incremental exercise (Wilcox et al. 2016) and its prominence during CWR exercise (Burnley and Jones, 2009; Poole et al. 1988). This finding supports the limited evidence that has shown that the accessible portion of W′ is determined by the rate of its utilisation (Chidnok et al. 2013b) and the link between the parameter estimates of the power-duration relationship and $\dot{V}O_2$ kinetics (Murgatroyd et al. 2011; Vanhatalo et al. 2011b). Similarly, the findings of Chapter 6 demonstrate that a self-paced fast-start strategy increases CP via speeded $\dot{V}O_2$ kinetics. Collectively, these studies demonstrate the plasticity of the power-duration relationship to changes in work-rate and its relationship with $\dot{V}O_2$ kinetics.

9.2.2 The mechanistic bases for fatigue and performance and its relationship with CP and W′

Conventionally, the assessment of in-vivo fatigue mechanisms have been conducted using twitch-interpolation and potentiated twitch techniques (Gandevia, 2001). However, it is not possible to utilize these techniques *during* whole-body exercise. Instead, previous researchers have evoked fatigue during whole-body exercise and assessed fatigue during single-legged knee-extension exercise (e.g., Martin et al. 2010; Morris et al. 2012; Thomas et al. 2015). The experimental setup

utilised in Chapter 7, which permitted the assessment of neuromuscular fatigue (via femoral nerve electrical stimulation) and muscle metabolic perturbation in a single whole-body exercise model, therefore, constitutes a major advance in our understanding of fatigue. Using this approach, it was revealed that the metabolic and neuromuscular determinants of fatigue during whole-body exercise differ according to whether they are performed above- or below-CP. Notably, a consistent metabolic milieu was achieved and neuromuscular fatigue developed to a similar extent at T_{lim} during exercise above-CP, suggesting that task failure above-CP is inherently linked to muscle metabolic perturbation and neuromuscular function. Chapter 8 aimed to investigate the influence of attenuating the decrease in muscle pH on the power-duration relationship. However, despite rigorous experimental control the supplementation regimen was ineffective in increasing muscle carnosine content and thus muscle buffering capacity. The potential influence of enhanced muscle buffering capacity on the power-duration relationship, therefore, remains unknown.

9.2.3 Summary

The findings of this thesis, particularly the plasticity of the parameter estimates of the power-duration relationship, should not be interpreted as providing evidence to invalidate the CP model. Rather, that the CP model is sensitive to subtle manipulations in the work-rate forcing function or pacing strategy utilised, and that the plasticity of the CP and W′ can be understood with respect to underlying physiological responses strengthens the validity of the CP model. The CP model describes the limits of human performance and provides *the* framework to

understand and investigate factors the shape the hyperbolic relationship between power and time.

Prior to the work presented in this thesis, exercise tolerance/performance above-CP was understood to be determined by the interaction of three physiological parameters, specifically, $\dot{V}O_2$ kinetics, $\dot{V}O_2$max, and W' (Burnley and Jones, 2007; Jones et al. 2011). The $\dot{V}O_{2max}$ dictates the extent to which the $\dot{V}O_2$ slow component and W′ can develop and the rate with which the $\dot{V}O_2$ slow component and W′ develop determines how rapidly $\dot{V}O_{2max}$ will be attained (Burnley and Jones, 2007; Jones et al. 2011). Therefore, an intervention that increases the CP with no change in $\dot{V}O_{2max}$ would delay the development of the $\dot{V}O_2$ slow component and the utilisation of the W′, thus increasing exercise tolerance. Consistent with this notion, the CP and W′ have been associated with parameters of $\dot{V}O_2$ kinetics (Murgatroyd et al. 2011; Vanhatalo et al. 2011b). Specifically, a faster phase II τ is related to a greater CP and a larger $\dot{V}O_2$ slow component is associated with an increased W′ (Murgatroyd et al. 2011). The interactive influence and the associations between the power-duration parameters and $\dot{V}O_2$ kinetics in determining exercise performance is supported by the findings of Chapter 6. It was shown that the speeding of $\dot{V}O_2$ kinetics during self-paced vs. CWR exercise increased the CP and improved exercise performance. The fast-start strategy implemented during the self-paced time trials speeded the $\dot{V}O_2$ MRT which increased the CP without altering the $\dot{V}O_{2max}$. When considered with the findings of Chapter 6, the increased CP reduced the reliance on the finite W′ and attenuated the development of the $\dot{V}O_2$ slow component thus delaying the attainment of a critical level of muscle metabolic perturbation (Chapter 7). The

increase in “sustainable power output” (i.e., CP) permitted exercise performance to be enhanced during self-paced relative to CWR exercise.

9.3 Implications for the practical application of the power-duration relationship: recommendations for best practice

This thesis has highlighted that determination of the power-duration relationship yields great insight(s) into muscle metabolic and neuromuscular responses to exercise (Chapter 7) and provides a useful tool to monitor and predict field based performance (Chapter 4). However, it is also shown that the power-duration relationship is not constant and is sensitive to changes in $\dot{V}O_2$ kinetics, which can be influenced by the work-rate forcing function and/or pacing strategy utilised (Chapters 5 and 6). Based on the findings presented in this thesis, the following recommendations are proposed:

For the applied practitioner;

- The findings of Chapter 4 demonstrate that a test lasting only 3-min can provide parameters that offer great insight(s) into the performance capabilities of an athlete, and should therefore be considered as a useful test to rank athletes, monitor fitness and predict performance. Specifically, it was demonstrated that the CP was a stronger predictor of 16.1-km TT performance that $\dot{V}O_2$max, ramp test peak power output, and power output at the GET (or the lactate threshold) and RCP. However, it should be noted that the regression equation derived from the lab measured variables and the field performance are course specific and the predicted time to completion is not transferable.

- BA supplementation has gained popularity for its purported ability to enhance muscle buffering capacity and improve high-intensity exercise performance. Whilst it is enticing to 'pop a few pills' to improve exercise performance, it should be noted that the evidence to support the ergogenicity of BA is very weak. Indeed, the most comprehensive and controlled study to-date, which included performance tests and direct assessment of muscle buffering capacity found no changes in either (Chapter 9). BA supplementation is therefore not recommended.

For the applied practitioner and researcher;

- It is important to carefully "match" the pacing conditions of the prediction trials, to the criterion performance task (i.e., the exercise which you are attempting to predict from the prediction trials). Failure to match the prediction trials to the performance task will influence the CP and W′ (Chapters 5 and 6), which are sensitive to subtle differences in pacing (Chapter 6), and will impair the predictive validity of the CP model.
- The CP is an important physiological threshold that differentiates between exercise intensities that are "sustainable" from those that are "not sustainable". The muscle metabolic and neuromuscular responses vary dependent on the position of the work-rate relative to CP (Chapter 7). It is therefore of paramount importance, to ensure an appropriate "physiological strain", that CP is considered when normalising exercise intensity.

9.4 Experimental limitations

A key focus of this thesis was to elucidate the mechanistic underpinnings of fatigue during whole-body exercise above and below CP. In Chapter 7, changes in the M-wave amplitude and M-wave area, and changes in neural drive (EMG RMS amplitude/M-wave amplitude) were quantified to broadly assess the contribution of peripheral and central fatigue, respectively. Whilst it would have been advantageous to quantify peripheral and central fatigue using interpolation and potentiated twitch techniques, the equipment needed to perform these assessments were not available. Moreover, it is not possible to utilise these techniques *during* whole-body exercise. Instead, previous researchers have evoked fatigue during whole-body exercise and assessed this fatigue during single-legged knee-extension exercise (e.g., Martin et al. 2010; Morris et al. 2012; Thomas et al. 2015). It should be recognised that the experimental approach utilised in Chapter 7 permitted the assessment of task-specific fatigue *during* whole-body exercise. The relationship between the neuromuscular fatigue assessment via twitch-interpolation and potentiated twitch techniques, and the method applied in the current thesis needs to be determined.

Chapter 8 aimed to explore the effect of muscle pH on the power-duration relationship via manipulation of the muscle buffering capacity. However, following an appropriate BA supplementation regimen, we observed no significant changes in muscle carnosine content, thus no improvement in muscle buffering capacity. As discussed, given the magnitude of change reported in the literature (see Figure 2.2) and the CV% in the assessment technique, it is unlikely that the ^{31}P-MRS method utilised in this thesis lacked sensitivity to detect meaningful changes in muscle carnosine content. Whilst it is not possible to confirm strict adherence to

the supplementation strategy, it is unlikely that subjects who willingly volunteered to participate in such an arduous and challenging experimental protocol would systematically fail to follow the supplementation regimen. Moreover, many of these subjects had previously participated in supplementation-based research at the University of Exeter and had closely followed the recommended supplementation protocol (confirmed via elevated plasma nitrate and nitrite values). Although subjects were instructed to continue their normal training routine and dietary habits, neither training nor diet was recorded during Chapter 8. All subjects verbally confirmed that they had maintained their normal levels of physical activity and that they had not consciously altered their diet. However, it remains possible that subtle alterations in meal composition between test visits and differences in training throughout the study may have influenced performance independent of any change in muscle carnosine content. Finally, it would have been advantageous to have included assessment of muscle pH during whole-body exercise using the muscle biopsy technique. Inclusion of this technique may have yielded further insight into the mechanistic bases for performance improvement in the ramp incremental test following BA supplementation.

Throughout this thesis the conventional (i.e., CWR) protocol and the 3-min all-out test protocol were used interchangeably to determine the parameters of the power-duration relationship. The 3-min all-out test has been shown to produce similar estimates to the conventional protocol with a standard error of estimate of 6 W and 2.8 kJ for the CP and W′, respectively (Vanhatalo et al. 2007). Furthermore, Simpson et al. (2015) also reported close agreement between protocols with a typical error of 10 ± 8 W and 2.5 ± 1.3 kJ for CP and W′, respectively. Similar vales have also been reported between the protocols in

hypoxia (Simpson et al. 2015). Given the close-agreement in the CP and W′ derived between the conventional and all-out protocols it was not considered necessary to establish the power-duration relationship in a single study using both techniques. The protocol used to establish the power-duration relationship was therefore carefully considered and dependent on the research question being addressed within each experimental chapter.

9.5 Future Directions

9.4.1 Alternative method to assess the power-duration relationship

Conventionally, the power duration relationship was derived from a series of CWR prediction trials (typically 3-5), performed on separate days, until T_{lim}. This arduous, experimentally taxing protocol may limit the design of research investigations and interfere with the normal training or competition of an athlete, restricting the assessment of the power-duration relationship. More recently, Burnley and colleagues (2006) developed a protocol which permits the estimation of the power-duration relationship in a 3-min all-out sprint test. This test requires the completion of a ramp incremental exercise test to normalise the fixed resistance, and requires expensive equipment typically only available to University researchers or elite athletes. Moreover, this test requires an extreme and sustained maximal effort that may not be favoured by the athlete. Given that the relationship between power and time is hyperbolic, such that power decreases as a function of time following the attainment of the peak power output (~6 s), it seems reasonable to suggest that a mathematical exponent could be used to predict CP and W' from a shorter maximal all-out sprint. This suggested method

may reduce the duration of the maximal effort required providing an easier means for monitoring in-season changes in fitness.

9.5.2 Mechanistic bases for fatigue

The findings of this thesis have extended our understanding of the muscle metabolic and neuromuscular responses evident during whole-body exercise above and below CP. For example, irrespective of the severe-intensity (>CP) work rate, a critical metabolic milieu was evident at T_{lim} (i.e., high [lactate] and pH, and low [PCr] and [ATP]). Manipulation of the factors associated with fatigue above CP may provide a means to prolong/improve severe-intensity exercise tolerance/performance. In addition to exercise training interventions, priming, pacing (Chapters 5 and 6), and nutritional supplementation have been shown to improve exercise tolerance and performance >CP. Research should continue to explore further strategies that can be implemented to attenuate the attainment of the critical metabolic milieu and thus offset the fatigue process.

The findings of Chapter 7 suggest that the attainment of a low muscle pH, in concert with other metabolic and systemic changes, results in the cessation of exercise. Chapter 8 sought to improve intramuscular buffering capacity to attenuate the decline in pH and determine whether enhanced buffering capacity may improve exercise performance and whether this improvement was associated with an increased W′. However, the role of pH in muscle fatigue and its influence on the power-duration relationship could also be investigated by increasing muscle acidosis prior to exercise via ingestion of ammonium chloride. In addition to looking at interventions to enhance exercise performance, future research

should also look to impair exercise capacity in healthy humans. This approach may provide further insight into the factors that are most important to exercise intolerance.

During exercise <CP, exhaustion did not coincide with the attainment of the critical metabolic milieu observed during severe-intensity exercise, but instead was associated with decreased muscle glycogen, and decreased muscle excitability. Muscle glycogen has been implicated in sarcoplasmic reticulum Ca^{2+} handling (Gejl et al. 2014), such that glycogen depletion is associated with impaired Ca^{2+} release. However, it remains unclear how impaired Ca^{2+} release may impact the power-duration relationship; whether it will decrease the CP, W′, or both. Furthermore, it remains unclear whether adherence to a diet high in carbohydrate and low in fat or vice-versa may alter Ca^{2+} release, muscle excitability, and the power-duration relationship.

9.5 Conclusion

Severe-intensity (>CP) exercise performance is characterised by the power-duration relationship. The findings of this thesis extend our understanding of the validity, plasticity and applicability of the power-duration relationship and provide greater insights into its mechanistic underpinnings during whole-body exercise. The results presented in this thesis support that severe-intensity exercise performance is dictated by the interplay between $\dot{V}O_2$ kinetics, the parameters of the power-duration relationship, and the $\dot{V}O_{2max}$.

It was shown that despite considerably different work-rate forcing functions and exercise durations, the CP derived from the laboratory-based 3-min all-out test was a powerful predictor of 16.1-km road TT performance (Chapter 4),

emphasising its importance to coaches and athletes. The predictive capability and thus applicability of the power-duration relationship was further explored in Chapters 5 and 6. Chapter 5 demonstrated that the ramp incremental exercise performance could not be accurately predicted from the power-duration relationship derived from CWR prediction trials, presumably due to differences in the work-rate forcing function and thus $\dot{V}O_2$ kinetics between the ramp incremental and CWR protocols. The relationship between $\dot{V}O_2$ kinetics and the power-duration relationship was explored further in Chapter 6. Here, the CP and W′ derived from a series of self-paced TT's resulted in a greater CP and no significant changes in W′, relative to the power-duration relationship established from a series of CWR prediction trials. The increase in CP observed during self-paced exercise was associated with the utilisation of a fast-start pacing strategy and a speeding in $\dot{V}O_2$ kinetics relative to the CWR prediction trials. Collectively, these studies provide empirical support for the association between $\dot{V}O_2$ kinetics and the power-duration relationship. That is, if an intervention speeds $\dot{V}O_2$ kinetics we can expect a concomitant increase in CP. The increased CP permits a higher power output to be sustained prior to the utilisation of W′ and the concomitant accumulation of fatigue related metabolites that ultimately result in exercise cessation, as evidenced by the consistent critically low muscle [PCr], [ATP] and pH, and high [lactate] at T_{lim} during whole-body severe-intensity exercise, irrespective of its duration, despite an increased neural drive to the muscle (Chapter 7). Furthermore, these findings highlight an important practical consideration for the accurate determination of the power-duration relationship; specifically, the importance of matching the work-rate forcing function and thus $\dot{V}O_2$ kinetics of the prediction trials with that of the performance.

In summary, the findings of this thesis demonstrate the predictive validity of the laboratory based 3-min all-out test CP to field-based performance, and extends our understanding of the plasticity and applicability of the power-duration relationship. Furthermore, this thesis provides novel insights into the underlying mechanisms that characterise the power-duration relationship during whole-body exercise which explains the plasticity and thus applicability of the power-duration relationship. Collectively, the findings of this thesis represent advancement in the methodological rigour and practical application of the CP model and contribute to a deeper understanding of its mechanistic underpinnings during whole-body exercise.

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Certificate of ethical approval for Chapter 4

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road
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United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 2011/63

Title: Relationship between laboratory measurement of critical power and time trial performance on the road

Applicants: Jacob Durrant (UG Student)

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until March 2012

Signature: 

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Statement of ethical approval for Chapter 5

Chapter 5 involved the reanalysis of data dervied from experiments that had ethical approval from Aberystwyth University and the University of Exeter and that had already been made available in the public domain.

Certificate of ethical approval for Chapter 6

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

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University of Exeter
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United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 2012/159

Title: Determining critical power: time-to-exhaustion vs. time trial protocols

Applicants: Matthew Black, Dr Anni Vanhatalo, Prof Andy Jones, Dr Stephen Bailey

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until January 2012

Signature:

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Certificate of ethical approval for Chapter 7

College of Life and Environmental Sciences
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United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 2013/438

Title: Muscle metabolic responses and fatigue mechanisms during moderate-, heavy-, and severe-intensity cycle exercise

Applicants: Matthew Black, Dr Anni Vanhatalo, Prof Joanna Bowtell, Dr Magni Mohr, Prof Andy Jones

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until August 2013

Signature:

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Certificate of ethical approval for Chapter 8

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

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United Kingdom

Certificate of Ethical Approval

Title: Effect of pH on the power-duration relationship

Applicants: Matthew Black

The proposal was reviewed by a Representative on the Committee.

Decision: This proposal has been approved until July 2014.

Signature:  Date: 08/11/13

Name/Title of Ethics Committee Reviewer: Dr Mark Wilson

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Documentation to certify attendance of Valid Informed Consent in Clinical Research training course.