University of Brighton

Neurophysiological Responses to Fatiguing Exercise above Critical Power in Healthy Humans

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ABSTRACT

Substantial levels of peripheral fatigue, i.e. reduction in the force-generating capacity induced by alterations at or distal to the neuromuscular junction, have been evidenced within the severe intensity domain. The primary aim of this thesis was to better understand the mechanism(s) of these exercise-induced neurophysiological alterations following severe intensity cycling exercise applying the critical power (CP) concept. The CP concept mathematically defines the relationship between power output and the tolerable duration of severe intensity exercise using a hyperbolic relationship with CP and W', the power asymptote and the curvature constant, respectively.

Study 1 examined the between-day reliability of key neuromuscular measures in the knee extensor muscles at rest and following severe intensity cycling exercise using femoral nerve stimulation. The reliability of key neuromuscular measures in the fresh and fatigued state were acceptable, with slightly greater variation in most measures following severe intensity cycling exercise. To our knowledge, Study 2 was the first study to demonstrate that neuromuscular fatigue observed after full depletion of W' is of similar magnitude whether supra-CP cycling exercise is performed close to the lower boundary for 12 min of exercise (P-12) or close to the upper boundary for 3 min of exercise (P-3) of the severe intensity domain. Further, exploratory analysis showed that smaller changes in the muscular force-generating capacity are seen in individuals with greater aerobic capacities (CP, $\dot{V}O_{2peak}$) for P-12, but greater changes in individuals with greater anaerobic capacities (W') for P-3. In Study 3, neuromuscular recovery following P-3 and P-12 was investigated. Both, central and peripheral alterations remained below baseline after 30 min of rest irrespective of exercise intensity and duration above CP. Based on the findings from Study 2, Study 4 aimed to increase W' and examine its effect on neuromuscular fatigue following severe intensity cycling. Creatine supplementation increased severe intensity cycling performance and thus, W'. However, despite the positive relationship between W' and neuromuscular fatigue found for P-3 in Study 2, the greater W' due to creatine supplementation did not lead to a greater level of neuromuscular fatigue at task failure in Study 4.

In conclusion, these findings suggest that similar levels of neuromuscular fatigue and a similar time course of neuromuscular recovery can be observed following severe intensity cycling exercise when W' is fully depleted, irrespective of the exercise intensity selected within the severe intensity domain. Further, the magnitude of neuromuscular fatigue at task failure does not depend on the size of W'.

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LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine triphosphate
Ca^{2+}	calcium ions
C_aO_2	arterial oxygen content
CMEP	cervicomedullary motor evoked potential
CNS	central nervous system
CO_2	carbon dioxide
CP	critical power
CT	contraction time
CV	coefficient of variation
EMG	surface electromyography
EP	end power
ERT	estimated resting twitch
F_iO_2	fraction of inspired oxygen
GET	gas exchange threshold
H^+	hydrogen ions
HR	heart rate
HR_{max}	maximum heart rate
HRT	one-half relaxation time
ICC	intraclass correlation
ITT	interpolated twitch technique
K^+	potassium ions
La^-	lactate
LoA	limit of agreement
LT	lactate threshold
MAOD	maximal accumulated oxygen deficit
MEP	motor evoked potential
MLSS	maximal lactate steady state
MRFD	maximal rate of force development
MRR	maximal rate of relaxation
MVC	maximal voluntary contraction (isometric)
M-wave	compound muscle action potential
n	sample size
Na^+	sodium ions

NMFA	neuromuscular fatigue assessment
O_2	oxygen
PCr	phosphocreatine
P_i	inorganic phosphate
PNS	peripheral nerve stimulation
PO	power output
PPA	peak-to-peak amplitude
P_{peak}	peak power
PS10	low-frequency paired stimulation at 10 Hz
PS100	high-frequency paired stimulation at 100 Hz
Q_{pot}	potentiated quadriceps twitch force
ROS	reactive oxygen species
RNS	reactive nitrogen species
RPE	rate of perceived exertion
SD	standard deviation
SE	standard error
SIT	superimposed twitch
SR	sarcoplasmic reticulum
TE	typical error
TMS	transcranial magnetic stimulation
TT	time trial
TTF	time to task failure
VA	voluntary activation
VA_{PNS}	peripheral voluntary activation
VA_{TMS}	voluntary activation evoked by TMS
VL	vastus lateralis
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2sc}$	slow component of oxygen uptake
$\dot{V}O_{2max}$	maximal oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
VT	ventilatory threshold
WEP	work done above end power
W'	curvature constant of the power-time relationship
^{31}PMRS	phosphorus magnetic resonance spectroscopy

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DECLARATION

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed:

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1. INTRODUCTION

Neuromuscular fatigue defined as any transient exercise-induced reduction in the maximal voluntary force of the skeletal muscle (Gandevia, 2001), is widely accepted as a phenomenon which affects exercise tolerance and represents not only a limiting factor during sporting competitions, but can also influence the quality of life for the elderly and patients with neurological diseases by restricting mobility. Understanding the underlying mechanism(s) of neuromuscular fatigue is essential to influence the limits of exercise tolerance.

Since the pioneering work of Angelo Mosso in the late 1800's and his publication 'La Fatica' (1891), the term 'fatigue' has been commonly categorised based on its peripheral vs. central origin. To date, the peripheral-central dichotomy is still widely adopted, however, more recent views argue that distinguishing between central and peripheral fatigue undermines the interplay between the nervous system and the muscles and therefore, should be avoided (Barry & Enoka, 2007). Instead, Enoka & Duchateau (2016) aimed to broaden this classic dichotomy approach and defined fatigue as a symptom, which is limited by the interaction between performance fatigability and perceived fatigability, a taxonomy first proposed by Kluger *et al.* (2013). Performance fatigability is understood as 'the decline in an objective measure of performance over a discrete period of time' which depends on the contractile function of the working muscle and muscle activation (Enoka & Duchateau, 2016). Perceived fatigability is understood as 'changes in the sensations that regulate the integrity of the performer' based on alterations related to the maintenance of homeostasis and the psychological state (Enoka & Duchateau, 2016). The research conducted in the present thesis aims to investigate what is understood as 'performance fatigability' according to the taxonomy proposed by Enoka & Duchateau (2016). However, the present thesis applied neurostimulation techniques to examine the origin of exercise-induced impairments in the force generating capacity according to their peripheral and central origin and therefore, fatigue will be discussed according to the traditional taxonomy as peripheral and central fatigue.

The interplay between exercise intensity and duration has been described as the most crucial factor determining exercise tolerance (Walsh, 2000), likely due to the specific physiological responses and energy demands associated with each intensity domain. Four exercise intensity domains can be distinguished: the moderate, heavy, severe and extreme intensity domains, whereby the lactate threshold (LT) or gas exchange threshold (GET) represents the boundary between the moderate and heavy intensity domains and critical power (CP) represents the boundary between the heavy and severe intensity domains (Whipp *et al.*, 2002; Whipp & Ward, 1992; Gaesser & Poole, 1996; Whipp, 1994). In contrast to severe intensity exercise where $\dot{V}O_{2max}$ is attained at exhaustion, exercise within

the extreme intensity domain includes power outputs at which exhaustion precedes the achievement of the $\dot{V}O_{2max}$ (Hill *et al.*, 2002). Distinct physiological responses, i.e. respiratory, muscle metabolic, blood lactate and blood acid-base responses, characterise each intensity domain (Black *et al.*, 2017; Vanhatalo *et al.*, 2016; Jones *et al.*, 2008; Gaesser & Poole, 1996; Whipp & Ward, 1992; Whipp, 1994; Poole *et al.*, 1988; Whipp & Wasserman, 1972). These intensity dependent physiological responses may induce peripheral and central alterations to neuromuscular function, making the origin of neuromuscular fatigue bespoke to each exercise intensity domain (Black *et al.*, 2017; Thomas *et al.*, 2015; Burnley *et al.*, 2012). Indeed, neuromuscular fatigue seems to be predominantly of peripheral origin following exercise within the severe intensity domain (Thomas *et al.*, 2015; Burnley *et al.*, 2012). Therefore, the present thesis focused primarily on peripheral alterations, however, central fatigue has been assessed and discussed in each experimental chapter (Chapter 4-7) and in the general discussion (Chapter 8).

The limits of tolerance for constant-load exercise within the severe intensity domain can be predicted using the critical power concept (Monod & Scherrer, 1965). The relationship between power output and duration of severe intensity exercise is characterised by a hyperbolic function, whereby the asymptote of this relationship (CP) represents the highest power output that can be sustained without continuously drawing on anaerobic energy stores and the curvature constant (W'), mathematically equivalent to a given amount of work that can be performed above CP (Poole *et al.*, 1988; Moritani *et al.*, 1981; Monod & Scherrer, 1965). According to the CP concept, task failure occurs when W' is fully depleted. Exercise above CP and thus, the depletion of W' has been associated with disturbances of muscle homeostasis, impairing muscle contractile function and ultimately, reducing the ability to produce force (Vanhatalo *et al.*, 2016; Murgatroyd *et al.*, 2011; Jones *et al.*, 2008; Burnley & Jones, 2007). The CP concept with its two key parameters CP and W', not only allows the prediction of performance but may also offer great potential for a better understanding of the interaction between physiological and neuromuscular alterations causing fatigue and ultimately, task failure.

The CP concept constitutes a potent framework for the investigation of the limits of exercise tolerance (Burnley & Jones, 2016; Poole *et al.*, 2016; Grassi *et al.*, 2015; Murgatroyd *et al.*, 2011) and its integration with electromyographic and mechanical measures of neuromuscular fatigue offers great potential for a better understanding of the limits of tolerance within the severe intensity domain. Therefore, the primary aim of the present thesis was to investigate the exercise-induced neurophysiological responses to fatiguing cycling exercise above critical power in healthy humans and to further examine a potential link between W' utilisation and the magnitude of neuromuscular fatigue during locomotor exercise. Research aims and hypotheses for each of the 4 studies of the present thesis are outlined in Chapters 4-7 (Study 1-4).

Below is presented a brief overview of the present thesis:

Chapter 2 reviews the relevant literature on the critical power concept and neuromuscular fatigue.

Chapter 3 describes the common methods used throughout this thesis / across multiple studies.

Chapter 4 examines between-day reliability of all neuromuscular measures undertaken in the present thesis, thus both at rest and following a fatiguing cycling exercise.

Chapter 5 investigates whether the development of neuromuscular fatigue within the severe intensity domain can be associated with the depletion of W'.

Chapter 6 investigates the time course of neuromuscular recovery following cycling exercise at different exercise intensities and durations above CP.

In the continuity of the findings from Chapter 5, the final experiment presented in Chapter 7 examines the effect of creatine supplementation on neuromuscular fatigue and exercise tolerance when cycling above CP.

Chapter 8 discusses the results from the experimental chapters, including principle findings, progression of the research area, limitations and assumptions and future directions.

Finally, Chapter 9 provides a conclusion of the present thesis.

2. LITERATURE REVIEW

The following chapter provides an overview of the literature on neurophysiological responses to fatiguing exercise above critical power and is separated into two main sections: the first section (2.1.) introduces exercise tolerance within the theoretical framework of the critical power concept and the second section (2.2.) provides an overview of the current understanding of neuromuscular fatigue, its underlying mechanisms and methods used to assess exercise-induced alterations. The third section introduces the study of fatigue within the present thesis (2.3.) and finally, research aims and hypotheses of the present thesis are presented at the end of the chapter (Section 2.4.).

2.1. The critical power concept as the theoretical framework of exercise tolerance within the severe intensity domain

The critical power concept, first described by Monod & Scherrer (1965), allows the mathematical prediction of an individual's ability to tolerate high-intensity exercise. The origins of this concept go back to Hill (1925) who characterised the velocity-time curve by plotting the world record performance times against performance speed for various sports (e.g. running, cycling, skating). Later on, Monod & Scherrer (1965) described a hyperbolic relationship between power output and time to exhaustion in a single muscle group which can be converted to a linear relationship between the 'limit work' (W_{lim}), the amount of work performed before exhaustion and the 'limit time' (t_{lim}), the tolerable exercise duration, whereby the slope (b) of this relationship represents the critical power (CP) and the y-intercept (a), the amount of work derived from the muscles energetic reserve:

Equation 2.1. The W_{lim} - t_{lim} relationship:

$$W_{lim} = a + b(t_{lim})$$

Moritani *et al.* (1981) was the first to extend this linear work-time relationship to whole-body exercise (cycle ergometry). This literature review focuses on cycle ergometry most exclusively with a few exceptions when deemed appropriate.

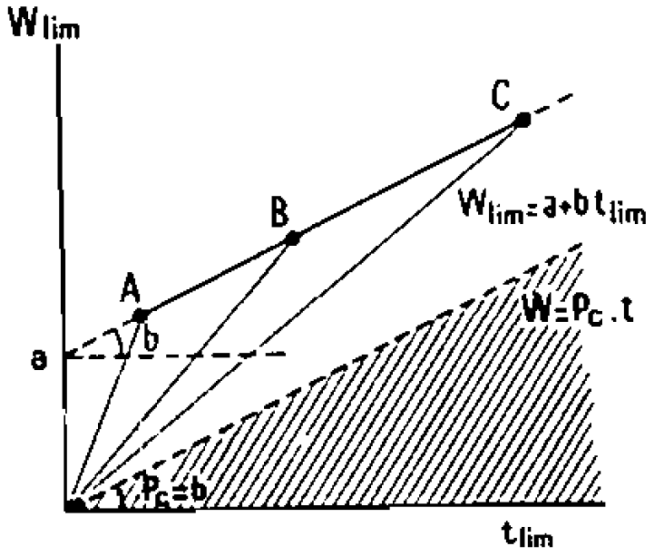

Figure 2.1. The W_{lim} -t_{lim} relationship. A, B and C represent three time to exhaustion trials which are located on a straight line defined by the relation between limit work and limit time (W_{lim} = a + b(t_{lim})). Critical power (P_c) represents the slope of the straight line going through the origin and parallel to the straight line defined by A, B and C. Exercise within the shaded zone can be sustained without fatigue (Monod & Scherrer, 1965).

The following section (2.1.1) will review the physiological responses during exercise above and below CP and discuss the physiological nature of the two parameters characterising the power-time relationship. Finally, the mathematical modelling of the two-parameter model and alternative models will be discussed in Section 2.1.2.

2.1.1. Exercise tolerance within the severe intensity domain according to a two-parameter model

2.1.1.1. Energy contribution during exercise

Exercise tolerance is limited by energy supply provided through three main energy systems: the anaerobic alactic energy system (or ATP-PCr system), the anaerobic lactic energy system (or glycolytic system) and the aerobic energy system (or oxidative system).

At exercise onset, immediate energy is provided through the breakdown of stored adenosine triphosphate (ATP) (Equation 2.2.) and the breakdown of high-energy phosphagens and phosphocreatine (PCr) (Equation 2.3.):

Equation 2.2. Breakdown of stored ATP (ATP hydrolysis)

$ATP \leftrightarrow ADP + P_i + H^+$

where ADP = adenosine 5'diphoshphate, P_i = inorganic phosphate and H^+ = hydrogen ion

Equation 2.3. Resynthesis of ATP via the ATP-PCr system

$PCr + ADP + H^+ \leftrightarrow ATP + Cr$

where Cr = creatine

The amount of stored ATP and the amount of ATP resynthesized by the phosphagen system is small and thus, only provides energy for 3 to 15 s (Gaitanos *et al.*, 2018; Conley *et al.*, 2001; Brooks *et al.*, 1996). If exercise continues, the contribution of energy from the glycolytic system becomes predominant. Anaerobic glycolysis describes the breakdown of glycogen or glucose to ATP and lactic acid in absence of oxygen (O_2). This energy system is the main energy provider between ~ 6 to 60 s (Withers *et al.*, 1991; Medbo & Tabata, 1989).

Equation 2.4. Resynthesis of ATP via breakdown of glucose and glycogen (anaerobic glycolysis)

$Glycogen + 3\ ADP + 3\ P_i \rightarrow 3\ ATP + 2\ lactate^- + 2\ H^+$

With increasing exercise duration, the importance of the oxidative system increases. This energy pathway involves the breakdown of fats and carbohydrates in the presence of oxygen and is the energy system that produces the greatest amount of ATP per mol of substrate (38 vs. 2 ATP molecules from one molecule of glucose), however, to a much slower rate in comparison to the anaerobic metabolism (Kenney *et al.*, 2015).

The relative contribution of each energy system to the total energy supply is dependent on exercise intensity and duration; no exercise is supported by one single energy system alone. Figure 2.2. illustrates the contribution of these three energy pathways during maximal exercise.

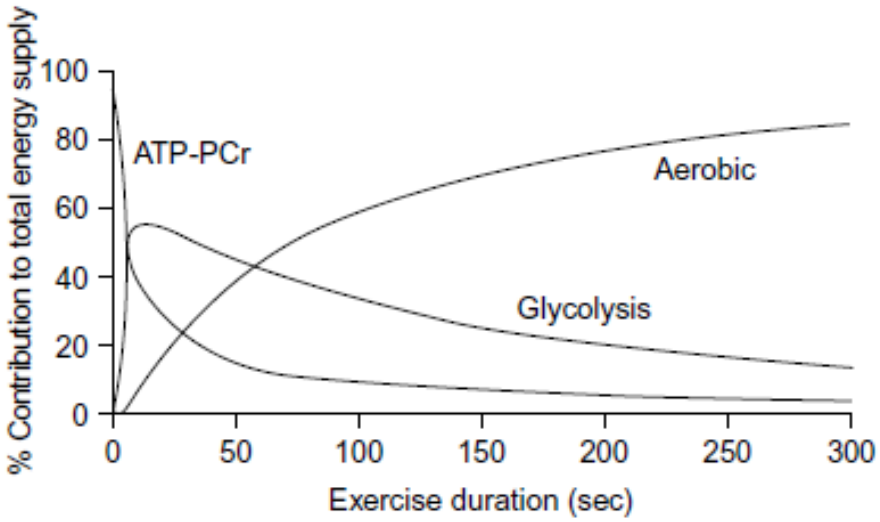

Figure 2.2. Relative contribution of the energy systems to the total energy supply for maximal exercise (Gastin, 2001).

During exhaustive severe intensity exercise, all three major energy systems (i.e. ATP-PCr, glycolysis, mitochondrial respiration) are involved in the replenishment of ATP (Baker *et al.*, 2010). Muscle [ATP] reduced by 30% and phosphate creatine by 60%, alongside an increase in muscle [La^-] from 2 to 28.1 mmol (kg wet wt)$^{-1}$ during intense, exhaustive single-leg knee extensor exercise lasting 3.2 min (Bangsbo *et al.*, 1990). The decline in PCr and increase in [La^-] originating from glycolysis are initiated rapidly at the onset of contraction (Greenhaff & Timmons, 1998). If skeletal muscle glycogen levels are low, ATP resynthesis relies initially to a relatively greater extent on the ATP-PCr energy system (Greenhaff & Timmons, 1998). The initiation of muscle contraction by Ca^{2+} alongside the accumulation of ADP, AMP, and P_i resulting from ATP and PCr hydrolysis, stimulate oxidative phosphorylation in the mitochondria but also the activity of creatine phosphokinase to convert PCr to ATP and the breakdown of glycogen, so that the rate of ATP utilisation is directly linked with its provision (Hargreaves & Spriet, 2006; Greenhaff & Timmon, 1998). The contribution of anaerobic energy to the total energy turnover during exhaustive ~3 min single leg exercise accounted for 80% at 0-30 s and declined to 30% after 120 s of exercise (Bangsbo *et al.*, 1990). On average, 45% of the total energy turnover was derived from the aerobic energy system, with an increasing contribution of muscle oxygen uptake to the total energy turnover from 20% at 0-30 s to 70% after 120 s of exercise (Bangsbo *et al.*, 1990). Thus, the decline in anaerobic energy contribution was accompanied by a relative increase in aerobic energy contribution (i.e. oxidative phosphorylation).

Different energy demands, depending on exercise intensity and duration, result in different physiological responses (i.e. depletion of ATP, PCr and glycogen; accumulation of metabolites, such as H^+, P_i, ADP, La^- or increased pulmonary $\dot{V}O_2$). These physiological responses are commonly used to separate exercise intensity domains as described in the following section.

2.1.1.2. Physiological responses above and below critical power

Figure 2.3. illustrates pulmonary $\dot{V}O_2$ and blood [La^-] responses for exercise within the severe, heavy and moderate exercise intensity domains. Whereas a steady state in $\dot{V}O_2$ and blood [La^-] is achieved rapidly during moderate intensity exercise and delayed during heavy intensity exercise, both $\dot{V}O_2$ and blood [La^-] continue to increase until task failure during severe intensity exercise (Jones *et al.*, 2019; Vanhatalo *et al.*, 2016; 2010; Jones *et al.*, 2008; Whipp & Ward, 1992; Poole *et al.*, 1988). Physiological thresholds demarcate these three exercise intensity domains, whereby the lactate threshold (LT) or gas exchange threshold (GET) separate the moderate and heavy exercise intensity domains and CP separates the heavy and severe intensity domains (Whipp *et al.*, 2002; Whipp & Ward, 1992; Gaesser & Poole, 1996; Whipp, 1994).

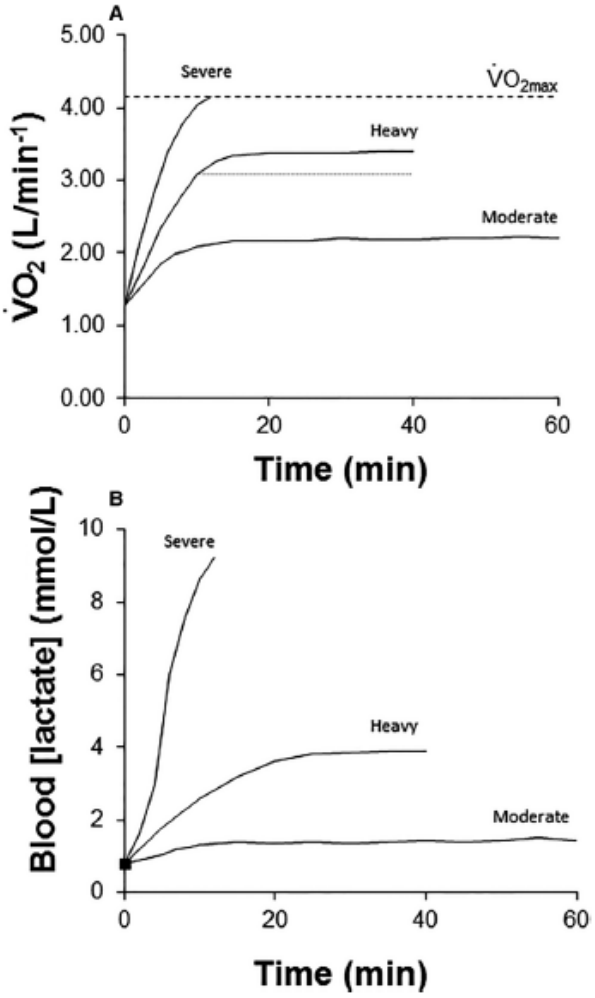

Figure 2.3. Pulmonary oxygen uptake ($\dot{V}O_2$) (A) and blood [La^-] responses (B) during moderate, heavy and severe intensity exercise (Jones *et al.*, 2019).

Since Monod & Scherrer (1965), numerous studies further investigated the physiological nature of CP. It has been described as the highest sustainable power output for which a steady state in oxygen uptake ($\dot{V}O_2$) and metabolic parameters (i.e. [PCr], [P_i], [H^+], blood and muscle [La^-]) can be achieved (Jones *et al.*, 2019; Black *et al.*, 2017; Vanhatalo *et al.*, 2016; 2010; Jones *et al.*, 2008; Poole *et al.*, 1988). CP can be understood as a transition separating the heavy and severe exercise intensity domains or sustainable and not-sustainable exercise intensities (Jones *et al.*, 2019).

Jones *et al.* (2008) compared the muscle metabolic response and fatigue mechanisms during single leg knee extensions at 10% below and 10% above CP. During exercise below CP, [PCr], [P_i] and pH stabilised within 3 min and remained stable until test termination (20 min). In contrast, during exercise above CP, a progressive increase in [P_i] and decrease in [PCr] and pH was found which ultimately, led to exhaustion (~15 min). Jones *et al.* (2008) concluded that CP is the highest sustainable power output without a progressive depletion of high-energy phosphates and accumulation of fatigue-related metabolites (i.e. H^+, P_i). Vanhatalo *et al.* (2016) expanded these

findings to whole body exercise and reported muscle [PCr], [La^-], plasma [K^+] and pH remained stable when cycling 5% below CP, but changed substantially over time when cycling 5% above CP (Figure 2.4.). However, muscle glycogen was not significantly different when comparing cycling cycling exercise at 5% above and 5% below CP (Vanhatalo *et al.*, 2016). Moreover, Black *et al.* (2017) provided further support for CP as a physiological threshold, with substantially greater muscle metabolic perturbations (i.e. low pH and [PCr], high [La^-]) during 4-5 severe intensity cycling bouts compared to cycling exercise in the heavy and moderate intensity domains.

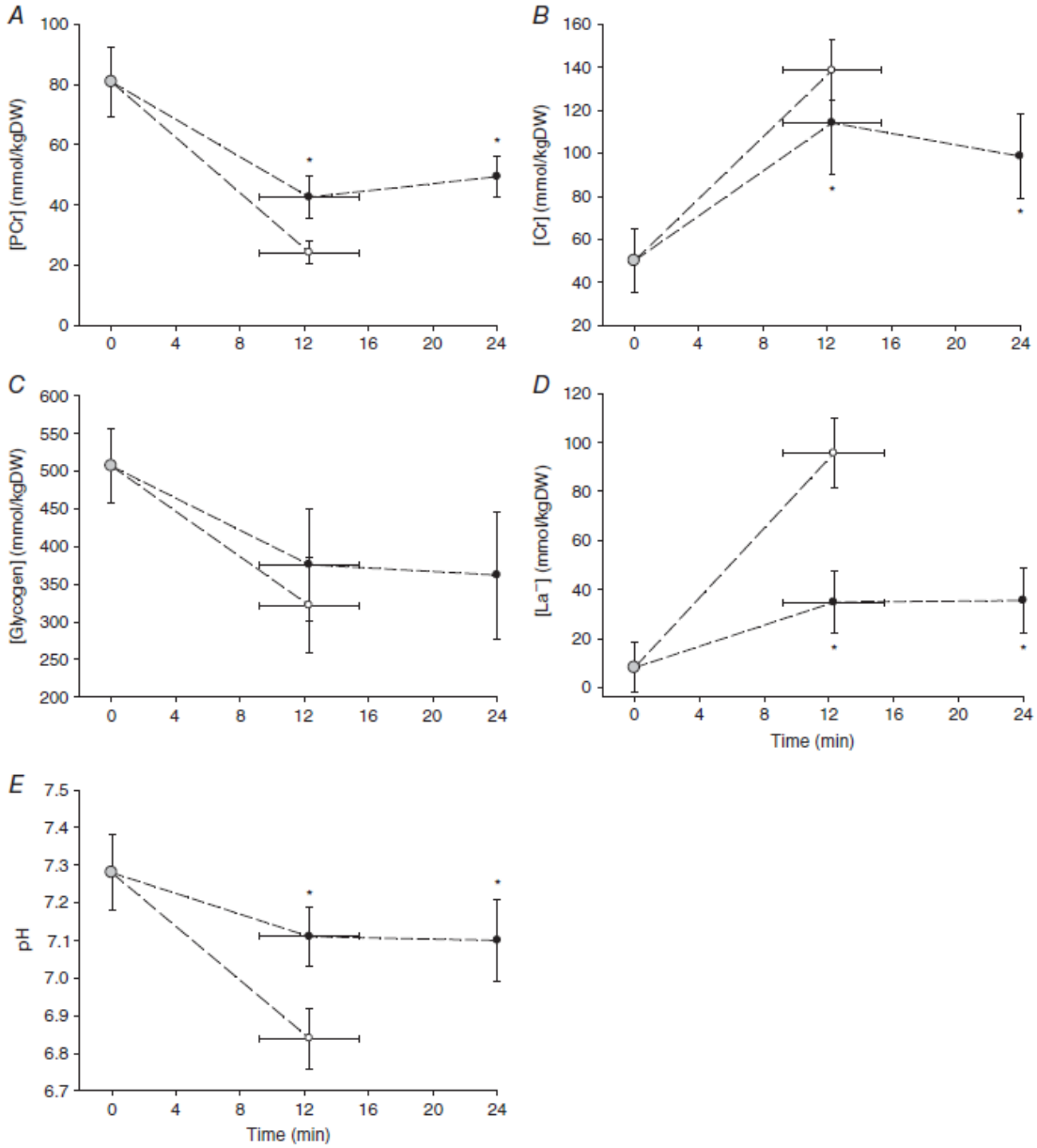

Figure 2.4. Muscle metabolic responses for phosphocreatine [PCr] (A), creatine [Cr] (B), glycogen (C), lactate [La^-] (D) and pH (E) at rest (grey circle) and during cycling exercise above (white circles) and below CP (black circles). *significant different from end-exercise value during >CP trial ($p < 0.05$) (Vanhatalo *et al.*, 2016).

2.1.1.3. The power asymptote: critical power

The duration CP can be sustained for has been discussed controversially, with studies reporting durations between ~16 to 65 min (De Lucas *et al.*, 2013; Carter *et al.*, 2005; Brickley *et al.*, 2002; Hill & Smith, 1999). These times to task failure (TTF) have been shown to depend on the mathematical model used to determine CP (Bergstrom *et al.*, 2014; Bull *et al.*, 2008; Housh *et al.*, 2001; Bull *et al.*, 2000) (see Section 2.1.2), the durations of the TTF included in the chosen model (Mattioni Maturana *et al.*, 2018; Jenkins *et al.*, 1998; Bishop *et al.*, 1998) (see Section 2.1.2.3), and familiarisation to the task (Hill & Smith, 1999).

The aerobic nature of CP is well evidenced through manipulation of O_2 delivery and/or utilization as summarized in Table 2.1. Briefly, (1) hyperoxia increased CP (Goulding *et al.*, 2019; Vanhatalo *et al.*, 2010); (2) hypoxia reduced CP (Parker Simpson *et al.*, 2015; Dekerle *et al.*, 2012; Moritani *et al.*, 1981); (3) endurance (Jenkins & Quigley (1992) and interval training (Vanhatalo *et al.*, 2008b; Poole *et al.*, 1990) increased CP; and (4) blood flow occlusion decreased CP (Broxterman *et al.*, 2015). Moreover, strong correlations were found between CP and traditional markers of aerobic function, such as VT (Smith *et al.*, 1999) and $\dot{V}O_{2peak}$ (Smith *et al.*, 1999; Moritani *et al.*, 1981). Mitchell *et al.* (2018) found a positive relation between CP and type I skeletal muscle fibre proportion, capillarity and cross-sectional area. Collectively, these findings provide strong evidence for CP as a marker of aerobic function.

Table 2.1. Manipulation of critical power and correlations with markers of aerobic function

	Participants	CP determination	Intervention	Change in CP	Correlation of CP with markers of aerobic function
Goulding *et al.* (2019)	8 healthy men	4 constant-load tests to task failure (supine cycling)	Normoxia vs. hyperoxia (F_iO_2 = 0.5)	Normoxia: 134 ± 27 W Hyperoxia: 148 ± 29 W*	The phase II time constant of pulmonary $\dot{V}O_2$ was related to CP in hyperoxia (r^2 = 0.89; $p < 0.001$)
Vanhatalo *et al.* (2010)	7 habitually active men	4 constant-load tests to task failure (knee extensions)	Normoxia vs. hyperoxia (F_iO_2 = 0.70)	Normoxia: 16.1 ± 2.6 W Hyperoxia 18.0 ± 2.3 W*	
Parker Simpson *et al.* (2015)	13 recreationally active women	5 constant-load tests to task failure (cycling)	Normoxia vs. hypoxia (F_iO_2 = 0.13)	Normoxia: 175 ± 25 W Hypoxia: 132 ± 17 W*	Relationship between the % change in CP relative to $\dot{V}O_{2peak}$ (r = 0.83; $p < 0.001$)
Dekerle *et al.* (2012)	5 active men and 6 women	3-5 constant-load tests to task failure (cycling)	Normoxia vs. hypoxia (F_iO_2 = 0.15)	Normoxia: 220 ± 25 W Hypoxia 190 ± 28 W*	
Moritani *et al.* (1981)	2 participants	4 constant-load tests to task failure (cycling)	Normoxia vs. hypoxia (F_iO_2 = 0.09)	Normoxia: 197 and 230 W Hypoxia: 110 and 102 W*	CP was strongly related to $\dot{V}O_{2max}$ (r = 0.87; $p < 0.01$) (16 participants – 8 women)
Jenkins & Quigley (1992)	12 healthy, active, but untrained men	3 constant-load tests to task failure (cycling)	8 weeks of endurance training	Pre-training: 196 ± 41 W Post-training: 255 ± 28 W*	
Vanhatalo *et al.* (2008b)	9 habitually active (1 woman)	3 constant-load tests to task failure (cycling)	4 weeks of interval training	Pre-training: 230 ± 53 W Post-training: 255 ± 50 W*	
Poole *et al.* (1990)	8 healthy men	5 constant-load tests to task failure (cycling)	7 weeks of interval training	Pre-training: 197 ± 12 W Post-training: 217 ± 11 W*	
Broxterman *et al.* (2015)	10 healthy men	4 constant-load tests to task failure (handgrip exercise)	Control blood flow vs. blood flow occlusion	Control: 4.1 ± 0.7 W Occlusion: -0.7 ± 0.4 W*	
Smith *et al.* (1999)	13 competitive cyclists (4 women)	4 constant-load tests to task failure (cycling)			CP was strongly related to VT (r = 0.73 to 0.90) and $\dot{V}O_{2max}$ (0.68 to 0.93) ($p < 0.01$).

*significant difference between conditions ($p < 0.05$).

2.1.1.4. The curvature constant *W′*

The physiological basis of the second parameter of the power-time relationship, the curvature constant *W′*, is not fully understood. Originally described by Monod & Scherrer (1965) as the 'muscles energy reserve', the term 'anaerobic work capacity' has been frequently used since then. It was originally proposed that the 'anaerobic work capacity' reflects a finite intramuscular energy store, mathematically equivalent to a given amount of work that can be performed above CP until task failure. This limited amount of work would derive energy from anaerobic metabolism, i.e. PCr hydrolysis, anaerobic glycolysis and a small aerobic contribution from myoglobin- and haemoglobin-bound O_2 stores (Moritani *et al.*, 1981; Monod & Scherrer, 1965).

These early assumptions were tested by investigating the changes in *W′* through manipulation of the anaerobic energy contribution and by relating traditional makers of anaerobic function to *W′*. An overview of these findings is summarized in Table 2.2. Briefly, (1) creatine supplementation increased *W′* (Miura *et al.*, 1999); (2) glycogen depletion reduced *W′* (Miura *et al.*, 2000); and (3) *W′* was related to markers of anaerobic function, such as the Wingate test (Hill, 1993; Vandewalle et al., 1989; Nebelsick-Gullett *et al.*, 1988) and the maximal O_2 deficit (Hill & Smith, 1993; Hill, 1993).

While Nebelsick-Gullett *et al.* (1988) concluded that the CP test is a valid protocol to determine anaerobic capacity based on a significant relation between *W′* and the anaerobic work capacity determined from a 30 s Wingate test, Vandewalle *et al.* (1989) suggested an underestimation of the maximal anaerobic capacity by *W′* as a consequence of disregarding the inertia of the aerobic metabolism. Furthermore, Bulbulian *et al.* (1996) suggested that *W′* may not comprise energy provided through anaerobic glycolysis and therefore, challenged the validity of *W′* as an indicator of the anaerobic capacity. The 30 s Wingate test has received critique for being too short to fully deplete the glycolytic system (Vandewalle *et al.*, 1989; Katch *et al.*, 1977) and for an aerobic contribution to the total work performed (Serresse *et al.*, 1988). Thus, Hill & Smith (1993) compared *W′* to the maximal oxygen deficit, which is an accepted alternative method to quantify the anaerobic capacity (Medbo *et al.*, 1988). The authors found no difference between the two and so concluded that *W′* accurately estimates the anaerobic capacity (Hill & Smith, 1993).

Moreover, *W′* appears insensitive to alterations in the fraction of inspired O_2 (Goulding *et al.*, 2019; Parker Simpson *et al.*, 2015; Dekerle *et al.*, 2012; Moritani *et al.*, 1981). Nonetheless, Vanhatalo *et al.* (2010) reported a reduction in *W′* (1.92 ± 0.70 kJ vs. 1.48 ± 0.31 kJ) following hyperoxia (F_iO_2 = 0.70) and pointed out that the decrease in *W′* was inversely correlated with the increase in CP in hyperoxia. This is in line with a negative relationship between the change in CP and the change in *W′* found by Dekerle *et al.* (2012) and Parker Simpson *et al.* (2015) in hypoxia and by (Gaesser & Wilson (1988) following a training intervention.

Table 2.2. Manipulation of the curvature constant *W′* and correlations with markers of anaerobic function

	Participants	*W′* determination	Intervention	Change in *W′*	Correlation of *W′* with markers of anaerobic function
Miura *et al.* (1999)	8 healthy men	4-5 constant-load tests to task failure (cycling)	5 days of creatine or placebo supplementation	Placebo: 10.9 ± 2.7 kJ Creatine: 13.7 ± 3.0 kJ*	
Miura *et al.* (2000)	7 healthy men	4 constant-load tests to task failure (cycling)	Glycogen depletion	Control: 12.8 ± 2.2 kJ Glycogen depletion: 10.3 ± 2.4 kJ*	
Goulding *et al.* (2019)	8 healthy men	4 constant-load tests to task failure (supine cycling)	Normoxia vs. hyperoxia (F_iO_2 = 0.5)	Normoxia: 13.9 ± 4.7 kJ Hyperoxia: 12.8 ± 4.7 kJ	
Vanhatalo *et al.* (2010)	7 habitually active men	4 constant-load tests to task failure (knee extensions)	Normoxia vs. hyperoxia (F_iO_2 = 0.70)	Normoxia: 1.92 ± 0.70 kJ Hyperoxia: 1.48 ± 0.31 kJ*	
Parker Simpson *et al.* (2015)	13 recreationally active women	5 constant-load tests to task failure (cycling)	Normoxia vs. hypoxia (F_iO_2 = 0.13)	Normoxia: 13.2 ± 2.2 kJ Hypoxia: 12.3 ± 2.7 kJ	
Dekerle *et al.* (2012)	5 active men and 6 women	3-5 constant-load tests to task failure (cycling)	Normoxia vs. hypoxia (F_iO_2 = 0.15)	Normoxia:11.7 ± 5.5 kJ Hypoxia: 12.1 ± 4.4 kJ	
Jenkins & Quigley (1992)	12 healthy, active, but untrained men	3 constant-load tests to task failure (cycling)	8 weeks of endurance training	Pre-training: 19.9 ± 4.1 kJ Post-training: 14.7 ± 3.2 kJ	
Poole *et al.* (1990)	8 healthy men	5 constant-load tests to task failure (cycling)	7 weeks of interval training	Pre-training: 14.6 ± 1.6 kJ Post-training: 14.8 ± 1.5 kJ	
Gaesser & Wilson (1988)	2 groups: 5 men (CE) and 6 men (IE)	5 constant-load tests to task failure (cycling)	6 weeks of low-intensity continuous (CE) or high-intensity interval training (IE)	Pre-training vs. post-training: CE: 14.7 ± 1.5 vs. 13.6 ± 2.2 kJ IE: 14.0 ± 1.1 vs. 13.1 ± 1.2 kJ	
Nebelsick-Gullett *et al.* (1988)	25 women	3 constant-load tests to task failure (cycling)			vs. 30 s Wingate test: $r = 0.74$; $p < 0.05$
Vandewalle *et al.* (1989)	9 men	4 constant-load tests to task failure (cycling)		*W′*: 205 ± 56 J·kg^{-1} vs. 30 s Wingate: 294 ± 23 J·kg^{-1}*	vs. 30 s Wingate: $r = 0.69$; $p < 0.05$
Bulbulian *et al.* (1996)	29 students (16 women)	3 constant-load tests to task failure (cycling)		*W′*: 184 ± 1.2 J·kg^{-1} vs. 30 s Wingate: 240 ± 31 J·kg^{-1}	vs. 30 s Wingate: $r = 0.07$; $p > 0.72$
Hill & Smith (1993)	26 students (13 women)	5 constant-load tests to task failure (cycling)		Women: 179 ± 10 vs. 177 ± 10 J·kg^{-1} Men: 224 ± 10 vs. 235 ± 9 J.kg^{-1}	vs. oxygen deficit: $r = 0.77$; $p < 0.01$

*significant difference between conditions ($p < 0.05$).

Moreover, *W′* has been associated with the development of the $\dot{V}O_{2sc}$ (Vanhatalo *et al.*, 2016; Vanhatalo *et al.*, 2011; Murgatroyd *et al.*, 2011), a characteristic of the severe exercise intensity domain which is described as a rise in $\dot{V}O_2$ above the steady-state value predicted from the $\dot{V}O_2$-power relationship observed during exercise below the gas exchange threshold (Roberts *et al.*, 2005; Paterson & Whipp, 1991; Poole *et al.*, 1988) and therefore, represents a loss in muscle efficiency (Rossiter *et al.*, 2002; Poole *et al.*, 1991). At exhaustion, the attainment of $\dot{V}O_{2peak}$ and the full depletion of *W′* coincide and studies have shown that the amplitude of the $\dot{V}O_{2sc}$ is related to the size of *W′* (Murgatroyd *et al.*, 2011; Vanhatalo *et al.*, 2011). The physiological basis of the $\dot{V}O_{2sc}$ remains under scrutiny with disagreement whether the additional O_2 cost reflects the recruitment of additional type II fibres (Endo *et al.*, 2007; Saunders *et al.*, 2003; 2000; Burnley *et al.*, 2002; Borrani *et al.*, 2001; Shinohara & Moritani, 1992) or a greater metabolic demand in already recruited muscle fibres (Perrey *et al.*, 2003; Tordi *et al.*, 2003; Scheuermann *et al.*, 2001). Nonetheless, evidence indicates that the origin of the $\dot{V}O_{2sc}$ is within the active muscle (Poole & Jones, 2005; Poole *et al.*, 1992; Poole *et al.*, 1991) and likely linked to type II fibres. This is supported by studies reporting a higher O_2 cost of force production in type II fibres (Krustrup *et al.*, 2008; Krustrup *et al.*, 2004; Pringle *et al.* 2003) and a larger $\dot{V}O_{2sc}$ in individuals with a greater proportion of type II fibres in the vastus lateralis (VL) (Pringle *et al.*, 2003; Barstow *et al.*, 1996). Given the relation between *W′* and $\dot{V}O_{2sc}$ (Murgatroyd *et al.*, 2011; Vanhatalo, *et al.*, 2011) and the association between $\dot{V}O_{2sc}$ and type II muscle fibres (Pringle, *et al.*, 2003; Barstow *et al.*, 1996), a link between type II muscle fibres and *W′* has been suggested, however, results failed to confirm this relation (Vanhatalo *et al.*, 2016).

The close relationship between the progressive reduction in muscle efficiency (existence of $\dot{V}O_{2sc}$) and the magnitude of *W′* has been linked via the accumulation of fatigue-related metabolites (Murgatroyd *et al.*, 2011). The depletion of *W′* has been associated with the accumulation of fatigue-related metabolites (i.e. P_i, H^+, ADP, La^-) to a critical level (Burnley *et al.*, 2010; Ferguson *et al.*, 2010; 2007; Poole *et al.*, 1988) and it is further believed, that these metabolic perturbations may also contribute to the continued reduction in muscle efficiency, proposed in the 'fatigue cascade' by Murgatroyd *et al.* (2011). Murgatroyd & Wylde (2011) suggested that the depletion of *W′* induces a 'fatigue cascade' (see Figure 2.4.): 1) the depletion of energy stores in substrate level phosphorylation leads to 2) an accumulation of metabolites which 3) challenges the cellular homeostasis and either directly or indirectly through the 4) recruitment of additional muscle fibres 5) increases the energy and oxygen costs of the exercise (reflected in the $\dot{V}O_{2sc}$) and ultimately, leads to the attainment of $\dot{V}O_{2max}$.

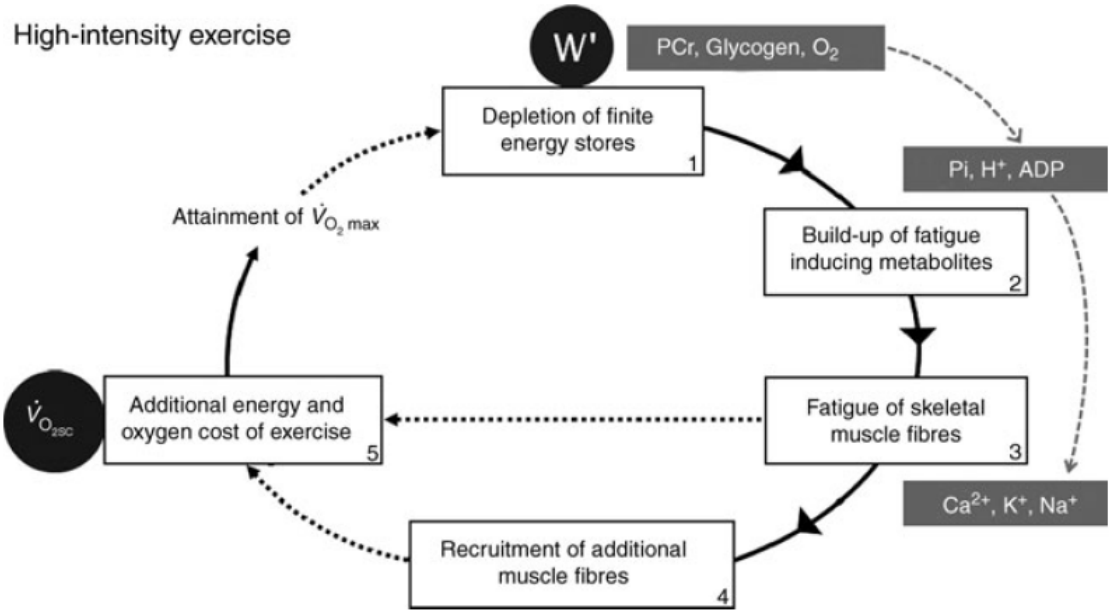

Figure 2.5. Schematic illustration of the putative physiological events represented by *W′* during severe intensity exercise (Murgatroyd & Wylde, 2011).

The rate of *W′* depletion is determined by the power output above CP, with a faster depletion and a greater rate of accumulation of fatigue-related metabolites within the upper boundary of the severe intensity domain compared to the lower boundary (Jones *et al.*, 2008). When *W′* is fully depleted, exercise will either be terminated or a reduction in power output to an intensity below CP is required for exercise to continue, and potentially for a reconstitution of *W′* (Coats *et al.*, 2003). Muscle metabolites indeed have been shown to recover back to baseline values only if exercise intensity is reduced to < CP or during passive recovery, whereas no recovery can be found during exercise > CP (Chidnok *et al.*, 2013; Skiba *et al.*, 2012).

The physiological nature of *W′* remains equivocal and to date, not fully understood, thus, its interpretation must be made with caution. Although the primarily anaerobic nature of *W′* is still widely accepted, its reliance solely on anaerobic energy has been recently challenged due to its sensitivity to interventions altering O_2 delivery (Dekerle *et al.*, 2012; Vanhatalo *et al.*, 2010) and its relation to changes in CP and/or $\dot{V}O_{2peak}$ (Parker Simpson *et al.*, 2015; Dekerle *et al.*, 2012; Vanhatalo *et al.*, 2010). Burnley & Jones (2007) suggested a close interplay between *W′*, $\dot{V}O_{2max}$ and the $\dot{V}O_{2sc}$ in determining exercise duration within the severe intensity domain. Increasing *W′* or $\dot{V}O_{2max}$, or decreasing the rate at which the $\dot{V}O_{2sc}$ develops while keeping the other two parameters constant would increase TTF (Burnley & Jones, 2007).

Overall, the CP concept represents a suitable framework to examine and likewise understand better exercise tolerance within the severe intensity domain, and more specifically its exercise-induced physiological responses associated with fatigue.

2.1.2. Modelling of the power-time relationship to study the severe intensity domain

The following section will provide an overview of the mathematical models commonly used to characterise the power-time relationship, with particular focus on the two-parameter models and their relevance for the present thesis.

2.1.2.1. The two-parameter model

Firstly, the three most widely used two-parameter models and their key assumptions will be listed. The hyperbolic relationship between power output and time was first described by Monod & Scherrer (1965):

Equation 2.5. The hyperbolic power-time model:

$$t_{lim} = W' / (P - CP)$$

where t_{lim} = the sustainable duration in s (time to task failure / time to exhaustion), W' = a finite amount of energy reserve expressed in J / a curvature constant, P = power output in W, and CP = the power asymptote in W (Figure 2.6., Panel A).

Equation 2.6. can be converted to a linear relationship between work and time:

Equation 2.6. The linear work-time model:

$$W = CP \cdot t_{lim} + W'$$

where W = the total amount of work performed (Figure 2.5., Panel B).

The power-inverse time relationship was introduced by Moritani *et al.* (1981):

Equation 2.7. The linear power-inverse time model (Figure 2.5., Panel C):

$$P = (1/t_{im}) \cdot W' + CP$$

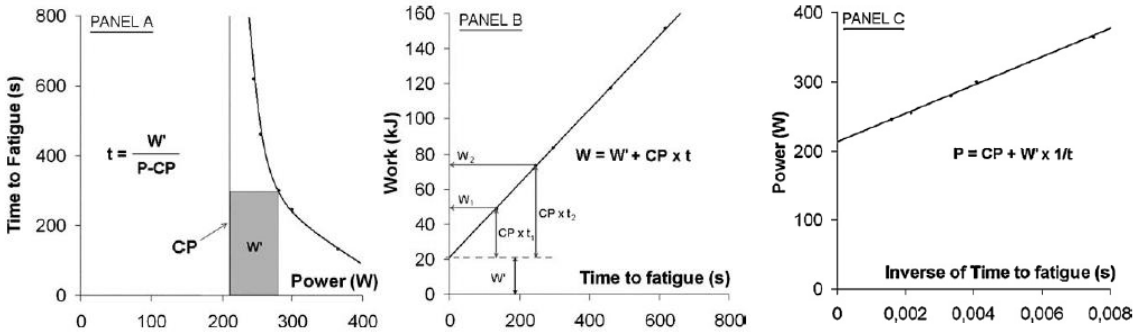

Figure 2.6. Schematic illustration of the hyperbolic power-time model (Panel A), the linear work-time model (Panel B) and the linear power-inverse time model (Panel C) (Dekerle *et al.*, 2008).

Differences in the mathematical models lead to differences in the values calculated for CP and *W′*. Bull *et al.* (2000) reported the largest estimate for CP for the linear power-inverse time model (208 ± 25 W) followed by the linear work-time model (196 ± 23 W) and the smallest estimate for the hyperbolic power-time model (192 ± 25 W). In the present thesis, the model with the 'best fit' (smallest standard error of the estimates, SE) was selected which is a common approach used in the literature (Black *et al.*, 2015; Dekerle *et al.*, 2015).

Four key assumptions associated with the two-parameter CP models (Hill, 1993) are listed below:

1. Only two sources of energy supply exist in humans, termed aerobic and anaerobic
2. The aerobic component, termed CP, is rate but not capacity limited
3. The anaerobic component, termed *W′*, is capacity but not rate limited
4. Exhaustion or per definition exercise termination occurs when *W′* is fully depleted

One may question the physiological validity of these assumptions, although mathematically accurate. According to the CP model, exercise below CP can be maintained for an indefinite time. However, from physiological investigations it is evident that no exercise can be performed indefinitely. The model also assumes that performance is solely dependent on two parameters, CP and *W′*; the latter being constant regardless of power output selected above CP. Vanhatalo *et al.* (2011) has demonstrated that both parameters can vary substantially due to health, age, fitness and training. In addition, alterations in *W′* and CP in opposite directions were observed following endurance training (Gaesser & Wilson, 1988) or when exercising in hyperoxia (Vanhatalo *et al.*, 2010). Moreover, the model assumes that exercise efficiency (i.e. converting metabolic energy to mechanical energy) remains constant across the full power and time domains (Morton, 2006; Di Prampero, 1999). This highlights that the physiological interpretation of the CP model and its two parameters may be more complex than initially assumed.

2.1.2.2. Three-parameter models and the 3 min all-out test

Based on the assumptions associated with the two-parameter models, several approaches have attempted to overcome these limitations and proposed more complex three-parameter models.

The two-parameter hyperbolic model assumes an infinite power as time approaches zero, with the consequence of an inaccuracy in particular in shorter prediction trials, ultimately overestimating CP and underestimating *W′* (Mattioni Maturana *et al.*, 2018; Bishop *et al.*, 1998; Morton, 1996; Housh *et al.*, 1990). This limitation led Morton (1996) to the introduction of a third parameter, referred as the 'maximal instantaneous power' (P_{max}), which limits the power as time approaches zero. More recently, Vinetti *et al.* (2019) did not find an agreement between the estimated power limit as time to exhaustion approaches zero and the measured maximal instantaneous power and therefore, proposed a further modification of the model which considers a TTF shorter than 1 min when modelling the power-time relationship.

Wilkie (1980) added a time constant (τ) to the model with the aim to correct for the delayed aerobic response between the onset and attainment of steady-state exercise. This correction seems to adjust appropriately for short durations but to a lesser extent for longer durations (Morton, 2006).

Hill (2004) compared the non-linear two-parameter model with the non-linear three-parameter model and the exponential model. The authors concluded that the two-parameter model described the power-time relationship well and provided good estimates for CP and *W′*, with no improvements when adding a third parameter.

More recently, Mattioni Maturana *et al.* (2018) reported that the non-linear and linear two-parameter models overestimated CP only if short times to exhaustion of duration < 10 min were selected. Further, non-linear and linear two-parameter models provided very accurate estimates for *W′* if two time to exhaustions of a duration between 12 and 20 min were considered (Mattioni Maturana *et al.*, 2018).

The development of protocols which allow the determination of CP and *W′* within one single visit in order to increase its practicality have gained growing interest. Brickley *et al.* (2007) reported 11% higher power output at the end of a single visit 90 s all-out isokinetic test compared to CP determined from the conventional method. Because 90 s was too short to achieve an end power equal to CP (Dekerle *et al.*, 2006), Burnley *et al*. (2006) extended the duration to 3 min which allowed a reliable levelling out of the power profile in the last 30-60 s at a power output close to where the boundary between the heavy and severe intensity domains would be expected; while still reaching $\dot{V}O_{2peak}$. Vanhatalo *et al.* (2007) found no difference between mean power output during the last 30 s of a 3 min all-out test (end power; EP) compared to CP. Work done above EP (WEP) was not different to and highly correlated with *W′* (Vanhatalo *et al.*, 2007). However, WEP was lower than *W′* in six out of ten participants which may indicate that the 3 min all-out test was too short to fully deplete *W′*

(Vanhatalo *et al.*, 2007). The 3 min all-out test has since been used in a variety of studies during which its sensitivity to detect changes in EP following a 4 week interval training (Vanhatalo *et al.*, 2008b) as well as its robustness to changes in the power profile has been demonstrated (Vanhatalo *et al.*, 2008a).

In general, contrasting findings were reported within the literature when comparing EP and WEP derived from the 3 min all-out test to CP and W' derived from the traditional protocol as summarized in Table 2.3. Briefly, EP appears to be a reliable and sensitive measure (Wright *et al.*, 2017; Karsten *et al.*, 2014; Johnson *et al.*, 2011; Vanhatalo *et al.*, 2008a; 2008b; 2007), however, its validity to estimate CP has been questioned (Wright *et al.*, 2017; Dekerle *et al.*, 2014; Bergstrom *et al.*, 2014; Karsten *et al.*, 2014). WEP has been demonstrated to be sensitive to changes in cadence (Dekerle *et al.*, 2014; Vanhatalo *et al.*, 2008a), however, the 3 min all-out test appears to consistently underestimate W', in both, the isokinetic and linear mode by ~10-40% compared to the conventional protocol and thus, it cannot be considered as a valid estimate of W' (Wright *et al.*, 2017; Bergstrom *et al.*, 2014; Dekerle *et al.*, 2014; Karsten *et al.*, 2014).

Table 2.3. Comparisons between the estimates of CP and *W′* with EP and WEP derived from the 3 min all-out test

	Participants	Cycle ergometer	Study design	EP	WEP
Wright *et al.* (2017)	12 male cyclists	Lode Excalibur Sport - isokinetic and linear mode	EP vs. CP WEP vs. *W′*	EP: 241 ± 23 (isokinetic) vs. 276 ± 41* (linear) vs. CP: 245 ± 26 W	WEP: 15.6 ± 5.6* (isokinetic) vs. 13.5 ± 4.7* (linear) vs. *W′* 22.7 ± 5.6 kJ
Bergstrom *et al.* (2014)	9 recreationally trained (5 women)	Lode cycle ergometer – linear mode	EP vs. CP WEP vs. *W′*	EP: 196 ± 49 vs. CP: 184 ± 43$^{\$}$ (P-1/t model) vs. 181 ± 42$^{\$}$ (W-t model) vs. 176 ± 40 W$^{\$}$ (P-t model)	WEP: 10.4 ± 2.6 vs. *W′*: 11.4 ± 6.1 (P-1/t model) vs. 12.2 ± 5.8 (W-t model) vs. 14.6 ± 5.5 kJ$^{\$}$ (P-t model)
Karsten *et al.* (2014)	13 trained cyclists (1 woman)	SRM - isokinetic mode	EP vs. CP WEP vs. *W′*	EP: 290 ± 41 vs. CP: 253 ± 41$^{\$}$ (W-t model) vs. 259 ± 38 W$^{\$}$ (P-1/t model)	WEP: 12.5 ± 4.3 vs. *W′*: 18.6 ± 4.8$^{\$}$ (W-t model) vs. 16.6 ± 4.8 kJ$^{\$}$ (1/t model)
Bergstrom *et al.* (2012)	12 moderately trained (6 women)	Quinton Corval 400 – linear mode	EP vs. CP WEP vs. *W′*	EP: 193 ± 54 vs. CP: 178 ± 47 W$^{\$}$	WEP: 10.9 ± 2.9 vs. *W′*: 13.4 ± 6.2 kJ
Vanhatalo *et al.* (2008a)	8 men + 1 woman	Lode Excalibur Sport - linear mode	Cadence: 88 ± 6 (Control) vs. 77 ± 5 (Low) vs. 95 ± 7 rpm (High)	Control: 254 ± 40 W Low: 251 ± 38 W High: 244 ± 41 W$^{\#}$	Control: 14.2 ± 3.7 kJ Low: 16.2 ± 4.4 kJ$^{\#}$ High: 12.9 ± 3.6 kJ$^{\#}$
Wright *et al.* (2019)	10 trained male cyclists	Lode Excalibur Sport - linear mode	Cadences ranging from pref -5 to pref +10 rpm	CP vs. EP pref: 268 ± 23 vs. 297 ± 26 W* vs. EP-5: 304 ± 24W* vs. EP+5: 290 ± 28 W* vs. EP+10: 279 ± 31 W	*W′* vs. WEP pref: 20.5 ±.5.1 vs. 11.2 ± 4.5 kJ* *W′* vs. WEP-5: 12.6 ± 4.0 kJ* *W′* vs. WEP+5: 11.0 ± 4.4 kJ* *W′* vs. WEP+10: 10.9 ± 4.8 kJ*
Dekerle *et al.* (2014)	9 active men	SRM – isokinetic mode	Cadence: 60 vs. 100 rpm	EP_{60}: 259 ± 40 vs. CP_{60}: 245 ± 38 W EP_{100}: 227 ± 57 vs. CP_{100}: 212 ± 44 W$^{\#}$	WEP_{60}: 14.7 ± 3.0 vs. W'_{60}: 16.2 ± 3.5 kJ WEP_{100}: 17.3 ± 3.1 vs. W'_{100}: 20.6 ± 6.4 kJ$^{\#}$
de Lucas *et al.* (2014)	14 healthy, physically active men	Lode Excalibur Sport - isokinetic	Cadence: 60 vs. 100 rpm	EP_{60} 197 ± 39 W EP_{100} 160 ± 44 W$^{\#}$	WEP_{60} 16.3 ± 14.1 kJ WEP_{100} 17.4 ± 2.9 kJ
Vanhatalo *et al.* (2008b)	9 habitually active (1 woman)	Lode Excalibur Sport - linear mode	4 weeks high-intensity training	Pre-training EP: 225 ± 52 vs. CP: 230 ± 53 W Post-training EP: 248 ± 46 vs. CP: 255 ± 50 W	Pre-training WEP: 16.7 ± 3.5 vs. *W′*: 17.2 ± 4.2 kJ Post-training WEP: 17.3 ± 3.3 vs. *W′*: 15.5 ± 3.8 kJ

*significantly different from estimates derived from the traditional model (i.e. CP and *W′*) ($p < 0.05$); $^{\$}$significantly different from estimates derived from the 3 min all-out test (i.e. EP and WEP); $^{\#}$significant difference between conditions / significantly different from control ($p < 0.05$); pref, preferred.

Considering the accuracy of the two-parameter model to estimate CP and *W′*, if appropriate power outputs and therefore, durations are selected for the prediction trials, the two-parameter models remain the most commonly used critical power models in sport and exercise physiology. Moreover, the CP concept allows to accurately separate exercise intensities during which a physiological homeostasis is achieved from exercise intensities during which no steady state is achieved (Jones *et al.*, 2019). Consequently, the two-parameter models allow to identify the boundaries of the severe intensity domain and therefore, represents a potent framework to study exercise tolerance within the severe intensity domain.

2.1.2.3. Methodological considerations when modelling the power-time relationship using the two-parameter model

The following section will review different aspects which may affect the calculation of estimates for CP and *W′* and therefore, require consideration when determining the severe intensity domain.

Number of times to task failure

The number of points (i.e. TTF) considered when modelling CP and *W′* using the two-parameter models, influences the accuracy of the estimates (i.e. standard error and confidence interval). An overview of numbers of TTF used within the literature and associated SE for CP and *W′* are summarized in Table 2.4.

Originally, three TTF trials were used for small muscle groups (Monod & Scherrer, 1965) and for whole-body exercise (Moritani *et al.*, 1981). The literature provides studies ranging from as little as two severe intensity exercise trials (Parker Simpson & Kordi, 2017) to as many as seven trials for the determination of CP and *W′* (Gaesser *et al.*, 1995), however, typically, three to five TTF are performed on separated days (Jones & Vanhatalo, 2017). Hill (1993) suggested that trained individuals, familiar to exhaustive exercise may only require as few as two trials Similarly, Parker Simpson and Kordi (2017) demonstrated that two exhaustive trials of 3 min and 12 min following two familiarisation sessions allow the reliable assessment of CP and *W′* in trained cyclists and therefore, may provide a time-efficient alternative test protocol for practitioners. However, the authors also highlighted that if performing only two exhaustive trials, the linear relationship between power and time will be ‘perfect’ and neither the determination of the goodness of fit of the linear relationship nor the errors associated with CP and *W′* can be determined (Parker Simpson & Kordi, 2017). Moreover, if only two exhaustive trials are used, the weighing of each is high, and an underperformance in one trial may have a greater effect on the CP and *W′* compared to the inclusion of three or more trials (Housh *et al.*, 1990). . More recently, Mattioni Maturana *et al.* (2018) reported

an overestimation of CP and an underestimation of *W′* if only two TTF of a duration < 12 min were selected. The authors stated that as few as two TTF may only be used under field conditions, where the feasibility of several predictive trials proves to be rather difficult, however, several predictive trials should be used in research studies to ensure maximal accuracy.

Collectively, dependent on the population and the priority of the task (feasibility vs. accuracy), a different amount of TTF may be appropriate. For athletes, the assessment of CP and *W′* from two TTF may be adequate for training purposes and advantageous due to its time-efficiency and the provision of reliable estimates if test durations of 3 min and 12 min are selected (Parker Simpson & Kordi, 2017). However, to allow the calculation of the accuracy of the estimates of CP and *W′* (i.e. SE), at least three TTF are required. Additional TTF may be considered to improve the fit of the model, thus decrease the SE associated with the estimates (Black *et al*., 2015).

Duration of times to task failure

The duration of TTF can substantially impact the estimates for CP and *W′* (Mattioni Maturana *et al.*, 2018; Jenkins *et al.*, 1998). An overview of durations for TTF used within the literature and associated SE for CP and *W′* are summarized in Table 2.4. Commonly, durations between ~2-15 min have been recommended (Hill, 1993; Poole *et al.*, 1988) and widely used (de Souza *et al.*, 2016; Burnley *et al.*, 2012; Dekerle *et al.*, 2009; Jones *et al.*, 2008; Carter *et al.*, 2005). Studies using shorter (Heubert *et al.*, 2005; Clingeleffer *et al.*, 1994) or longer durations (Sawyer *et al.*, 2014; Pringle *et al.*, 2011) may risk incorporating exercise intensities outside the range of the severe intensity domain and as a consequence over- or underestimate CP and *W′*. Durations should be selected to allow the attainment of $\dot{V}O_{2max}$ (Di Prampero, 1999), which commonly occurs 2-3 min following exercise onset (Poole *et al.*, 1988). Very short exercise durations (≤ 2-3 min) have been associated with aerobic inertia (Bishop *et al.*, 1998), whereas prolonged durations (≥ 15-20 min) may not allow participants to attain their $\dot{V}O_{2max}$ (Morton & Billat, 2000) and may be impacted by muscle glycogen depletion, reduced motivation and/or dehydration (Hill & Smith, 1999) and thus, should be avoided.

To confidently identify the severe intensity domain, one must ensure to select durations which lead to a full depletion of *W′* and the attainment of $\dot{V}O_{2max}$. Therefore, power outputs which are confidently above CP (+ 95% CI) and of durations > 2-3 min are recommended.

Table 2.4. Summary of exercise protocols used to assess CP and *W′* and associated standard error of the estimates (SE)

	Number of trials	Exercise mode	Range of times	CP (W) Mean (SD)	SE-CP (%)	*W′* (kJ) Mean (SD)	SE-*W′* (%)
Dekerle *et al.* (2015)	4-5	TTF	~2 to 15 min	208(19)	1.2	21.4(4.2)	5.1
Mattioni Maturana *et al.* (2018)	3-5	TTF	~2 to 19 min	W-t: 265(47;3); 259(44;4); 256(45;5)[*] P-1/t: 263(45;3); 268(47;4); 261(45;5)[*] P-t: 263(45;3); 256(42;4); 253(44;5)[*]	W-t: 2.3(3); 1.5(4); 0.90(5)[#] P-1/t: 2.9(3); 2.2(4); 1.7(5)[#] P-t: 1.9(3); 1.1(4); 0.63(5)[#]	W-t: 15.9(5.7;3); 17.1(5.7;4); 7.9(5.7;5)[*] P-1/t: 15.2(6.0;3); 15.8(5.9;4); 16.1(6.0;5)[*] P-t: 16.4(5.7;3); 18.7(6.7;4); 20.3(5.9;5)[*]	W-t: 10.1(3); 9.9(4); 8.4(5)[#] P-1/t: 7.2(3); 6.3(4); 5.0(5)[#] P-t: 9.8(3); 10.2(4); 8.4(5)[#]
Sawyer *et al.* (2014)	4	TTF	~2 to 15 min	W-t: 162(24) P-1/t: 173(25) P-t: 149(21)	W-t: 6.1 P-1/t: 6.0 P-t: 6.4	14.5(5.8) 10.6(6.4) 22.7(8.0)	W-t: 37.2 P-1/t: 17.9 P-t: 32.2
Black *et al.* (2017)	4-5	TTF	~2 to 14 min	W-t: 253(54) P-1/t: 252(52) P-t: 248(52)	W-t: 2.6 P-1/t: 3.0 P-t: 2.2	W-t: 22.5(5.3) P-1/t: 20.7(5.2) P-t: 22.4(3.8)	W-t: 11.0 P-1/t: 9.5 P-t: 11.3
Black *et al.* (2015)	3-4	TTF	~2 to 15 min	W-t: 248(44) P-1/t: 251(47) P-t: 247(43)	W-t: 1.7 P-1/t: 1.9 P-t: 1.9	W-t: 21.4(7.0) P-1/t: 19.1(5.1) P-t: 20.6(6.0)	W-t: 9.5 P-1/t: 7.3 P-t: 12.4
	3-4	TT (linear mode)	matched for total work from TTF	W-t: 265(45) P-1/t: 265(44) P-t: 264(45)	W-t: 2.1 P-1/t: 2.3 P-t: 2.6	W-t: 18.5(6.8) P-1/t:18.0(5.7) P-t: 18.4(8.4)	W-t: 12.3 P-1/t: 8.9 P-t: 20.7
Coakley & Passfield (2018)	3	TTF	~2 to 14 min	W-t: 268(196-347)[$] P-1/t: 277(207-347)[$] P-t: 267(194-347)[$]	W-t: 4.8 P-1/t: 4.4 P-t: 5.1	W-t: 9.5(6.0-18.2)[$] P-1/t: 9.5(6.0-16.1)[$] P-t: 10.0(6.0-29.1)[$]	W-t: 11.3 P-1/t: 11.6 P-t: 16.9
	3	TT	matched for time from TTF	W-t: 259(190-337)[$] P-1/t: 261(198-342)[$] P-t: 258(189-336)[$]	W-t: 4.8 P-1/t: 4.8 P-t: 4.8	W-t: 11.7(9.4-17.6)[$] P-1/t: 11.6(6.0-18.2)[$] P-t: 11.9(8.9-20.0)[$]	W-t: 6.9 P-1/t: 8.6 P-t: 7.5
Karsten *et al.* (2018)	3 3	TTF TT	~3 to 11 min 3, 7 and 12 min	279(52) 276(50)	TTF: 2.4 TT: 1.2	14.8(3.4) 16.3(4.3)	TTF: 11.2 TT: 5.6
Triska *et al.* (2017)	3	TT	3, 7 and 12 min	302(28)	1.3	15.0(3.1)	7.3
Parker Simpson & Kordi (2017)	3	TT	3, 5 and 12 min	282(65)	0.44	18.3(6.29)	5.8

*standard deviation and the number of trials used to estimate CP and *W′* in brackets (i.e. 47; 3); [#]number of trials in brackets; [$]reported as median(range)

Exercise mode: time to task failure vs. time trial

Methodological constraints associated with the selected type of performance task (i.e. TT, TTF, TTE) may considerably influence the performance outcome and therefore the estimates for CP and *W′* (see Table 2.4.). Time trials represent real-life performance with fluctuations in power output throughout exercise and have a clearly defined endpoint (closed-loop); either participants attempt to cover a given distance as fast as possible or accumulate as much work as possible within a given time. In contrast, constant-load performance tests do not allow participants to adapt pacing strategies and have no known endpoint (open-loop). The literature often refers to open-loop performance tests as 'time to exhaustion' or 'time to task failure'. The latter term has been used within the present thesis because 'task failure' in contrast to 'exhaustion' can be precisely defined as a failure to maintain a task/criterion (e.g. cadence).

Traditionally and until today most commonly, TTFs are used to determine CP and *W′* (Mattioni Maturana *et al.*, 2018; Black *et al.*, 2017; Dekerle *et al.*, 2015; Monod & Scherrer, 1965). More recently, the ecological validity of TTF has been questioned (Currell & Jeukendrup, 2008; Laursen *et al.*, 2007) and the implementation of TT for the determination of CP and *W′* has been discussed (Karsten *et al.*, 2018; Coakley & Passfield, 2018; Black *et al.*, 2015). A comparison between estimates for CP and *W′* for TT and TTF protocols and associated standard errors of the estimates are summarized in Table 2.4. Briefly, comparisons between CP derived from TT vs. TTF protocols reveal contradictory results, with higher CP (+6%) for TT reported by Black *et al.* (2015), lower CP (~ −4%) for TT reported by Coakley & Passfield (2018) and no differences in CP found by Karsten *et al.* (2018). For *W′*, Coakley & Passfield (2018) as well as Karsten *et al.* (2018) described higher *W′* for the TT protocol (~15-17%), whereas Black et al (2015) found no significant difference. These findings indicate that the estimates of the two-parameter model derived from TTF and TT may not be used interchangeably. Moreover, Black *et al.* (2015) reported slightly lower SE for CP and *W′* for TTF in comparison to TT for the best fit model, whereas Karsten *et al.* (2018) and Coakley & Passfield (2018) found comparably larger SE for *W′* for TTF for the best fit model (> 10%). Black *et al.* (2015) controlled for the standard error to be < 5% and < 10% for CP and *W′*, respectively and a fourth TTF was performed if this was not the case. This may have resulted in a better fit of the model and consequently, may explain why the authors found lower SE for *W′*.

Collectively, these results indicate that CP and *W′* derived from TT and TTF protocols cannot be used interchangeably. For the present thesis, it was of major importance to ensure that participants cycled consistently within the severe intensity domain to allow a controlled depletion of *W′* and a comparison of neuromuscular fatigue between participants and conditions. Therefore, TTF were used to ensure participants cycled at a constant exercise intensity above CP. In contrast, during TT, participants can regulate exercise intensity and therefore, may adapt pacing strategies which lead to differences in the time course of *W′* depletion between participants, but which may also result in a

full depletion prior to the end of the TT, so that participants must decrease exercise intensity to reconstitute W' and continue exercise. Further aspects regarding the behavioural component of performance tests such as TTF and TT and their challenge to ensure a full depletion of W' will be discussed in Section 2.2.5.

The reliability of TTF and the estimates of the power-time relationship is presented in the following section.

2.1.2.4. Reliability of the estimates of the power-time relationship

The reliability of a measure is crucial for a scientist to differentiate between changes originating from an intervention and changes due to measurement error.

Tables 2.5. and 2.6. provide an overview of reliability measures associated with TTF and the estimates of the power-time relationship, respectively.

Table 2.5. Reliability of time to task failure within the severe intensity domain

	Participants	Number of trials	Performance measure	Fam	Duration	CV (%)	Test-retest difference (%)	Test-retest correlation (r)
Cycling								
Krebs & Powers (1980)	10 M	2	80%$\dot{V}O_{2max}$	NA	NA	NA	~20	0.51
McLellan *et al.* (1995)	15 M	5	80%$\dot{V}O_{2max}$	NA	14-18 min	~17	NA	NA
Running								
Laursen *et al.* (2007)	8M, runners	2	at mean 1500 m TT	Yes	6-7 min	~13*	−0.18	NA
Billat *et al.* (1994)	8 M, sub-elite	2	100%$\dot{V}O_{2max}$	NA	6-7 min	~8	0.50	0.86
Laursen *et al.* (2007)	8M, runners	2	at mean 5 km TT	Yes	~18 min	~15*	−9.7	NA

Fam, familiarisation; CV, coefficient of variation (reported as the mean CV calculated from the individuals' CVs. Individuals' CVs were calculated from the standard deviation of an individual's repeated measurement, expressed as a percent of the individual's mean score; unless otherwise stated); NA, not applicable; M, male; F, female; *the authors calculated CV according to newstats.org/xrely.xls.

Table 2.6. Reliability of CP and W' for cycling exercise

	Participants	Number and mode of trials	Number of TTF and durations	Fam	CP CV (%)	Test-retest difference (%)	Test-retest correlation (r)	W' CV (%)	Test-retest difference (%)	Test-retest correlation (r)
Bulbulian *et al.* (1996)	6 F + M	2; TTF	3; 1-10 min	NA	7.6*	2.1	0.89	8.7	0.46	0.81
Bishop & Jenkins (1995)	9 F, untrained	3; TTF	3; 1-10 min	Yes	2.4;2.3*	3.4;0.6	0.99;0.99	13; 8.4	−1.8;11.1	0.64;0.88
Smith & Hill (1993)	13 M, untrained 13 F, untrained	2; TTF	5; 1-10 min	NA	5.5* 6.4*	5.4 6.0	0.92 0.90	~7.0 ~14	−1.6 1.9	0.80 0.64
Gaesser & Wilson (1988)	5 M runners, 6 M	2; TTF	5; 1-10 min	NA	2.8	3.2	0.92	7.5	2.1	0.62
Nebelsick-Gullett *et al.* (1988)	25 F	2; TTF	3; ≥1 min	NA	5.6*		0.94	11		0.87

Two CV or changes in the mean in a study of 3 trials are for consecutive pairs or trials (1+2, 2+3). Fam, familiarisation; CV, coefficient of variation (reported as the mean CV calculated from the individuals' CVs. Individuals' CVs were calculated from the standard deviation of an individual's repeated measurement, expressed as a percent of the individual's mean score; unless otherwise stated); M, male; F, female; taken from Hopkins *et al*., (2001; Table VI), where mean CV was calculated from individuals' CVs.

The amount of studies investigating reliability of TTF for cycling exercise above CP is surprisingly small. In general, the reliability of TTF in the severe intensity domain has been associated with large variations (Currell & Jeukendrup, 2008; McLellan *et al.* 1995). Hopkins *et al.* (2001) argues that larger CVs found for TTF are an artefact of the relation between power output and duration because small changes in power output have a great effect on exercise duration and suggested that constant-load tests are more reliable than other type of tests (e.g. constant-work or constant-duration tests), most likely due to removing the effect of pacing on performance.

The variations in TTF may be partly explained by the level of familiarisation of participants to the testing protocol. The meta-analysis of Hopkins *et al.* (2001) highlights the learning effect between the first two trials of a test and recommends the performance of at least one familiarisation trial.

Despite the relative large variation in TTF, the CV for CP is small (~2-8%) and slightly greater for W' (~7-14%) (see Table 2.6.). Smith & Hill (1993) found an increase in CP by 5 to 6% when determined from the second set of five TTF compared to the first set, due to improvements in the longer TTF compared to the shorter TTF. Similarly, Bishop & Jenkins (1995) reported a significant increase in CP between the first set of TTF (familiarisation trial) compared to the second and third sets of TTF (3.4 and 4.0%), with no difference between the second and third sets.

Collectively, a minimum of one familiarisation trial is highly recommended to minimize the error of the estimates for CP and W'.

2.2. Neuromuscular fatigue and the models of exercise regulation

The following section will outline neurophysiological responses to fatiguing exercise in healthy humans. More specifically, definitions and distinctions regarding the key terminology used will be provided (Section 2.2.1), followed by two sections outlining the mechanisms of and methods to assess, peripheral (Section 2.2.2) and central fatigue (Section 2.2.3). Finally, the intensity- and duration dependent development of neuromuscular fatigue (Section 2.2.4) as well as models of exercise regulation based on the interaction between central and peripheral fatigue (Section 2.2.5) will be reviewed.

2.2.1. Terminology

The term 'fatigue' has caused much controversy in the research area of sport and exercise physiology due to a lack of scientific consensus about its underlying mechanism, the diversity of methods applied for its quantification and its broad usage within the literature. A generic use of the term 'fatigue' seems problematic considering its complex, multifactorial nature.

For the purpose of the proposed thesis, fatigue will be defined as any transient exercise-induced reduction in the maximal voluntary force of the skeletal muscle (Gandevia, 2001) and is most commonly quantified by comparing the force produced during maximal voluntary contractions (MVC) before and after a fatiguing exercise (Millet et *al*., 2011a). During the performance of an MVC, both the nervous system and the muscular system are involved, which explains why the term 'neuromuscular fatigue' can be considered as most appropriate. The mechanism(s) causing neuromuscular fatigue may involve any changes within the motor pathway leading to impairments in force production, i.e. alterations in central motor drive or excitation-contraction coupling. Therefore, neuromuscular fatigue has been traditionally categorised into peripheral fatigue, originating at or distal to the neuromuscular junction and central fatigue, located proximal to the neuromuscular junction and defined as a progressive decline in central motor drive (Gandevia, 2001). Figure 2.6. illustrates the numerous processes involved in regulating force production and thus, representing potential origins of neuromuscular fatigue.

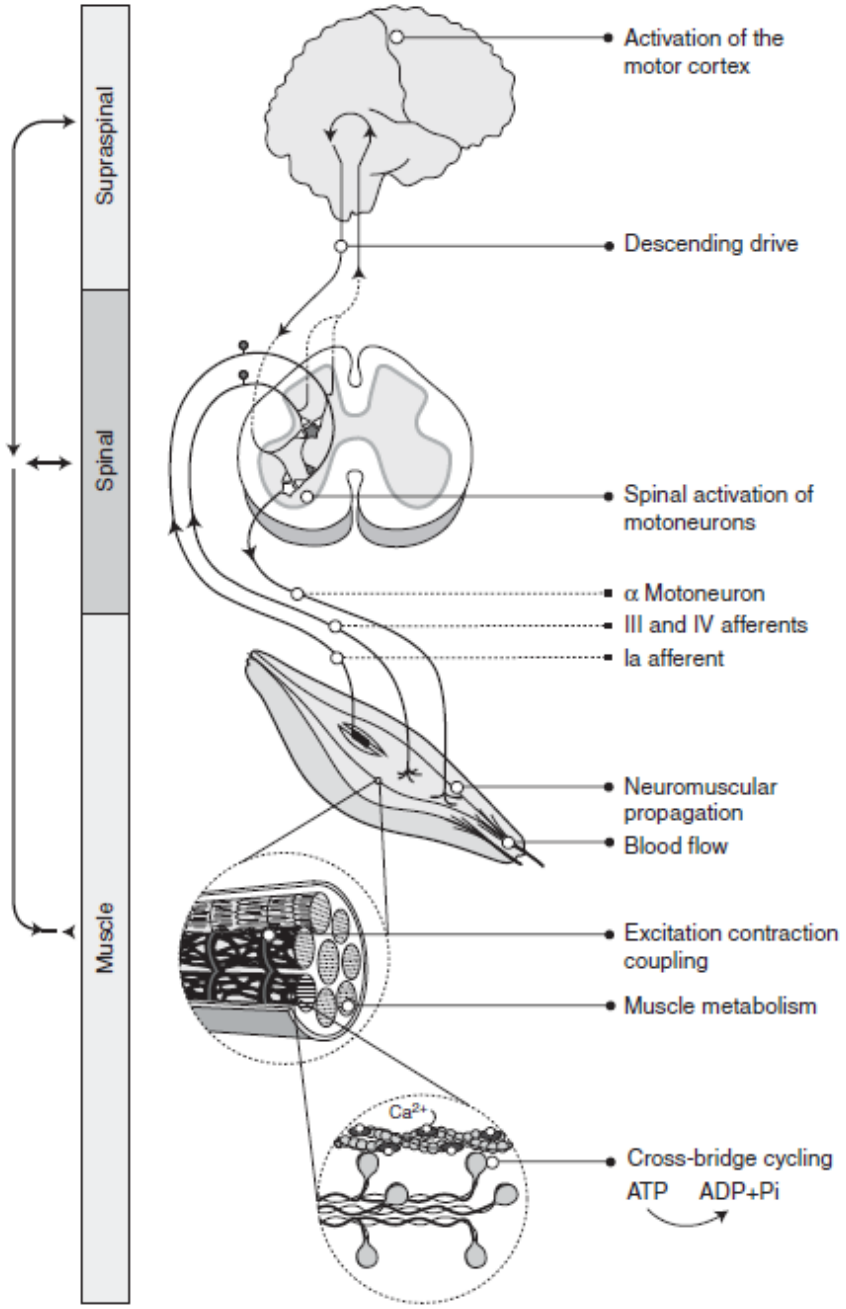

Figure 2.7. Schematic summarising the different sites of central (supraspinal and spinal) and peripheral fatigue affecting voluntary force production and potential feedback mechanisms from afferent muscle fibres (Hunter, 2017).

Different theories and approaches have been developed to understand the origin of neuromuscular fatigue and numerous mechanisms have been proposed within the literature. A description of the primary mechanisms causing exercise-induced fatigue will be outlined in the following section.

2.2.2. Peripheral fatigue

2.2.2.1. Definition

Peripheral fatigue is defined as a reduction in MVC due to processes occurring at or distal to the neuromuscular junction (Gandevia, 2001; Bigland-Ritchie *et al.*, 1979).

2.2.2.2. Mechanism of peripheral fatigue

Any process within the long chain of events involved in the excitation-contraction coupling can be altered during exercise and impair force production. Thus, numerous potential sites causing peripheral fatigue do exist which may explain why the precise mechanisms are still not fully understood.

Neuromuscular transmission

Neuromuscular transmission at the skeletal muscle is initiated by the arrival of an action potential at the distal part of the motor nerve, which induces the opening of voltage-gated calcium ion (Ca^{2+}) channels and the increase of intracellular Ca^{2+} (Catterall, 2011; Martyn *et al.*, 2009; Cohen-Cory, 2002). This results in the release of the neurotransmitter acetylcholine from the nerve ending into the synaptic cleft, acetylcholine's binding to cholinoreceptors on the sarcolemma and an influx of sodium ions into the muscle cell which subsequently, causes its depolarisation (Martyn *et al.*, 2009; Sudhof, 2004; Cohen-Cory, 2002) (see Figure 2.7.).

In vitro and *in situ* animal models demonstrated the existence of neuromuscular transmission failure following stimulation of the motor nerve with trains of pulses (Pagala *et al.*, 1984; Magleby & Pallotta, 1981), however, an accurate understanding at molecular level of how neuromuscular transmission failure evolves in the intact human remains speculative.

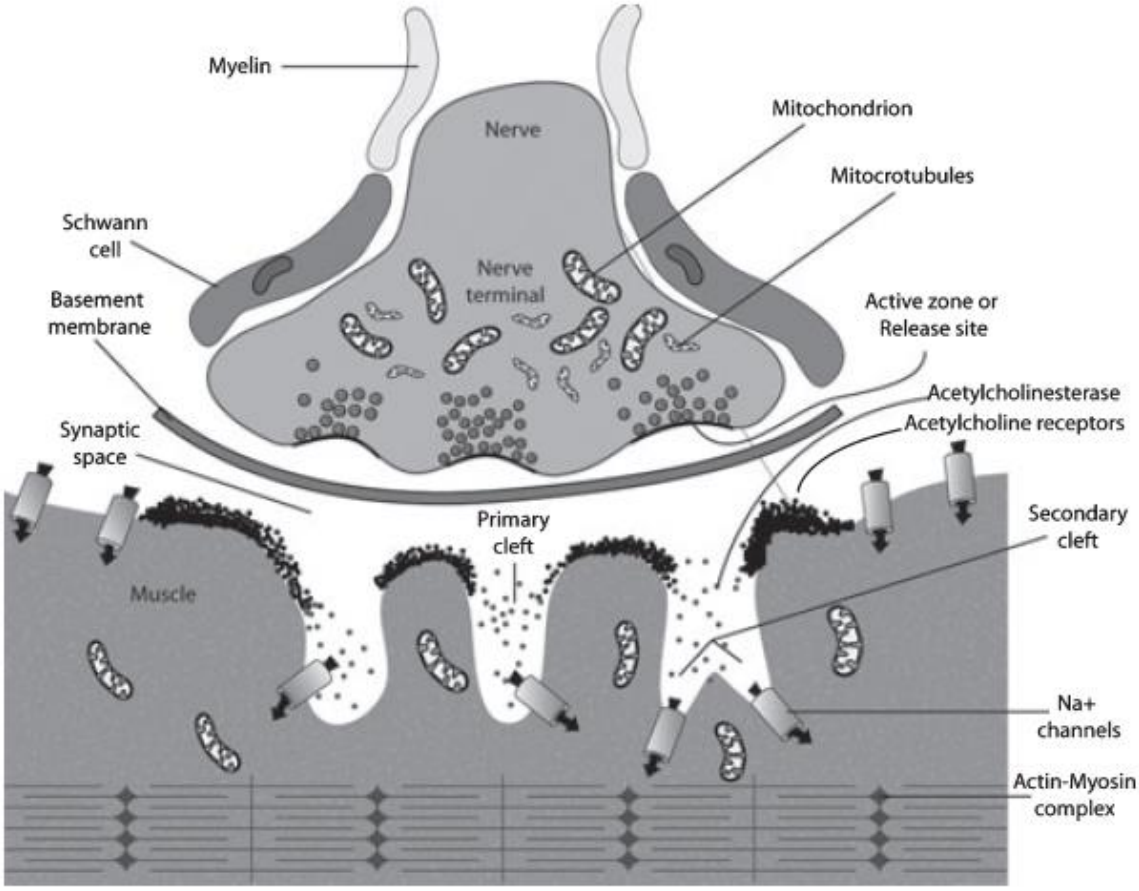

Figure 2.8. The structure of the neuromuscular junction. The figure illustrates the motor neuron and muscle fibre separated by the synaptic cleft. The muscle membrane is provided with a high density of acetylcholin receptors at the top and sodium channels at the bottom of the clefts to allow depolarisation (Martyn *et al.*, 2009).

Propagation of the muscle action potential

The action potential initiated by the binding of acetylcholine to nicotinic acetylcholine receptors (Na^+ channels) on the muscle membrane propagates longitudinally along the sarcolemma and radially into the transverse (t)- tubular system (Martyn *et al.*, 2009; Lamb, 2009; Stephenson *et al.*, 1998; Adrian *et al.*, 1969; Adrian & Peachey, 1973), deep into the muscle fibre where it triggers the opening of voltage-gated channels from the sarcoplasmic reticulum (SR) of the muscle cell to release Ca^{2+} (Fauler *et al.*, 2012). The movement of ions (i.e. Na^+, K^+, Ca^{2+}) is essential for the propagation of action potentials as this induces depolarisation of the membrane by opening voltage-gated Na^+ ion channels (Na^+ influx) and repolarisation by inactivating voltage-gated Na^+ ion channels and opening of K^+ and Cl^- channels (K^+ efflux and Cl^- influx) (Fauler *et al.*, 2012; Allen *et al.*, 2008a; Jurkat-Rott *et al.*, 2006). Alterations in ATP availability during exercise may impair the functioning of ion pumps (i.e. Na^+-K^+ pumps and in particular SR Ca^{2+} pumps) due to their reliance on energy in the form of ATP (Allen *et al.*, 2008a; Clausen, 2003; Okamoto *et al.*, 2001; Davies *et al.*, 1992).

In particular during high-intensity exercise, an elevated firing rate of the motor neuron challenges muscle homeostasis due to incomplete restoration of the membrane potential within the refractory period, the time window following the action potential and until a second action potential can be propagated (Fauler *et al.*, 2012). Specifically, extracellular K^+ increases to a critical level (> 10 mM) which reduces the trans-sarcolemmal K^+ gradient, reduces sarcolemmal excitability (the amount of current needed to elicit an action potential; Fauler *et al.*, 2012) by reducing action potential amplitude or conduction velocity and ultimately, impair the force generating capacity (Banks *et al.*, 2018; Cairns *et al.*, 1997; 1995; Cairns & Lindinger, 2008; McKenna *et al.*, 2008). Indeed, exposure to increased extracellular [K^+] accelerated the development of muscle fatigue in isolated rat soleus muscles (Clausen and Nielsen, 2007). However, a large number of studies were conducted in isolated muscle fibres and therefore, more complex physiological mechanisms may be expected to influence the ion fluxes in the working muscles. Nonetheless, there is some evidence that the accumulation of extracellular [K^+] *in vivo* is higher than previously assumed. The accumulation of extracellular [K^+] in working rat extensor digitorum longus muscles resulted in a distinct decline in membrane excitability (Clausen, 2013).

Overall, alterations in sarcolemmal or t-tubular excitability are not considered as the primary factor of peripheral fatigue; instead, metabolic changes within the muscle fibre, such as Ca^{2+} release from the sarcoplasmic reticulum as discussed in the next paragraph, are thought to be stronger candidates for the development of peripheral fatigue during exercise in humans (Allen *et al.*, 2008b).

Calcium release from the sarcoplasmic reticulum

The arrival of an action potential in the t-tubule system is detected by voltage-sensors (dihydropyridine receptors, DHPR) located on the sarcolemma, near the terminal cisternae of the SR (i.e. triad junction or triad region), which subsequently interact with the nearby ryanodine receptors (RyR) located on the SR and trigger the release of Ca^{2+} from the SR (Allen *et al.*, 2008b; Favero, 1999; Melzer *et al.*, 1995). The triad junction is an area of high ATP demand due to a large density of Ca^{2+} pumps on the SR and Na^+-K^+ pumps in the t-system; both requiring ATP for their functioning (Allen *et al.*, 2008b; Clausen, 2003; Okamoto *et al.*, 2001; Davies *et al.*, 1992). The role of Ca^{2+} and its contribution to a decline in the force generating capacity has been central to many debates on the cause underlying peripheral fatigue (Allen *et al.*, 2008b). The release of Ca^{2+} from the SR and its binding to troponin enables cross-bridge formation and thus, plays a major role in maintaining muscle function. However, the precise mechanisms through which Ca^{2+} release is impaired remain unclear. It has been suggested that Ca^{2+} release from the SR may be impaired by changes in voltage sensor activation, RyR or alterations in metabolite concentrations (Allen *et al.*, 2008b). In principle, any

alteration in the chain preceding the release of Ca^{2+} from the SR could be affected and ultimately, either inhibit SR Ca^{2+} release and/or Ca^{2+} binding to troponin.

Lamb *et al.* (1995) demonstrated that structural alterations of the triad junction may impair excitation-contraction coupling by preventing the coupling between DHPR and RyR, thus inhibiting release of Ca^{2+} from the SR (Figure 2.8.). Moreover, a progressive depletion of cellular ATP during exercise may be detected within the triad junction by preventing the ATP-dependent SR Ca^{2+} pump activity and consequently causing a failure or reduction of SR Ca^{2+} release (Balog, 2010; Allen *et al.*, 2008b). Chin and Allen (1997) studied force recovery under full and reduced muscle glycogen concentration and demonstrated that a prolonged depression in Ca^{2+} release was partly associated with glycogen dependent processes. Furthermore, not only energy depletion *per se*, but also an increase in the products of ATP hydrolysis (i.e. ADP, AMP, P_i) may considerably alter Ca^{2+} release (Sahlin et al, 1998). An association between P_i accumulation and a reduction in free Ca^{2+} has been suggested (Fauler *et al.*, 2012; Allen & Trajanovska, 2012; Allen *et al.*, 2011; Dutka *et al.*, 2005; Allen & Westerblad, 2001). The anaerobic metabolism relies on the hydrolysis of phosphocreatine (PCr) to creatine and P_i to generate ATP, the main energy source for the majority of cellular functions. During intense exercise, P_i accumulates and is transported into the SR, where the releasable pool of Ca^{2+} is reduced, either by Ca^{2+} - P_i precipitation in the SR or through a direct inhibition of RyR channels (Fauler *et al.*, 2012; Allen & Trajanovska, 2012; Allen *et al.*, 2011; Dutka *et al.*, 2005; Allen & Westerblad, 2001).

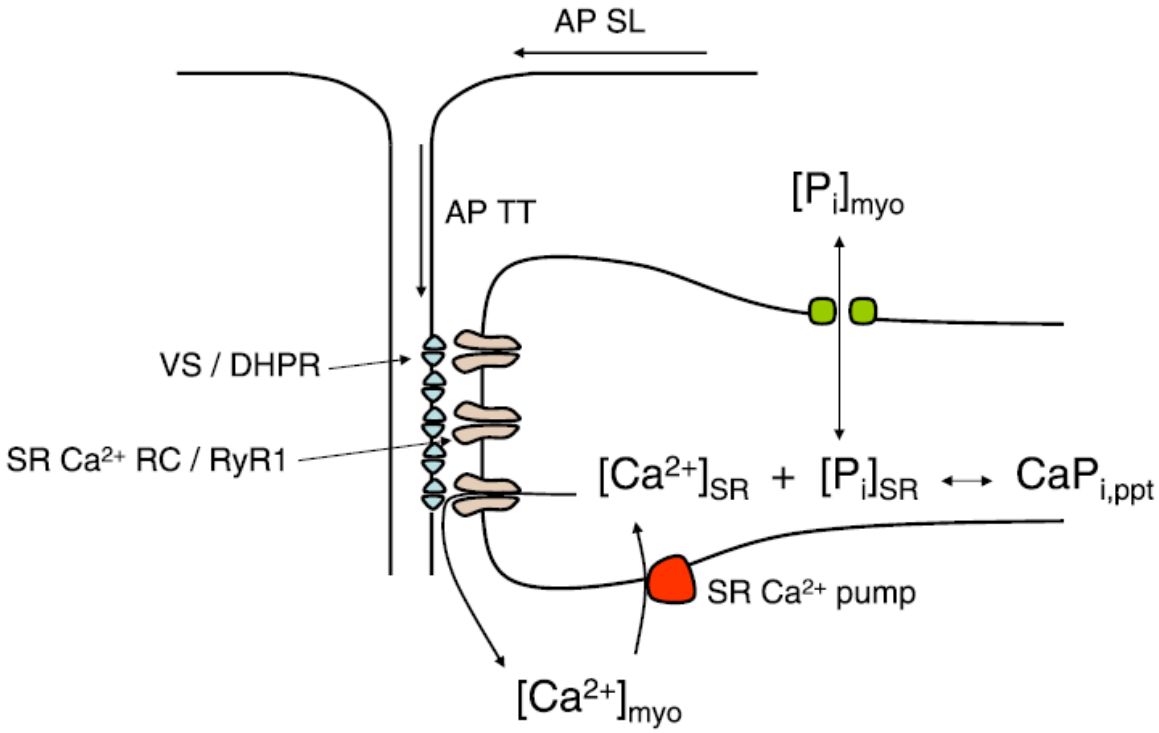

Figure 2.9. The interaction between the t-tubule system and the sarcoplasmic reticulum. The figure illustrates the propagation of action potentials along the sarcolemma into the t-tubule system, where the coupling between the voltage-sensors or dihydropyridine receptors (VS/DHPR) in the t-tubule system and the SR Ca^{2+} release channels (SR Ca^{2+} RC), also known as ryanodine receptors (RyR) causes the release of Ca^{2+} and a subsequent increase in myoplasmic calcium concentration ($[Ca^{2+}]_{myo}$). Also shown is a channel in the longitudinal SR which is phosphate permeable and allows equilibration of the myoplasmic P_i ($[P_i]_{myo}$) with the SR P_i ($[P_i]_{SR}$). When the solubility product is exceeded calcium phosphate precipitates in the SR ($CaP_{i,ppt}$) (Allen *et al.*, 2008b).

The cross bridge cycle

Following the Ca^{2+} release from the SR into the cytosol, Ca^{2+} binds to the thin filament troponin which induces the tropomyosin movement, reveals the site of actin to which myosin binds and allows the formation of strong cross-bridges to generate force (Tanner *et al.*, 2012; Gordon *et al.*, 2000). Impairments of the cross-bridge cycle may include reduced Ca^{2+} sensitivity and or dysfunction of the contractile proteins (Debold, 2012).

For many decades, the accumulation of hydrogen ions (H^+) as a result of ATP hydrolysis and the anaerobic breakdown of glycogen (see Section 2.1.1.1.) has been controversially discussed as a major cause of peripheral fatigue. Due to early work on isolated muscle fibres, it was believed that acidosis inhibits muscle function (Fabiato & Fabiato, 1978; Metzger & Moss, 1987). This link has been further supported by a temporal congruent decline in force and pH during exercise (Dawson *et al.*, 1978). However, later research suggested that the effect of acidosis on muscle function have been overestimated, as early studies on skinned muscle fibres were traditionally performed at temperatures

<20°C and similar results for physiologically more realistic temperatures could not be confirmed (Westerblad *et al.*, 1997a; Pate *et al.*, 1995). Furthermore, force and pH recovered differently over time after a bout of intense exercise (Cady *et al.*, 1989; Sahlin & Ren, 2017). Although it is generally accepted that acidosis may not be the primary cause of peripheral fatigue, evidence has suggested that it may have indirect effects on the muscle function by interfering the cross-bridge cycle by reducing Ca^{2+} sensitivity of troponin (Stackhouse *et al.*, 2001; Ball *et al.*, 1994) or decreasing the SR Ca^{2+} release as presented in the previous section (Westerblad & Allen, 1991; Stackhouse *et al.*, 2001). Debold *et al.* (2008) reported that low pH reduced actin filament velocity due to a slower rate of ADP release from myosin and an increased amount of non-productive actomyosin interactions. Similarly, a direct effect of acidosis on the actomyosin interaction and as a consequence, a reduced velocity of detachment and possibly also reduced attachment rate from actin was found by Longyear *et al.* (2014).

The impact of reactive oxygen species (ROS) on peripheral fatigue has been investigated in a progressing number of studies. Increased oxygen uptake and mitochondrial activity during exercise has been associated with an accelerated production of ROS (Ferreira & Reid, 2008; Alessio *et al.*, 2000; Steinberg *et al.*, 2007). Different sites within the excitation-contraction coupling, such as Na^+-K^+ pumps, contractile proteins and in particular, Ca^{2+} sensitivity may be susceptible for ROS, which ultimately, could impair muscle function (Bruton *et al.*, 2008; Moopanar & Allen, 2005; Andrade *et al.*, 2001; Andrade *et al.*, 1998). An increased production of reactive oxygen/nitrogen species (ROS/RNS) during intense and prolonged exercise has been associated with reduced SR Ca^{2+} release and/or myofibrillar Ca^{2+} sensitivity (Cheng *et al.*, 2016; Place *et al.*, 2015). The mechanisms through which ROS affect muscle function remains unclear, however, it has been indicated that the discharge frequency of group IV muscle afferents may be affected (Delliaux *et al.*, 2009).

2.2.2.3. Measuring peripheral fatigue

Even though changes in MVC from pre- to post-exercise indicate the existence of neuromuscular fatigue, additional techniques, such as the application of electrical or magnetic stimulation either directly to the muscle or to the corresponding motor nerve, are required to localise neuromuscular fatigue more precisely. In regards to peripheral fatigue, this procedure allows to by-pass influences from the central nervous system (CNS) and to ascertain muscular contractile properties exclusively. Most commonly, a muscle twitch is evoked by delivering supramaximal stimulation to the motor nerve of a resting muscle. Changes in evoked twitch force give an indication of the site of muscular impairment (Merton, 1954).

A twitch force can be evoked from the knee extensor muscles (i.e. the quadriceps femoris), which contribute substantially to force production during locomotor exercise and in particular to power production during cycling, by stimulating the femoral nerve as first proposed by Polkey *et al.* (1996). The amplitude of the twitch force is affected by prior activation, which can reduce (fatigue) or enhance (potentiation) the contractile response (Rassier & MacIntosh, 2000). The mechanism underlying potentiation effects is not fully understood, but likely due to an increased Ca^{2+} concentration during muscle activation and an increased Ca^{2+} sensitivity of the contractile proteins (Rassier & MacIntosh, 2000). Potentiated twitch force has been demonstrated to be as reproducible as unpotentiated twitch forces and to be more sensitive for the detection of early muscle fatigue (Kufel *et al.*, 2002). The co-existence of both fatigue and potentiation may complicate the interpretation of changes in twitch force. Therefore, it is essential to assess fatigue under maximally potentiated conditions (Kufel *et al.*, 2002). It has been recommended to perform at least two conditioning contractions of 5-10s at an intensity of 70-100% MVC prior to any recordings of evoked twitch (Rodriguez-Falces *et al.*, 2015; Froyd *et al.*, 2013; Kufel *et al.*, 2002) and the first two measurements are typically discarded (e.g. Amann *et al.*, 2013; Romer *et al.*, 2007).

Reductions in the potentiated quadriceps force (Q_{pot}) following exercise indicate the existence of peripheral fatigue, however, numerous sites at or distal to the neuromuscular junction as outlined in Section 2.2.2.2 may contribute to the impaired force-generation capacity and therefore, additional measures are required to decipher the underlying mechanism(s) more precisely.

The Q_{pot} can be further analysed for within-twitch characteristics, such as contraction time (CT), maximal rate of force development (MRFD), maximal rate of relaxation (MRR) and one-half relaxation time (HRT). These additional measures reveal changes in muscle shortening velocity (CT and MRFD) and muscular relaxation (MRR, HRT). Prolonged CT is believed to indicate changes in SR Ca^{2+} release (Klitgaard *et al.*, 1989; Klug *et al.*, 1988) and reduced MRFD has been associated with a reduced rate of cross-bridge formation (Stein & Parmiggiani, 1981; Drachman & Johnston, 1973). Reduced MRR has been linked to decreases in the maximal rate of cross-bridge kinetics (Westerblad *et al.*, 1997b; Jones, 2010), whereas prolonged HRT has been associated with impaired reuptake of Ca^{2+} to the SR (Klug *et al.*, 1988) and a reduced rate of cross-bridge detachment (Jones, 2010; Westerblad *et al.*, 1997b).

In addition, investigating the force-frequency relationship may reveal further insights into the mechanisms underlying peripheral fatigue (Figure 2.9.). The force-frequency relationship describes the sigmoidal relationship between the frequency of an electrical stimuli and the force it evokes when stimulating a muscle or motor nerve (Macefield *et al.*, 1996) (Figure 2.10.).

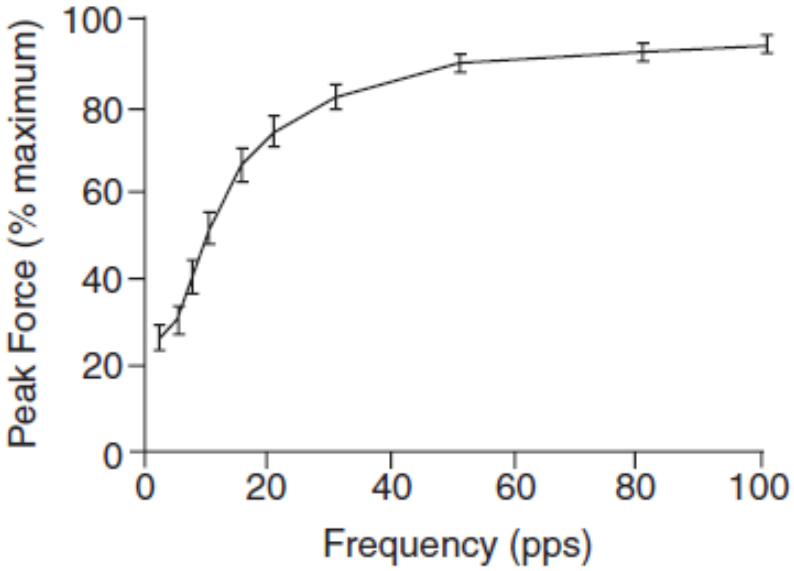

Figure 2.10. Relation between stimulus frequency in pulse per second (pps) and the normalised peak force expressed as percentage of maximum value in toe extensor (Macefield *et al.*, 1996; modified by Heckman & Enoka, 2012).

Low-frequency fatigue (LFF), defined as a reduction in force evoked by low-frequency stimulations, accompanied by a delayed force recovery, was first described by Edwards *et al.* (1977). It is hypothesised that LFF indicates an impairment of SR Ca^{2+} release and excitation-contraction coupling (Keeton & Binder-Macleod, 2006). In contrast, reductions in force generated by high-frequency stimulations are associated with attenuations in action potential propagation due to an accumulation of extracellular $[K^+]$ (Jones, 1996). Generally, the force-frequency relationship is examined by delivering paired stimuli at low (e.g. 10-30 Hz) and high frequencies (e.g. 80-100 Hz), which in comparison to tetanic stimulations alleviate the discomfort (Millet *et al.*, 2003) and reduce the degree of random variation and the influence of potentiation compared to single stimulations (Blacker *et al.*, 2013).

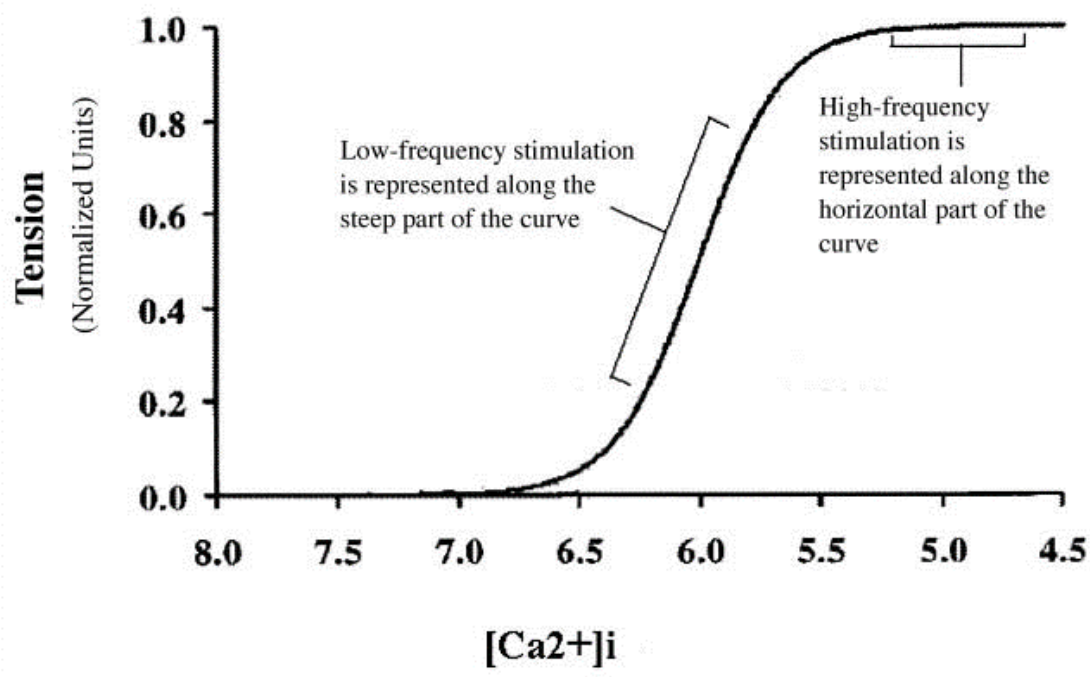

Figure 2.11. A hypothetical $[Ca^{2+}]_i$-tension curve. During low-frequency stimulation, moderate falls in $[Ca^{2+}]_i$ cause distinct reductions in force production as reflected by the steep part of the $[Ca^{2+}]_i$-tension curve. In contrast, high-frequency stimulations are located at the plateau of the curve, so that changes in $[Ca^{2+}]_i$ have only minimal effect on tension development (Keeton & Binder-Macleod, 2006).

Finally, changes in membrane excitability during fatiguing exercise are reflected in changes of the compound muscle action potential (M-wave), which represents a change in EMG following supramaximal stimulation (Place *et al.*, 2010; Millet *et al.*, 2011a; Millet & Lepers, 2004). Most commonly M-wave amplitude and M-wave area are reported, with the amplitude being suggested as a better indicator of transmembrane potential (Rodriguez-Falces & Place, 2018). Reductions in twitch force without changes in M-wave properties (M-wave area and amplitude) indicate that peripheral disturbances must be located distal to the sarcolemma (Allen *et al.*, 2008a).

2.2.3. Central fatigue

2.2.3.1. Definition

Central fatigue can be defined as a progressive reduction in voluntary activation or central motor drive to the muscle during exercise, causing a reduction in the force generating capacity (Gandevia, 2001; Taylor *et al.*, 2006; Place *et al.*, 2010).

2.2.3.2. Mechanism of central fatigue

In comparison to peripheral fatigue, the mechanism(s) underlying central fatigue are less well understood. The central mechanisms contributing to force reductions are complex and can arise at a supraspinal and spinal level (Gandevia, 2001) (see Figure 2.6.).

Supraspinal fatigue

Supraspinal fatigue has traditionally been described as a suboptimal output from the motor cortex, insufficient to activate all motor units to produce maximal force and is suggested to contribute to the development of central fatigue (Gandevia *et al.*, 1996; Taylor & Gandevia, 2008). Supraspinal fatigue may be caused by processes which either (1) reduce the descending output from the motor cortex, due to changes in the input to or the properties of corticospinal neurons, and/or by processes which (2) reduce the efficacy of the output from the motor cortex in producing force, due to alterations in motor neuron behaviour and muscle contractile properties (Taylor & Gandevia, 2008; Taylor *et al.*, 2006).

Increases in cortical excitability and in intracortical inhibition were reported during a sustained MVC (Sidhu *et al.*, 2009; Taylor *et al.*, 1996; McKay *et al.*, 1996; Mills & Thomson, 1995) and during sustained cycling exercise (Weavil *et al.*, 2015; Sidhu *et al.*, 2013), which may contribute to supraspinal fatigue by impairing the voluntary descending drive. However, changes in voluntary activation can be found regardless of these alterations in the cortex and thus, other factors must be involved (Taylor *et al.*, 2000; Gandevia *et al.*, 1996). Gandevia *et al.* (1996) found no recovery in supraspinal fatigue (i.e. central motor drive) when the muscle was held ischaemic following fatiguing exercise which indicates that peripheral alterations may impair central motor drive via afferent feedback. Indeed, pharmacological blockade of metabosensitive group III/IV muscle afferents confirmed that metabolic alterations are detected by group III/IV muscle afferents which indirectly reduce the central motor drive via a negative feedback loop (Amann, 2011). Moreover, reductions in the motor cortex output have been linked to reduced cerebral O_2 availability in severe hypoxia (Goodall *et al.*, 2012; 2010) and to limited cerebral blood flow and thus, O_2 delivery to the brain during vigorous exercise (Rasmussen *et al.*, 2010; Nybo & Rasmussen, 2007).

Spinal fatigue

Fatigue arising at the spinal level has been associated with alterations in excitatory and inhibitory processes which affect the spinal motor neuron discharge rate and ultimately, reduce muscle force (Gandevia *et al*., 1996; 1998). However, McNeil *et al.* (2011) reported that changes in the intrinsic properties of the motor neuron and not the decrease in muscle spindle firing rate substantially

contribute to the reduction in motor neuron excitability. Moreover, a reduced responsiveness of motor neurons to synaptic input has been demonstrated during fatiguing contractions (Butler *et al.*, 2003), which may result in additional descending central motor drive in order to maintain activation (Taylor *et al.*, 2006). More recently, Amann *et al.* (2013) showed that peripheral fatigue during fatiguing exercise and associated metabosensitive group III/IV muscle afferents reduced spinal motoneuronal output and thus, limited single-limb performance.

Neurobiological mechanism of central fatigue

The relevance of brain neurotransmitters as a mechanism for the development of central fatigue has been first proposed by Newsholme *et al.* (1987). Neurotransmitters play a key role in the communication between neurons and have been suggested to mediate central fatigue (Meeusen et al, 2006). Elevated serotonin levels in the brain have been associated with sensations of sleepiness and lethargy, causing a reduction in force (Davis et al, 2000; Meeusen et al, 2006). It has been suggested that serotonin (5-hydroxytryptamine, 5-HT) increases the gain of motor neurons and thus, enhances muscle contraction during moderate exercise, whereas motor neuron firing and thus, muscle contraction is inhibited during intense exercise (Perrier, 2016). The role of other neurotransmitters and/or neuromodulators in the development of central fatigue, such as dopamine, acetylcholine, cytokines, noradrenaline and ammonia have been reviewed (Davis & Bailey, 1997; Klass *et al.*, 2012; Roelands *et al.*, 2010; Wilkinson *et al.*, 2010). It is unlikely that one single neurotransmitter induces central fatigue considering the complexity of brain functioning. Instead, the interplay of various brain neurotransmitters with other levels of the nervous system (i.e. spinal cord, motor output, sensory input and autonomic function) appears most plausible in the regulation of exercise tolerance (Taylor *et al.*, 2016)

2.2.3.3. Measuring central fatigue

Merton (1954) introduced the interpolated twitch technique (ITT) to quantify the central component of neuromuscular fatigue. Femoral nerve stimulation as described in Section 2.2.2.3 is superimposed during an isometric MVC and any additional evoked force is suggested to indicate a failure of the CNS to voluntary activate all motor units to produce maximal force. The amplitude of this 'superimposed twitch' force (SIT) is related to the amplitude of the twitch force evoked by the same stimulus to the relaxed muscle (potentiated twitch force; Q_{pot}) and expressed in percent to quantify voluntary activation (VA) (Merton, 1954).

Equation 2.8. Calculation of peripheral voluntary activation:

$$VA_{PNS}\ (\%) = [1 - (SIT / Q_{pot})] \cdot 100$$

Merton & Morton (1980) were the first to activate the motor cortex via non-invasive, electrical stimulation through the intact skull. Later on, Barker *et al.* (1985) introduced that a change in magnetic field excites the underlying neural tissue and thus, allows the activation of the motor cortex via magnetic stimulation, which is considered less painful. Transcranial magnetic stimulation (TMS) has since been accepted as a safe technique (Rossi *et al.*, 2009) and widely applied to quantify cortical voluntary activation using the ITT. The existence of a SIT using TMS indicates supraspinal fatigue and reflects a suboptimal output of the motor cortex to produce maximal force (Todd *et al.*, 2016; Taylor & Gandevia, 2008). The calculation of VA evoked by TMS requires an estimation instead of a measure of a resting twitch because motor cortical and motoneuronal excitability increases during muscle activation (Todd *et al.*, 2003; Di Lazzaro *et al.*, 1998; Ugawa *et al.*, 1995). Traditionally, a linear regression of the size of the SIT against voluntary forces at 50, 75 and 100% MVC was performed and the y-intercept was taken as the estimated resting twitch (ERT) (Todd *et al.*, 2003):

Equation 2.9. Calculation of voluntary activation evoked by TMS:

$$VA_{TMS}\ (\%) = (1 - SIT/ERT) \cdot 100$$

Several methodological issues have been raised concerning the ITT, such as an overestimation of the true voluntary activation and as a consequence an underestimation of the maximal force capacity (de Haan *et al.*, 2009), timing of control and superimposed twitches, type of twitch stimulus and type of extrapolation utilised (Folland & Williams, 2007). Contessa *et al.* (2016) not only questioned the methods to assess central fatigue but also its interpretation because according to the equation to calculate VA, central fatigue (i.e. reductions in VA) could be theoretically solely explained by peripheral factors (i.e. reduction in Q_{pot}). Moreover, the reliability and face validity of the traditional three-contraction protocol for the estimation of VA_{TMS} has been recently challenged (Dekerle *et al.*, 2019). Mira *et al.* (2017) criticised the traditional approach due to 5-10 s of relaxation between each contraction to determine VA and proposed a continuous method instead. To date, the most appropriate method of VA_{TMS} calculation remains to be determined.

Corticospinal excitability can be quantified by delivering single-pulse TMS to the motor cortex at rest and during contraction and measuring the evoked electrical response in the target muscle, termed the motor evoked potential (MEP) using surface EMG (Taylor & Gandevia, 2001). Changes in MEP amplitude are commonly used as a marker of state-changes in the cortical motor system (Bestmann & Krakauer, 2015; Rothwell *et al.*, 1991), whereby a reduced MEP amplitude indicates reduced responsiveness of the corticomotor pathway (Brasil-Neto *et al.*, 1993). However, the descending excitation evoked by single-pulse TMS may not always reflect the activation of descending pathways by volitional motor command (Bestmann & Krakauer, 2015) and therefore, comparisons between evoked and voluntary motor commands must be made with caution.

The existence of central fatigue may include spinal alterations, however, the separation of spinal and cortical alterations is rather complex and requires additional neurostimulation techniques to non-invasively electrically stimulate the spinal tract at the level of the thoracic spine or cervicomedullary junction (Gruet *et al.*, 2013; McNeil *et al.*, 2013; Sidhu *et al.*, 2012a; Martin *et al.*, 2008; Taylor, 2006). Changes in the resulting cervicomedullary evoked potential (CMEP) indicate altered excitability of spinal motoneurons (Taylor, 2006). However, cervicomedullary stimulation is considered to cause discomfort or even pain in the majority of participants and its valid measurement has been questioned (McNeil *et al.*, 2013).

The preceding sections details common techniques used to measure neuromuscular fatigue in humans at rest. Further detail and additional measures quantifying central mechanisms are out of the scope of the present thesis. For a review see Todd *et al.* (2016), Rossini *et al.* (2015) and McNeil *et al.* (2013).

In summary, the multifaceted phenomenon fatigue is unlikely to be a consequence of one singular isolated mechanism. It is suggested that the contribution of numerous processes along the motor pathway may be involved in exercise-induced reductions in the force generating capacity of the active muscles. The application of TMS to assess VA_{TMS} in addition to femoral nerve stimulation requires a modified and more time consuming NMFA protocol which results in a delayed assessment of peripheral fatigue. Considering the immediate recovery of neuromuscular function post-exercise (Froyd *et al.*, 2013) and the primary focus of the present thesis on peripheral alterations, and in addition to the recent critique regarding the face validity and reliability associated with VA_{TMS} (Dekerle *et al.*, 2019), TMS was not applied in the present thesis.

2.2.4. Exercise intensity and duration dependent development of neuromuscular fatigue

The nature of fatigue is multifactorial and its primary cause dependent on the characteristics of the task undertaken. Central or peripheral alterations are dependent on numerous task-related factors, such as type of muscle activation, exercise intensity and duration or exercise mode (Barry & Enoka, 2007; Enoka *et al.*, 1992). The following section provides an overview of exercise intensity- and duration-dependent development of neuromuscular fatigue.

The relevance of exercise intensity and duration when localising the primary site of exercise-induced fatigue has been widely accepted (Barry & Enoka, 2007; Enoka & Stuart, 1992). Neuromuscular fatigue has been investigated across the whole spectrum of exercise intensity domains, from

prolonged, moderate exercise (Temesi *et al.*, 2014; Jubeau *et al.*, 2014; Millet & Lepers, 2004; Lepers *et al.*, 2000; Lepers *et al.*, 2002; Millet *et al.*, 2002) to severe intensity exercise (Temesi *et al.*, 2017; O'Leary *et al.*, 2016; Thomas, *et al.*, 2016; 2015; Johnson *et al.*, 2015) and sprint exercise (Goodall *et al.*, 2015; Hureau *et al.*, 2016a; Fernandez-del-Olmo *et al.*, 2013; Girard *et al.*, 2013). Table 2.7. provides an overview of studies investigating neuromuscular fatigue in the knee extensors across exercise intensity domains.

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Table 2.7. Neuromuscular fatigue in the knee extensors following exercise across exercise intensity domains (ordered by exercise duration)

				% change pre- to post-exercise						
	Exercise mode	Exercise duration	Exercise intensity	MVC	Q_{pot}	PS10: PS100	PS100	VL M-wave PPA	VA_{PNS}	VA_{TMS}
Martin *et al.* (2010)	Running	24 h	Self-paced	−41	−25			ns	−33	
Temesi *et al.* (2014)	Running	~20 h 17 min	110 km ultratrail	−34	−11			ns	−26	−16
Millet *et al.* (2002)	Running	~480 min	65 km ultramarathon	−30	+19			ns	−28	
Lepers *et al.* (2002)	Cycling	300 min	55% P_{peak}	−18	−16			ns	−8	
Place *et al.* (2004)	Running	300 min	55% max aerobic velocity	−28	+18			−38	−16	
Jubeau *et al.* (2014)	Cycling	3x 80 min	45% P_{peak}	−25	−28	−28	−23	−25	−14	−8
Lepers *et al.* (2000)	Cycling	120 min	65% P_{peak}	−13	−24			ns		
Ross *et al.* (2010)	Running	~91 min	20 km all out	−15	ns			ns	−13	
Sarre *et al.* (2015)	Cycling	60 min	65% P_{peak}	−14	−20				−5	
Jeffers *et al.* (2015)	Cycling	45 min	70% P_{peak}	−17	−19			−12	−9	
Theurel & Lepers (2008)	Cycling	33 min	70% P_{peak}	−7	−9			−6.5	−0.7	
Lepers *et al.* (2008)	Cycling	30 min	75% P_{peak}	−9	−11			reduced (no data provided)	−5	
Lepers *et al.* (2001)	Cycling	30 min	80% P_{peak}	−12	−16			ns	−16	
Decorte *et al.* (2012)	Cycling	~28 min	Intermittent bouts of 6 min at 80% P_{peak} (4 min break) until task failure	−20	−45	−29	−25		−6	−7

Romer *et al.* (2007)	Cycling	~13 min	TTF at 92% P_{peak}	−24	−39			ns	−4	
Goodall *et al.* (2015)	Cycling	~11 min	60%Δ	−9	−21			ns	−7	−3
Sidhu *et al.* (2014)	Cycling	~10 min	TTF at 80% P_{peak}	−16	−46			ns	−10	
Amann *et al.* (2011)	Cycling	~9 min	TTF at 80% P_{peak}	−10	−34			ns	ns	
Goodall *et al.* (2012)	Cycling	~8 min	TTF at 77% P_{peak}	−17	−19				−5	−8
Amann et al. (2009)	Cycling	~8 min	5 km TT	−8	−32				ns	
Amann & Dempsey (2008)	Cycling	~ 7 min	5 km TT	−10	−36				ns	
Johnson *et al.* (2015)	Cycling	~7 min	TTF at 85% P_{peak}	−16	−39				−5	
Pageaux *et al.* (2015)	Cycling	6 min	80% P_{peak}	−15	−32		−13	ns	−6	
Temesi et al. (2017)	Cycling	6 min	80% P_{peak}	−34	−55	−30	−43		−8	
O'Leary et al. (2016)	Cycling	~118 and 18 min	TTF at 90% LT and at 50%Δ	−17, −19	−17, −31				−7, +7	−7, +7
Thomas *et al.* (2016)	Cycling	~ 42, 11 and 3 min	TTF at 64%, 76% and 100% P_{peak}	−15, −15, −18	−11, −16, −33			ns	−9, −6, −3	−9, −8, −3
Thomas *et al.* (2015)	Cycling	~66, 32 and 6 min	40, 20 and 4 km TT	−10, -11, -18	−29, −31, -40			ns	−10, −11, −7	−10, −12, −6
Burnley *et al.* (2012)	Knee extension	~60, 57, 18, 9, 6, 4, 3 min	29, 31, 38, 42, 46, 50 and 55% MVC	−30, −35, −56, −50, −50, −44, −41	−26, −29, −38, −34, −36, -33, −32		−26, −29, −38, −34, −36, −33, −32		−9, −24, −29, −20, −12, −7, −6	

MVC, maximal voluntary contraction; Q_{pot}, potentiated twitch force; PS10, paired low-frequency (10Hz) twitch force; PS100, paired high-frequency (100 Hz) twitch force; VL, vastus lateralis; PPA, peak-to-peak amplitude; VA, voluntary activation; PNS, peripheral nerve stimulation; TMS, transcranial magnetic stimulation; ns, not significant; P_{peak}, peak power; TT, time trial; TTF, time to task failure; GET, gas exchange threshold; LT, lactate threshold; Δ, % of the difference between the LT and $\dot{V}O_{2max}$

It is generally accepted that peripheral fatigue increases with increasing exercise intensity whereas central fatigue becomes more predominant during prolonged, moderate exercise. A 65 km ultramarathon led to similar reductions in MVC and VA_{PNS} but no changes in M-wave amplitude for KE and surprisingly, an increase in Q_{pot} (Millet *et al.*, 2002). Thus, the reduction in MVC during prolonged moderate exercise was primarily associated with the development of central fatigue (i.e. decrease in VA) (Millet *et al.*, 2002). Lepers *et al.* (2002) found significant reductions in contractile properties (i.e. Q_{pot}, CT) during the first hour, with changes in M-wave duration for VM after 4 h and reductions in VA_{PNS} and MVC following 5 h of cycling exercise at 55% P_{peak}. The authors concluded that alterations in contractile properties occur during the first hour of exercise, while sarcolemma excitability and voluntary activation are greater affected towards the end of the cycling bout (Lepers *et al.*, 2002). The importance of central drive and excitability during the latter stages of prolonged exercise (i.e. running) was also highlighted by Place *et al.* (2004). Central fatigue as a limiting factor during moderate intensity exercise has been demonstrated during a 20 km all-out self-paced treadmill run, with reductions in MVC, VA_{PNS} and EMG during MVC, but no changes in Q_{pot} or M-wave amplitude (Ross *et al.*, 2010). Moreover, evidence for a supraspinal contribution to fatigue during prolonged exercise was indicated by reductions in VA_{TMS} (Temesi *et al.*, 2014; Jubeau *et al.*, 2014; Decorte *et al.*, 2012).

Collectively, these studies support that multiple sites contribute to fatigue during prolonged moderate exercise, however, the majority of studies highlight that impairments in the central component of fatigue appear to be the predominant factor limiting performance.

The contribution of peripheral fatigue (i.e. reductions in Q_{pot}) to reductions in MVC appears to become predominant with increasing exercise intensity (see Table 2.7.). Indeed, exercise to the limit of tolerance in the severe intensity domain is characterised by substantial peripheral alterations whereas the central contribution to fatigue appears less pronounced (Thomas *et al.*, 2016; 2015; Burnley *et al.*, 2012). Thomas *et al.* (2015) compared central and peripheral fatigue after 4-, 20-, and 40-km TTs and found no differences in reductions in MVC between the three conditions. However, greater levels of peripheral fatigue were reported after 4-km compared to 20- and 40-km TTs, whereas greater reductions in VA_{PNS} and VA_{TMS} were reported in longer TTs compared to the 4-km TT. Substantial reductions in Q_{pot} and moderate reduction in VA_{PNS} following severe intensity cycling were reported in numerous studies (Temesi *et al.*, 2017; Pageaux *et al.*, 2015; Johnson *et al.*, 2015; Sidhu *et al.*, 2014; Romer *et al.*, 2007). Greater changes in peripheral fatigue for severe intensity cycling were accompanied by a higher metabolic and physiological demand (i.e. higher HR, $[La^-]$ and RER) compared to moderate intensity cycling (O'Leary *et al.*, 2016).

The intensity and duration dependent development of neuromuscular fatigue across exercise intensities seems to be widely accepted, however, to the best of the author's knowledge, Thomas *et*

al. (2016) was the first to investigate an intensity-dependent development of neuromuscular fatigue even within the severe intensity domain for constant-load cycling. Reductions in MVC were not different following cycling exercise at 100% or 76% P_{peak} until task failure. However, greater levels of peripheral fatigue and a tendency for less central fatigue was found at the exercise bout closer to the upper boundary of the severe intensity domain. The authors suggested that differences are due to differences in the force and/or motor unit recruitment between conditions (Thomas *et al.*, 2016). This is in line with findings of Burnley *et al.* (2012), who reported an increase in central fatigue with decreasing exercise intensity following intermittent knee extensor contractions at different exercise intensities and durations above critical torque. Substantial changes in VA_{PNS} were found near the lower boundary of the severe intensity domain, whereas only moderate reductions were reported near the upper boundary of the intensity domain (Burnley *et al.*, 2012). However, Black *et al.* (2017) found similar changes for muscle excitability (i.e. M-wave amplitude and M-wave area), voluntary activation (measured as EMG RMS amplitude) and neural drive (measures as RMS relative to M-wave amplitude) at task failure between three different severe exercise intensities. Incongruent results may be due to different approaches to assess central fatigue or due to differences between the characteristics of the physical task, as further examined in the following section (see Section 2.2.5.) and investigated further in experimental chapters 5 and 7 in the present thesis.

Nonetheless, peripheral fatigue (reductions in Q_{pot}) appeared to reduce to a similar extent across exercise intensities above critical torque (32 to 38%) (Burnley *et al.*, 2012). Interestingly, similar changes in evoked twitch forces (~35%) have been reported immediately after supra-CP exercise across a wide range of severe exercise intensities, which was described as a 'critical threshold of peripheral fatigue' (Johnson *et al.*, 2015; Thomas *et al.*, 2015; Amann, 2011; Amann *et al.*, 2009; Amann & Dempsey, 2008; Romer *et al.*, 2007). The 'critical threshold' of peripheral fatigue as well as additional models aiming to explain the interaction between peripheral and central components in regulating fatigue and thus, exercise tolerance are explained in Section 2.2.5.

In conclusion, peripheral mechanism(s) appear to primarily limit short high intense exercise whereas central fatigue becomes more predominant with increasing exercise duration likely due to distinct physiological characteristics for each exercise intensity domain (see 2.1.1.2.).

2.2.5. Models of exercise regulation based on the interaction between central and peripheral fatigue

A reductionist approach where solely one single origin is proposed to explain the mechanism of fatigue has been commonly used, however, this may not adequately take into account the complex nature of fatigue and the system as a whole (Lambert *et al.*, 2005). Instead, an interplay of multiple peripheral and central mechanisms (reviewed in Section 2.2.2.2. and 2.2.2.3.), which ultimately limit the ability to generate force and thus, induce task failure, appears most likely (Amann, 2011; Lambert *et al.*, 2005; Nybo & Secher, 2004; Gandevia, 2001; Enoka *et al.*, 1992). Numerous models to explain the complex nature of fatigue have been proposed and some of the most relevant physiological approaches to the present thesis are summarized below.

Cardiovascular, anaerobic or catastrophic model

Hill *et al.* (1924) proposed that an inadequate O_2 supply as a result of a limited cardiac capacity and/or cardiac output limits muscular exercise. According to this first 'model of fatigue', a 'governor' located within the heart muscle or central nervous system would regulate exercise to prevent an oxygen deficiency of the heart (Hill *et al.*, 1924). Noakes (2000) proposed that the 'governor' is located in the CNS because maximal exercise at extreme altitudes is terminated before the heart or the skeletal muscles develop 'anaerobiosis'. The same research group also postulated that the existence of a skeletal muscle recruitment reserve at task failure provides further support that the CNS reduces or terminates skeletal muscle activation before the development of myocardial ischemia (Noakes, 2000; Noakes & St Clair Gibson, 2004). Thus, this research group introduced the 'central governor model'.

The central governor model

The central governor model emphasises the importance of the CNS as the regulator of performance in order to prevent a catastrophic physiological failure (Noakes *et al.*, 2004; Noakes *et al.*, 2005). This model proposes that the CNS regulates exercise intensity and thus, metabolic demand by modifying the recruitment of skeletal muscle motor units (Noakes *et al.*, 2004). In this model, fatigue is described as a conscious sensation which originates from the subconscious parts of the brain rather than the periphery (St. Clair Gibson *et al.*, 2003). The sensation of fatigue is understood as an interpretation of current and previous muscle activity against the potential severe threats to homeostasis arising from this activity and the subsequent anticipatory regulation of muscle activity (Noakes & St Clair Gibson, 2004; Tucker *et al.*, 2004; St. Clair Gibson *et al.*, 2003). The incorporation of prior experience on this interpretation (Tucker, 2009) was considered in an updated version of the central governor model (Noakes, 2012). Moreover, the latest theory, the 'integrative

governor theory', also considers decision-making processes during exercise regulation (St Clair Gibson *et al.*, 2018).

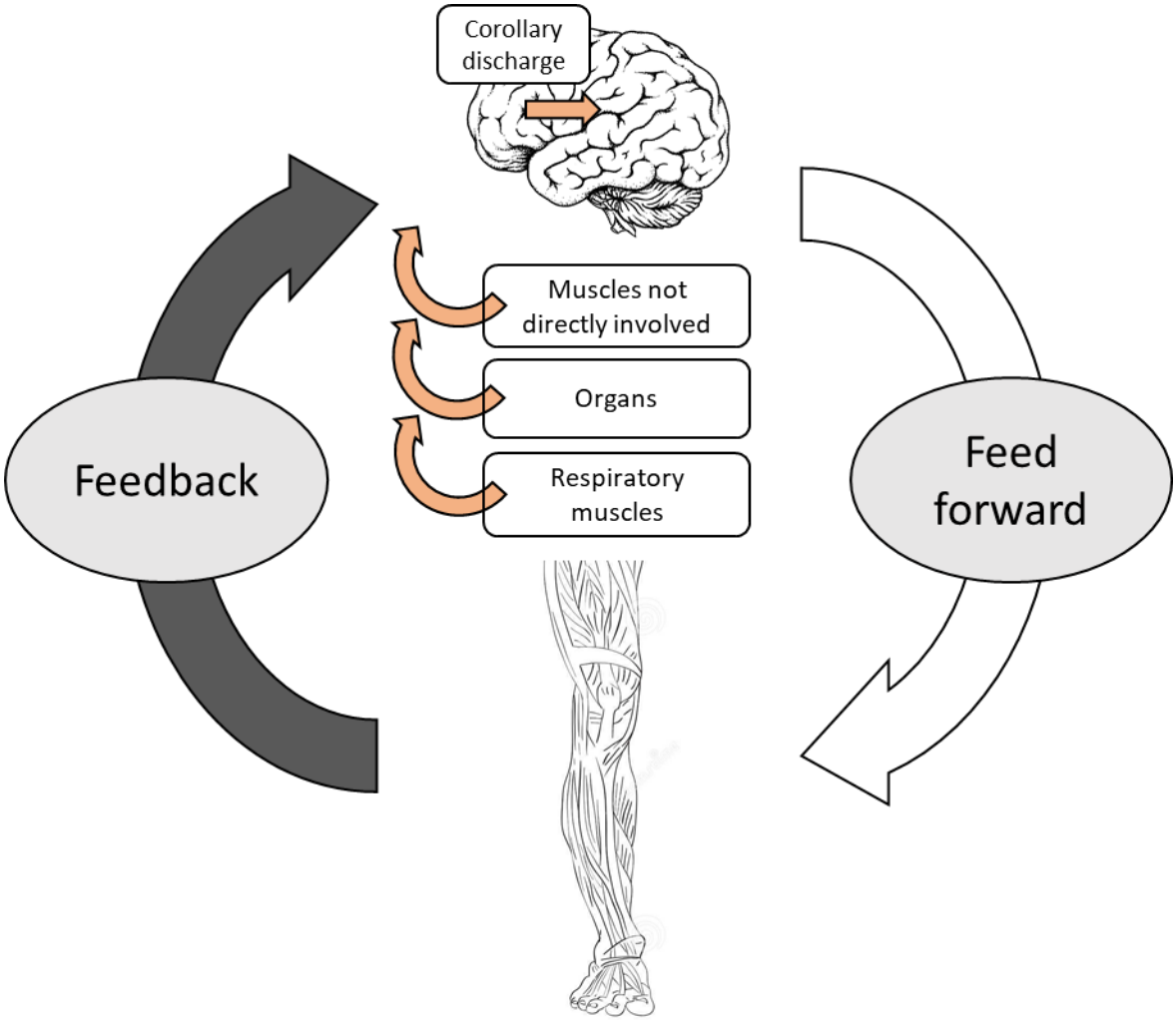


Figure 2.12. A schematic summary of three different models of exercise regulation. The central governor model of exercise regulation proposed by Noakes *et al.* (2004) emphasizes the relevance of the CNS and its feedforward mechanism (white arrow) while also considering feedback mechanisms (black arrow). The 'critical threshold of peripheral fatigue' proposed by Amann *et al.* (2006) focuses on the afferent feedback from the exercising muscles (black arrow). The 'sensory tolerance limit' by Hureau *et al.* (2016b) extends the model of Amann *et al.* (2006) by additionally considering afferent feedback from other areas of the body and corollary discharge (orange arrows).

The central governor model received criticism for lacking convincing experimental evidence and for supporting the existence of the feed-forward mechanism. The model also argues against a ceiling of O_2 consumption, even though this has been widely demonstrated in healthy individuals (Shephard, 2009). In addition, the relevance of a subconscious instance has been questioned and considered as unnecessarily increasing the complexity of the model (Marcora, 2008). Moreover, the central governor model disregards the regulation of performance at the spinal level and aims to explain

fatigue across all exercise modalities, without considering the task-dependent nature of fatigue (Weir *et al.*, 2006).

The afferent feedback model

The afferent feedback model, proposed by Amann *et al.* (2006), suggests that central motor drive to the working muscles regulates performance via metabosensitive group III/IV muscle afferent feedback in order to not surpass a 'critical level of peripheral fatigue'. This model is of interest for the entire thesis and has particular relevance for two experimental chapters (Chapter 5 and 7).

Amann *et al.* (2006a) compared neuromuscular fatigue following a 5 km cycling TT performed under four different levels of arterial oxygen content (C_aO_2). It has previously been shown that the development of peripheral fatigue is sensitive to changes in C_aO_2 induced by varying fractions of inspired O_2 from hypoxia to hyperoxia (Amann *et al.*, 2006b). Although increasing the inspired O_2 fraction (0.15-1.0) increased the central neural output (measured as iEMG of VL) and power output, and thus, improved cycling performance, the level of peripheral fatigue (i.e. reductions in Q_{pot}) post-exercise was not different between the four conditions (Amann *et al.*, 2006a). Similarly, increasing C_aO_2 improved TTF (at 80-100% $\dot{V}O_{2max}$) but had no effect on peripheral fatigue (Amann *et al.*, 2006a). These findings led the authors conclude that central motor drive and therefore, performance is regulated to a critical level of peripheral fatigue.

It has further been suggested that changes in C_aO_2 influence the metabolic milieu in the working muscle(s) (i.e. changes in SR Ca^{2+} (Duhamel *et al.*, 2004); Na^+-K^+-ATPase (Sandiford *et al.*, 2004); intramuscular P_i, PCr and H^+ levels (Hogan *et al.*, 1999); plasma lactate (Amann *et al.*, 2006a)). These exercise-induced metabolic alterations are then detected by group III/IV muscle afferents which indirectly reduce the central motor drive (i.e. central fatigue) via a negative feedback loop to limit the level of peripheral fatigue and ultimately, performance in order to prevent a catastrophic failure of muscle and overall homeostasis (Amann, 2011; see Figure 2.11.).

Interestingly, similar substantial levels of peripheral fatigue (i.e. reductions in evoked twitch forces of ~35%) have been reported following supra-CP exercise across a wide range of severe exercise intensities (Thomas *et al.* 2015; Johnson *et al.* 2015; Amann *et al.* 2011; 2009; Romer *et al.* 2007; Amann & Dempsey, 2008) (see Table 2.7. in Section 2.2.4.), which would support the existence of a 'critical threshold of peripheral fatigue'.

More recently, the concept of an individual critical threshold of peripheral fatigue has been challenged. Johnson *et al.* (2015) found shorter performance times and smaller reductions in twitch forces following a TTF at 85% P_{peak} when preceded by a high-intensity arm-cranking exercise. The existence of a critical threshold has also been questioned by Froyd *et al.* (2016a,b) who found

different levels of peripheral fatigue following the second bout of repeated sets of 10 x 5 s isometric knee extensions performed until task failure compared to the first bout. In addition, Rossman *et al.* (2014) reported a higher magnitude of peripheral fatigue following single-leg knee extensions performed until task failure compared to knee extensions performed with both legs at 85% of peak workload or compared to cycling exercise. The authors concluded that the CNS tolerates greater levels of peripheral fatigue and associated metabolic disturbances in smaller muscle mass when strong afferent feedback is restricted to one muscle group and/or a smaller muscle mass compared to the sum of weaker afferent feedback originating from more than one muscle group and/or a larger muscles mass. According to Thomas *et al.* (2018), greater levels of peripheral fatigue (performance fatigability) following single- vs. both-leg exercise, as reported by Rossman *et al.* (2014), were found due to a greater recruitment of the active skeletal muscle and a more local site of group III/IV afferent feedback while limiting the demand on other physiological systems and restricting alterations predominantly to one muscle group. This reduces the potential threat to overall homeostasis and therefore, allows a greater level of peripheral fatigue to be perceived as tolerable.

The interpretation of studies where participant did not reach similar levels of peripheral fatigue has caused much controversy. The validity of a critical threshold of peripheral fatigue remains unclear based on the fact that simply reaching or not reaching a certain magnitude of peripheral fatigue is not a sufficient evidence to disprove the concept because other reasons, such as task disengagement may cause an individual to stop. Both, open-loop (i.e. TTF) and closed-loop (i.e. TT) performance tests include a behavioural component which may result in a premature test termination before a 'true' physiological limit is reached. The present thesis aimed to remove this decision-making process (i.e. task disengagement) of a behavioural test in Chapter 5 (Study 2) by controlling for power output and exercise duration, and therefore, work done.

Sensory tolerance limit

Based on the original concept of an individual critical peripheral fatigue threshold and the more recent findings reporting differences in end-exercise levels of peripheral fatigue between different exercise modalities, Hureau *et al.* (2016b) modified the original concept and re-introduced the 'sensory tolerance limit', initially described by Gandevia (2001). The 'sensory tolerance limit', a more global negative feedback loop, emphasises that in addition to inhibitory afferent feedback from the working muscle(s), sensory afferents from muscles indirectly involved in exercise, limit peripheral fatigue and thus, performance, such as (see Figure 2.11.; Hureau *et al.*, 2016b): (1) Respiratory muscle work/fatigue; (2) Possibly organs; (3) Neural feedback from remote muscles

previously or simultaneously exercising and not directly involved in the exercise; (4) Corollary discharges associated with central command.

Hureau *et al.* (2016b) explained that smaller levels of peripheral fatigue in the right knee extensor following both-legs vs. single-leg exercise (Rossman *et al.*, 2014) are likely due to afferent feedback arising not only from one, but two legs, alongside a greater cardiovascular and ventilatory demand, so that the sensory tolerance limit was reached with smaller metabolic perturbations in the right knee extensor, but a similar overall level of sensory feedback. Moreover, according to Hureau *et al.* (2016b), shorter cycling TTF and smaller levels of peripheral fatigue with prior arm-cranking exercise (Johnson *et al.*, 2015) are due to afferent feedback arising not only from the active lower limbs but also from the recovering upper limbs simultaneously, so that the sensory tolerance limit is reached at a quicker rate. Similar conclusions might be drawn from shorter performance times and smaller levels of peripheral fatigue following one leg knee-extensions with prior fatigue of the contralateral knee extensor as reported by Amann *et al.* (2013).

Collectively, these models highlight that exercise tolerance appears to be regulated by the interaction of multiple central and peripheral mechanisms which ultimately induce task failure. A ‘universal' model applicable to all exercise design may not always hold true due to the task-dependent nature of fatigue.

2.3. Introduction to the study of fatigue within the present study

The present thesis investigated neuromuscular fatigue during severe intensity cycling exercise using the critical power concept as described in Section 2.1 of the literature review. According to this concept, task failure occurs when W' is fully depleted. The depletion of W' has been associated with disturbances of muscle homeostasis, impairing muscle contractile function and ultimately, reducing the ability to produce force (Murgatroyd *et al.*, 2011). Interestingly, peripheral fatigue above CP has been associated with intramuscular disturbances, i.e. H^+, P_i, ADP, La^- (Blain *et al.*, 2016; Burnley *et al.*, 2010; Allen *et al.*, 2008a; Jones *et al.*, 2008; Poole *et al.*, 1988) and similar changes in muscle metabolites have been reported following exercise above CP (Black *et al.*, 2017; Chidnok *et al.*, 2013). Blain *et al.* (2016) found a positive relationship between exercise-induced increases in intramuscular metabolites (i.e. P_i, H^+) and decreases in evoked twitch forces (i.e. $Q_{pot.}$ PS10, PS100) following attenuation of group III/IV muscle afferent feedback via intrathecal fentanyl. The authors concluded that muscle afferents have a regulatory role to prevent muscle damage due to severe intramuscular metabolic perturbations. Consequently, it may be speculated that the full depletion of W' across different exercise conditions leads to similar substantial muscle metabolic disturbances and thus, similar levels of peripheral fatigue at task failure. This would be in accordance with the ‘critical

threshold of peripheral fatigue' proposed by Amann *et al.* (2006a) (see Section 2.2.5.) and the findings of Black *et al.* (2017), who provided evidence that similar metabolic disturbances and similar magnitudes of peripheral and central fatigue may coincide at task failure, where W' should be fully depleted, assuming that individuals did not disengage with the task prior to its full depletion.

In attempt to link the depletion of W', the accumulation of metabolites and an increased muscle inefficiency of O_2 utilisation ($\dot{V}O_{2sc}$), Murgatroyd *et al.* (2011) proposed a 'fatigue cascade', as described in Section 2.1.1.4. (Figure 2.4.). However, to the best of the author's knowledge, there is still a lack of experimental evidence to support a causal link between $\dot{V}O_{2sc}$, W' and neuromuscular fatigue.

In summary, the CP concept constitutes a potent framework for the investigation of exercise tolerance in the severe intensity domain (Burnley *et al.* 2016; Poole *et al.* 2016; Grassi *et al.* 2015; Murgatroyd *et al.* 2011). Its integration with electromyographic and mechanical measures of neuromuscular fatigue offers great potential for a better understanding of the limits of tolerance within the severe intensity domain (Burnley *et al.* 2012; Black *et al.*, 2017). However, to date, only Burnley *et al.* (2012) and Black *et al.* (2017) have combined the CP concept with neurostimulation techniques. Burnley *et al.* (2012) found similar levels of peripheral fatigue at different exercise intensities within the severe intensity domain for single-leg knee extensions. Whether similar relationships hold true for whole body exercise remains to be established. Moreover, the present thesis aimed to explore further and experimentally challenge the relationship between W' and the magnitude of neuromuscular fatigue. Therefore, the present thesis conducted four studies in order to address the identified research gaps and provide support for a potential link between W' and neuromuscular fatigue during cycling exercise above CP.

2.4. Thesis aims and hypotheses

The present thesis includes four experimental studies (Chapter 4 - 7) in consideration of the review of literature as outlined below:

Study 1 (Chapter 4)

Reliability of neuromuscular measurements in the non-fatigued and fatigued knee extensors using femoral nerve stimulation.

Aim: The aim of this study was to examine the between-day reliability of muscle contractile properties, M-wave properties and peripheral voluntary activation in the knee extensor muscles at rest before and 1, 6, 15 and 30 min following fatiguing locomotor exercise.

Hypotheses: It was hypothesised that key neuromuscular measures would provide good between-day reliability in the fresh and fatigued knee extensors for voluntary and evoked twitch forces and voluntary activation and moderate reliability for M-wave properties.

Study 2 (Chapter 5)

Neuromuscular fatigue following cycling exercise at different intensities above critical power.

Aim: The aim of this study was to test whether the development of neuromuscular fatigue within the severe intensity domain can be linked to the depletion of *W′*.

Hypotheses: It was hypothesised that exercise above CP would lead to reductions in MVC, and the development of peripheral fatigue (e.g. Q_{pot}, PS10, PS100) and central fatigue (VA), without differences between the 3 min and 12 min trials.

Study 3 (Chapter 6)

Neuromuscular recovery following cycling exercise above critical power

Aim: The aim of this study was to examine the time course of neuromuscular recovery following cycling exercise at different exercise intensities and durations above CP.

Hypotheses: It was hypothesised that (1) no significant difference would be observed in all neuromuscular measures at any time point during recovery between conditions; (2) the ability to voluntarily produce force would recover rapidly, but only partially within the first few minutes following exercise termination; (3) both central and peripheral fatigue would recover substantially but not fully within the first few minutes following exercise; (4) twitch forces evoked by low-frequency stimulations would recover at a slower rate compared to twitch forces evoked by high-frequency stimulations; (5) a faster neuromuscular recovery would be observed in individuals with greater aerobic capacities.

Study 4 (Chapter 7)

Creatine supplementation improves performance above critical power but does not influence the magnitude of neuromuscular fatigue at task failure.

Aim: The aim of this study was to provide experimental evidence for an association between the use of W' and the development of neuromuscular fatigue using creatine supplementation.

Hypotheses: It was hypothesised that (1) creatine supplementation would improve performance (i.e. time to task failure) by increasing the amount of work done above CP; (2) a greater amount of work done above CP would increase the magnitude of neuromuscular fatigue observed at task failure; (3) the same absolute amount of work completed above CP (i.e. exercise time in control vs. 'isotime') would lead to a similar magnitude of neuromuscular fatigue regardless of creatine supplementation.

3. GENERAL METHODS

This chapter describes the methods, materials and equipment commonly used within the experimental chapters of this thesis. If alternative, additional and/or modified methods were used, details are provided within the methods section of the specific experimental chapter. Data collection was combined for Studies 1, 2 and 3. Therefore, participants, methods and equipment used in these chapters were the same. All data was collected in the British Association of Sport and Exercise Sciences (BASES) accredited laboratories at the University of Brighton (Welkin Human Performance Laboratories, Eastbourne, UK).

3.1. Health and safety procedures

All experimentation was conducted according to the University of Brighton's standard operating procedures, health and safety procedures and risk assessment laboratory guidelines. Biological materials and waste and all sharps, such as needles and lancets were disposed of in designated biohazard waste and/or sharps containers in line with the relevant guidelines. Any equipment in contact with the skin was cleaned with warm soapy water or an alcohol wipe after use to avoid contamination. Equipment used for gas collection was soaked in a biocide solution (Virkon, Day-Impex, Essex, UK) for a minimum of 10 min, rinsed with water and dried prior to further usage. All other equipment, such as cycle ergometers and isometric rig were cleaned following each session using disinfectant surface spray (Bioguard, UK).

Exercise was terminated at any time if participants requested this and without the obligation to provide a reason or if participants displayed any evidence of signs of disorientation, nausea, vomiting and/or syncope. Following the termination of exercise, participants were monitored until physiological responses returned to baseline.

3.2. Ethical approval

All studies in this transfer document were approved by the University of Brighton Research Ethics and Governance Committee and conducted in accordance with the guidelines outlined in the Declaration of Helsinki 1964, as revised in 2013 except for registration in a database.

3.3. Participants

Healthy males aged 18-35 years and familiar to regular, recreational physical activity were recruited via an introductory email or flyer to the University of Brighton students and members of staff.

Information material included a summary of the study and contact details of the lead investigator to request further information if interested. Prior to attending the laboratory, participants were provided with a study-specific participant information sheet, outlining the background of the study, details regarding each laboratory visit as well as an explanation of requirements, risks and benefits associated with the study. Prospective participants were able to take time to thoroughly consider their participation and it was explicitly pointed out that participation was on an entirely voluntary basis with the right to withdraw at any time and without reason.

Participants were given appropriate time during the preliminary visit to discuss the purpose of the research undertaken and any queries associated with the study. In addition, details of the Doctoral College were provided to discuss any issues with a contact independent of the research team. Upon arrival in the laboratories, participants were asked to complete a medical questionnaire and an informed consent form to participate in the study.

Participants were excluded if they had any recent history of injuries or respiratory, cardiac or haematological disease. Any identified medical condition that contradicted participation in maximal exercise led to exclusion from the study.

3.4. Confidentiality and data protection

All research was conducted in accordance with the Data Protection Act 1998 and data was collected and stored confidentially and anonymously under a numerical code for each participant on a locked institutional computer with password access restricted only to the lead investigator. All data and written forms will be kept locked and disposed of as confidential waste after a period of ten years. The privacy, rights and dignity of the participants was maintained at all times.

3.5. Pre-trial diet and exercise standardisation

Participants were asked to refrain from strenuous exercise (48 h), alcohol (24 h) and caffeine (12 h) (De Carvalho *et al.*, 2010; Meyers & Cafarelli, 2005) consumption prior to each laboratory visit and to arrive at the laboratories in a rested and hydrated state, at least 2 h postprandial. To limit the influence of circadian variation on physiological variables (Atkinson & Reilly, 1996), knee extensor force (Guette *et al.*, 2005) and exhaustive severe-intensity cycling (Hill, 2014), all trials were completed at the same time of day ± 2 h for each participant. Participants performed exercise in cycling or running trainers and appropriate clothing (i.e. sports shorts, t-shirt).

3.6. Preliminary visit and familiarisation

Upon participants' first visit at the laboratories anthropometric data for stature (cm) was measured to nearest 0.5 cm using a stadiometer and body mass was recorded to the nearest 0.1 kg using calibrated electronic scales (Seca 220, Seca Limited, Birmingham, UK) were taken.

Participants then completed a maximal ramp incremental test where power was initially set to 50-125 W depending on individual fitness level and increased by 5 W every 12 s until task failure. Participants were instructed to maintain a cadence of 80 rpm throughout every laboratory session and task failure was defined as a drop in cadence twice below 75 rpm for more than 5 s despite strong verbal encouragement. At task failure, the power was reduced to 20 W for 5 min of baseline pedalling, followed by an increase to 105% P_{peak} performed to task failure in order to confirm if a true $\dot{V}O_{2max}$ was reached (Rossiter *et al.*, 2006). All cycling trials were performed on an electromagnetically-braked, computer-controlled cycle ergometer (SRM High Performance Ergometer with 8 strain gauges; Schoberer Rad Meßtechnik, Jülich, Germany). The zero offset calibration procedure was performed on the SRM Powermeter according to the manufacturer's guidelines prior to each cycling test. Seat height, handle bar height and distance from seat to the handlebar were adjusted and recorded to replicate the set-up for each participant for the duration of the study.

During a second visit, participants were familiarised with constant-load trials performed to task failure and neuromuscular function assessment (NMFA) (see Section 3.8.) to ensure a quick and smooth transition from the cycle ergometer to the isometric rig and, also, to allow adequate practice of performing MVCs. Each constant-load session was preceded by a warm-up protocol, consisting of 3 min rest, 5 min baseline pedalling at 50 W, 3 min rest and 4 min baseline pedalling at 20 W. Cadence was fixed at 80 rpm throughout every session and participants were given adequate time to familiarise themselves. Visual feedback of cadence was provided, and verbal instructions were given if cadence increased or dropped by $\geq$ 3 rpm for $\geq$ 5 s. Participants were instructed to stay seated throughout cycling.

Figure 3.1: The SRM cycle ergometer.

3.7. Determination of CP and *W′*

Participants completed a randomised series of four to five constant-load tests on a cycle ergometer to elicit task failure within ~3 and 15 min (Hill, 1993; Poole *et al.*, 1988). Participants were blinded for elapsed time, power output and heart rate throughout testing and not informed about any other measure than cadence until the entire study had been completed.

Three different models, the 'hyperbolic power-time' model (see Equation 2.5.), the 'linear work-time' (see Equation 2.6.) and the 'linear power-inverse time' model (see Equation 2.7.) were used to obtain estimates of both CP and *W′*.

The SE for CP and *W′* derived from the three regression models were compared and the model with the 'best fit' (i.e. lowest SE) was selected (Black *et al.*, 2015). The SE was used as a quality control measure to ensure a good fit of the mathematical model to the data and therefore, an additional fifth trial was performed if these SE were above 2% and 10% of CP and *W′*, respectively (Dekerle *et al.*, 2015). The 95% CI for the CP estimate was calculated to ensure that the power output for the main trials was truly above CP.

3.8. Neuromuscular function assessment

3.8.1. Neuromuscular assessment protocol

Prior to the first neuromuscular function assessment of each visit, participants performed a standardised isometric warm-up with their right knee extensors, which consisted of ten 3 s isometric contractions with progressively increasing contraction intensity and maximal efforts during the last three contractions (3 s on – 7 s off; adapted from Girard *et al.*, 2013). Neuromuscular function

assessment involved the completion of five isometric 3 s MVCs separated by 20 s rest. The first two MVCs were performed in order to ensure adequate potentiation of the knee extensor muscles (Kufel *et al.*, 2002). Paired stimuli at 100 Hz (PS100) were delivered during and 2 s after the subsequent three contractions, followed by paired stimuli at 10 Hz (PS10) and a single stimulus (Q_{pot}). The time window between exercise termination and the start of the first MVC for NMFA was standardised to 60 s for every participant and every session to avoid different magnitudes of neuromuscular function recovery due to different time windows between test termination and the start of NMFA. Real-time visual feedback was displayed throughout each effort as recommended by Gandevia (2001).

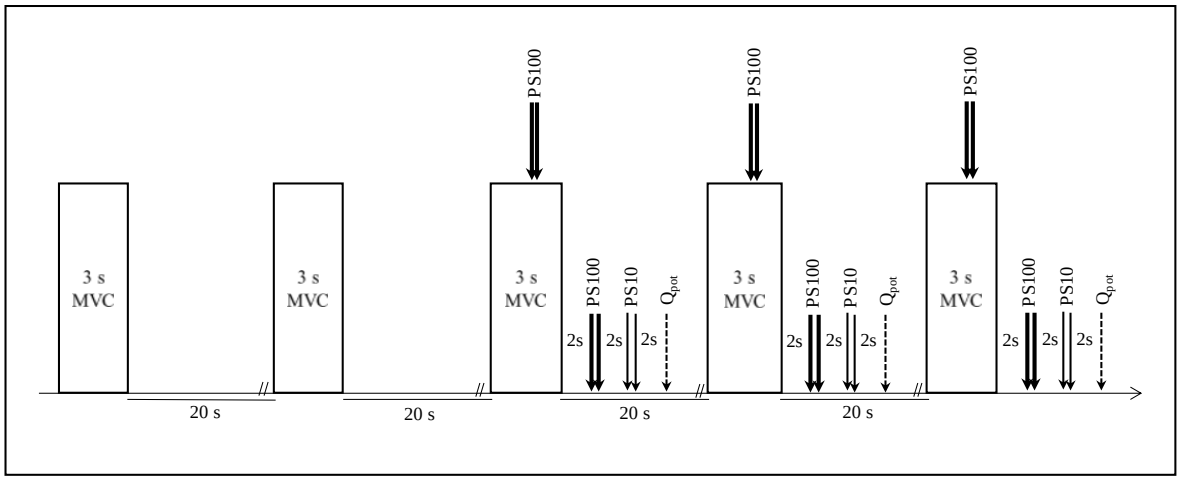


Figure 3.2. Schematic of neuromuscular function assessment. Three types of stimulation were delivered either during an isometric maximal voluntary contraction (MVC) or on the relaxed potentiated muscle: high-frequency paired stimulations at 100 Hz (PS100), low-frequency paired stimulations at 10 Hz (PS10) and a single twitch (Q_{pot}).

3.8.2. Force and EMG recording

For neuromuscular function assessment, participants were seated on a custom-built isometric chair (Figure 3.3) to record voluntary and evoked force production. The isometric chair was adjusted to enable hip and knee joint angles of 90° (Becker & Awiszus, 2001). Upper body movement was minimised via two cross-shoulder straps. Participants were asked to keep their hands in the same position during each NMFA, crossed in front of the body, holding the chest strapping. A cuff attached 1 – 2 cm superior to the right ankle malleoli was connected to a load cell (Model 615, Tedea, Basington, UK) and the set-up was adjusted individually to ensure a direct line between the load cell and the applied force. The load cell was connected to a custom-built bridge amplifier and calibrated by applying known masses across a range of 1 - 85 kg and recording raw analogue signal in Volts

(V). Regression analysis between kg and V was used to convert the raw signals to a measure of force in Newton.

EMG activity of the VL was recorded using surface electrodes (Kendall H59P, Coviden, Massachusettes, USA). Electrodes were positioned based on the SENIAM recommendations (Hermens *et al.*, 1999). The reference electrode was fixed to the right patella. All electrodes were marked with indelible ink to ensure consistent electrode placement between trials but also within-sessions in case electrodes had to be replaced due to reduced adhesiveness.

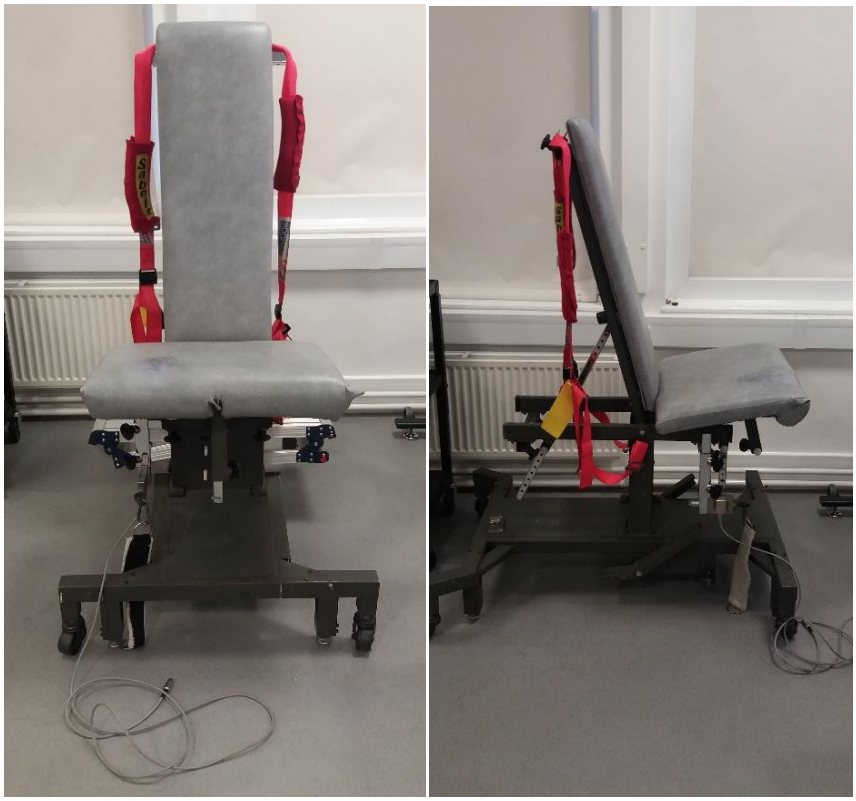


Figure 3.3. The custom-built isometric rig at the Welkin Laboratories, University of Brighton

3.8.3. Electrical femoral nerve stimulation

Single and paired square-wave electrical stimulations (200 μs pulse width) were delivered via adhesive surface electrodes (ValuTrode, Axelgaard, Fallbrook, USA) to the femoral nerve. Therefore, the cathode was positioned in the femoral triangle and the anode midway between the iliac crest and the greater trochanter. Positioning was adjusted with regard to optimal responses in resting twitch force and M-wave PPA. This position was marked with indelible ink and kept consistent throughout each subsequent visit to minimize effects through variable placement on different trials. Stimulation threshold was determined by delivering two single stimuli separated by 5 s to the femoral nerve and current was increased progressively (+ 20 mA) starting at 10 mA until no further increase in M-wave PPA and resting twitch force was evoked. Stimulation intensity was

set at 130% to ensure full spatial motor-unit recruitment. The determination of stimulation threshold was conducted prior to each first NMFA of every subsequent trial.

3.8.4. Data capture

All raw EMG data was amplified (gain x1000), digitized at 4 kH and filtered using a digital band-pass filter with high cut-off frequency of 2 kHz and a low cut-off frequency of 20 Hz. All data was recorded and processed off-line using a data acquisition system (PowerLab 15T with LabChart 7, ADInstrument Ltd, Oxford, UK).

3.8.5. Neuromuscular data analysis

Peak MVC was defined as the greatest 0.5 s mean force produced prior to electrical stimulation and reported as the mean of five MVCs. Potentiated twitch force was measured as the peak twitch force minus the onset force of the twitch evoked in response to supramaximal stimulation. Low-frequency fatigue was determined as the ratio between twitch forces evoked by low- and high-frequency paired stimuli (PS10:PS100).

For within-twitch measures, i.e. contraction time (CT), maximal rate of force development (MRFD), maximal rate of relaxation (MRR) and half relaxation time (HRT) were derived from each resting twitch. CT was defined as the duration from stimulus artefact to peak twitch force. MRFD and MRR were defined as the maximal slope of the incline and decline of the twitch force, respectively. HRT was defined as the time taken for the force to decline to half of peak twitch force.

EMG recording allowed the analysis of the potentiated compound M-wave elicited from a single stimulus in the relaxed VL. M-wave PPA was measured as the absolute difference of the maximum and minimum point of the biphasic M-wave, and the M-wave area was determined as the integral of the absolute value of the M-wave (Fowles *et al.*, 2002).

Voluntary activation (VA) was calculated using the interpolated paired stimulation technique (Merton, 1954) (see Equation 2.8. for VA_{PNS}).

For twitch force, within-twitch measures, M-wave properties and VA, the mean of three was reported for each NMFA.

3.9. Physiological measurements

3.9.1. Respiratory measurements

For Study 1, 2 and 3, ventilatory and pulmonary gas exchange were measured using a breath-by-breath open-circuit system (MediSoft Ergocard®, Sorinnes, Belgium) which was calibrated prior to each test according to the manufacturer's instructions against ambient air, gases of known

concentrations and for flow using a 3 L syringe. Participants breathed through a low-dead space (90 mL), low-resistance (0.65 mm H_2O $L^{-1} \cdot s^{-1}$ at 8 $L \cdot s^{-1}$) mouthpiece. Expired air was analysed for $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$ following its interpolation on a second-by-second basis. Due to equipment failure during the ongoing study, a different breath-by-breath system (Metalyzer Sport, Cortex Biophysik, Leipzig, Germany) was used for participants 10, 11 and 12 in Study 1-3 and for all participants in Study 4. Thus, no participant was tested using two different breath-by-breath systems within one study. Likewise, the system was calibrated according to the manufacturer's guidelines, which involved the input of the barometric pressure, determined from a portable barometer (Weather Station, Oregon Scientific, Oregon, USA), the calibration of the O_2 and CO_2 sensors against gases of known concentrations (15% O_2, 5% CO_2 sourced from Brin Oxygen Company, UK) and finally, the calibration of the volume flow sensor using a manual syringe (3 L, Hans Rudolph Inc., Kansas, USA) for 5 cycles that elicited an acceptable flow rate between 2-4 $L \cdot s^{-1}$.

The maximal rate of oxygen uptake is considered as the gold standard measurement of cardiorespiratory function (Howley *et al.*, 1995). BASES defines $\dot{V}O_{2max}$ as the 'maximum volume of oxygen consumed by the body each minute during large muscle group exercise at sea level'. Traditionally, a plateau in $\dot{V}O_2$ (< 150 $mL \cdot min^{-1}$ or < 2 $mL \cdot kg^{-1} \cdot min^{-1}$) despite a further increase in exercise intensity has been established as the primary criterion to classify whether a true $\dot{V}O_{2max}$ has been achieved (Taylor *et al.*, 1955; Hill *et al.*, 1924). However, many individuals fail to show a plateau in $\dot{V}O_2$ and therefore, the following secondary criteria have been introduced to verify a maximal effort (ACSM / *BASES guidelines):*

- RER of 1.15 or above
- HR_{max} within ± 10 beats$\cdot min^{-1}$ of age-predicted maximum (220 – age)
- Blood [La^-] of > 8 $mmol \cdot L^{-1}$

Despite its wide application, the $\dot{V}O_{2max}$ criteria have been discussed controversially (Poole & Jones, 2017; Poole *et al.*, 2008; Midgley *et al.*, 2007). To address the low percentage of individuals achieving the suggested $\dot{V}O_2$ criteria, previous research has suggested a verification trial, consisting of a constant-load trial following a ramp test to confirm whether the $\dot{V}O_2$ achieved can be classified as true $\dot{V}O_{2max}$ (Poole & Jones, 2017; Nolan *et al.*, 2014; Rossiter *et al.*, 2006). In the present thesis a verification trial was performed according to the protocol proposed by Rossiter *et al.* (2006) as described in Section 3.6.

The $\dot{V}O_{2max}$ in the present thesis was analysed as the highest 15 s moving average recorded during the ramp test (Dekerle *et al.*, 2015; Rossiter *et al.*, 2006) and verified during the following constant load trial.

3.9.2. Heart rate

Heart rate was measured using a telemetric heart rate monitor (RS800, Polar Electro Oy, Kempele, Finland). A heart rate monitor strap was tied around the chest and heart rate was recorded through an integrated receiver at the SRM cycle ergometer during exercise or recorded manually during the period on the isometric rig.

3.9.3. Visual analogue fatigue scale (VAFS)

Visual analogue scales have been commonly used to assess subjective feelings or sensations (Wewers & Lowe, 1990), such as pain (Sihvonen *et al.*, 1998) or fatigue (Leung *et al.*, 2004; Brunier & Graydon, 1996). In the present thesis, participants were presented with a 100 mm visual analogue scale to rate how fatigued they felt. The scale ranged from 'no fatigue at all' to 'totally fatigued/exhausted, the highest level of fatigue ever experienced'. Participants were asked to respond honestly and to carefully select the rating that best reflects how they feel. The scales were presented pre-exercise with the instruction to respond 'how they feel right now, at this moment in time' and post-exercise, immediately following neuromuscular function assessment, with the instruction to reflect retrospectively as good as possible 'how they felt within the last 20 s of exercise'. Participants marked on the line which best described their global fatigue level. Examples were provided if needed to ensure participants were confident with their judgement.

3.9.4. Blood sampling and analysis

Blood lactate concentration ($[La^-]$) was determined from an arterialized fingertip capillary blood sample using lithium-heparin coated microvette tubes (CB300, Sarsedt, Germany). Prior to collection, the fingertip was cleaned with an alcohol wipe, left to air dry and punctured using a single use lancet (Accu-Chek Safe T-Pro, Roche Diagnostics, West Sussex, UK). Blood samples were analysed for $[La^-]$ using an automated, electrochemical lactate and glucose analyser (YSI 2300, Yellow Springs Instruments, Ohio, USA).

3.10. Statistical analysis

All data was analysed using theStatistical Package for the Social Sciences (SPSS version 22-24 for Windows, IBM Corporation, New York, USA) and reported as mean ± standard deviation, unless otherwise stated. Significance was accepted at the level of $p < 0.05$. Prior to data collection G*Power analysis (v3.1) revealed the sample size required for each experimental chapter, using an alpha (α) level of 0.05 and a beta (β) level of 0.20.

All data was assessed for normal distribution using Shapiro-Wilk's test and sphericity was checked using the Mauchly's test. Any significant F-ratios were adjusted according to Greenhouse-Geisser if the sphericity assumption could not be confirmed.

Effect sizes for the primary dependent variable within each individual chapter were used to assess the magnitude of reported effects. They were either taken from published literature using similar experimental designs where possible or, calculated from means and SD. Partial eta squared (η_p^2) was calculated for main and interaction effects and Cohen's *d* was used for differences between two related samples (Lakens, 2013), where 0.2 represents 'small' effects, 0.5 'medium' effects and 0.8 'large' effects (Cohen, 1998).

4. STUDY 1: Reliability of neuromuscular measurements in the non-fatigued and fatigued knee extensors using femoral nerve stimulation.

4.1. ABSTRACT

The purpose of the present study was to examine the between-day reliability of muscle contractile properties, M-wave properties and peripheral voluntary activation in the knee extensor muscles at rest before and 1, 6, 15- and 30-min following fatiguing locomotor exercise. Although previous research has demonstrated good reliability of neuromuscular measures in the fresh state and after fatiguing single-leg knee extensions (Blacker *et al.*, 2013; Del Coso *et al.*, 2011; Place *et al.*, 2007), evidence that this holds true after severe intensity locomotor exercise remains to be established. Therefore, 12 recreationally active males performed two identical 12 min cycling sessions above critical power (P-12.1 vs. P-12.2) and neuromuscular function was assessed at rest before and 1, 6, 15- and 30-min following exercise using electrical femoral nerve stimulation. Test-retest reliability was examined for a battery of neurophysiological parameters. Resting measures showed moderate to good reliability, with no significant difference between trials ($p > 0.05$) and coefficient of variations (CV) ranging from ~4 to 6% (MVC, 5.4% CV, typical error [TE] = 41 N; Q_{pot}, 6.2% CV, TE = 11 N; PS10, 6.1% CV, TE = 16 N; PS100, 4.0% CV, TE = 12 N; VA, 3.4% CV, TE = 4%). There were no significant differences between-days at 1 min following severe intensity cycling exercise and CVs ranging from ~5 to 9% (MVC, 5.9% CV, TE = 36 N; Q_{pot}, 8.6% CV, TE = 10 N; PS10, 7.9% CV, TE = 17 N; PS100, 5.4% CV, TE = 13 N; VA, 3.0% CV, TE = 2%) ($p > 0.05$). Within-twitch measures and M-wave PPA showed moderate to good between-day reliability in the fresh and fatigued state. M-wave area showed low to moderate reliability in the fresh (17.1% CV, TE = 5.5 $\mu V \cdot s^{-1}$) and fatigued state (13.2% CV; TE = 5.3 $\mu V \cdot s^{-1}$). In conclusion, these findings demonstrate that key markers for muscle force and muscle contractile properties provide reliable measures of peripheral fatigue following locomotor severe intensity cycling exercise. Further, the variability in M-wave area needs to be considered cautiously when interpreting results.

4.2. INTRODUCTION

The measure of maximal voluntary force is of great relevance in exercise physiology and the inability to generate force can be a factor limiting performance of high-intensity exercise, including a wide range of athletic events. It is commonly accepted that neuromuscular fatigue affects the ability to tolerate severe intensity exercise since the maximal force generating capacity of the exercising muscles is reduced (see Section 2.2.4.). These exercise-induced neurophysiological changes can be of central and/or peripheral origin depending on exercise intensity and duration (Thomas *et al.*, 2015; 2016). A reduction in MVC is the most commonly used measure to quantify exercise-induced

changes in the maximal force generating capacity. However, a change in MVC pre- to post-exercise does not provide information regarding the origin of fatigue development. Constant technological and methodological advances to non-invasively assess neurophysiological changes during exercise allow for a progressive enhancement of our understanding of neuromuscular fatigue. The application of surface EMG in combination with neuro-stimulation techniques, to magnetically or electrically evoke and record twitch forces and compound muscle action potentials, enables the study of contractile properties, M-wave properties and muscle activation in healthy and clinical populations. These measures allow to further localise the origin of neurophysiological changes and may provide essential information to manipulate and delay neuromuscular fatigue development. The delivery of a single electrical stimulus, directly to the relaxed muscle or alternatively to the peripheral nerve innervating the muscle, evokes a twitch force by bypassing central contributions and enables us to investigate peripheral mechanisms exclusively (see Section 2.2.2.). The analysis of the contractile properties of the skeletal muscle, such as Q_{pot}, CT, MRFD, MRR and HRT provide further insight into the exercise-induced peripheral alterations (i.e. excitation-contraction coupling) causing neuromuscular fatigue. Low frequency fatigue describes a relatively greater loss in force evoked by low-frequency compared to high-frequency paired or tetanic stimulations and is suggested to be related to changes in Ca^{2+} release from the sarcoplasmic reticulum (Edwards *et al.*, 1977). In addition, M-wave characteristics (i.e. peak-to-peak amplitude or M-wave area) indicate changes in membrane excitability and can be recorded using surface EMG. The traditional technique to evaluate exercise-induced changes in central activation is known as the interpolated twitch technique. This technique involves the delivery of an electrical or magnetic stimulus during a maximal voluntary contraction. The magnitude of the force increment evoked during the MVC is related to a twitch force evoked by the same stimulus to the resting muscle and used to quantify VA (Merton, 1954; Gandevia, 2001) (see Equation 2.8.).

To assess the effect of an intervention on exercise-induced fatigue, it is crucial that neurophysiological variables can be reliably measured and reflect changes pre- to post-exercise resulting from the intervention *per se* rather than being affected by measurement error originating from the participant, the testing, the experimenter or the equipment (Thomas *et al.*, 2015).

Previous studies have assessed reliability either in the fresh state or after single-leg fatiguing exercise. A summary of their findings is displayed in Table 4.1. Numerous studies have analysed the reliability of maximal force measurements in a variety of muscle groups and good between-day reliability was reported for MVCs ($CV \leq 5\%$; $ICC > 0.89$) (Del Coso *et al.*, 2011; Zech *et al.*, 2008; Morton *et al.*, 2005) and voluntary and evoked contractions in the knee extensor muscles (Blacker *et al.*, 2013). However, these results are limited to the fresh muscle and similar levels cannot be assumed after fatiguing exercise. Place *et al*. (2007) were the first to investigate neuromuscular function measured in the fatigued condition and found high levels of between-day reliability for MVC, evoked twitch

forces and VA after a 2 min fatiguing MVC with the knee extensor muscles (CV < 10%; ICC ≥ 0.91). Similar results were observed for voluntary and evoked twitch forces after exhausting isometric single contractions with the knee extensor muscles (Bachasson *et al.*, 2013; see Table 4.1.). Measurements involving surface EMG (i.e. M-wave PPA) showed moderate reliability in the fatigued state (Place *et al.*, 2007).

Although previous studies have demonstrated moderate to high levels of reliability for peripheral and central measures of neuromuscular fatigue at the fresh state and following single-leg contractions, it is remarkable that to the authors' knowledge, reliability has not been assessed after severe intensity locomotor exercise despite the widespread use of neuro-stimulation techniques within sport and exercise science. Further, most studies have solely focused on the primary markers of neuromuscular fatigue (i.e. twitch forces, VA), whereas secondary markers (i.e. within-twitch and M-wave properties) have received far less attention.

Therefore, the aim of this study was to determine the between-day reliability of neuromuscular measures before and after high-intensity cycling exercise. We hypothesised that key neuromuscular measures would provide good between-day reliability in the fresh and fatigued knee extensors for voluntary and evoked twitch forces and voluntary activation and moderate reliability for M-wave properties.

Table 4.1. Between-day reliability of neuromuscular function measures before and following single-leg contractions of the knee extensor muscles

	MVC (N)			Q_{pot} (N)			PS100 (N)			VL M-wave PPA (mV)		VA (%)	
	CV (%) (95% CI)	ICC (95% CI)	LoA	CV (%) (95% CI)	ICC (95% CI)	LoA	CV (%) (95% CI)	ICC (95% CI)	LoA	CV (%)	ICC (95% CI)	CV (%)	LoA
Blacker *et al*. (2013)			± 12.7			± 32.0			±13.9				± 5.4
Bachasson *et al.* (2013)	5.1 (3.9;7.4)	0.98 (0.94;0.99)		5.7 (4.3;8.3)	0.96 (0.90;0.98)		6.1 (4.5;8.8)	0.96 (0.90;0.98)					
	5.3[#] (3.5;6.9)	0.88[#] (0.72;0.95)		5.1[#] (3.9;7.4)	0.88[#] (0.73;0.95)		5.1[#] (3.8;7.4)	0.85[#] (0.67;0.94)					
Del Coso *et al.* (2011)	3.6 ± 0.6	0.89 (0.68;0.96)										17.0 ± 3.3	
Zech *et al.* (2008)	4.2 - 5.0	0.92 - 0.97											
Place *et al.* (2007)	3.5 ± 4.2	0.90 (0.75;0.97)		5.6 ± 5.0	0.92 (0.74;0.98)		5.2 ± 4.5	0.93 (0.78;0.98)		14.6 ± 13.5	0.71 (0.27;0.91)	3.1 ± 4.2	
	7.4 ± 7.4[#]	0.91[#] (0.77;0.97)		9.3 ± 10.2[#]	0.94[#] (0.82;0.98)		8.3 ± 10.1[#]	0.92[#] (0.75;0.98)		17.0 ± 13.8[#]	0.70[#] (0.21;0.91)	6.0 ± 5.4[#]	
Morton *et al.* (2005)	4.3 ± 1.1		± 76.0									3.4 ± 1.3	± 4.4

CV, Coefficient of variation; ICC (95% CI), Intra-class Correlation Coefficient (95% Confidence Interval); LoA, 95% Limits of Agreement; VL, Vastus Lateralis; PPA, peak-to-peak amplitude; [#]post-exercise

4.3. METHODS

4.3.1. Participants

Twelve active male participants (mean ± SD: age, 23 ± 4 years; body mass 77 ± 11 kg; $\dot{V}O_{2peak}$ 3.84 ± 0.56 L.min^{-1}, peak power output (P_{peak}) 337 ± 46 W) volunteered for this study. Participants completed a medical health questionnaire and provided written, informed consent prior to testing (see Section 3.3.). All experimental procedures were approved by the University of Brighton Research Ethics & Governance Committee (see Section 3.2.). Participants were asked to refrain from strenuous exercise (48 h), alcohol (24 h) and caffeine (12 h) (De Carvalho *et al.*, 2010; Meyers & Cafarelli, 2005) consumption prior to each laboratory visit and to arrive at the laboratories in a rested and hydrated state, at least 2 h postprandial. Participant details are shown in Table 4.2.

Table 4.2. Participant characteristics

Participant	Age (years)	Body mass (kg)	Height (cm)	$\dot{V}O_{2peak}$ (L·min^{-1})
01	19	72.1	174	3.82
02	24	91.9	190	3.57
03	22	94.5	191	5.06
04	35	66.0	174	3.57
05	22	61.4	170	4.58
06	23	71.0	167	3.70
07	22	91.0	192	3.78
08	27	71.0	174	4.45
09	22	82.4	187	3.57
10	22	78.6	186	3.37
11	22	73.6	180	3.44
12	21	73.7	180	3.18
Mean ± SD	23 ± 4	77.3 ± 10.6	180 ± 9	3.84 ± 0.56

$\dot{V}O_{2peak}$, peak oxygen consumption

4.3.2. Experimental design

The study required eight visits to the laboratory over a three to five week period, with visits 1 to 6 separated by a minimum of 24 h and the main trials (visit 7 and 8) separated by a minimum of 48 h. All sessions were performed at the same time of day (± 2 h) to control effects of diurnal variation (Atkinson & Reilly, 1996). The tests involved: a ramp incremental test for the determination of $\dot{V}O_{2peak}$ (see Sections 3.6. and 3.9.1.), a familiarisation to the neuromuscular function assessment and to constant-load cycling to task failure (see Section 3.6.), four to five constant-load trials

performed to task failure for the determination of CP and *W′* (see Section 3.7.) and subsequently, two trials to test for between-day reliability of neuromuscular function measures of the fresh and fatigued knee extensors. During the two main trials, the power output for each individual was selected to deplete 100% *W′* within 12 min from interpolation of the P-t_{lim} relationship and neuromuscular function was assessed before and immediately after exercise termination.

4.3.3. Incremental test and familiarisation

Incremental tests were performed as described in the General Methods (see Section 3.6.). Briefly, power output was initially set to 50-125 W depending on individual fitness level and increased by 5 W every 12 s until task failure, followed by a verification trial at 105% P_{peak} (Rossiter *et al.* 2006). $\dot{V}O_{2peak}$ was defined as the highest 15 s moving average. Peak power was calculated as the highest 15 s moving average during the ramp test.

During a second visit, participants were familiarised with constant-load trials performed to task failure, NMFA and a quick transition from the cycle ergometer to the isometric rig.

4.3.4. Determination of CP and *W′*

The participants completed a semi-randomised series of four to five constant-load tests to elicit task failure within ~3 and 15 min (Poole *et al.*, 1988; Hill, 1993) as described in Section 3.7. For an overview displaying individual data for characterisation of the P-t relationship see Table 4.3.

4.3.5. Experimental trials

Power output was predicted for each participant from interpolation of the power - time relationship and set to induce full depletion of *W'* within 12 min.

4.3.6. Neuromuscular function assessment

Neuromuscular function assessment was performed before and 1, 6, 15- and 30-min post-exercise (POST 1, 6, 15 and 30) (Section 3.8.) during the two main trials.

4.3.7. Data analysis

Neuromuscular function measures, critical power and pulmonary gas exchange were analysed as described in Section 3.8.5., Section 3.7. and Section 3.9.1., respectively.

One participant was excluded from the data analysis for all neuromuscular measures except for MVC due to technical issues at 30 min post-exercise (n = 11). One further participant was excluded from the data analysis for VA after values were identified as outliers (> 2 SD from the mean; n = 10).

4.3.8. Statistical analysis

All data were first checked for normal distribution and sphericity as outlined in Section 3.10. Two-way repeated measures ANOVA on the factors 'experimental condition' (P-12.1, P-12.2) and 'time' (pre, 1, 6, 15- and 30-min post-exercise) were used to test for differences in neurophysiological measures. The level of significance was set at $p < 0.05$. Absolute reliability was assessed by the 95% LoA (Bland & Altman, 1986) and displayed using Bland-Altman plots, and assessed by the typical error of measurement (TE), calculated as the standard deviation of the mean difference for each pair of measurements / √2. The coefficient of variation (CV) was calculated as the standard deviation of the two measurements / mean of the two measurements ·100. Relative reliability was assessed by calculating the intra-class correlation coefficient (ICC) with 95% confidence intervals (a two-way mixed effects model with single measures), except for VA due to the ceiling effect. All data are expressed as mean ± SD. Statistical analysis was performed using SPSS 22 (SPSS Inc, Chicago USA). In the present chapter, CV ≤ 10% would be deemed as acceptable (Atkinson & Nevill, 1998) and ICC would be classified as 'excellent' if close to 1, as 'high' if close to 0.90, as 'moderate' between 0.70-0.80 and as 'low' if < 0.70 (Vincent, 1994).

4.4. RESULTS

4.4.1. Incremental test and determination of CP and *W′*

P_{peak} was 337 ± 46 W. There was no significant difference in $\dot{V}O_{2peak}$ achieved during the fast ramp test (3.84 ± 0.56 L·min^{-1}) compared to the subsequent verification trial (3.72 ± 0.43 L·min^{-1}) ($t_{(11)}$ = 2.17; p = 0.053; *d* = 0.24). CP and *W′* were 220 ± 46 W (65.1 ± 8.1% P_{peak}) and 19.9 ± 6.0 kJ with associated standard errors of 2.7 ± 1.3 W and 1.2 ± 0.6 kJ. Mean power output for P-12.1 and P-12.2 was 248 ± 45 W (73 ± 6% P_{peak}).

4.4.2. Peak oxygen uptake ($\dot{V}O_{2peak}$) across trials

There was no significant difference in $\dot{V}O_{2peak}$ achieved during the fast ramp test (3.84 ± 0.56 L·min^{-1}), the four constant-load trials to determine CP and *W′* (1: 3.79 ± 0.52; 2: 3.84 ± 0.58; 3: 3.58 ± 0.57; 4: 3.70 ± 0.57 L·min^{-1}) and the two main trials, P-12.1 (3.65 ± 0.55 L·min^{-1}) and P-12.2 (3.67 ± 0.59 L·min^{-1}) ($F_{(2.633,28.958)}$ = 1.91; p = 0.16; η_p^2 = 0.15).

Table 4.3. Characterisation of the P-t_{lim} relationship

Participant	Model	Number of tests	CP (W)	SE-CP (W)	*W′* (kJ)	SE-*W′* (kJ)
1	P-1/t	4	275	4.5	9.6	1.0
2	P-1/t	5	274	2.9	17.0	1.3
3	P-1/t	5	216	3.6	26.5	1.1
4	P-1/t	5	242	3.4	14.9	1.2
5	W-t	5	292	4.7	28.6	2.7
6	P-1/t	4	191	1.9	22.6	0.6
7	W-t	5	160	2.4	27.0	1.4
8	P-1/t	5	256	1.9	14.3	0.9
9	P-1/t	4	201	0.6	22.7	0.3
10	W-t	4	197	2.2	17.0	1.4
11	P-t	5	179	1.0	15.3	1.1
12	W-t	4	160	2.8	22.8	1.8
Mean ± SD			220 ± 46	2.7 ± 1.3	19.9 ± 6.0	1.2 ± 0.6

CP, critical power; *W′*, curvature constant

4.4.3. Reliability of neuromuscular function measures

There was no main effect for condition in neurophysiological variables at rest and following fatiguing severe intensity cycling exercise ($p > 0.05$). However, there was a significant main effect for time in all measures taken ($p < 0.001$), except for M-wave PPA ($p = 0.809$) (see Table 4.4.). A summary of reliability statistics for key markers of neuromuscular fatigue is presented in Table 4.5. and 4.6.

Table 4.4. Neuromuscular measures for P-12.1 and P-12.2 at baseline (PRE) and following exhaustive constant-load cycling at 1, 6, 15- and 30-min post (POST 1, 6, 15 and 30)

	Condition	PRE	POST 1	POST 6	POST 15	POST 30	Main and interaction effects
Neuromuscular fatigue							
MVC (N)	P-12.1	563 ± 110	473 ± 77	515 ± 91	534 ± 110	531 ± 120	Time: baseline vs. POST 1, 6 and 30
	P-12.2	570 ± 120	481 ± 88	530 ± 106	553 ± 118	554 ± 117	
Peripheral fatigue							
Q_{pot}[#] (N)	P-12.1	150 ± 19	103 ± 27	111 ± 26	115 ± 24	121 ± 20	Time: baseline vs. POST 1, 6, 15 and 30
	P-12.2	156 ± 18	109 ± 30	114 ± 29	118 ± 26	125 ± 24	
PS10[#] (N)	P-12.1	218 ± 33	136 ± 48	144 ± 49	150 ± 48	164 ± 44	Time: baseline vs. POST 1, 6, 15 and 30
	P-12.2	227 ± 41	148 ± 60	154 ± 60	157 ± 56	175 ± 54	
PS100[#] (N)	P-12.1	225 ± 26	194 ± 25	201 ± 26	206 ± 27	209 ± 22	Time: baseline vs. POST 1, 6, 15 and 30
	P-12.2	229 ± 27	199 ± 27	206 ± 26	207 ± 26	212 ± 25	
CT[#] (ms)	P-12.1	76 ± 5	67 ± 6	67 ± 7	67 ± 6	72 ± 4	Time: baseline vs. POST 1, 6, 15 and 30
	P-12.2	74 ± 4	67 ± 5	66 ± 6	66 ± 5	69 ± 5	
MRFD[#] ($N \cdot ms^{-1}$)	P-12.1	4.96 ± 1.00	3.23 ± 1.39	3.51 ± 1.22	3.98 ± 1.55	3.97 ± 1.19	Time: baseline vs. POST 1, 6, 15 and 30
	P-12.2	5.14 ± 1.32	3.18 ± 1.15	3.64 ± 1.29	3.79 ± 1.28	4.24 ± 1.21	
MRR[#] ($N \cdot ms^{-1}$)	P-12.1	−1.74 ± 0.46	−1.36 ± 0.36	−1.51 ± 0.30	−1.58 ± 0.33	−1.49 ± 0.33	Time
	P-12.2	−1.77 ± 0.44	−1.40 ± 0.35	−1.55 ± 0.36	−1.51 ± 0.25	−1.50 ± 0.26	
HRT[#] (ms)	P-12.1	74.8 ± 10.9	61.2 ± 14.6	54.7 ± 13.3	56.8 ± 14.7	61.0 ± 12.2	Time: baseline vs. POST 1, 6, 15 and 30
	P-12.2	77.8 ± 15.7	61.2 ± 13.3	56.7 ± 14.9	58.7 ± 17.5	66.7 ± 17.6	
Central fatigue							
Peripheral VA[†] (%)	P-12.1	90 ± 6	80 ± 7	84 ± 6	86 ± 5	85 ± 6	Time: baseline vs. POST 1 and 6
	P-12.2	90 ± 5	83 ± 6	85 ± 5	88 ± 5	89 ± 4	
Surface EMG							
M-wave PPA[#] (mV)	P-12.1	8.6 ± 2.4	8.7 ± 2.0	8.4 ± 2.3	8.0 ± 2.4	7.9 ± 2.3	
	P-12.2	9.0 ± 2.5	8.6 ± 2.3	9.1 ± 2.5	8.4 ± 2.8	8.5 ± 2.6	
M-wave area[#] ($\mu V \cdot s^{-1}$)	P-12.1	30.1 ± 10.9	30.8 ± 11.8	27.6 ± 10.0	25.0 ± 9.4	26.1 ± 10.2	Time: baseline vs. POST 1, 6 and 30
	P-12.2	34.5 ± 11.6	30.6 ± 12.5	29.5 ± 11.1	27.8 ± 10.0	29.4 ± 11.3	

Data are presented as mean ± SD. MVC, Maximal voluntary contraction; Q_{pot}, Potentiated twitch force; PS10, Low-frequency (10 Hz) doublet force; PS100, High-frequency (100 Hz) doublet force; CT, Contraction time; MRFD, Maximal rate of force development; MRR, Maximal rate of relaxation; HRT, Half-relaxation time; M-wave PPA, M-wave peak-to-peak amplitude; peripheral VA, Voluntary activation; where a main effect for time or interaction effects have been indicated, the pairwise comparisons are displayed subsequent if significant. [#]n = 11; [†]n = 10

Table 4.5. Between-day reliability of neuromuscular function measures at rest

Dependent variable	ICC (95% CI)	CV (%)	TE	SDC_{sample}
MVC (N)	0.87 (0.61;0.96)	5.4	41	33
$Q_{pot}^{\#}$ (N)	0.64 (0.10;0.89)	6.2	11	9
PS10$^{\#}$ (N)	0.82 (0.47;0.90)	6.1	16	12
PS100$^{\#}$ (N)	0.81 (0.43;0.94)	4.0	12	9
CT$^{\#}$ (ms)	0.37 (-0.26;0.78)	3.8	4	3.0
MRFD$^{\#}$ ($N \cdot ms^{-1}$)	0.84 (0.51;0.95)	6.0	0.47	0.37
MRR$^{\#}$ ($N \cdot ms^{-1}$)	0.80 (0.42;0.94)	8.4	0.20	0.16
HRT$^{\#}$ (ms)	0.71 (0.22;0.91)	6.7	7.4	5.9
M-wave PPA$^{\#}$ (mV)	0.78 (0.36;0.93)	9.9	1.2	1.0
M-wave area$^{\#}$ ($\mu V \cdot s^{-1}$)	0.76 (0.33;0.93)	17.1	5.5	4.4
Peripheral VA† (%)	NA	3.4	4	3

SD, Standard deviation; ICC, Intra-class coefficient correlation; CV, Coefficient of variation; TE, Typical error, SDC_{sample}, smallest detectable change; MVC, Maximal voluntary contraction, Q_{pot}, Potentiated twitch force; PS10, Low-frequency (10 Hz) doublet force; PS100, High-frequency (100 Hz) doublet force; CT, Contraction time; MRFD, Maximal rate of force development; MRR, Maximal rate of relaxation; HRT, Half-Relaxation Time; M-wave PPA, M-wave peak-to-peak area; peripheral VA, Voluntary activation; NA, not applicable. $^{\#}n = 11$; $^{\dagger}n = 10$

Table 4.5. Between-day reliability of neuromuscular function measures following fatiguing cycling exercise

	POST 1				POST 6				POST 15				POST 30			
Dependent variable	ICC (95% CI)	CV (%)	TE	SDC_{sample}	ICC (95% CI)	CV (%)	TE	SDC_{sample}	ICC (95% CI)	CV (%)	TE	SDC_{sample}	ICC (95% CI)	CV (%)	TE	SDC_{sample}
MVC (N)	0.81 (0.47;0.94)	5.9	36	29	0.91 (0.70;0.97)	4.6	31	24	0.91 (0.72;0.97)	4.9	34	27	0.92 (0.76;0.98)	5.6	32	26
Q_{pot}[#] (N)	0.88 (0.60;0.97)	8.6	10	8	0.92 (0.73;0.84)	6.3	10	7	0.92 (0.73;0.98)	4.7	7	6	0.93 (0.75;0.98)	4.3	6	5
PS10[#] (N)	0.90 (0.67;0.97)	7.9	17	14	0.96 (0.85;0.92)	5.3	11	9	0.96 (0.87;0.93)	4.5	10	8	0.95 (0.82;0.99)	5.6	11	9
PS100[#] (N)	0.77 (0.34;0.93)	5.4	13	10	0.90 (0.66;0.97)	3.6	8	7	0.93 (0.78;0.98)	2.9	7	6	0.88 (0.62;0.97)	3.1	8	6
CT[#] (ms)	0.82 (0.45;0.95)	3.0	2.5	2.0	0.70 (0.20;0.91)	3.8	3.5	2.8	0.76 (0.33;0.93)	2.9	2.7	2.1	0.48 (-0.14;0.83)	4.3	3.1	2.5
MRFD[#] ($N.ms^{-1}$)	0.88 (0.62;0.97)	12.5	0.44	0.35	0.93 (0.77;0.98)	8.0	0.77	0.62	0.70 (0.22;0.91)	9.9	0.77	0.62	0.97 (0.91;0.99)	6.3	0.19	0.15
MRR[#] ($N.ms^{-1}$)	0.83 (0.49;0.95)	8.3	0.14	0.12	0.74 (0.29;0.92)	8.1	0.17	0.13	0.57 (-0.02;0.86)	8.3	0.19	0.15	0.89 (0.66;0.97)	4.8	0.10	0.08
HRT[#] (ms)	0.85 (0.53;0.96)	6.2	5.5	4.4	0.90 (0.67;0.97)	5.0	4.4	3.6	0.89 (0.65;0.97)	5.0	5.3	4.2	0.88 (0.61;0.97)	8.0	5.3	4.3
M-wave PPA[#] (mV)	0.83 (0.48;0.95)	7.6	0.9	0.7	0.87 (0.58;0.96)	8.5	0.9	0.7	0.88 (0.61;0.97)	7.6	0.9	0.7	0.85 (0.53;0.96)	9.8	1.0	0.8
M-wave area[#] ($\mu V \cdot s^{-1}$)	0.81 (0.44; 0.95)	13.2	5.3	4.2	0.75 (0.30;0.93)	17.2	5.3	4.2	0.77 (0.34;0.93)	16.4	4.7	3.7	0.74 (0.28;0.92)	17.6	5.5	4.4
Peripheral VA[†] (%)	NA	3.0	2	2	NA	3.4	4	3	NA	2.6	3	2	NA	4.3	3	3

SD, Standard deviation; ICC, Intra-class coefficient correlation; CV, Coefficient of variation; TE, Typical error, MVC, Maximal voluntary contraction, Q_{pot}, Potentiated twitch force; PS10, Low-frequency (10 Hz) doublet force; PS100, High-frequency (100 Hz) doublet force; CT, Contraction time; MRFD, Maximal rate of force development; MRR, Maximal rate of relaxation; HRT, Half-Relaxation Time; M-wave PPA, M-wave peak-to-peak area; peripheral VA, Voluntary activation; NA, not applicable. [#]n = 11; [†]n = 10

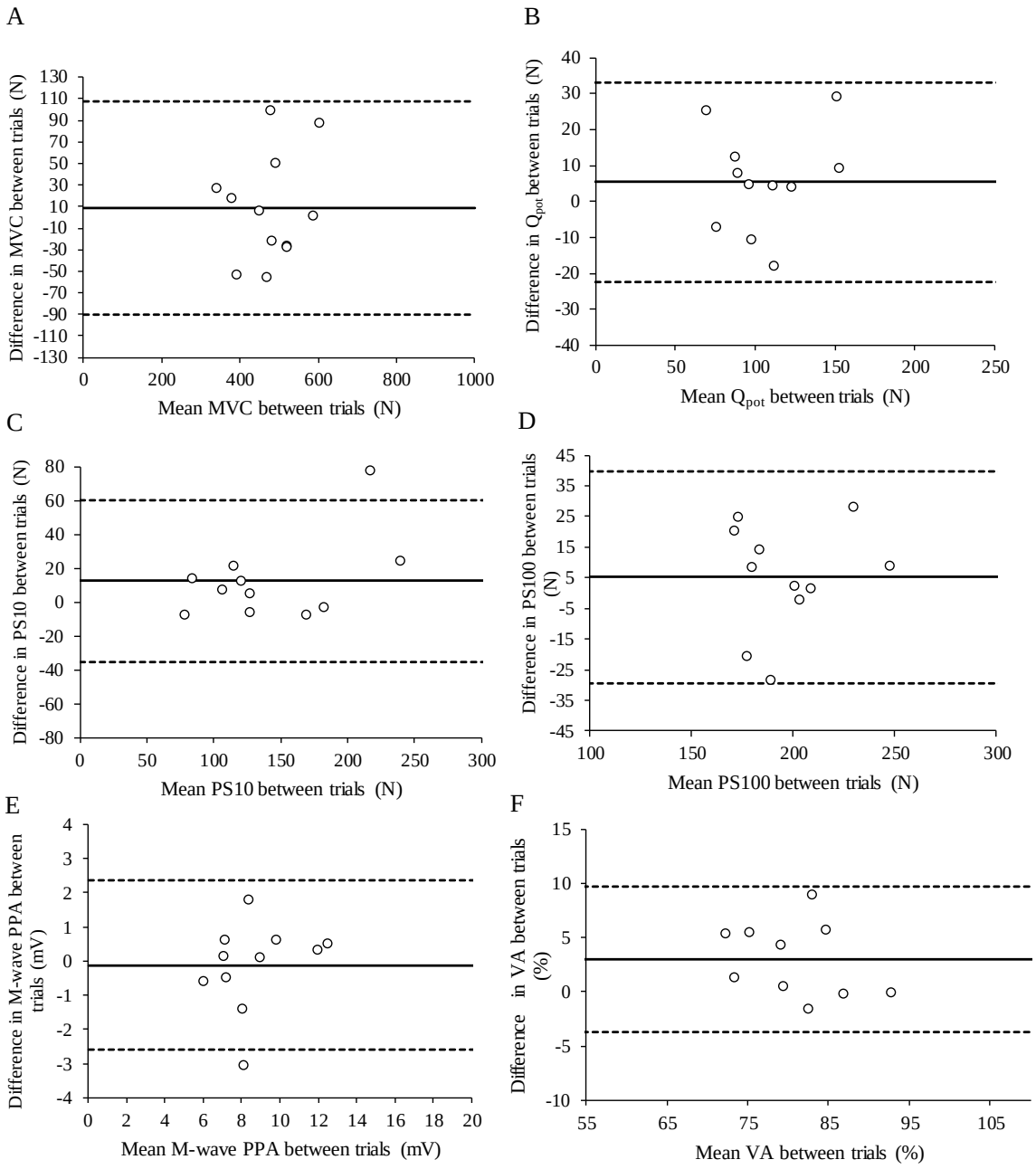


Figure 4.1. Bland-Altman plots with mean bias (solid line) and 95% limits of agreement (dashed line) for maximal voluntary contraction (MVC; A), potentiated twitch force (Q_{pot}; B), low-frequency (10 Hz) doublet force (PS10; C), high-frequency (100 Hz) doublet force (PS100; D), M-wave peak-to-peak amplitude (PPA; E) and voluntary activation (VA; F). n = 12 for MVC; n = 11 for Q_{pot}, PS10, PS100, M-wave PPA

4.5. DISCUSSION

The purpose of this study was to examine between-day reliability of neuromuscular measures before and following high-intensity cycling exercise. The main finding from the present study demonstrated for the first time that neuromuscular function measures show moderate to good between-day reliability after fatiguing severe intensity cycling exercise. Although Place *et al.* (2007) reported

good reliability after a 2 min fatiguing MVC performed on an isokinetic dynamometer, these findings cannot be generalised to locomotor exercise. Thus, the present study examined reliability following cycling exercise, which is highly relevant when mechanisms underlying neuromuscular fatigue are to be investigated.

Maximal voluntary contraction force is the most commonly used measure of muscle function. Previous studies have assessed reliability of MVCs and reported good within-participant between-day reliability with ≤ 5% CV and ICC > 0.89 (Del Coso *et al*., 2011; Zech *et al*., 2008; Morton *et al.*, 2005) in the fresh muscle and ≤ 10% CV and ICC ≥ 0.91 following fatiguing single-leg contractions (Place *et al.*, 2007). This is in line with the present results with 5.4% CV and ICC = 0.87 in the fresh muscle and < 6% CV and ICC ≥ 0.81 after the fatiguing cycling exercise. Good reliability within the field has been considered as ≤ 10% CV (Atkinson & Nevill, 1998). Hopkins (2000) suggested < 5% CV to be acceptable in sport science studies. Intra-class correlation coefficients close to 1 indicate 'excellent' reliability. ICC between 0.70-0.80 can be considered as 'moderate' (Vincent, 1994). Therefore, the MVC can be considered as a reliable measure. In addition, MVC measures taken during neuromuscular recovery at 6, 15- and 30-min post-exercise showed similar good reliability with < 5.9% CV and ICC > 0.81.

For evoked twitch forces, no significant differences were found between trials. The data indicates moderate to good absolute reliability for evoked twitch forces between repeated trials at fresh state (< 6.2% CV) and throughout neuromuscular recovery from severe intensity cycling exercise, with slightly greater variation at 1 min post compared to 6, 15- and 30-min post. Relative reliability can be considered as good in the fresh state (ICC > 0.81), except for Q_{pot} (ICC 0.64), and as good to high in the fatigued state for all evoked forces (ICC 0.77-0.96). These results are in accordance with previous findings reported following single-leg contractions (ICC 0.88-0.94; 5-10% CV) (Place *et al.*, 2007; Bachasson *et al.*, 2013). Blacker *et al.* (2013) reported greater random variation in peak force evoked by single compared to paired stimulation in the fresh muscle (95% LoA ± 14 vs. ± 32). In contrast, Place *et al.* (2007) showed similar between-day reliability for twitch forces evoked by single and high-frequency paired stimulations in the fresh (ICC 0.92 vs. 0.93; 6% vs. 5% CV) and fatigued state (ICC 0.94 vs. 0.92; 9% vs. 8% CV). In the present study, smaller within-participant variation was found for PS100 compared to Q_{pot} in the fresh state (4.0% vs. 6.2% CV) and in the fatigued state at 1 min following fatiguing severe intensity cycling exercise (5.4% vs. 8.6% CV). Twitch forces evoked by low-frequency stimulations described smaller variation compared to Q_{pot} and greater variation compared to PS100 in the fresh (6.1% CV) and fatigued state (7.9% CV). A better absolute reliability of high-frequency stimulations compared to twitch forces evoked by single stimulations as demonstrated in the present study may promote the application of tetanic stimulations. However, although tetanic stimulations represent a very effective way to recruit knee

extensor muscles maximally and can be used to evaluate low-frequency fatigue, the high level of associated discomfort, prevent it from being the most established method. In a study by Millet *et al.* (2003), 7 out of 11 cyclists refused to have measured neuromuscular function after a road race due to the associated pain (Millet *et al.*, 2003). Consequently, paired pulses have been recommended as a good compromise between single and tetanic stimulation and seem to represent the preferred stimulation technique in the most recent published fatigue-related studies (e.g. Jubeau *et al.*, 2017; Temesi *et al.*, 2017; Froyd *et al.*, 2016a,b).

Within-twitch measures demonstrated moderate to good absolute reliability with < 9% CV in the fresh state and < 10% CV in the fatigued state and throughout neuromuscular recovery, except for MRFD at 1 min post-exercise (12.5% CV). Further, moderate to high relative reliability was found in the fresh and fatigued state (ICC 0.70-0.97), except for CT in the fresh state (ICC 0.37) and at 30 min post (ICC 0.48) and for MRR at 15 min post (ICC 0.57). To the authors' knowledge no other study has reported between-day reliability of within-twitch parameters to date and therefore, no comparison can be made. Further studies examining absolute and relative reliability for within-twitch measures are required to reveal whether similar results can be found, in particular, when using larger samples sizes.

M-wave PPA showed moderate to good levels of absolute and relative reliability, both in the fresh state and 1 min following severe intensity cycling exercise (9.9 vs. 7.6% CV; ICC 0.78 vs. 0.83). Similarly, moderate to good levels of reliability were found 6, 15- and 30-min post-exercise (< 9.8% CV, ICC > 0.85). Place *et al.* (2007) reported slightly higher variations for M-wave PPA in the VL in the fresh (~15% CV) and fatigued state (17% CV). Both, the present study and Place *et al.* (2007) used femoral nerve stimulation, controlled for the site of stimulation, prepared the skin and controlled for EMG placement, however, differences in reliability post-exercise may be due to the exercise *per se* (12 min severe intensity cycling vs. 2 min sustained MVC). The present study controlled for power output and exercise duration, and therefore, work done, removing the behavioural component of a behavioural test. In contrast, Place *et al.* (2007) controlled for duration but not torque, so that participants were required to contract maximally which might have differed between trials, even though the authors reported no significant differences in torque at start, middle and end of the contraction between the two sessions. The greater variation at rest reported by Place *et al.* (2007) in comparison to the present study remains unclear. Moderate relative and low absolute reliability were found for M-wave area in the fresh (17.1% CV, ICC 0.76) and fatigued state (13.2-17.6% CV, ICC 0.74-0.81). These variations need to be considered carefully when interpreting results and might be too large to detect changes.

For VA, good absolute reliability was found at rest (3.4% CV) and at 1, 6, 15 and 30 min following severe cycling exercise (< 4.3%). These findings are comparable to ~3% CV at rest (Morton *et al.*, 2005; Place *et al.*, 2007) and ~6% following single-leg contractions reported previously (Place *et al.*,

2007). Weak within-participant between-day reliability (17% CV) has been demonstrated by Del Coso *et al.* (2011). The interpolated twitch technique has caused much controversy since its introduction and numerous studies have questioned its application (Neyroud *et al.*, 2016; Huang *et al.*, 2010; Folland & Williams, 2007; Shield & Zhou, 2004). It is known that central measures can be affected by other limiting factors, e.g. time of the day, electrode placement or caffeine intake (De Carvalho *et al.*, 2010; Tamm *et al.*, 2009); however, as data collection was conducted at the same time of day and controlled for electrode placement and caffeine intake, it is very unlikely that these variables affected the outcome.

4.6. CONCLUSION

In conclusion, neurophysiological measures assessed using femoral nerve stimulation describe moderate to good reliability in the fresh state, with slightly higher CVs 1 min following severe cycling exercise in most measures. The results suggest that key neuromuscular measures (i.e. MVC, Q_{pot}, PS10, PS100 and VA) were highly repeatable after fatiguing cycling exercise on different days. However, low reliability of M-wave area and some within-twitch measures (i.e. CT, MRR, MRFD) need to be considered. Overall, the magnitude of changes induced through an intervention must be related to the measurement error, as assessed in the present study, before interpreting the results.

5. STUDY 2: Neuromuscular fatigue following cycling exercise at different intensities above critical power

5.1. ABSTRACT

The aim of the second study was to test whether the development of neuromuscular fatigue within the severe intensity domain could be linked to the depletion of *W′*. Twelve recreationally active men completed tests to determine $\dot{V}O_{2peak}$, CP and *W′*, followed by two randomly assigned constant-load supra-CP trials set to deplete *W′* fully in 3 (P-3) and 12 min (P-12). Pre- to post-exercise changes in MVC, Q_{pot}, PS10, PS100 and VA were determined. Cycling above CP reduced MVC (P-3, −20 ± 10% vs. P-12, −15 ± 7%), measures associated with peripheral fatigue (Q_{pot}, −35 ± 13 vs. −31 ± 14%; PS10, −38 ±13 vs. −37 ± 17%; PS100, −18 ± 9 vs. −13 ± 8% for P-3 and P-12, respectively) and VA (P-3: −12 ± 3% vs. P-12: −13 ± 3%) ($p < 0.05$), with no significant difference between trials ($p > 0.05$). Changes in MVC and evoked twitch forces were inversely correlated with CP and $\dot{V}O_{2peak}$ following P-12, while *W'* was positively correlated with changes in Q_{pot} and PS10 following P-3 ($p < 0.05$). Therefore, the magnitude of neuromuscular fatigue does not depend on exercise intensity when *W′* is fully exhausted during severe intensity exercise, yet exploration of inter-individual variations suggests that mechanisms underpinning exercise tolerance within this domain differ between short- vs. long-duration exercise.

5.2. INTRODUCTION

The relationship between power output and duration of severe intensity exercise is characterised by a hyperbolic function (Poole *et al.* 1988; Moritani *et al.* 1981; Monod & Scherrer, 1965). The asymptote of this relationship (critical power, CP) separates the heavy (< CP) from the severe (> CP) exercise intensity domain and represents the highest power output that can be sustained without continuously drawing on anaerobic energy stores (see Section 2.1.1.3.). The aerobic nature of CP is well evidenced through manipulation of O_2 delivery and/or utilisation (Parker Simpson *et al.* 2015; Dekerle *et al.* 2012; Vanhatalo *et al.* 2010). The curvature constant *W′* was originally described as a fixed anaerobic work capacity, mathematically equivalent to a given amount of work that can be performed above CP; according to the CP model, exercise intolerance occurs once this energy store is fully depleted (Moritani *et al.* 1981; Monod & Scherrer, 1965). Although its reliance solely on anaerobic energy stores has been questioned due to its sensitivity to interventions altering O_2 delivery (Dekerle *et al.* 2012; Vanhatalo *et al.* 2010), its primarily anaerobic nature is still widely accepted (Miura *et al.* 2000; Miura *et al.* 1999; Smith *et al.* 1998; Jenkins & Quigley, 1993) (see Section 2.1.1.4.).

Peripheral fatigue, i.e. a reduction in the force generating capacity induced by alterations at or distal to the neuromuscular junction, has been evidenced within the severe intensity domain whereas central fatigue, i.e. a reduction in the ability to voluntarily activate motor neurons and muscle fibres (Gandevia, 2001), seems less pronounced when exercising above CP (Thomas *et al.* 2016; 2015; Lepers *et al.* 2002; Place *et al.* 2004) (see Section 2.2.4.).

The development of peripheral fatigue during exercise above CP has been associated with substantial intramuscular metabolic disturbances (Burnley *et al.*, 2010; Jones *et al.*, 2008; Allen *et al.*, 2008a). Similar changes in muscle metabolic response (i.e. low pH and [PCr] and high [La]) have been reported following continuous and intermittent whole-body exercise performed at different intensities above CP (Black *et al.* 2017; Chidnok *et al.*, 2013) and plantar flexion exercise performed to exhaustion at different fractions of inspired O_2 (Hogan *et al.*, 1999). Interestingly, similar changes in evoked twitch forces (~35%) have also been reported immediately following supra-CP exercise across a wide range of severe exercise intensities, which was described as a 'critical threshold' of peripheral fatigue (Thomas *et al.* 2015; Johnson *et al.* 2015; Amann *et al.* 2011; 2009; Amann & Dempsey, 2008; Romer *et al.* 2007). Accordingly, Burnley *et al.* (2012) found similar levels of peripheral fatigue (i.e. reductions in potentiated twitch force; Q_{pot}) following single limb exercise and suggested that critical torque represents a critical threshold for neuromuscular fatigue development. In contrast, Thomas *et al.* (2016) reported different levels of peripheral fatigue following whole-body exercise within the severe intensity domain performed to task failure. More specifically, Thomas *et al.* (2016) reported similar reductions in MVC following supra-CP constant-load cycling (−15 to −18%), but with more predominant peripheral alterations when cycling in the upper part of the severe intensity domain. Central fatigue was conversely more predominant when cycling nearer or within the upper boundary of the heavy intensity domain. Unfortunately, in this study, the full depletion of W' was not controlled and therefore, an earlier termination of the voluntary task, due to behavioural effects before reaching 'true' physiological limits, could have confounded the results. An improved study design controlling for the use of W' and removing the potential confounding effect of participants' decision-making processes associated with performance may be warranted. In addition, the use of a more sophisticated neuromuscular assessment, i.e. application of paired low-frequency (PS10) and high-frequency (PS100) stimulations, may provide further insight into the mechanisms underlying peripheral fatigue (Verges *et al.*, 2009).

The CP concept constitutes a potent framework for the investigation of exercise tolerance in the severe intensity domain (Burnley *et al.* 2016; Poole *et al.* 2016; Grassi *et al.* 2015; Murgatroyd *et al.* 2011). Its integration with electromyographic and mechanical measures of neuromuscular fatigue offers great potential for a better understanding of the limits of exercise tolerance within the severe intensity domain (Burnley *et al.* 2012). The aim of the present study was therefore to investigate the aetiology of neuromuscular fatigue following severe cycling exercise leading to full and controlled

depletion of *W′*, thus at two different power outputs above CP calculated to exhaust 100% of *W′* in 3 and 12 min. We hypothesised that exercise above CP would lead to reductions in MVC, and the development of peripheral (i.e. reductions in Q_{pot}, PS10, PS100) and central fatigue (i.e. reductions in VA_{PNS}), without differences between the 3 min and 12 min trials. In addition, inter-individual variations in the development of neuromuscular fatigue were further explored against classic determinants of aerobic (CP and $\dot{V}O_{2peak}$) and anaerobic capacities (*W′*).

5.3. METHODS

Data collection for the present study and the study presented in Chapter 4 and Chapter 6 (Study 1 and 3) was conducted collectively.

5.3.1. Participants

Following ethical approval (see Section 3.2.) and informed consent (see Section 3.3.), twelve recreationally active males (mean ± SD: age, 23.4 ± 4.1 years; body mass 77.3 ± 10.6 kg; peak O_2 consumption ($\dot{V}O_{2peak}$), 3.84 ± 0.56 L·min^{-1}, peak power output (P_{peak}), 337 ± 46 W) volunteered for this study. All participants were young healthy individuals who were familiar with cycle ergometry and the exercise procedures used in our laboratory. For participant characteristics (see Table 4.2., Section 4.3.1.).

5.3.2. Experimental design

The participants reported to the laboratory on eight different occasions over a three to five week period. The tests included a ramp incremental test for the determination of $\dot{V}O_{2peak}$, a familiarisation to the experimental protocol (Section 3.6.), four to five constant-load trials performed to task failure for the determination of CP and *W'* (Section 3.7.) and subsequently, two randomised visits to assess neuromuscular fatigue before and 1 min following constant-load cycling above CP. All tests were performed at the same time of day (± 2 h) to control for the effect of diurnal variation (Atkinson & Reilly, 1996) and separated by a minimum of 24 h. The two randomly assigned main trials (visit 7 and 8) were separated by a minimum of 48 h. Participants were instructed to report to the laboratory in a fully rested and well hydrated state and to refrain from alcohol (24 h) and caffeine consumption (12 h) prior to testing (see Section 3.5.).

5.3.3. Incremental test and familiarisation

Incremental tests were performed as described in the Chapter 3 (Section 3.6.). Briefly, power output was initially set to 50-125 W depending on individual fitness level and increased by 5 W every 12 s

until task failure, followed by a verification trial at 105% P_{peak} (Rossiter *et al.* 2006). $\dot{V}O_{2peak}$ was defined as the highest 15 s moving average. Peak power was calculated as the highest 15 s moving average during the ramp test.

During a second visit, participants were familiarised with constant-load trials performed to task failure, NMFA and a quick transition from the cycle ergometer to the isometric rig.

5.3.4. Determination of CP and *W′*

The participants completed a semi-randomised series of four to five constant-load tests to elicit task failure within ~3 and 15 min (Hill, 1993; Poole *et al.*, 1988) as described in Section 3.7.

5.3.5. Experimental trials

Power output was predicted for each participant from interpolation of the power - time relationship and set to induce full depletion of *W'* within 12 min (P-12) and 3 min (P-3). Neuromuscular function assessment was performed before and 1 min post-exercise. Ventilation and pulmonary gas exchange were recorded continuously throughout cycling exercise.

5.3.6. Neuromuscular function assessment

Neuromuscular function assessment (NMFA) was performed before (PRE) and 1 min post-exercise (POST 1) as described in Section 3.8.

5.3.7. Peak oxygen uptake ($\dot{V}O_{2peak}$) across trials

Oxygen uptake was recorded continuously at rest and throughout exercise and $\dot{V}O_{2peak}$ was determined as described in Section 3.9.1.

5.3.8. Heart rate

Heart rate was recorded continuously at rest and throughout exercise (see Section 3.9.2.).

5.3.9. Visual analogue fatigue scale

Subjective feelings of fatigue were assessed using a visual analogue fatigue scale at rest and after neuromuscular function assessment, as described in Section 3.9.3.

5.3.10. Data analysis

Neuromuscular function measures, critical power and pulmonary gas exchange were analysed as described in Section 3.8.5., Section 3.7. and Section 3.9.1., respectively.

One participant was excluded from the data analysis for MRFD, MRR and HRT after values were identified as outliers (> 2SD from the mean; n = 11). One participant was excluded from the data analysis for M-wave parameters due to technical issues (n = 11).

5.3.11. Statistical analysis

All data was analysed using the Statistical Package for the Social Sciences (SPSS version 22 for Windows, IBM Corporation, New York, USA) and reported as mean ± SD, unless stated otherwise. Each data set was assessed for normal distribution and sphericity as outlined in Section 3.10. Two-way repeated measures ANOVA on the factors 'condition' (P-3 vs. P-12) and 'time' (pre- vs. post-exercise) were used to test for differences in neuromuscular, physiological and perceptual-measures. *Post hoc* analysis was performed following a significant main or interaction effect using Bonferroni *post hoc* adjusted pairwise comparisons. Paired-samples *t*-tests were used to compare the $\dot{V}O_{2peak}$ achieved during the ramp incremental test and the verification trial. Relationships were investigated using Pearson's product-moment correlations or partial correlations. The level of significance was set at $p < 0.05$. Partial eta squared (η_p^2) was calculated for main and interaction effects (Cohen, 1998).

5.4. RESULTS

5.4.1. Incremental test and determination of CP and *W′*

Data from the incremental test as well as a summary of individual data characterising the P-t_{lim} relationship is presented in Section 4.4.1. (Table 4.3.). Mean power outputs for P-3 and P-12 were 329 ± 47 W (98 ± 4% P_{peak}) and 248 ± 45 W (73 ± 6% P_{peak}), respectively.

5.4.2. Maximal voluntary force

MVC decreased significantly from pre- to 1 min post-exercise by −20 ± 10% and −15 ± 7% for P-3 and P-12, respectively ($F_{(1,11)} = 35.23$; $p < 0.001$; $\eta_p^2 = 0.76$), with no significant main effect for condition ($F_{(1,11)} = 0.34$, $p = 0.57$, $\eta_p^2 = 0.03$) and no interaction effect ($F_{(1,11)} = 3.64$; $p = 0.08$; $\eta_p^2 = 0.25$) (Figure 5.1. and Table 5.1.).

5.4.3. Potentiated twitch force and doublet twitch forces

Potentiated twitch force, PS10, PS100 and PS10:100 were significantly reduced by −35 ± 13%, −38 ± 13%, −18 ± 9% and −26 ± 11% following P-3 and by −31 ± 14%, −37 ± 17%, −13 ± 8% and −27 ± 15% following P-12 (Q_{pot}: $F_{(1,11)} = 95.96$; $p < 0.001$; $\eta_p^2 = 0.90$; PS10: $F_{(1,11)} = 109.30$; $p < 0.001$; $\eta_p^2 = 0.91$; PS100: $F_{(1,11)} = 52.64$; $p < 0.001$; $\eta_p^2 = 0.83$, PS10:100: $F_{(1,11)} = 71.33$; $p < 0.001$; $\eta_p^2 = 0.87$) (Figure 5.1. and Table 5.1.). There was no significant main effect for condition ($p > 0.05$; Figure 5.1. and Table 5.1.) and no interaction effect ($p > 0.05$).

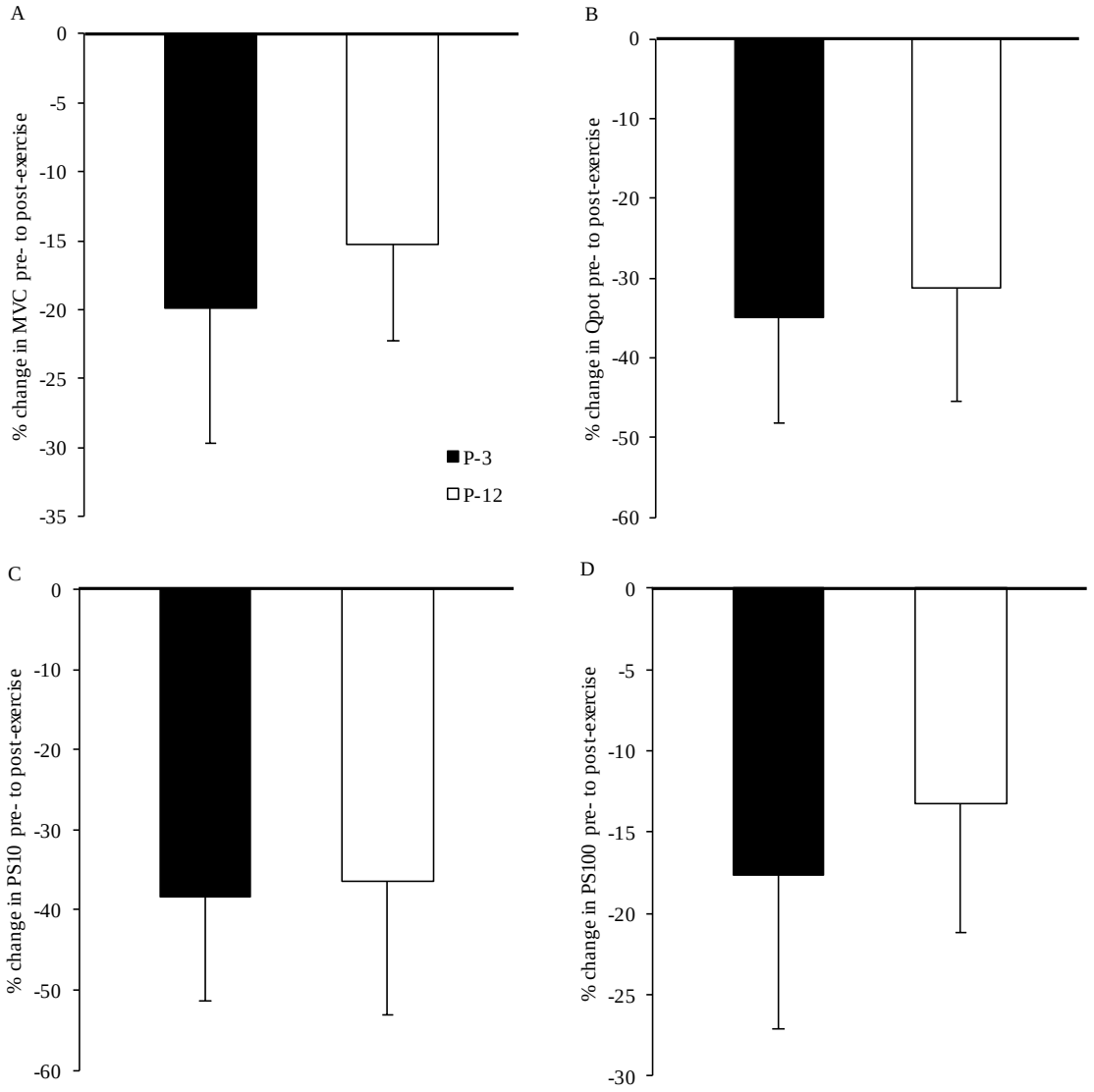


Figure 5.1. Pre- to post-trial percentage change in maximal voluntary contraction (MVC; A), potentiated twitch force (Q_{pot}; B), low-frequency (10 Hz) doublet force (PS10; C) and high-frequency (100 Hz) doublet force (PS100; D) for 3 (P-3) and 12 min (P-12).

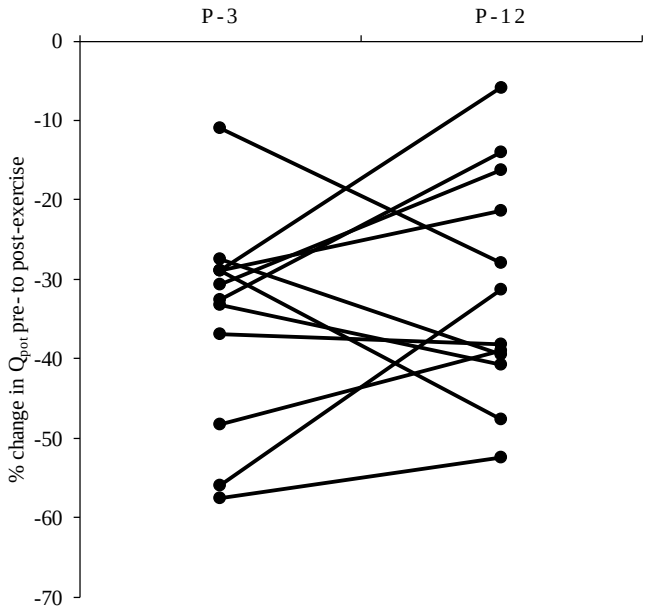


Figure 5.2. Pre- to post-trial percentage change in potentiated twitch force (Q_{pot}) for 3 (P-3) and 12 min (P-12). Individual data are shown.

5.4.4. M-wave properties

M-wave PPA and M-wave area showed no significant main effect for time ($F_{(1,10)} = 0.47$; $p = 0.509$; $\eta_p^2 = 0.05$ and $F_{(1,10)} = 4.40$; $p = 0.062$; $\eta_p^2 = 0.31$) or condition ($F_{(1,10)} = 2.31$; $p = 0.160$; $\eta_p^2 = 0.19$ and $F_{(1,10)} = 0.61$; $p = 0.454$; $\eta_p^2 = 0.06$; Table 5.1.) and no interaction effect ($F_{(1,10)} = 1.92$; $p = 0.196$; $\eta_p^2 = 0.16$ and $F_{(1,10)} = 3.97$; $p = 0.074$; $\eta_p^2 = 0.28$).

5.4.5. Voluntary activation

VA decreased significantly pre- to 1 min post-exercise by −11% and −12% for P-3 and P-12 ($F_{(1,11)} = 53.51$; $p < 0.001$; $\eta_p^2 = 0.83$), with no main effect for condition (Table 5.1.) and no interaction effect ($F_{(1,11)} = 0.13$; $p = 0.73$; $\eta_p^2 = 0.01$).

Table 5.1. Neuromuscular measures at pre-exercise (PRE) and 1 min following severe intensity cycling exercise (POST 1) for 3 (P-3) and 12 min (P-12).

Parameter	P-3		P-12	
	PRE	POST 1	PRE	POST 1
Neuromuscular fatigue				
MVC (N)	573 ± 128	451 ± 72*	563 ± 110	473 ± 77*
Peripheral fatigue				
Q_{pot} (N)	156 ± 23	100 ± 19*	150 ± 18	104 ± 26*
PS10 (N)	224 ± 44	137 ± 36*	212 ± 36	136 ± 46*
PS100 (N)	228 ± 25	186 ± 18*	222 ± 26	192 ± 25*
PS10:PS100	0.98 ± 0.13	0.73 ± 0.17*	0.96 ± 0.11	0.70 ± 0.17*
CT (ms)	77 ± 12	70 ± 8*	74 ± 8	67 ± 7*
MRFD$^{\#}$ ($N \cdot ms^{-1}$)	4.92 ± 0.98	2.61 ± 0.70*	4.96 ± 1.00	3.23 ± 1.39*
MRR$^{\#}$ ($N \cdot ms^{-1}$)	−1.77 ± 0.47	−1.07 ± 0.28*	−1.74 ± 0.46	−1.36 ± 0.36*†
HRT$^{\#}$ (ms)	75.5 ± 13.2	78.2 ± 18.1	74.8 ± 10.8	61.2 ± 14.6†
Central fatigue				
Peripheral VA (%)	88 ± 5	77 ± 15*	88 ± 8	76 ± 9*
Surface EMG				
M-wave PPA$^{\#}$ (mV)	9.3 ± 2.5	9.2 ± 3.9	8.7 ± 2.4	8.9 ± 2.0
M-wave area$^{\#}$ ($\mu V \cdot s^{-1}$)	30.5 ± 12.9	34.9 ± 14.9	29.8 ± 11.4	30.3 ± 12.3

Data are presented as mean ± SD (n = 12). Abbreviations: MVC, maximal voluntary contraction; Q_{pot}, potentiated twitch force; PS10, low-frequency (10 Hz) doublet force; PS100, high-frequency (100 Hz) doublet force; CT, contraction time; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; HRT, half-relaxation time; M-wave PPA, M-wave peak-to-peak area; VA, voluntary activation; *$p < 0.05$ vs. PRE, $^{\dagger}p < 0.05$ vs. P-3 at POST 1; $^{\#}n = 11$.

5.4.6. Peak oxygen uptake ($\dot{V}O_{2peak}$) across trials

There was no significant difference in $\dot{V}O_{2peak}$ achieved during the fast ramp test (3.84 ± 0.56 $L \cdot min^{-1}$), the four constant-load trials to determine CP and *W′* (1: 3.79 ± 0.52; 2: 3.84 ± 0.58; 3: 3.58 ± 0.57; 4: 3.70 ± 0.57 $L \cdot min^{-1}$) and the two main trials, P-3 (3.86 ± 0.59 $L \cdot min^{-1}$) and P-12 (3.65 ± 0.55 $L \cdot min^{-1}$) ($F_{(2.902, 31.918)} = 2.04$; $p = 0.13$; $\eta_p^2 = 0.16$).

5.4.7. Heart rate

There was no difference between trials in resting HR between trials ($t_{11} = 0.371$; $p = 0.718$; $d = 0.104$). Heart rate increased significantly over time in both P-3 ($F_{(1.053,9.475)} = 23.73$; $p = 0.001$; $\eta_p^2 = 0.73$) and P-12 ($F_{(1.281,14.092)} = 58.64$; $p < 0.001$; $\eta_p^2 = 0.84$). There was a significant main effect for condition ($F_{(1,9)} = 8.88$; $p = 0.016$; $\eta_p^2 = 0.50$) and time ($F_{(1,9)} = 8.88$; $p = 0.016$; $\eta_p^2 = 0.50$) between P-3 in comparison to the same time point during P-12, but no interaction effects ($F_{(1.051,9.462)} = 2.056$; $p = 0.18$; $\eta_p^2 = 0.19$). The end HR taken at the final 30 s was not significantly different between trials ($t_{11} = -0.393$; $p = 0.703$, $d = 0.145$; Figure 5.2.).

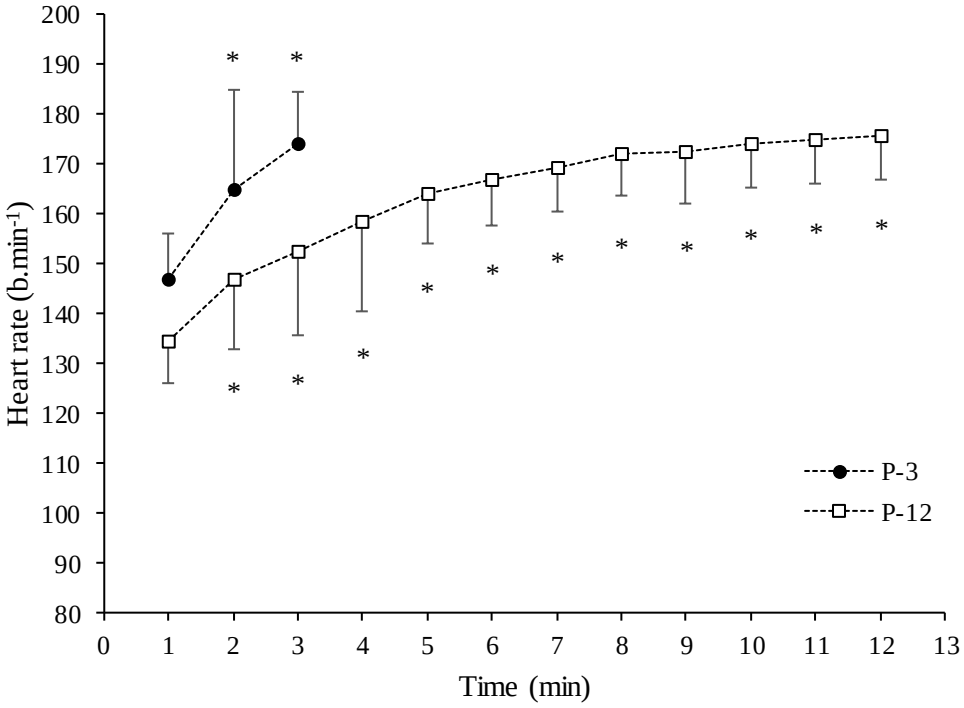


Figure 5.3. Heart rate response following severe intensity cycling exercise for 3 (P-3; closed circles) and 12 min (P-12; open squares). *p < 0.05 vs. minute 1

5.4.8. Visual analogue fatigue scale

There was a significant difference between pre- and post-exercise ($F_{(1,11)} = 118.43$; $p < 0.001$; $\eta_p^2 = 0.92$) with no difference between conditions ($F_{(1,11)} = 0.761$; $p = 0.40$; $\eta_p^2 = 0.07$) and no interaction effect ($F_{(1,11)} = 2.887$; $p = 0.12$; $\eta_p^2 = 0.21$).

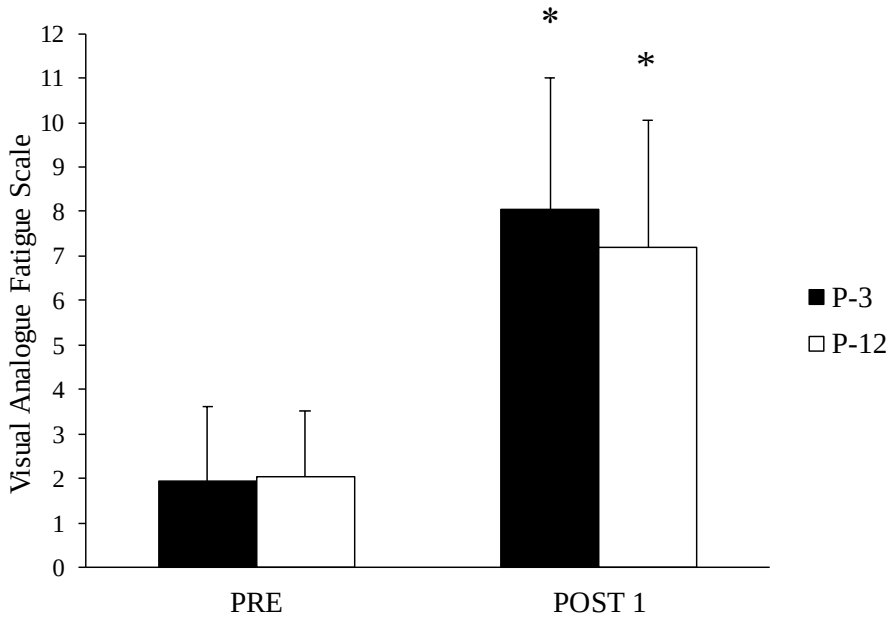


Figure 5.4. Visual Analogue Fatigue Scale following severe intensity cycling exercise for 3 (P-3; closed bars) and 12 min (P-12; open bars). *p < 0.05 vs. PRE.

5.4.9. Bivariate correlations between changes in neuromuscular function and measures of aerobic and anaerobic capacities

A larger *W′* was associated with larger reductions in Q_{pot} (r = 0.65; p = 0.022) and PS10 (r = 0.62; p = 0.033) for P-3 (Figure 5.4.). No significance was obtained for relationships between aerobic capacity (CP and $\dot{V}O_{2peak}$) and measures of neuromuscular fatigue for P-3 (p > 0.05), except an inverse relationship between CP and PS10 (r = −0.63; p = 0.028). In contrast for P-12, the changes in neuromuscular function did not correlate with *W′* for P-12 (p > 0.05). However, changes in MVC were inversely related to CP (r = −0.74; p = 0.006) and $\dot{V}O_{2peak}$ (r = 0.66; p = 0.019), so that individuals with greater aerobic capacities showed smaller changes in MVC (Fig. 3). Similar relationships were found between changes in evoked twitch forces and $\dot{V}O_{2peak}$ for P-12, with smaller changes in Q_{pot} (r = 0.65; p = 0.023) and PS10 (r = 0.58; p = 0.047) for individuals of higher $\dot{V}O_{2peak}$ (Figure 5.5.).

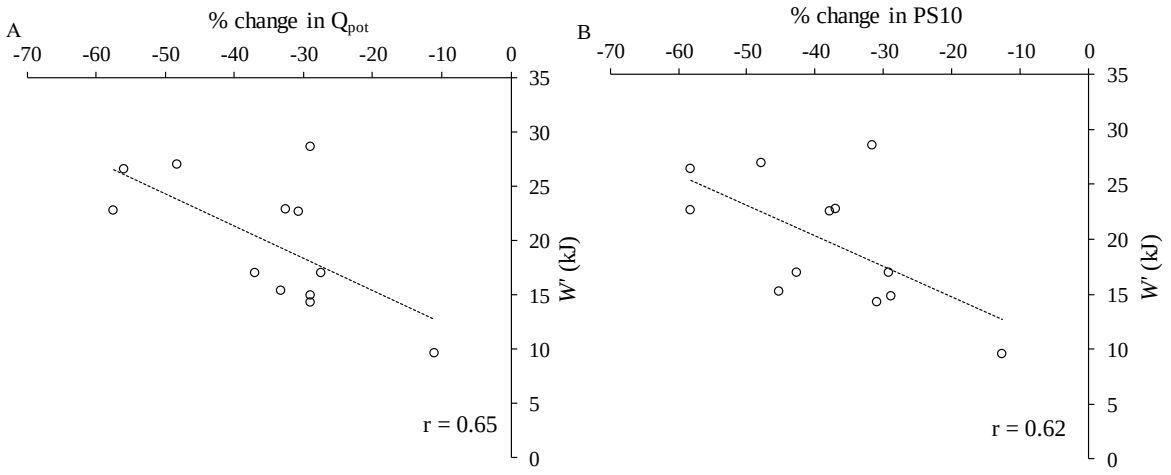


Figure 5.5. Correlations between *W′* and % change in maximal voluntary contraction, MVC (A) and between *W′* and % change in potentiated twitch force, Q_{pot} (B) for P-3. Pearson's correlation coefficient (r) are displayed. Correlations were significant ($p < 0.05$).

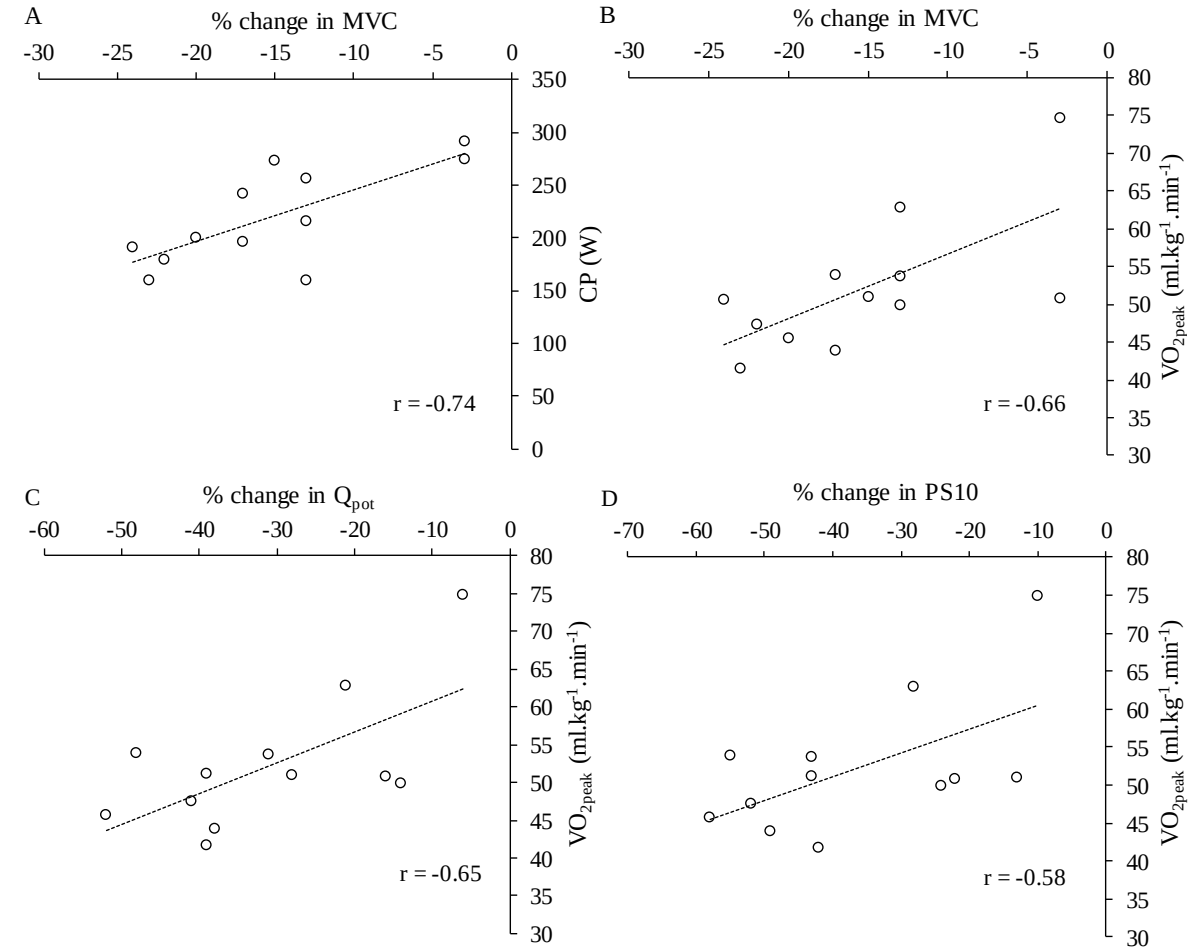


Figure 5.6. Correlations between CP and % change in MVC (A), $\dot{V}O_{2peak}$ and % change in MVC (B), $\dot{V}O_{2peak}$ and % change in Q_{pot} (C), $\dot{V}O_{2peak}$ and % change in PS10 (D) for P-12. Pearson's correlation coefficient (r) are displayed. All correlations were significant ($p < 0.05$)

5.5. DISCUSSION

The present study is the first to demonstrate that neuromuscular fatigue observed following full depletion of *W′* is of similar magnitude whether supra-CP cycling exercise is performed close to the lower vs. upper boundary of the severe intensity domain. The level of peripheral fatigue in the severe intensity domain does therefore not depend on power output or exercise duration when 100% of *W′* has been exhausted above CP.

5.5.1. Peripheral fatigue following cycling above CP

Peripheral fatigue has previously shown to be duration- and intensity-dependent, with greater loss of evoked twitch forces following shorter, highly intense exercise when compared to longer, low-intensity exercise (Temesi *et al.* 2017; Thomas *et al.* 2016; 2015; O'Leary *et al.* 2015; Burnley *et al.* 2012). The present study found reductions in Q_{pot} following severe cycling exercise of 30-35%, which is in line with previously reported reductions of −20 to −40% following whole-body high-intensity exercise (Dominelli *et al.* 2017; Goodall *et al*. 2015; O'Leary *et al.* 2015; Johnson *et al.* 2015; Thomas *et al.* 2015; Amann, 2011), but with no difference between P-3 and P-12. These differences between studies may be due to the design of the task (e.g. open-end vs. closed-end test, exercise mode). This has been considered in the 'sensory tolerance limit theory', refined from the 'critical threshold' concept of peripheral fatigue. The sensory tolerance limit theory described a more global negative feedback loop, taking the sum of numerous factors into account (locomotor muscles, respiratory muscles, organs and muscles not directly involved in exercise) (Hureau *et al.*, 2016b).

The present study confirms and expands the findings of Burnley *et al.* (2012) to whole-body exercise. The authors reported substantial reductions in the force generating capacity following single-leg contractions at different intensities above critical torque (~40-55%). These impairments were predominantly associated with alterations at or distal to the neuromuscular junction (PS100: ~32-35%). These changes are greater than those of the present study, which may be due to an underestimation of the magnitude of fatigue as a result of a delayed NMFA following cycling exercise in the present study or due to differences in exercise modalities *per se* (knee extensions vs. cycling exercise). Indeed, greater magnitudes of peripheral fatigue have been demonstrated following exercise involving smaller muscle mass by Rossman *et al.* (2014; 2012).

Interestingly, the reductions in potentiated twitch force observed following P-12 (−31%) are substantially greater than those following a ~11 min constant-load trial performed until task failure reported by Thomas *et al.* (2016) (−16%). The decision-making behaviour involved in the performance of a time to task failure may lead to a premature end of the task, i.e. before 100% of *W'* is depleted, and participants may therefore stop before reaching their 'true' physiological limits. This would further lead to an underestimation of the magnitude of neuromuscular fatigue. However, blood

[La^-] as well as $\dot{V}O_2$ reached near maximum levels in Thomas *et al.* (2016) and therefore, a premature test termination may be excluded. Furthermore, the set-up for the assessment of neuromuscular function was similar between studies (e.g. 90° hip and knee angle, motor nerve stimulation at 130% of stimulation threshold, pulse duration etc.); apart from the additional application of paired low- and high-frequency stimulations used in the present study. Thus, it remains unclear why reductions in Q_{pot} were substantially smaller in Thomas *et al.* (2016) compared the present work.

In the present study, PS10 and PS100 were significantly reduced following exercise, but with no significant difference between P-3 and P-12. A proportionally greater reduction in twitch force was observed for PS10 (~27%), described as low-frequency fatigue (LFF) (Verges *et al.*, 2009; Edwards et al., 1977). These findings are in accordance with the reduction in PS10:100 (~30%) reported by Temesi *et al.* (2017) following 6-min cycling exercise within the severe intensity domain (80% P_{peak}). LFF has been associated with a reduction of Ca^{2+} release from the SR (Balog, 2010; Allen *et al.* 2008b; Keeton & Binder-Macleod, 2006; Rassier & MacIntosh, 2000).

Reductions in Q_{pot} may be mediated by alterations of the sarcolemmal excitability, measured by changes in M-wave PPA and M-wave area. Whereas M-wave PPA did not differ significantly pre- to post-exercise in both trials, changes in M-wave area were more pronounced following P-3 compared to P-12 (+14% vs. +2%). This would suggest a greater disturbance in the propagation or transmission of action potentials following high-intensity cycling exercise in the upper part of the severe intensity domain. In contrast, Black *et al.* (2017) found changes in M-wave amplitude and area of greater extent following moderate and heavy exercise compared to severe intensity exercise, suggesting that sarcolemmal excitability is more affected after longer, low-intensity exercise. Previous studies described controversial results with some studies reporting increases, decreases or no changes in M-wave properties (for review see Rodriguez-Falces & Place, 2018). These discrepancies may be explained by differences in the muscle studied and/or methodological differences (i.e. stimulation technique, electrode placement). Furthermore, the modest reliability reported for surface EMG recordings of voluntary and evoked contractions (Ball & Scurr, 2010; Buckthorpe *et al.* 2012; Rota *et al.* 2013) may be considered when discussing meaningful change.

5.5.2. Central fatigue following cycling above CP

Changes in VA of 5 to 10% have been found following exercise within the severe intensity domain (Temesi *et al.* 2017; Thomas *et al*. 2016; 2015; Johnson *et al.* 2015; Goodall *et al.* 2015; Sidhu *et al.* 2014), with greater reductions in the lower part of the domain (Thomas *et al.*, 2016; Burnley *et al.*, 2012). The present study is in line with these findings, observing moderate reductions in VA but with no difference between the trials (P-3: −11%; P-12: −12%). Exercise-induced reduction in VA implies that central fatigue develops due to a suboptimal neural drive from the motor cortex (supra-spinal

fatigue) and/or changes in the intrinsic properties of the motor neurons (spinal fatigue) (Taylor *et al.* 2006; Gandevia, 2001; 1998).

5.5.3. Exploratory research using bivariate correlation between changes in neuromuscular function and indices of aerobic and anaerobic capacities

The magnitude of the changes in neuromuscular fatigue showed large variability between participants (reduction in MVC: CV ~50% for P-3; CV ~46% for P-12). De Souza *et al.* (2016) reported similar results with great between-participant variability (>50% CV) in peak torque reduction after fatiguing cycling exercise (70% W' depletion in 3 and 10 min) and suggested that this may be due to an inverse relationship between CP and the change in peak torque. Further, Coelho *et al.* (2015) reported an inverse relationship between the reduction in isokinetic power and $\dot{V}O_{2peak}$ following a maximal incremental cycling test. In agreement, in the present study, changes in MVC following P-12 were inversely related to CP ($r = -0.74$; $p = 0.006$) and $\dot{V}O_{2peak}$ ($r = -0.66$; $p = 0.019$; Figure 5.5.). Participants of high $\dot{V}O_{2peak}$ also displayed smaller changes in Q_{pot} ($r = -0.65$; $p = 0.023$) and PS10 ($r = -0.58$; $p = 0.047$; Figure 5.5.). Aerobically fitter participants seem to be coping better with the development of peripheral fatigue during severe intensity exercise of longer duration. This may be because of a faster and greater O_2 delivery alongside structural adaptations within the exercising muscles (Murgatroyd *et al.* 2011; Rossiter, 2011) which may reduce or delay the accumulation of fatigue-related metabolites or, expedite their removal.

In contrast, for P-3, the changes in MVC were not significantly correlated with $\dot{V}O_{2peak}$ or CP ($p > 0.05$). A larger W' was associated with larger reductions in Q_{pot} ($r = 0.65$; $p = 0.022$) and PS10 ($r = 0.62$; $p = 0.033$; Figure 5.4.). Although bivariate correlations do not prove a causal relationship, these significant relationships support the links between utilisation of W' and development of peripheral fatigue as suggested by Murgatroyd *et al.* (2011). It would be worth noting here that individuals with greater MVC pre-exercise also showed greater reductions following P-12 ($r = 0.85$; $p < 0.001$) and P-3 ($r = 0.79$; $p = 0.002$) but did not have greater W' (P-12: $r = 0.35$; $p = 0.27$; P-3: $r = 0.37$; $p = 0.24$). It may be suggested that aerobic capacity is of greater relevance during P-12 as the aerobic contribution relative to the total energy turnover increases with increasing exercise duration, whereas during P-3 the anaerobic energy turnover contributes to a relatively larger extent to the total energy turnover.

5.5.4. Limitations

In the protocol of the present study, muscular activation must have differed between P-3 and P-12 (intensity · time effect) for all flexors and extensors contributing to external power production, and most likely so for the knee extensors, i.e. the muscle group tested during the NMFA protocol.

According to the relationship between exercise intensity and percentage and type of muscle fibres engaged in cycling exercise, it might be assumed that the contribution of type II muscle fibres to the total force required was greater in P-3 compared to P-12, during which a relatively smaller force was applied over a longer duration (Sale, 1987). An intensity x time effect on VL activation during the two cycling trials would have affected the physiological processes underpinning muscle force generation of both MVC and twitch forces recorded following cycling. This would likely interfere with the association proposed in the present framework between the use of W' and the key measures of NMF.

A major methodological limitation in studies investigating neuromuscular fatigue following locomotor exercise lies in the time delay between exercise termination and neuromuscular assessment due to the transition from the cycle ergometer or treadmill to the dynamometer. However, the present study standardised the time window for the transition to 60 s for each trial and all neuromuscular assessments were completed within 100 s. The delayed assessment of neuromuscular measures likely caused an underestimation of the magnitude of neuromuscular fatigue due to significant recovery of neuromuscular function within the first 1-2 min after exercise termination (Froyd *et al.* 2013). Furthermore, the isometric contraction used to assess muscular force generating capacity does not represent the dynamic contraction pattern during cycling exercise. Therefore, results should be interpreted with caution.

Moreover, the study was designed with the assumption that exercise is terminated when W' is fully depleted. It needs to be acknowledged that this mathematical assumption of the CP model may not fully hold true physiologically; considering the variation in TTF reported when exercising at CP (~16 to 65 min; de Lucas *et al.*, 2013; Carter *et al.*, 2005; Brickley *et al.*, 2002; Hill & Smith, 1999) or exercising above CP (Study 4: 154 to 302 s). This discrepancy may be explained by the variation associated with the W' and the TTF to predict CP.

5.6. CONCLUSION

In conclusion, the present study demonstrates for the first time that the magnitude of neuromuscular fatigue observed following full depletion of W' is similar when supra-CP exercise is performed close to the lower or upper boundary of the severe intensity domain. Further exploratory analysis showed that smaller changes in the force-generating capacity are seen in individuals with greater aerobic capacities for the longer severe-intensity exercise, but in individuals with greater anaerobic capacities for the shorter severe-intensity cycling exercise. Thus, despite no difference in the magnitude of neuromuscular fatigue following a short vs. long bout of severe intensity exercise, the present results suggest different physiological mechanisms underlie exercise tolerance within the lower vs. upper boundary of this intensity domain. Future research should aim to provide experimental evidence for

a causal relationship between *W′*, CP and neuromuscular fatigue in order to further understand exercise tolerance within the severe intensity domain.

6. STUDY 3: Neuromuscular recovery following cycling exercise above critical power

6.1. ABSTRACT

The third study examined the time course of neuromuscular recovery following cycling exercise at different exercise intensities and durations above CP. Twelve recreationally active male participants completed an incremental cycling test to determine $\dot{V}O_{2peak}$, 4-5 constant-load trials to task failure to calculate CP and W' and two randomly assigned experimental trials, where power output was set to fully deplete W' in either 3 (P-3) or 12 min (P-12). Neuromuscular function assessment of the right knee extensors was performed before and 1, 6, 15- and 30-min post-exercise to measure voluntary and evoked twitch forces, M-wave properties and VA using femoral nerve stimulation. A significant main effect for time was found for MVC and evoked twitch forces as well as for VA ($p < 0.05$; pre- to post-exercise changes are presented in Chapter 5). MVC fully recovered within 15 min after test termination ($p > 0.05$), however, central (VA) and peripheral fatigue (Q_{pot}, PS10, PS100) remained present after 30 min of rest ($p < 0.05$). There was no significant difference during neuromuscular recovery between conditions for MVC ($F_{(1,10)} = 0.11$; $p = 0.752$), Q_{pot} ($F_{(1,9)} = 3.27$; $p = 0.104$; $\eta_p^2 = 0.27$), PS100 ($F_{(1,9)} = 3.17$; $p = 0.109$; $\eta_p^2 = 0.26$) and VA ($F_{(1,9)} = 0.02$; $p = 0.888$; $\eta_p^2 = 0.002$). PS10 was significantly greater in P-3 compared to P-12 ($F_{(1,9)} = 6.91$; $p = 0.027$; $\eta_p^2 = 0.43$). No interaction effects were found for MVC, Q_{pot}, PS10, PS100 and VA ($p < 0.05$). Both M-wave PPA and area did not change over time ($p = 0.146$; $p = 0.157$) with no significant difference between conditions ($p = 0.146$; $p = 0.209$). Individuals with a larger CP presented a slower recovery in Q_{pot} ($r = -0.678$, $p = 0.031$) and PS10 ($r = -0.705$; $p = 0.023$) at POST 30 for P-3 and a greater $\dot{V}O_{2peak}$ was associated with a slower recovery in PS10 ($r = -0.707$; $p = 0.022$) at POST 30 for P-12. However, when controlling for the magnitude of neuromuscular fatigue at POST 1, the relationship between aerobic capacity and neuromuscular recovery of PS10 disappeared ($p > 0.05$). These results indicate that impairments in muscle function outlast the exercise bout irrespective of exercise intensity and duration above CP. Further, the magnitude of neuromuscular fatigue post-exercise and not aerobic capacity seems to predominantly determine the time course of neuromuscular recovery following severe intensity cycling exercise.

6.2. INTRODUCTION

The development of neuromuscular fatigue, measured as a reduction in the maximal force generating capacity has been subject of numerous research studies over more than a century, yet surprisingly little is known about the time course and the mechanism(s) underpinning neuromuscular recovery. Although a variety of interventions have been investigated, aiming to accelerate recovery following

an exercise bout (e.g. carbohydrate/protein supplementation (Betts & Williams, 2010; Goh *et al.*, 2012), cold therapy (Pointon *et al.*, 2011), hyperoxia (Sperlich *et al.*, 2017), active recovery (Giboin *et al.*, 2018)), only a limited number of studies provides further understanding of the physiological mechanisms driving neuromuscular recovery (Vernillo *et al.*, 2018; Froyd *et al.*, 2018; Carroll *et al.*, 2017; Brownstein *et al.*, 2017; Thomas *et al.*, 2017; Froyd *et al.*, 2013; Decorte *et al.*, 2012). A better understanding of neuromuscular recovery may enable us to determine more precisely the time point at which maximal force should be fully regained.

Time course of neuromuscular recovery has previously been shown to depend on exercise modality, intensity and duration (Vernillo *et al.*, 2018; Carroll *et al.*, 2017), however, the limited amount of studies published and the diversity in testing modalities make it difficult to draw comparisons. In general, a major proportion of volitional force recovers typically within 1-2 min following exercise termination, yet, complete recovery may take several hours or even days depending on the task undertaken (Carroll *et al.*, 2017; Froyd *et al.*, 2013; Millet *et al.*, 2011b; Ross *et al.*, 2007). During prolonged low-intensity exercise, this rapid initial force recovery is thought to be predominantly due to the recovery of the ability to voluntarily activate muscles (Carroll *et al.*, 2017). Following high-intensity exercise, a quick recovery of spinal and supraspinal components of fatigue can also be observed, however, the contribution of peripheral processes linked to excitation-contraction coupling and reperfusion of the muscles has been considered as the driving force behind the rapid initial restitution of force (Carroll *et al.*, 2017; Hunter *et al.*, 2008; Gandevia, 2001; Taylor *et al.*, 2000). For instance, fatigue-induced low-frequency impairments in muscle function have been associated with alterations in intracellular Ca^{2+} release or sensitivity and required protracted timescales for full restoration, whereas high-frequency force tends to recover within seconds likely due to the muscle reperfusion and the clearance of K^+ (Carroll *et al.*, 2017; Allen *et al.*, 2008a). Interestingly, Froyd *et al.* (2018) reported a substantial recovery in MVC and neuromuscular activation (measured as RMS during MVC normalized to M-wave PPA) with no recovery in evoked twitch force 5-10 s following three consecutive isometric times to task failure, which led to the conclusion that the quick recovery in the force generating capacity immediately following fatiguing exercise was likely due to increases in the central drive and not due to peripheral mechanisms.

To date, no systematic approach has been undertaken to evaluate whether the recovery kinetics of neuromuscular function depend on exercise intensity, and therefore duration, following severe-intensity exercise performed to task failure. Consistent changes in muscle metabolic responses (i.e. pH, [PCr], $[La^-]$) have been found following whole-body exercise of different intensities above CP (Black *et al.*, 2017). These metabolic perturbations at or distal to the neuromuscular junction (i.e. peripheral fatigue) have been put forward to explain the substantial reductions in the force-generating

capacity of the exercising muscles, particularly evident when exercising above CP. In Chapter 5, Study 2, similar magnitudes of neuromuscular fatigue when exercising at different intensities above CP were reported, but peripheral alterations were larger for individuals of greater anaerobic capacity following the shorter exercise and smaller in individuals of greater aerobic capacity following the longer exercise bout, suggesting that the physiological mechanisms underpinning neuromuscular fatigue may be dependent on exercise intensity even within the severe intensity domain. Interestingly, Vernillo *et al.* (2019) reported a faster recovery of voluntary force following 2 min of sustained isometric knee extension until 8 min post-exercise in individuals with greater aerobic capacities. Whether the time course of neuromuscular fatigue depends on exercise intensity following supra-CP exercise, and whether the ability to recover is related to an individual's aerobic and anaerobic capacities remains to be explored.

Therefore, the present study aims to investigate the effect of exercise intensity and duration on the time course of neuromuscular recovery following exercise above CP. To the best of this author's knowledge, this is the first study investigating the effect of different exercise intensities above CP on the time course of neuromuscular recovery. We hypothesised that (1) no significant difference would be observed in all neuromuscular measures at any time point during recovery between conditions, (2) the ability to voluntarily produce force recovers rapidly, but only partially within the first few minutes following exercise termination, (3) both, central and peripheral fatigue recover substantially but not fully within the first few minutes following exercise, (4) twitch forces evoked by low-frequency stimulations recover at a slower rate compared to twitch forces evoked by high-frequency stimulations, and (5) a faster neuromuscular recovery can be observed in individuals with greater aerobic capacities.

6.3. METHODS

Data collection for the present study and the study presented in Chapter 4 and Chapter 5 (Study 1 and 2) was conducted collectively.

6.3.1. Participants

Following ethical approval (see Section 3.2.) and collection of informed consent (see Section 3.3.), twelve recreationally active males (mean ± SD: age, 23.4 ± 4.1 years; body mass 77.3 ± 10.6 kg; peak O_2 consumption ($\dot{V}O_{2peak}$), 3.84 ± 0.56 $L \cdot min^{-1}$, peak power output (P_{peak}), 337 ± 46 W) volunteered for this study. All participants were young healthy individuals who were familiar with cycle ergometry and the exercise procedures used in our laboratory. For participant characteristics (see Table 4.2., Section 4.3.1.).

6.3.2. Experimental design

Participants reported to the laboratory on eight different occasions over a three to five week period, with each test performed at the same time of day (± 2 h) to control effects of diurnal variation (Atkinson & Reilly, 1996) and separated by a minimum of 24 h (48 h for the main trials). The tests included a ramp incremental test for the determination of $\dot{V}O_{2peak}$, a familiarisation to the experimental protocol (see 3.6.), four to five constant-load trials performed to task failure for the determination of CP and *W'* (see 3.7.) and two randomised experimental visits to investigate neuromuscular recovery following constant-load cycling with neuromuscular function assessment at rest and post-exercise and throughout recovery.

6.3.3. Neuromuscular function assessment

Neuromuscular function assessment was performed before (PRE) and 1, 6, 15- and 30-min following exercise (POST 1, 6, 15 and 30) as described in Section 3.8.

6.3.4. Visual analogue fatigue scale

Subjective feelings of fatigue were assessed using a visual analogue fatigue scale at rest and after each neuromuscular function assessment, as described in Section 3.9.3.

6.3.5. Data analysis

Voluntary and evoked twitch forces (MVC, Q_{pot}, PS10 and PS100), within-twitch measures (CT, MRFD, MRR and HRT), M-wave properties (M-wave PPA, M-wave area) and VA were analysed as described in Section 3.8.5.

Recovery was expressed as the change from POST 1 to POST 30, relative to the corresponding PRE value (Δrecovery).

One participant was excluded from the data analysis for MVC due to technical issues (n = 11). Two participants were excluded from the data analysis for evoked twitch forces (i.e. Q_{pot}, PS10, PS100), within-twitch measures (i.e. CT, MRFD, MRR, HRT) and VA due to technical issues (n = 10). Three participants were excluded from the data analysis for M-wave parameters due to technical issues (n = 9).

6.3.6. Statistical analysis

All data was checked for normal distribution as outlined in Section 3.10. Two-way repeated measures ANOVA on the factors 'condition' (P-3 vs. P-12) and 'time' (PRE, POST 1, POST 6, POST 15 and POST 30) were used to test for differences in neuromuscular and perceptual measures. *Post hoc* analysis was performed following a significant main or interaction effect using Bonferroni *post hoc*

adjusted pairwise comparisons. Effect sizes are presented as partial eta squared (η_p^2) for main and interaction effects (Section 3.10.). The level of significance was set at $p < 0.05$. All data are expressed as mean ± SD. Relationships between Δrecovery and measures of aerobic and anaerobic capacities were investigated using Pearson's product–moment correlations.

6.4. RESULTS

6.4.1. Maximal voluntary force

There was a significant main effect for time ($F_{(1.488,14.878)} = 22.31$; $p < 0.001$; $\eta_p^2 = 0.69$), but no significant main effect for condition ($F_{(1,10)} = 0.11$; $p = 0.752$; $\eta_p^2 = 0.01$). MVC was significantly reduced POST 1 ($p = 0.001$), remained significantly reduced POST 6 ($p = 0.012$) and recovered back to baseline at POST 15 ($p = 0.120$) (Figure 6.1., Table 6.1.). A significant interaction was observed between condition and time ($F_{(2.487, 24.871)} = 3.40$; $p = 0.040$; $\eta_p^2 = 0.25$), however follow-up pairwise comparison revealed no significant differences ($p > 0.05$).

6.4.2. Potentiated twitch force and doublet twitch forces

Q_{pot}, PS10, PS100 and PS10:100 showed a significant effect for time (Q_{pot}: $F_{(1.417,12.749)} = 77.11$; $p < 0.001$; $\eta_p^2 = 0.90$; PS10: $F_{(1.899,17.092)} = 95.81$; $p < 0.001$; $\eta_p^2 = 0.91$; PS100: $F_{(1.892,17.024)} = 29.70$; $p < 0.001$; $\eta_p^2 = 0.77$; PS10:100: $F_{(2.001,18.011)} = 96.28$; $p < 0.001$; $\eta_p^2 = 0.92$). All evoked twitch forces remained significantly reduced at POST 30 (Q_{pot}: $p < 0.001$; PS10: $p < 0.001$; PS100: $p = 0.002$; PS10:100: $p = 0.018$) (Figure 6.1., Table 6.1.). No significant differences between the two conditions were found for Q_{pot} and PS100 (Q_{pot}: $F_{(1,9)} = 3.27$; $p = 0.104$; $\eta_p^2 = 0.27$; PS100: $F_{(1,9)} = 3.17$; $p = 0.109$; $\eta_p^2 = 0.26$). PS10 was however found to be significantly greater for P-3 when compared to P-12 ($F_{(1,9)} = 6.91$; $p = 0.027$; $\eta_p^2 = 0.43$). A tendency for a greater PS10:100 ratio was observed for P-3 compared to P-12 ($F_{(1,9)} = 4.62$; $p = 0.06$; $\eta_p^2 = 0.34$). No interaction effects were found for all these variables ($p > 0.05$).

6.4.3. Within-twitch measures

CT, MRFD, MRR and HRT changed significantly over time (CT: $F_{(2.181,19.629)} = 7.32$; $p = 0.004$; $\eta_p^2 = 0.45$; MRFD: $F_{(2.121,19.092)} = 41.80$; $p < 0.001$; $\eta_p^2 = 0.82$; MRR: $F_{(2.130,19.169)} = 11.66$; $p < 0.001$; $\eta_p^2 = 0.56$; HRT: $F_{(4,36)} = 13.98$; $p < 0.001$; $\eta_p^2 = 0.61$) (Table 6.1.), with only HRT showing a significant difference between conditions (P-3 > P-12; $F_{(1,9)} = 12.41$; $p = 0.006$; $\eta_p^2 = 0.58$). MRFD and HRT were still significantly reduced POST 30 (MRFD: $p < 0.001$; HRT: $p = 0.007$). In contrast, CT and MRR returned to baseline levels within 30 min and 6 min of rest (CT: $p = 0.458$; MRR: $p = 0.103$). Interaction effects were only found for HRT ($F_{(2.099,18.890)} = 8.31$; $p = 0.002$; $\eta_p^2 = 0.48$) and pairwise

comparison revealed significantly greater values for P-3 at POST 6 (63 ± 15 ms vs. 55 ± 13 ms; $t_{(10)}$ = 3.30; p = 0.009; *d* = −0.57) and POST 15 (63 ± 16 ms vs. 57 ± 14 ms; $t_{(10)}$ = 3.10; p = 0.011; *d* = −0.40).

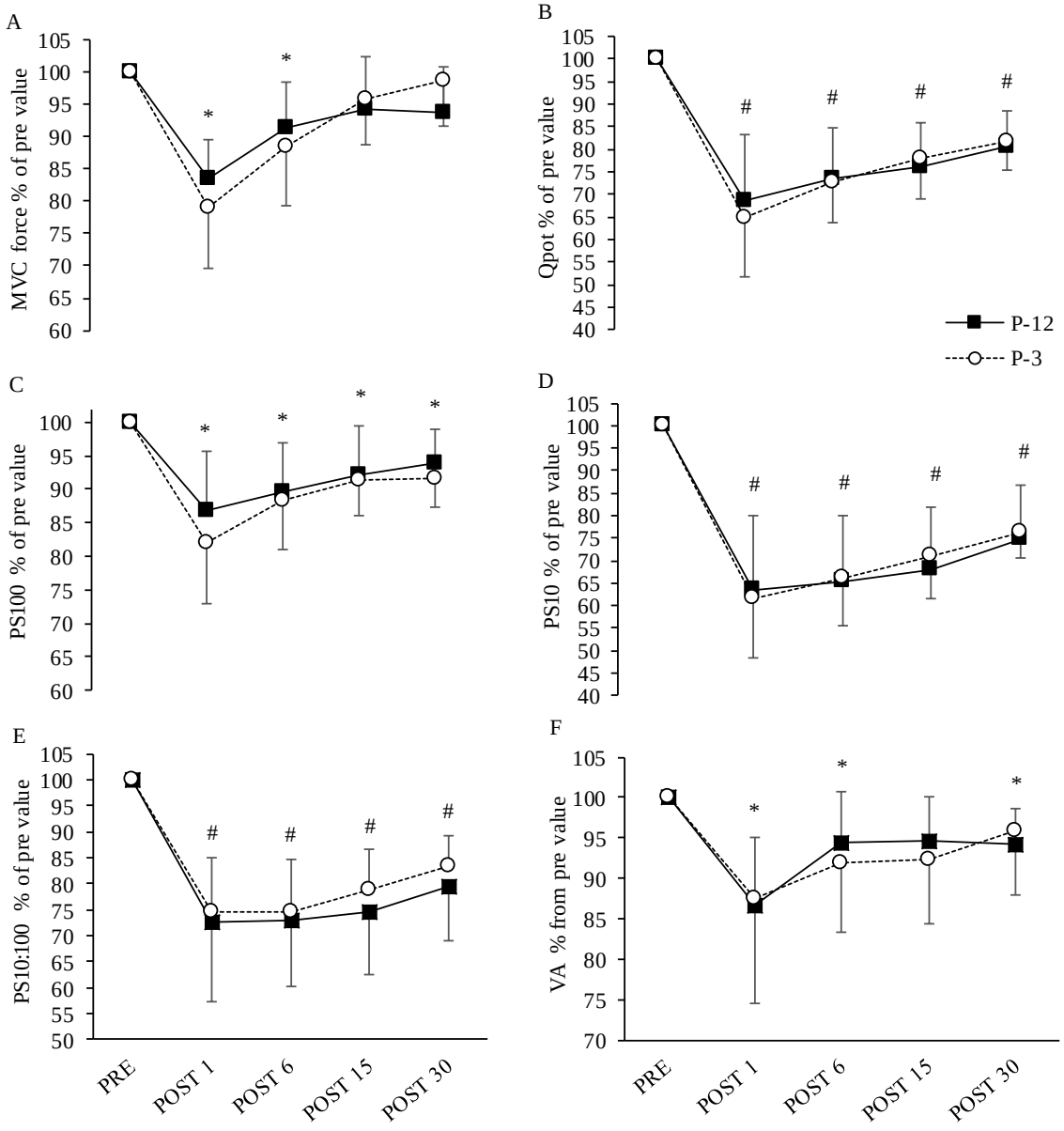


Figure 6.1. Percentage change from PRE values for maximal voluntary contraction (MVC; n = 11) (A), twitch forces evoked from single stimulations (Q_{pot}; n = 10) (B), paired stimulation at 100 Hz (PS100; n = 10) (C) and 10 Hz (PS10; n = 10) (D), the ratio between PS10:PS100 (n = 10) (E) and voluntary activation (VA; n = 10) (F) at POST 1, 6, 15 and 30; *p < 0.05 vs. PRE; #p < 0.001 vs. PRE.

6.4.4. M-wave properties

M-wave PPA and M-wave area showed no significant main effect for time (M-wave PPA: $F_{(2.041,16.329)}$ = 2.17; p = 0.146; η_p^2 = 0.21; M-wave area: $F_{(1.696,13.571)}$ = 2.16; p = 0.157; η_p^2 = 0.21; ; Table 6.1.), condition (M-wave PPA: $F_{(1,8)}$ = 2.59; p = 0.146; η_p^2 = 0.25; M-wave area: $F_{(1,8)}$ = 1.87; p = 0.209;

η_p^2 = 0.19) or interaction (M-wave PPA: $F_{(2.212,17.700)}$ = 1.00; p = 0.396; η_p^2 = 0.11; M-wave area: $F_{(1.429,11.436)}$ = 1.73; p = 0.220; η_p^2 = 0.18).

6.4.5. Voluntary activation

Voluntary activation changed significantly over time ($F_{(4,36)}$ = 11.40; p < 0.001; η_p^2 = 0.56) with no difference between conditions ($F_{(1,9)}$ = 0.021; p = 0.888; η_p^2 = 0.002) and no interaction effect ($F_{(4,36)}$ = 0.77; p < 0.549; η_p^2 = 0.08). Voluntary activation did not recover fully back to baseline after 30 min of recovery (p = 0.038; Table 6.1.).

Table 6.1. Neuromuscular measures for P-3 and P-12 at PRE and following exhaustive constant-load cycling at 1, 6, 15- and 30-min post

	Condition	PRE	POST 1	POST 6	POST 15	POST 30	Main and interaction effects
Neuromuscular fatigue							
MVC (N)	P-3	593 ± 112	462 ± 63	520 ± 80	565 ± 93	584 ± 104	Time: baseline vs. POST 1 and 6; Interaction
	P-12	583 ± 89	486 ± 66	531 ± 75	550 ± 98	549 ± 109	
Peripheral fatigue							
Q_{pot} (N)	P-3	157 ± 20	97 ± 18	114 ± 18	119 ± 18	125 ± 16	Time: baseline vs. POST 1, 6, 15 and 30
	P-12	147 ± 17	99 ± 24	107 ± 21	111 ± 20	117 ± 17	
PS10 (N)	P-3	222 ± 31	132 ± 35	148 ± 37	160 ± 41	169 ± 36	Time: baseline vs. POST 1, 6, 15 and 30; Condition
	P-12	210 ± 23	127 ± 39	134 ± 38	140 ± 38	155 ± 35	
PS100 (N)	P-3	229 ± 18	187 ± 19	202 ± 19	209 ± 18	210 ± 19	Time: baseline vs. POST 1, 6, 15 and 30
	P-12	218 ± 15	190 ± 20	196 ± 21	201 ± 22	205 ± 16	
CT (ms)	P-3	76 ± 5	71 ± 6	68 ± 6	71 ±7	72 ± 6	Time: baseline vs. POST 1, 6 and 15
	P-12	76 ± 6	67 ± 6	67 ± 7	68 ± 6	72 ± 4	
MRFD ($N \cdot ms^{-1}$)	P-3	4.77 ± 0.88	2.53 ± 0.67	3.36 ± 0.73	3.55 ± 0.66	3.76 ± 0.79	Time: baseline vs. POST 1, 6, 15 and 30
	P-12	4.79 ± 0.88	3.07 ± 1.36	3.27 ± 0.97	3.76 ± 1.44	3.74 ± 0.98	
MRR ($N \cdot ms^{-1}$)	P-3	-1.68 ± 0.41	-1.04 ± 0.28	-1.32 ± 0.28	-1.43 ± 0.30	-1.47 ± 0.34	Time: baseline vs. POST 1
	P-12	-1.69 ± 0.46	-1.29 ± 0.27	-1.46 ± 0.26	-1.51 ± 0.26	-1.41 ± 0.20	
HRT (ms)	P-3	73.4 ± 11.5	75.1 ± 16.3	63.4 ± 15.7	60.8 ± 16.1	63.5 ± 14.4	Time: baseline vs. POST 6, 15 and 30; Condition;
	P-12	73.5 ± 10.7	59.0 ± 13.3	52.4 ± 11.8	55.1 ± 14.4	59.2 ± 11.4	Interaction: P-3 vs. P-12 at POST 1, 6 and 15
Central fatigue							
Peripheral VA (%)	P-3	88 ± 6	79 ± 15	81 ± 11	82 ± 12	85 ± 10	Time: baseline vs. POST 1, 6 and 30
	P-12	89 ± 8	78 ± 8	84 ± 8	84 ± 9	83 ± 8	
Surface EMG							
M-wave PPA (mV)	P-3	9.8 ± 2.8	9.9 ± 3.0	9.7 ± 3.1	9.6 ± 3.1	9.4 ± 2.9	
	P-12	9.2 ± 2.3	9.0 ± 2.0	8.9 ± 2.3	8.5 ± 2.3	8.5 ± 2.2	
M-wave area ($\mu V \cdot s^{-1}$)	P-3	31.7 ± 13.3	35.9 ± 15.3	34.9 ± 11.4	32.4 ± 11.1	30.5 ± 11.1	
	P-12	32.0 ± 11.1	32.3 ± 12.6	29.2 ± 10.4	26.6 ± 9.6	28.3 ± 10.0	

Data are presented as mean ± SD. MVC, Maximal voluntary contraction; Q_{pot}, Potentiated twitch force; PS10, Low-frequency (10 Hz) doublet force; PS100, High-frequency (100 Hz) doublet force; CT: Contraction time; MRFD, Maximal rate of force development; MRR, Maximal rate of relaxation; HRT, Half-relaxation time; M-wave PPA, M-wave peak-to-peak amplitude; peripheral VA, Voluntary activation; where a main effect for time or interaction effects have been indicated, the pairwise comparisons are displayed subsequent in the last column if significant.

6.4.6. Visual analogue fatigue scale

There was a significant main effect for time ($F_{(4,44)} = 40.2$; $p < 0.001$; $\eta_p^2 = 0.79$) with no difference between conditions ($F_{(1,11)} = 0.04$; $p = 0.846$; $\eta_p^2 = 0.004$) and no interaction effect ($F_{(4,44)} = 1.50$; $p = 0.219$; $\eta_p^2 = 0.12$). Although not significantly different, the data still reveals a trend towards an elevated level of perceptual fatigue for both conditions following 30 min of rest ($p = 0.074$).

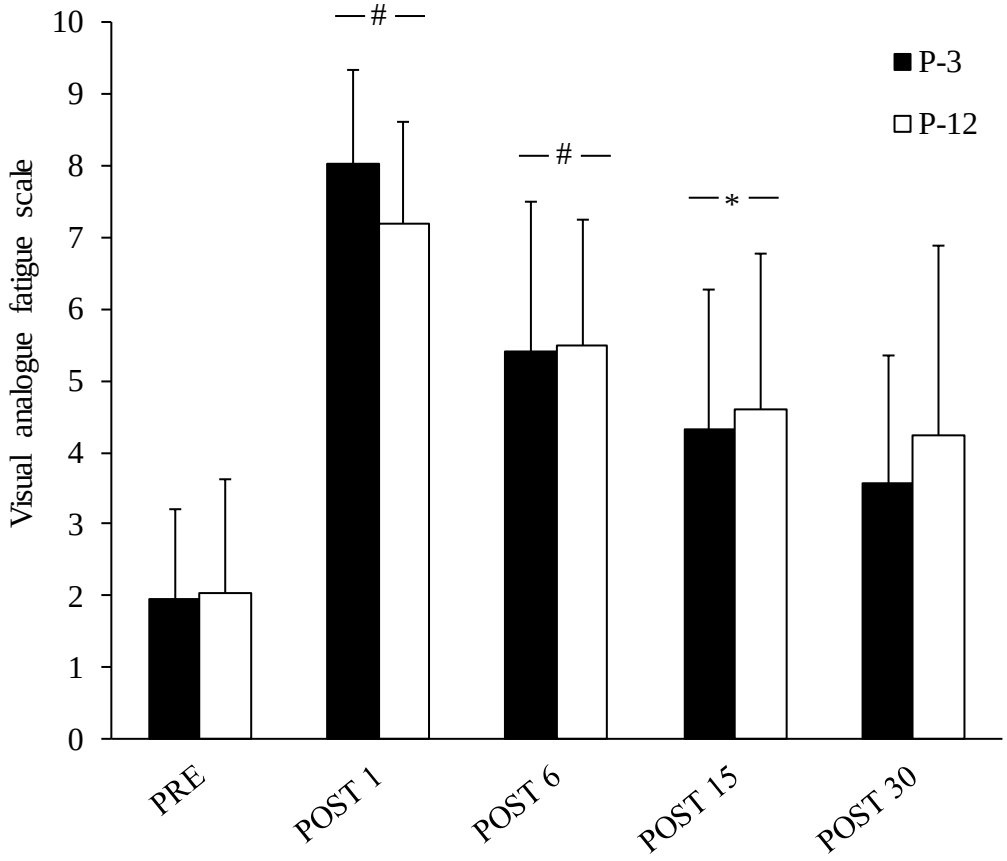


Figure 6.2. Perceptual fatigue measures via visual analogue fatigue scale at PRE and at POST 1, POST 6, POST 15 and POST 30 for 3 (P-3; closed bars) and 12 min (P-12; open bars). *$p < 0.05$ vs. PRE; $^{\#}p < 0.001$ vs. PRE.

6.4.7. Bivariate correlations between neuromuscular recovery and measures of aerobic and anaerobic capacities

A larger CP was associated with a slower Δrecovery in Q_{pot} (r = −0.678, p = 0.031) and PS10 (r = −0.705, p = 0.023) for P-3 (Figure 6.3.). A greater $\dot{V}O_{2peak}$ was associated with a slower Δrecovery in PS10 (r = −0.707, p = 0.022) for P-12.

Further, an inverse relationship was found between the level of NMF at POST 1 (% of PRE) and Δrecovery for P-3. Greater reductions at POST 1 (i.e. smaller % of PRE) were associated with a greater recovery in MVC (r = −0.794, p = 0.004), Q_{pot} (r = −0.947, p < 0.001), PS10 (r = −0.840, p = 0.002) and PS100 (r = −0.884, p = 0.001) at POST 30 for P-3 (Figure 6.4.). Similarly, greater reductions at POST 1 were associated with a greater recovery in MVC (r = −0.657, p = 0.028), Q_{pot} (r = −0.878, p = 0.001) and PS100 (r = −0.812, p = 0.004) for P-12.

In addition, partial correlations revealed that the effect of aerobic capacity on Δrecovery in PS10 disappeared when controlling for the magnitude of NMF at POST 1 (CP vs. Δrecovery, r = −0.503, p = 0.168; $\dot{V}O_{2peak}$ vs. Δrecovery, r = −0.507, p = 0.163).

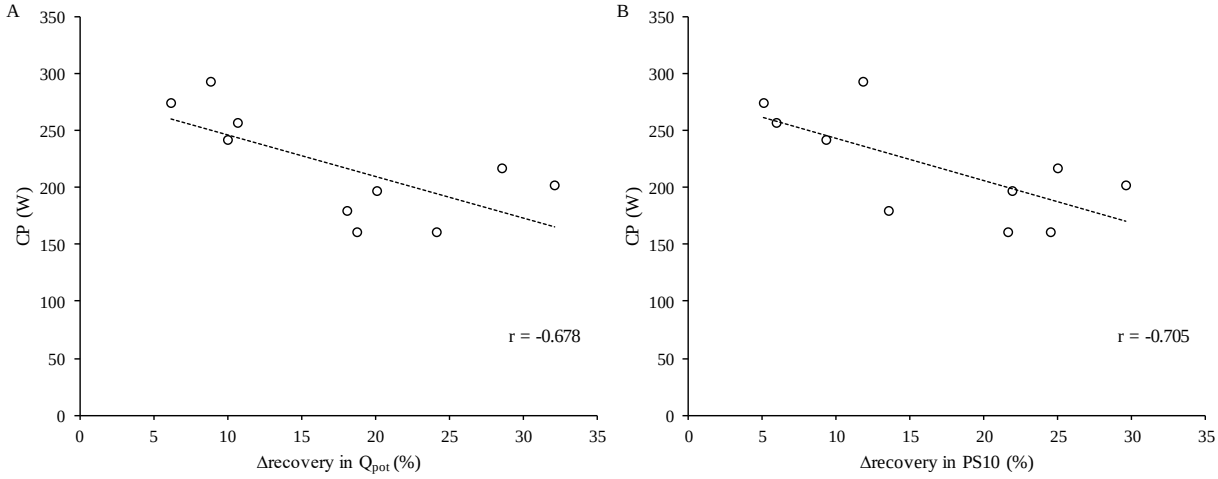


Figure 6.3. Correlations between critical power (CP) and Δrecovery in potentiated twitch force (Q_{pot}; A) and CP and Δrecovery in low-frequency (10 Hz) doublet force (PS10; B) for P-3. Pearson's correlation coefficients (r) are displayed. All correlations were significant (p < 0.05).

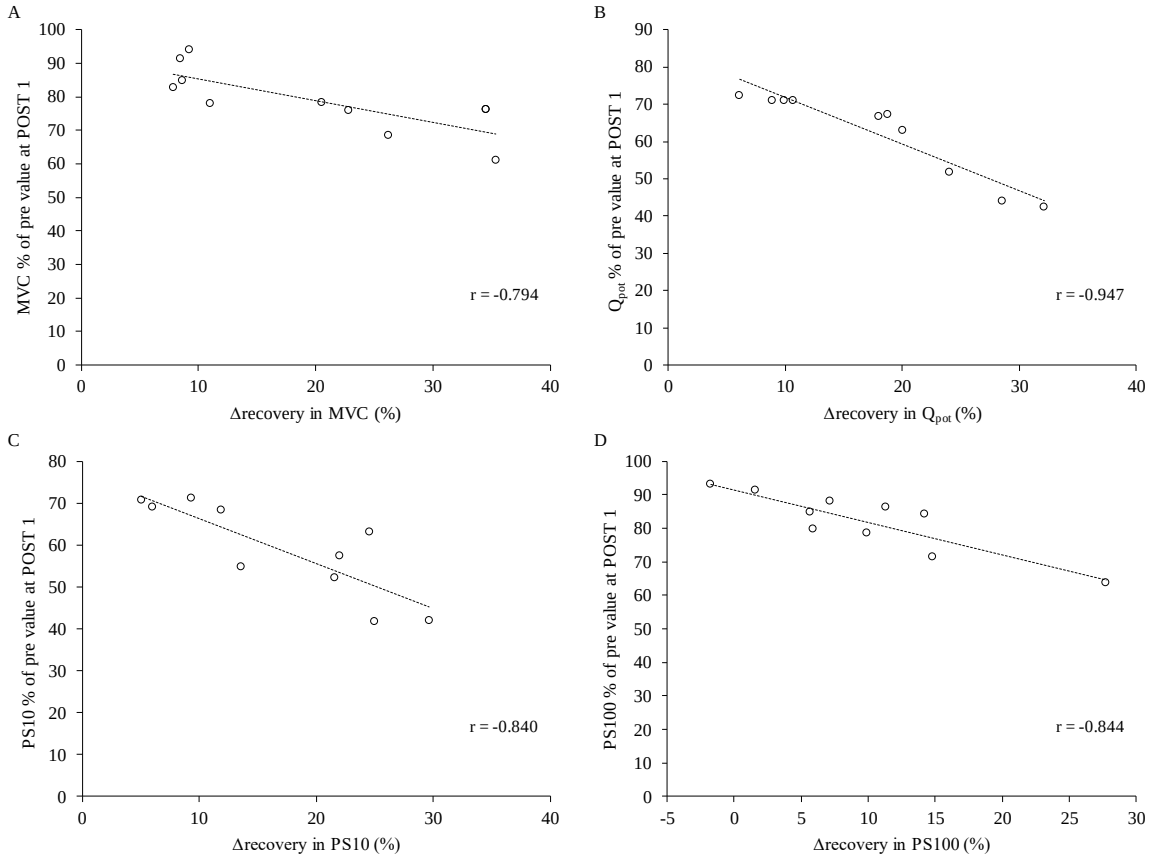


Figure 6.4. Correlations between the magnitude of neuromuscular fatigue at POST 1 (% of PRE) and Δrecovery (%) for maximal voluntary contraction (MVC; A), potentiated twitch force (Q_{pot}; B), low-frequency (PS10; C) and high-frequency (PS100; D) doublet force for P-3. Pearson's correlation coefficients (r) are displayed. All correlations were significant ($p < 0.05$).

6.5. DISCUSSION

This is the first study investigating the effect of exercise intensity, and therefore duration on the time course of neuromuscular recovery following severe intensity exercise performed to task failure. The present study found (1) no differences in most key neuromuscular measures at any time point during a 30-min recovery period between two different exercise intensities set to fully deplete W' in 3 (P-3) vs. 12 min (P-12), except for PS10, which was higher for P-3 throughout the recovery; (2) the ability to voluntarily produce force recovers substantially but not fully within the first 6 min post-exercise (P-3: 88%; P-12: 91%) and returns to baseline levels within 15 min; (3) this is despite central and peripheral fatigue still remaining significantly reduced following 30 min of rest, (4) evoked low- and high-frequency forces were substantially reduced post-exercise and remained reduced following 30 min of rest, however, low-frequency forces were reduced to a greater extent at 30 min post, (5) against our hypothesis, a slower recovery of evoked twitch forces was found in individuals with greater aerobic capacities in both P-3 and P-12.

6.5.1. Recovery of peripheral fatigue following cycling above CP

In the present study, the ability to produce force volitionally was substantially impaired by peripheral fatigue, as indicated by large reductions in evoked twitch forces. Whereas MVC returned to baseline within less than 15 min (~95% of baseline), Q_{pot}, PS10 and PS100 approached baseline values, although remained significantly reduced after 30 min of rest (~80%, 75% and 93% of baseline, respectively). In line with the present findings, Vernillo *et al.* (2018) reported near baseline values for MVC after 8 min of recovery from a 2 min sustained MVC with the knee extensor muscles (>~90% vs. ~89% after 6 min in the present study). However, in contrast to our findings, Q_{pot} also approached pre-fatigued levels already within 8 min post-exercise (Vernillo *et al.*, 2018; ~80% vs 73% after 6 min in the present study). Further, Froyd *et al.* (2013) demonstrated an initial phase of rapid recovery in MVC and evoked twitch forces (i.e. Q_{pot}, PS10, PS100) within the first 2 min after intense, self-paced, concentric extensions and flexions of the right knee, however, in line with the present findings, all measures remained significantly reduced 8 min into recovery (MVC: ~74% of baseline; Q_{pot}: ~59%; PS10: 50%; PS100: ~66%). The task-dependent nature of neuromuscular recovery has previously been discussed (Carroll *et al.*, 2017). The relevance of reperfusion of the exercising muscle post-exercise increases with increasing muscle force and duty cycle and has been described as a key factor for the initial, rapid recovery in neuromuscular function following high-intensity exercise as opposed to submaximal contractions during which muscle reperfusion can be maintained and therefore, an accumulation of fatigue-related metabolites reduced (Carroll *et al.*, 2017). This is supported by the findings of Husmann *et al.* (2018) who reported an elevated magnitude of muscle fatigue during and up to 2 min following knee extension exercise when combined with blood flow restriction. The reperfusion of the contracting muscles enables the clearance of K^+ and consequently, the repolarisation of the t-tubule membrane following sustained maximal contractions (Carroll *et al.*, 2017; Allen *et al.*, 2008a). However, this should be less relevant during locomotor exercise where a continuous alteration between contraction and relaxation allows an almost uninterrupted perfusion during exercise.

The accumulation of muscle metabolites (e.g. ADP, PCr, P_i, H^+, K^+) has been discussed controversially as a contributor to fatigue development and recovery (Clausen, 2013; Allen *et al.*, 2008a; Balog & Fitts, 2001; Allen & Westerblad, 2001). Chidnok et al. (2013) reported a substantial recovery of muscle metabolites (i.e. PCr, ATP, pH) within the first 2 min following exhausting single-leg, knee extension exercise of severe intensity (~180 s) and a recovery back to pre-fatigued levels within 10 min. It may be suggested that a temporal link between metabolic perturbation and neuromuscular recovery drives the reconstitution of force depending on exercise intensity and duration. This is in agreement with findings of Baker *et al.* (1993), who reported a close correlation between force and P_i recovery within ~5 min following a 2 min sustained MVC. In contrast, Saugen *et al.* (1997) reported a temporal dissociation between changes in muscle force and metabolic

mechanisms, with PCr, P_i and pH recovering near pre-fatigue levels 5 min following exhaustion (repetitive isometric single-leg knee extensions at 40% MVC: 6 s on – 4 s rest until exhaustion) whereas force was not fully regained following 30 min of recovery. Metabolic perturbations and the time course of their return to baseline following exercise may not be the sole mechanism inducing force decrements and/or might not reflect the time course of the functional recovery in voluntary force because physiological responses to exercise may directly or indirectly contribute and/or compensate for neuromuscular fatigue. Ultimately, an interplay of these physiological processes might regulate the recovery of an individual's ability to voluntarily produce force.

The pronounced and sustained existence of peripheral fatigue throughout recovery found in the present study, in particular the distinct changes in Q_{pot} and PS10 in combination with relatively smaller changes in PS100, indicate that excitation-contraction coupling failure is likely to be the predominant factor impairing the ability to produce force. Decorte et al. (2012) reported similar results following intensive constant-load cycling exercise, with evoked twitch forces (Q_{pot}, PS10, PS100) remaining reduced after 30 min of rest, but Q_{pot} and PS10 being substantially more affected than PS100. The substantially greater reductions in twitch forces evoked by low-frequency stimulations (e.g. 10-20 Hz) relative to high-frequency stimulations (e.g. 50-100 Hz) has been described as low-frequency fatigue (Edwards *et al.*, 1977). LFF has been associated with alterations in Ca^{2+} uptake or Ca^{2+} ATP-ase activity (Balog, 2010; Allen et al., 2008b; Keeton & Binder-Macleod 2006; Rassier & MacIntosh 2000; Booth et al., 1997). The recovery of force can take up to hours or days (Keeton & Binder-Macleod, 2006). Although underlying mechanism(s) are yet to be fully clarified, fluctuations in intracellular P_i and ATP concentrations have been suggested to affect Ca^{2+} release sensitivity (Stackhouse *et al.* 2001; Westerblad *et al.* 1998). In contrast, twitch forces evoked by high-frequency stimulations recover rapidly following exercise (Carroll *et al.*, 2017; Edwards *et al.*, 1977) and have been associated with impairments in action potential propagation induced by accumulation of extracellular K^+ (Bigland-Ritchie *et al.* 1979). Besides, no alterations and differences in sarcolemmal membrane excitability, reflected by changes in M-wave properties were observed during neuromuscular recovery between the conditions which is in line with findings from Decorte *et al.* (2012).

Similar recovery kinetics were observed between the two different exercise intensities and durations above CP. Interestingly, a significant main effect for condition was only revealed for PS10, but no interaction effect could be found. Differences in power output between P-3 and P-12 might have led to alterations in muscle fibre recruitment, with P-3 requiring a greater target force over a shorter duration to complete the same amount of work done above CP compared to P-12; when controlled for the same cadence between conditions. According to the relationship between exercise intensity

and percentage and type of muscle fibres engaged in cycling exercise, it might be assumed that the contribution of type II muscle fibres to the total force required was greater in P-3 compared to P-12, during which a relatively smaller force was applied over a longer duration (Sale, 1987). Vernillo *et al.* (2018) suggested that muscle fibre distribution might influence the contractile properties, with a greater reduction in Q_{pot} in muscle groups presenting a higher proportion of type II fibres. In accordance, Hamada *et al.* (2003) reported a greater susceptibility for fatigue-induced changes in contractile properties in muscle groups presenting a higher proportion of type II fibres. This would imply, if P-3 induced a higher demand on type II fibres, that PS10 would be reduced more in P-3 compared to P-12 which is not the case in our study (PS10 was greater in P-3 at each time point compared to P-12). However, the absolute differences in PS10 found between conditions were 5, 14, 20 and 14 N over the four neuromuscular assessments performed following exercise (POST 1, POST 6, POST 15, POST 30) which is greater than the SDC_{sample} reported for the fatigued knee extensor muscles for the last three assessments (14, 9, 8 and 9 N) (see Chapter 4, Study 1). Nonetheless, a difference in PS10 between conditions (12 N) already existed at baseline and therefore, it may be assumed that excitation-contraction coupling is impaired to a similar extent regardless of exercise intensity and duration above CP.

With regards to the within-twitch measures, only HRT revealed differences between conditions at 6 min and 15 min following exercise termination (more greatly reduced in P-12). Half relaxation time has been associated with changes in the rate of cross-bridge binding and detachment (Jones, 2010; Westerblad *et al.*, 1997b). The absolute differences in HRT between conditions were greater than the SDC_{sample} reported for the fatigued knee extensor muscles except 30 min post-exercise (1 min: 16.1 vs 4.4 ms; 6 min: 11.0 vs. 3.6 ms; 5.7 vs. 4.2 ms; 30 min: 4.3 vs. 4.3 ms) (see Chapter 4 - Study 1). Therefore, it may be assumed that cross-bridge binding and detachment is affected to a greater extent following exercise at the lower boundary of the severe intensity domain.

6.5.2. Recovery of central fatigue following cycling above CP

As for peripheral fatigue, similar recovery kinetics were observed following the two bouts of exercise performed above CP for central fatigue. VA gradually increased throughout recovery but remained below pre-fatigued values at 30 min post-exercise. This is in line with findings from Decorte *et al.* (2012) who reported a partial recovery in VA 30 min after performing severe-intensity intermittent cycling exercise until exhaustion (6 min at 80% of maximal power followed by 4 min rest). Typically, a rapid initial but incomplete restoration of MVC force following high-intensity exercise has been demonstrated (Carroll et *al.*, 2017; Froyd et *al.*, 2013; Gruet et *al.*, 2014) and has been associated with a substantial recovery of central alterations within the first 2 min following exercise termination (Carroll et *al.*, 2017; Hureau et *al.*, 2016a; Gruet et *al.*, 2014; Eichelberger & Bilodeau, 2007). Froyd

et al. (2018) reported a simultaneous recovery in MVC force and neuromuscular activation following isometric knee extensions, irrespective of the changes in evoked twitch forces and concluded that the rapid recovery of force was primarily due to increased central recruitment. In addition, Gruet *et al.* (2014) reported a rapid recovery in VA 2 min following high-intensity single-joint exercise (Post: 85% of baseline vs. Post 2 min: 91% of baseline) and Vernillo *et al.* (2018) found an increase in VA from 41% post-exercise to ~82% at 2 min post-exercise and to ~94% at 8 min following a sustained isometric MVC. The slower rate of recovery in the present study (from 78 and 79% post to 84 and 81% post 6 min for P-12 and P-3, respectively) is likely due to differences in exercise modalities (i.e. isometric single-joint contractions vs. locomotor exercise and exercise duration). Immediately following sustained maximal isometric contractions, the reperfusion of the contracting muscles enables the clearance of fatigue-related metabolites and ultimately, alters the central motor drive by decreasing the inhibitory effect of group IV muscle afferents, whereas during locomotor exercise muscle reperfusion can be maintained (Carroll *et al.*, 2017; Allen *et al.*, 2008a; Amann & Dempsey, 2008).

6.5.3. Exploratory research using bivariate correlation between neuromuscular recovery and measures of aerobic and anaerobic capacities

In Chapter 5, Study 2, an inverse relationship between an individual's aerobic capacity and reductions in MVC following P-12 was reported. In accordance with these findings, Vernillo *et al.* (2019) reported a faster recovery of voluntary knee extensor force in aerobically fitter participants following a 2 min sustained isometric MVC. It might be suggested that a faster and greater O_2 delivery due to structural adaptations within the exercising muscles (e.g. improved muscle capillarisation (Klausen *et al.*, 1981); greater mitochondrial content (Holloszy & Coyle, 1984)), enhances the clearance of fatigue-related metabolites (Dubouchaud *et al.*, 2000; Tomlin & Wenger, 2001), increases PCr resynthesis in aerobically fitter individuals (Bogdanis & Nevill, 1996; Tomlin & Wenger, 2001) and ultimately, accelerates the restoration of the force generating capacity. In contrast to these findings, the present study found that individuals with a larger CP seem to recover slower after severe intensity exercise of both short (P-3), as revealed by the significant inverse relation between CP and the recovery of Q_{pot} and PS10, and long duration exercise (P-12), as indicated by the significant inverse relationships between $\dot{V}O_{2peak}$ and the recovery of PS10. However, strong correlations between the magnitude of changes from pre- to 1 min post-exercise and Δrecovery seem to indicate that it is predominantly the level of neuromuscular fatigue at test termination that determines the time course of neuromuscular recovery. Partial correlations confirmed these findings, revealing that the correlations between recovery in PS10 and CP/$\dot{V}O_{2peak}$ disappear when controlling for NMF at POST 1. Therefore, it is not surprising that individuals with

a greater aerobic capacity presented a slower recovery because these individuals show smaller reductions post-exercise in P-12 (see Study 2, Chapter 5).

6.5.4. Limitations

The time window between neuromuscular assessments post-exercise and following 6 min of recovery may have prevented the capture of more rapid neuromuscular changes. However, considering the duration of the NMFA protocol (100 s) and the number of voluntary and evoked contractions involved, a quicker rate of re-assessing neuromuscular function might have risked a potential fatiguing effect from repeated NMFA *per se*.

As both VA and evoked twitch forces remained below baseline even 30 min post-exercise, one should expect a reduced ability to voluntarily produce force, which paradoxically was not the case at 15- and 30-min post-exercise. It remains unclear why peripheral and central alterations outlast changes in the ability to produce force. It may be that other muscle groups along with the knee extensor muscles were activated in the fatigued state and contributed to the MVC force measured, although upper body and hip movement was minimised via two cross-shoulder straps. In contrast, during evoked twitch forces solely the knee extensors were recruited in both, fresh and fatigued states. This might provide an explanation for the discrepancy in recovery between MVC and measures of central and peripheral fatigue. Furthermore, it may be argued that reductions in MVC cannot solely be explained by the traditional measures of central and peripheral fatigue. Instead alterations associated with the maintenance of homeostasis and the psychological state may change the sensations which adjust the integrity of the individual during fatiguing exercise (Enoka & Duchateau, 2016).

6.6. CONCLUSION

To the best of our knowledge this study is the first to report similar neuromuscular recovery kinetics when W' is fully depleted irrespective of the power output selected above CP. The present study found that severe intensity cycling exercise performed to fully deplete W' induces substantial impairments in the ability to produce voluntary force which outlast the exercise bout regardless of exercise intensity and duration above CP. Both central and peripheral alterations remained below baseline after 30 min of rest. This knowledge is crucial to determine more precisely the time point at which maximal force is fully regained and to better understand the physiological mechanism(s) underpinning neuromuscular recovery to inform the development of training interventions or recovery strategies.

7. STUDY 4: Effect of creatine supplementation on high-intensity cycling performance above CP and neuromuscular fatigue

7.1. ABSTRACT

The present study examined the effect of creatine supplementation on neuromuscular fatigue and exercise tolerance when cycling above CP. Eleven recreationally active male participants performed an incremental cycling test to determine $\dot{V}O_{2peak}$, 4-5 constant-load trials to task failure to obtain asymptote (CP) and curvature constant (*W′*) of the power-duration relationship, followed by three constant-load supra-CP trials set to fully deplete *W′* in 3 min: 1) one trial to task failure (TTF) following placebo supplementation (PLA); 2) one TTF following creatine supplementation (CRE); and 3) one trial of equal duration to PLA following creatine supplementation (iso-time; ISO). Neuromuscular assessment of the right knee extensors was performed pre- and post-exercise to measure maximal voluntary contraction (MVC), twitch forces evoked by single (Q_{pot}) and paired high- (PS100) and low-frequency (PS10) stimulations and voluntary activation. Creatine supplementation increased time to task failure in CRE vs. PLA (205 ± 65 s vs.184 ± 46 s; $p = 0.017$) and accordingly the amount of work done above CP (CRE: 21.2 ± 4.2 kJ vs. PLA: 19.3 ± 4.0 kJ; $p = 0.015$), but with no difference in the reductions in MVC (CRE: −24 ± 8% vs. PLA: −20 ± 9%), evoked twitch forces (CRE vs. PLA: Q_{pot}, −39 ± 13% vs. −32 ± 14%; PS10, −42 ± 14% vs. −36 ± 13%; PS100, −25 ± 10% vs. −18 ± 12%) and voluntary activation (CRE: −7 ± 8% vs. PLA: −5 ± 7%; $p > 0.05$). Further, no significant difference in neuromuscular changes were found between ISO and both PLA and CRE ($p > 0.05$). These findings suggest that similar levels of neuromuscular fatigue can be found following cycling to task failure above CP despite increases in performance time and amount of work done above CP in the CRE condition. Therefore, a greater *W′* does not lead to a higher level of neuromuscular fatigue, supporting the notion of a critical level of neuromuscular fatigue at task failure when exercising within the severe intensity domain and challenging a direct causative link between utilisation of *W′* and neuromuscular fatigue.

7.2. INTRODUCTION

When using the two-parameter model, it has been assumed that exercise above CP depletes *W′*, with task failure occurring when this mathematically finite amount of work is fully utilised (Poole *et al.*, 1988; Moritani *et al.*, 1981; Monod & Scherrer, 1965) (see Section 2.1.). *W′* has long been associated with the use of an anaerobic energy store (Miura *et al.*, 2000; Miura *et al.*, 1999; Smith *et al.*, 1998; Jenkins & Quigley, 1993) although its solely anaerobic nature has been questioned due to its sensitivity to interventions altering O_2 delivery (Dekerle *et al.*, 2012; Vanhatalo *et al.*, 2010).

More recently, a continuous decline in muscle [PCr] has been demonstrated during exercise above CP (Jones *et al.*, 2008). It has been suggested that task failure within the severe intensity domain, i.e. when exercising above CP, occurs when a critical level of intramuscular [PCr], [P_i] and/or pH is reached (Vanhatalo *et al.*, 2010; Jones *et al.*, 2008). These intramuscular metabolic disturbances have been associated with the development of substantial levels of peripheral fatigue, i.e. a reduction in the force-generating capacity of the muscle induced by alterations at or distal to the neuromuscular junction (Burnley *et al.*, 2010; Allen *et al.*, 2008a).

Interestingly, similar magnitudes of peripheral fatigue, i.e. reductions in evoked twitch forces (~35%), have been observed following exercise across a wide range of supra-CP intensities performed until task failure (Johnson *et al.*, 2015; Thomas *et al.*, 2015; Amann *et al.*, 2011; 2009; Amann & Dempsey, 2008; Romer *et al.*, 2007) and this has led some authors to introduce the concept of a 'critical threshold of peripheral fatigue' (Amann *et al.*, 2011; 2009; Amann & Dempsey, 2008; Romer *et al.*, 2007). The theory behind this concept proposes that group III/IV muscle afferents detect fatigue-related metabolites within the exercising muscles and regulate the central motor drive accordingly to limit the magnitude of peripheral fatigue and maintain muscle and overall homeostasis of the organism (see Section 2.2.5.).

The link between exercise tolerance and fatigue has historically found broad interest in the field of sport and exercise physiology, whereby understanding and manipulating the mechanism(s) underlying fatigue to ultimately improve exercise tolerance, has been the focus of numerous studies. However, only recently have studies combined the CP concept with neuro-stimulation techniques to further understand the neurophysiological limits of severe intensity exercise (Burnley *et al.*, 2012; Study 2 - Chapter 5). Study 2 (Chapter 5) found a positive correlation between an individual's *W′* and changes in neuromuscular function (i.e. maximal voluntary contraction, MVC; potentiated twitch force, Q_{pot}; twitch forces evoked by low-frequency stimulations at 10 Hz, PS10) following cycling exercise above CP. This suggests a greater level of peripheral fatigue at task failure in individuals able to accumulate a larger amount of work above CP.

Creatine supplementation has the potential to challenge the relationship between *W′* and neuromuscular fatigue through manipulation of an individual's anaerobic work capacity. Interventions aiming to increase total creatine stores ([TCr]; i.e. sum of phosphocreatine [PCr] and free creatine [Cr]) may facilitate the resynthesis of ATP from PCr, decelerate or delay the depletion of PCr and accumulation of ADP and consequently, delay the onset of fatigue during high-intensity exercise. An increase in muscle [TCr] by up to 20% ($^1/_3$ in form of PCr) following creatine supplementation has previously been demonstrated (Finn *et al.*, 2001; Casey *et al.*, 1996; Greenhaff *et al.* 1994; Harris *et al.*, 1992). The effect of creatine supplementation on high-intensity performance has been intensively studied since the 1990s (Miura *et al.*, 1999; Smith *et al.*, 1998; McNaughton *et*

al., 1998; Jacobs *et al.*, 1997; Rossiter *et al.*, 1996). Improvements in time to task failure of up to 24% have been observed, with greater changes observed following shorter, more intense exercise during which the contribution of the anaerobic energy turnover becomes more predominant (Jacobs *et al.*, 1997; Prevost *et al.*, 1997; Smith *et al.*, 1998; Branch, 2003). In addition, creatine supplementation increased *W′* by 10-25%, without affecting CP (Smith *et al.*, 1998; Miura *et al.*, 1999; Eckerson *et al.*, 2005). These findings provide support for a significant role of muscle Cr/PCr content in high-intensity performance and evidence the primarily anaerobic nature of *W′*.

Whereas the effect of creatine on performance is well-established, very little is known about its effect on neuromuscular fatigue. Creatine supplementation has been reported to influence neuromuscular measures (Stout *et al.* 2000; Smith *et al.*, 2007). Stout *et al.* (2000) reported a greater physical working capacity at the fatigue threshold (+ 20%), which indicates a delay in the onset of neuromuscular fatigue, following five days of creatine loading. Similarly, Smith *et al.* (2007) found an increase in the electromyographic fatigue threshold during cycle ergometry (+ ~15%). However, whether creatine supplementation alters neuromuscular fatigue at task failure following exercise above CP remains unclear. The integration of the CP concept with electromyographic and mechanical measures of neuromuscular fatigue may offer further insights into the limits of exercise tolerance within the severe intensity domain.

Therefore, the aim of the present study was to provide experimental evidence for an association between the use of *W′* and the development of neuromuscular fatigue using creatine supplementation. It was hypothesised that: (1) creatine supplementation would improve performance (i.e. time to task failure) by increasing the amount of work done above CP; (2) a greater amount of work done above CP would increase the magnitude of neuromuscular fatigue observed at task failure; (3) the same absolute amount of work completed above CP (i.e. exercise time in control vs. 'iso-time') would lead to the same magnitude of neuromuscular fatigue regardless of creatine supplementation.

7.3. METHODS

7.3.1. Participants

Eleven recreationally active, non-vegetarian male participants (mean ± SD: age, 22.6 ± 2.8 years; body mass, 75.8 ± 11.5 kg; $\dot{V}O_{2peak}$, 3.86 ± 0.47 L·min^{-1}; P_{peak}, 311 ± 37 W) volunteered for this study. All participants were familiar with cycle ergometry and the exercise procedures used in our laboratory. Participants completed a medical health questionnaire and provided written, informed consent prior to testing (Section 3.3.). All experimental procedures were approved by the University of Brighton Research Ethics & Governance Committee (Section 3.2.). Participants were instructed to report to the laboratory in a fully rested and well hydrated state, to avoid vigorous activity within

the previous 24 h and to refrain from alcohol (24 h) and caffeine consumption (12 h) prior testing (Kalmer & Cafarelli, 2004; Gandevia & Taylor, 2006). Participant details are shown in Table 4.2

Table 7.1. Participant characteristics

Participant	Age (years)	Body mass (kg)	Height (cm)	$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)
01	24	76.5	175	3.75
02	23	81.0	176	3.55
03	28	79.0	179	4.07
04	25	72.0	167	4.04
05	22	78.0	170	3.90
06	20	68.7	173	3.47
07	20	67.3	183	4.22
08	23	102.7	184	4.12
09	19	68.2	177	4.72
10	25	82.5	185	3.79
11	20	58.1	163	2.88
Mean ± SD	23 ± 3	75.8 ± 11.5	176 ± 7	3.86 ± 0.47

$\dot{V}O_{2peak}$, peak oxygen consumption

7.3.2. Experimental design

The participants reported to the laboratory on nine to ten different occasions over a five to six week period. The tests included a ramp incremental test for the determination of $\dot{V}O_{2peak}$, a familiarisation to the experimental protocol (Section 3.6.), four to five constant-load trials performed to task failure for the determination of CP and *W'* (Section 3.7.) and subsequently three constant-load trials to investigate the effect of creatine supplementation on neuromuscular function in the fresh state and following constant-load cycling above CP. All tests were performed at the same time of day (± 2 h) to control for the effect of diurnal variation (Atkinson & Reilly, 1996) and separated by a minimum of 24 h. The three main trials were separated by five to seven days.

7.3.3. Incremental test and familiarisation

Incremental tests were performed as described in the General Methods (Section 3.6.). Briefly, power output was initially set to 50-125 W depending on individual fitness level and increased by 5 W every 12 s until task failure, followed by a verification trial at 105% P_{peak} (Rossiter *et al.* 2006). $\dot{V}O_{2peak}$ was defined as the highest 15 s moving average. Peak power was calculated as the highest 15 s moving average during the ramp test.

During a second visit, participants were familiarised with constant-load trials performed to task failure, NMFA and a quick transition from the cycle ergometer to the isometric rig.

7.3.4. Determination of CP and *W′*

The participants completed a semi-randomised series of four to five constant-load tests to elicit task failure within ~3 and 15 min (Poole *et al.*, 1988; Hill, 1993) as described in Section 3.7.

7.3.5. Experimental trials

Power output was predicted for each participant from interpolation of the power - time relationship and set to induce full depletion of *W'* within 3 min. Trials were performed 1) until task failure following placebo supplementation (PLA); 2) until task failure following creatine supplementation (CRE); and 3) for an equal duration to PLA following creatine supplementation (ISO). CRE and ISO were performed in a randomised order. Neuromuscular function assessment was performed before and 1 min post-exercise.

7.3.6. Neuromuscular function assessment

Neuromuscular function assessment was performed before (PRE) and 1 min post-exercise (POST 1) according to Section 3.8. during familiarisation and the three main trials.

7.3.7. Supplementation, urinary creatinine and body mass

All participants ingested 4 x 5 $g \cdot d^{-1}$ of dextrose (PLA) during the first 5 day supplementation period. Prior to the second main trial, participants ingested 4 x 5 $g \cdot d^{-1}$ of creatine monohydrate for five successive days and during the third supplementation period, participants ingested a maintenance dose of 2 $g \cdot d^{-1}$ of creatine for each day between the second and the third main trial. This supplementation protocol (4 x 5 $g \cdot d^{-1}$ creatine) has been shown to increase muscle total creatine concentration by ~20% (Hultman *et al.*, 1996). Moreover, an elevated concentration can be maintained when supplementation is continued at 2 $g \cdot d^{-1}$ of creatine (Hultman *et al.*, 1996). Each dose was dissolved in 200 mL of warm water and flavoured with no added sugar orange squash. Supplements were taken at regular intervals equally spread throughout the day. Participants were blinded to the supplementation condition and were asked to log the times supplements were taken for each supplementation period. The self-reported compliance across participants was 100%.

Participants collected a 24 h urine sample on day 5 of the first (PLA) and second (CRE or ISO) supplementation period. Urinary volume was determined and urinary creatinine concentration was determined calorimetrically using the Jaffe reaction (Jaffe, 1886).

Body mass was measured during the first visit and prior to each of the three main trials.

7.3.8. Peak oxygen uptake ($\dot{V}O_{2peak}$) across trials

Oxygen uptake was recorded continuously at rest and throughout exercise and $\dot{V}O_{2peak}$ was determined as described in Section 3.9.1.

7.3.9. Blood lactate concentration

Capillary blood samples were collected at rest and immediately following the neuromuscular function assessment at 1 min post-exercise and analysed for blood lactate concentration as outlined in Section 3.9.4.

7.3.10. Heart rate

Heart rate was recorded continuously at rest and throughout exercise (see Section 3.9.2).

7.3.11. Visual analogue fatigue scale

Subjective feelings of fatigue were assessed using a visual analogue fatigue scale at rest and after each neuromuscular function assessment, as described in Section 3.9.3.

7.3.12. Data analysis

Neuromuscular function measures, critical power and pulmonary gas exchange were analysed as described in Section 3.8.5., Section 3.7. and Section 3.9.1., respectively.

One participant was excluded from the data analysis for VA after values were identified as outliers (values were > 2SD from the mean; n = 10).

7.3.13. Statistical analysis

All data were first checked for normal distribution and sphericity as outlined in Section 3.10. Two-way repeated measures ANOVA on the factors ‘condition’ (CRE, PLA, ISO) and ‘time’ (PRE, POST

1) were used to test for differences in neurophysiological measures. The level of significance was set at $p < 0.05$. *Post hoc* analysis was performed following a significant main or interaction effect using Bonferroni *post hoc* adjusted pairwise comparisons. Student's paired-sample *t*-tests were used to compare performance times and work done above CP between PLA and CRE. Effect sizes are presented as partial eta squared (η_p^2) for main and interaction effects and Cohen's *d* was calculated to estimate effect sizes for pair-wise comparisons. All data are expressed as mean ± SD. Statistical analysis was performed using SPSS 22 (SPSS Inc, Chicago USA).

7.4. RESULTS

7.4.1. Incremental test and determination of CP and *W′*

P_{peak} was 311 ± 37 W. There was a significant difference in $\dot{V}O_{2peak}$ achieved during the fast ramp test (3.86 ± 0.47 L·min^{-1}) compared to the subsequent verification trial (3.49 ± 0.35 L·min^{-1}) ($t_{(10)}$ = 6.16; $p < 0.001$; d = 0.89). CP and *W′* were 191 ± 37 W (61.3 ± 5.9% P_{peak}) and 19.9 ± 6.2 kJ with associated standard errors of 2.2 ± 0.9 W and 1.1 ± 0.7 kJ. Mean power outputs for the main trials was 302 ± 38 W (97 ± 7% P_{peak}). A summary of individual data characterising the P-t_{lim} relationship see Table 7.2.

Table 7.2. Characterization of the P-t_{lim} relationship.

Participant	Model	Number of tests	CP (W)	SE-CP (W)	*W′* (kJ)	SE-*W′* (kJ)
1	W-t	4	196	2.17	16.1	1.0
2	P-1/t	5	165	0.98	27.1	0.4
3	P-1/t	4	219	2.38	10.3	0.7
4	P-1/t	5	225	2.45	14.6	1.0
5	W-t	5	156	2.88	27.0	1.3
6	P-1/t	4	186	1.06	13.8	0.5
7	P-t	5	175	3.45	26.2	2.1
8	P-t	5	219	3.45	26.9	2.7
9	P-t	4	263	0.69	16.5	0.6
10	P-1/t	4	158	2.60	23.2	0.9
11	P-1/t	5	144	2.11	17.3	0.8
Mean ± SD			191 ± 37	2.20 ± 0.94	19.9 ± 6.25	1.1 ± 0.7

CP, critical power; SE, standard error; *W′*, curvature constant

7.4.2. Time to task failure

Time to task failure improved significantly with creatine supplementation (PLA: 184 ± 46 s vs. CRE: 205 ± 65 s; $t_{(10)} = -2.85$, p = 0.017, *d* = 0.37). Work done above CP increased significantly from 19.3 ± 4.0 kJ for PLA to 21.2 ± 4.2 kJ for CRE ($t_{10} = -2.95$, p = 0.015, *d* = 0.46). $\dot{V}O_{2peak}$ was not significantly different between experimental trials (PLA: 3.72 ± 0.42 $L \cdot min^{-1}$ vs. CRE: 3.64 ± 0.48 $L \cdot min^{-1}$ vs. ISO 3.61 ± 0.41 $L \cdot min^{-1}$) ($F_{1.335,13.353} = 1.81$, p = 0.204, $\eta_p^2 = 0.15$).

7.4.3. Maximal voluntary force

MVC decreased significantly from pre- to 1 min post-exercise by −20 ± 9% for PLA, −24 ± 8% for CRE and −20 ± 9% for ISO ($F_{(1,10)} = 102.30$; p < 0.001; $\eta_p^2 = 0.91$), with no significant main effect for condition ($F_{(2,20)} = 1.82$, p = 0.19, $\eta_p^2 = 0.15$) and no interaction effect ($F_{(2,20)} = 1.75$; p = 0.20; $\eta_p^2 = 0.15$) (Figure 7.1. and Table 7.3.).

7.4.4. Potentiated twitch force and doublet twitch forces

Potentiated twitch force, PS10 and PS100 were significantly reduced after PLA (−32 ± 14, −36 ± 13 and −18 ± 12%, respectively), CRE (−39 ± 13, −42 ± 14 and −25 ± 10%, respectively) and ISO (−34 ± 16, −38 ± 17 and −24 ± 16%) (Q_{pot}, $F_{(1,10)} = 78.71$, p < 0.001, $\eta_p^2 = 0.89$; PS10, $F_{(1,10)} = 95.51$, P < 0.001, $\eta_p^2 = 0.91$; PS100, $F_{(1,10)} = 70.31$, p < 0.001, $\eta_p^2 = 0.88$; Figure 7.1.; Table 7.3.). There was no significant main effect for condition for these variables (Q_{pot}, $F_{(2,20)} = 0.33$, p = 0.721, $\eta_p^2 = 0.03$; PS10, $F_{(2,20)} = 0.83$, p = 0.449, $\eta_p^2 = 0.08$; PS100, $F_{(2,20)} = 0.71$, p = 0.505, $\eta_p^2 = 0.07$) and no significant interaction effect for PS10 ($F_{(2,20)} = 3.34$, p = 0.056, $\eta_p^2 = 0.25$) and PS100 ($F_{(2,20)} = 2.12$, p = 0.146, $\eta_p^2 = 0.18$). However, a significant interaction effect was found for Q_{pot} ($F_{(2,20)} = 6.11$, p =0.009, $\eta_p^2 = 0.38$). At baseline, Q_{pot} was significantly greater in CRE compared to PLA ($t_{(10)} = -4.27$; p = 0.002, *d* = 0.45) and ISO ($t_{(10)} = 2.89$; p = 0.016, *d* = 0.33).

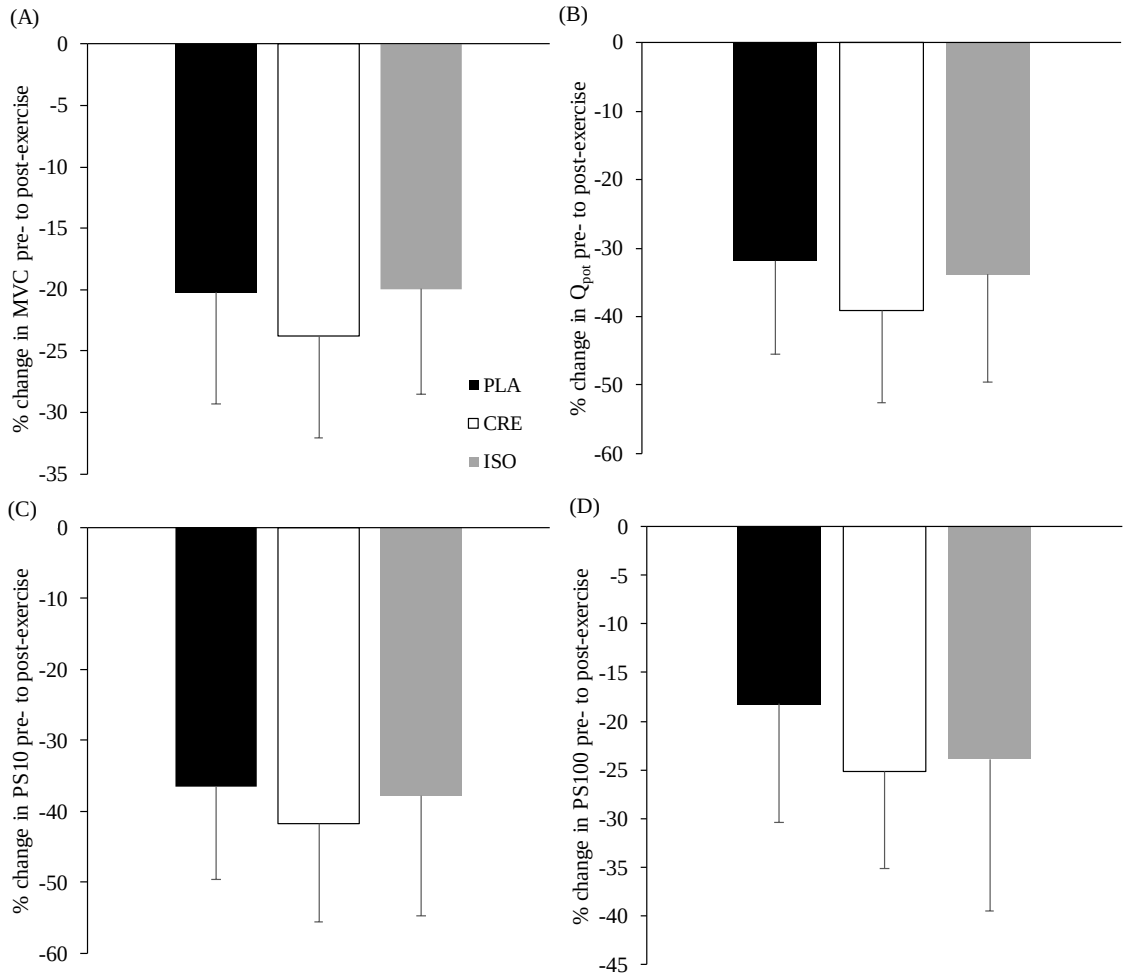


Figure 7.1. Pre- to post-trial percentage change in maximal voluntary contraction (MVC; A), potentiated twitch force (Q_{pot}; B), low-frequency (10 Hz) doublet force (PS10; C) and high-frequency (100 Hz) doublet force (PS100; D) for Placebo (PLA), Creatine (CRE) and Iso-time (ISO).

7.4.5. M-wave properties

M-wave PPA showed no significant main effect for time ($F_{(1,10)}$ = 2.47, p = 0.147, η_p^2 = 0.20) or condition ($F_{(2,20)}$ = 0.23, p = 0.799, η_p^2 = 0.02) and no interaction effect ($F_{(2,20)}$ = 0.84, p = 0.446, η_p^2 = 0.08; Table 7.3.). M-wave area was significantly greater following exercise ($F_{(1,10)}$ = 9.48, p = 0.012, η_p^2 = 0.49) with no significant difference between conditions ($F_{(2,20)}$ = 0.26, p = 0.775, η_p^2 = 0.03) and no interaction effect ($F_{(2,20)}$ = 1.85, p = 0.183, η_p^2 = 0.16; Table 7.3.).

7.4.6. Voluntary activation

Voluntary activation decreased significantly pre- to post-exercise by 5 ± 7, 7 ± 8 and 7 ± 9% for PLA, CRE and ISO ($F_{(1,9)}$ = 7.53, p = 0.023, η_p^2 = 0.46), with no main effect for condition ($F_{(2,18)}$ = 1.82, p = 0.190, η_p^2 = 0.17) and no interaction effect ($F_{(2,18)}$ = 1.31, p = 0.295, η_p^2 = 0.13; Table 7.3.).

Table 7.3. Neuromuscular measures at pre-exercise (PRE) and after exhaustive constant-load cycling (POST) for placebo (PLA), creatine (CRE) and iso-time (ISO)

	PLA		CRE		ISO	
	PRE	POST 1	PRE	POST 1	PRE	POST 1
Neuromuscular fatigue						
MVC (N)	566 ± 128	451 ± 105*	584 ± 124	447 ± 113*	583 ± 127	472 ± 134*
Peripheral fatigue						
Q_{pot} (N)	171 ± 23†	117 ± 29*	182 ± 26	111 ± 30*	174 ± 23†	116 ± 32*
PS10 (N)	251 ± 50	160 ± 48*	267 ± 49	158 ± 56*	259 ± 48	161 ± 52*
PS100 (N)	242 ± 33	198 ± 41*	248 ± 37	187 ± 43*	242 ± 32	188 ± 55*
PS10:PS100	1.04 ± 0.14	0.80 ± 0.16*	1.08 ± 0.10	0.83 ± 0.16*	1.07 ± 0.13	0.87 ± 0.21*
CT (ms)	76 ± 8	70 ± 3*	78 ± 7	73 ± 5*	77 ± 7	73 ± 6*
MRFD ($N \cdot ms^{-1}$)	5.98 ± 1.05	3.87 ± 1.54*	5.95 ± 1.40	3.21 ± 1.05*	5.47 ± 0.65	3.41 ± 1.02*
MRR ($N \cdot ms^{-1}$)	−1.81 ± 0.35	−1.12 ± 0.27*	−1.69 ± 0.29	−0.94 ± 0.30*	−1.71 ± 0.23	−0.98 ± 0.23*
HRT (ms)§	82.5 ± 8.1	91.6 ± 11.5*	85.8 ± 12.4	96.8 ± 9.8*	88.5 ± 11.7	94.8 ± 17.8*
Surface EMG						
M-wave PPA (mV)	7.8 ± 1.9	8.1 ± 2.3	8.1 ± 2.1	8.7 ± 2.7	8.0 ± 2.2	8.2 ± 2.5
M-wave area ($\mu V \cdot s^{-1}$)	33.5 ± 11.1	36.8 ± 12.5*	33.2 ± 7.6	39.0 ± 11.4*	32.7 ± 10.8	35.5 ± 11.0*
Central fatigue						
Peripheral VA (%)#	88 ± 6	84 ± 7*	93 ± 4	86 ± 9*	89 ± 5	84 ± 10*

Data are presented as mean ± SD (n = 12). Abbreviations: MVC, maximal voluntary contraction; Q_{pot}, potentiated twitch force; PS10, low-frequency (10 Hz) doublet force; PS100, high-frequency (100 Hz) doublet force; CT, contraction time; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; HRT, half-relaxation time; M-wave PPA, M-wave peak-to-peak area; VA, voluntary activation; *p < 0.05 vs. PRE, †p < 0.05 vs. CRE at PRE, §main effect for condition p = 0.031; #n = 10

7.4.7. Urinary creatinine and body mass

Urinary creatinine excretion following 5 days of placebo supplementation was 115 ± 61 $mg \cdot dL^{-1}$ and tended to increase but not significantly following 5 days of creatine supplementation to 140 ± 86

mg·dL^{-1} ($t_{(10)}$ = −0.90; p = 0.391, *d* = 0.34). Urinary volume did not change with creatine supplementation (PLA 108 ± 43 mL·h^{-1} vs. CRE 105 ± 48 mL·h^{-1}, $t_{(10)}$ = −0.40; p = 0.699, *d* = 0.07).

No significant changes in body mass were found following 5 days of creatine supplementation (75.7 ± 11.4 kg vs. 76.3 ± 11.7 kg; $t_{(10)}$ = −1.51; p = 0.163; *d* = 0.05).

7.4.8. Peak oxygen uptake ($\dot{V}O_{2peak}$) across trials

There was no significant difference in $\dot{V}O_{2peak}$ achieved during the fast ramp test (3.86 ± 0.47 L·min^{-1}), the four constant-load trials to determine CP and *W′* (1: 3.70 ± 0.53; 2: 3.65 ± 0.60; 3: 3.63 ± 0.47; 4: 3.66 ± 0.60 L·min^{-1}) and the three main trials, PLA (3.69 ± 0.44 L·min^{-1}), CRE (3.66 ± 0.47 L·min^{-1}) and ISO (3.61 ± 0.41 L·min^{-1}) ($F_{(7,70)}$ = 1.90; p = 0.08; η_p^2 = 0.16).

7.4.9. Blood lactate concentration

Blood lactate concentrations increased significantly pre- to post-exercise from 1.57 ± 0.34 to 9.05 ± 1.66 mmol.l^{-1}, 1.51 ± 0.32 to 9.02 ± 2.11 mmol.l^{-1} and 1.53 ± 0.39 to 9.16 ± 2.10 mmol.l^{-1} for PLA, CRE and ISO ($F_{(1,10)}$ = 200.64, p < 0.001, η_p^2 = 0.95), with no main effect for condition ($F_{(2,20)}$ = 0.08, p = 0.846, η_p^2 = 0.01) and no interaction effect ($F_{(2,20)}$ = 0.05, p = 0.949, η_p^2 = 0.01).

7.4.10. Heart rate

There was a main effect for time ($F_{(1,10)}$ = 802.22; p < 0.001; η_p^2 = 0.99), but no main effect for condition ($F_{(2,20)}$ = 1.10; p = 0.353; η_p^2 = 0.10) and no interaction effects ($F_{(1.701,17.014)}$ = 0.26; p = 0.262; η_p^2 = 0.03).

7.4.11. Visual analogue fatigue scale

There was a significant difference between pre- and post-exercise ($F_{(1,10)}$ = 125.50; p < 0.001; η_p^2 = 0.93) with no difference between conditions ($F_{(2,20)}$ = 1.90; p = 0.176; η_p^2 = 0.16) and no interaction effect ($F_{(1.328,13.279)}$ = 0.61; p = 0.492; η_p^2 = 0.06).

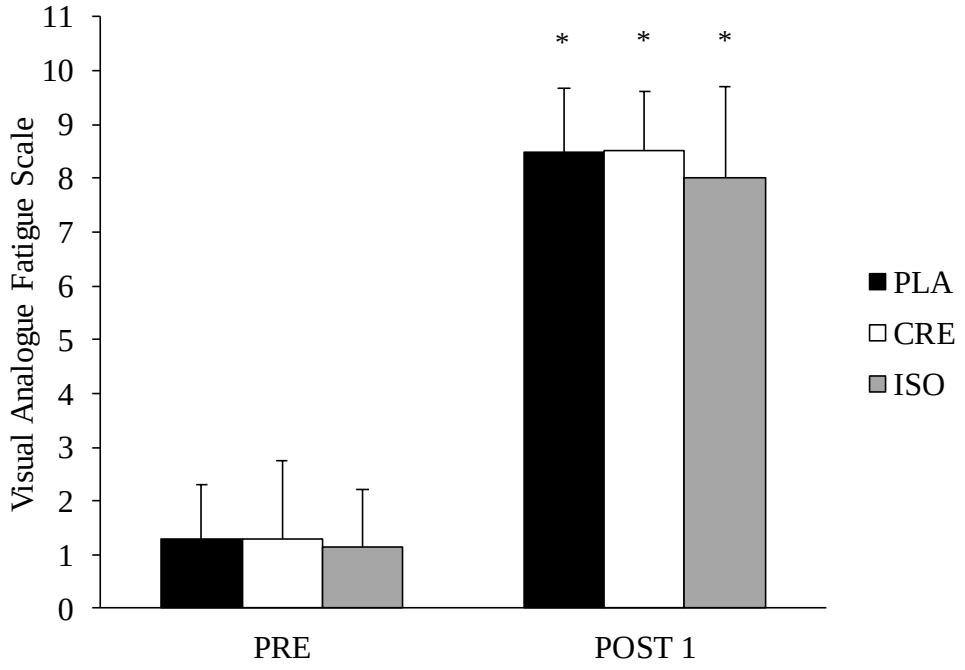


Figure 7.2. Visual analogue fatigue scale at rest, before (PRE) and 1 min following severe intensity cycling exercise (POST 1) for PLA, CRE and ISO. *p < 0.05 vs. PRE.

7.5. DISCUSSION

The present study is the first to demonstrate that an improvement in high-intensity cycling performance above CP following creatine supplementation does not influence the magnitude of neuromuscular fatigue at task failure. The magnitude of neuromuscular fatigue does therefore not seem to depend on the amount of work done above CP.

7.5.1. Creatine and cycling performance above CP

Numerous studies have investigated the performance enhancing effect of creatine, in particular on high-intensity and sprint performance (Skare *et al.*, 2001; Mujika *et al.*, 2000; Prevost *et al.*, 1997; Jacobs *et al.*, 1997; Aaserud *et al.*, 1998). In the present study, time to task failure (~97% P_{peak}) improved by ~11% (184 vs. 205 s, p = 0.017, *d* = 0.37) following 5 days of creatine supplementation. Jacobs *et al.* (1997) reported an improvement of 8% following short-term creatine supplementation when cycling to exhaustion at 125%$\dot{V}O_{2max}$ (130 vs. 141 s, p < 0.001, *d* = 1.57). Prevost *et al.* (1997) reported a larger mean improvement in TTF of 24% at a higher exercise intensity (150%$\dot{V}O_{2max}$). A similar observation of greater improvements at higher exercise intensities was described by Smith *et al.* (1998) with an increase of ~11% (93 vs. 103 s) and ~7% (236 vs. 253 s) in TTF for power outputs

eliciting task failure in ~90-250 s. The efficacy of creatine seems greater for shorter efforts, i.e. when the relative contribution of the anaerobic pathways to the total energy turnover becomes more predominant (Branch, 2003). Performance improvements might be attributed to an increase in muscular [PCr] concentration and therefore, a greater accessibility of immediate energy storage (ATP) (Greenhaff *et al.*, 1994).

In contrast, some studies failed to support performance enhancing effects of creatine supplementation during all-out cycling bouts of 15 s to 3 min (Vanhatalo & Jones, 2009; Finn *et al.*, 2001; Schneider *et al.*, 1997; Cooke *et al.*, 1995). Febbraio *et al.* (1995) found no differences in TTF when cycling at 115 or 125%$\dot{V}O_{2max}$ following creatine loading. Possible explanations for no performance enhancing effects in these studies may include differences in the exercise design (i.e. all-out vs. time to task failure), duration, sample size, and the sensitivity of the protocol to detect changes in performance and/or anaerobic capacity.

W′ is mathematically equivalent to a given amount of work that can be performed above CP (Poole *et al.*, 1988; Moritani *et al.*, 1981; Monod & Scherrer, 1965) and is greater in CRE compared to PLA (~ +10%). One may therefore assume creatine supplementation successfully increased *W′* in the present study. Accordingly, similar supplementation protocols have previously been shown to increase *W′* by 10-25% (Eckerson *et al.*, 2005; Miura *et al.*, 1999; Smith *et al.*, 1998). However, some may argue that the relatively small contribution of PCr to severe intensity performance of ~3 min duration challenges whether additional creatine supplementation would indeed, improve performance or instead, result in a negligible small effect, which in relation to the error associated with TTF and *W′* may be questionable. Nonetheless, the reported improvements in performance and work done above CP, even though small, support the efficacy of creatine supplementation in the present study and are in line with previously reported results (Eckerson *et al.*, 2005; Miura *et al.*, 1999; Smith *et al.*, 1998)

Interestingly, large variations between participants in performance improvements and changes in work done above CP (−8 to +27%) were found. The major reason put forward to explain the discrepancy in creatine's efficacy between participants is likely due to individual differences in initial muscle [TCr] (responders vs. non-responders), so that individuals with low initial [TCr] show greater responses to creatine supplementation compared to individuals with high initial [TCr]. Greenhaff *et al.* (1994) classified individuals with [TCr] of close to or <120 mmol·kg^{-1} dry weight (dw) prior to creatine ingestion as 'responders', showing substantial increases in muscle [TCr] (~25%; + 29 ± 3 mmol·kg^{-1} dw) compared to 'non-responders' (~6%; + 8-9 mmol·kg^{-1} dw). Syrotuik & Bell (2004) have identified three responder's types: true responders (>20 mmol·kg^{-1} dw from preload levels), quasi responders (>10 and <20 mmol·kg^{-1} dw from preload levels) and non-responders (<10 mmol.kg^{-1} dw from preload levels). In the present study, [TCr] was not measured, however,

significant changes in TTF may indicate good responsiveness of the tested participants to creatine supplementation.

Inferences regarding individual responsiveness to creatine supplementation might be drawn from further physiological measures, such as creatinine excretion and/or changes in body mass. During the first few days of creatine supplementation, the majority of the ingested creatine remains within the body until the muscle's capacity to extract creatine from the blood is exhausted. Despite continuous supplementation, ~90% of ingested creatine is excreted into the urine (Terjung *et al.*, 2000; Chanutin & Guy, 1926). The rate of creatine degradation to creatinine, the end product of the creatine metabolism, approximates 2 $g \cdot d^{-1}$ (Walker, 1979). An increase in urinary creatinine excretion has previously been demonstrated following 5 to 6 days of creatine supplementation (60% Hultman *et al.*, 1996; ~22% Mujika *et al.*, 2000). Syrotuik & Bell (2004) reported that individuals classified as responders showed the lowest urinary creatine concentrations at baseline and the greatest absolute increase after 5 days of supplementation compared to non-responders. However, data for urinary creatinine did not show a clear trend between the groups (Syrotuik & Bell, 2004). In the present study, small to medium (d = 0.34) but non-significant increases in urinary creatinine excretion following 5 days of creatine supplementation of ~22% were found, probably due to small sample size and large inter-individual variability. Similarly to Hultman *et al.* (1996), large variations in urinary creatinine excretion between participants were observed, with 6 out of 11 showing an increase of up to +142% and 5 showing a decrease of up to −60%. However, neither initial levels of urinary creatinine (PLA) nor changes in urinary creatinine levels following creatine supplementation did correlate with changes in performance times between PLA and CRE ($r = -0.31$; $p = 0.352$; $r = 0.29$, $p = 0.390$) and thus, increases in urinary creatinine cannot be used in the present study as an indicator of performance effects.

7.5.2. Creatine and neuromuscular fatigue following cycling above CP

In line with our third hypothesis, no difference in neuromuscular fatigue was found following ISO and CRE, i.e. when the same total work / the same duration of exercise was performed. This may support a relationship between the amount of work done above CP and the level of neuromuscular fatigue. However, creatine supplementation did not lead to greater levels of neuromuscular fatigue at task failure despite greater amount of work performed above CP, which contradicts our second hypothesis.

The effect of creatine on neuromuscular fatigue is less well understood, with only a few studies investigating changes in surface EMG during submaximal and supramaximal cycling exercise following short-term creatine loading (Stout *et al.*, 2000; Smith *et al.*, 2007). Creatine supplementation delayed the onset of neuromuscular fatigue when measured as the highest power

output leading to no increase in EMG activity during a constant-load exercise bout (Stout *et al.*, 2000; Smith *et al.*, 2007). During fatiguing high-intensity exercise, the predominant reliance on anaerobic glycolysis and the subsequent changes in intramuscular metabolites have been suggested to impair excitation-contraction coupling and ultimately, alter motor unit recruitment, measured as an increase in EMG amplitude, so that either additional motor units are recruited or the firing rate of already active motor units is increased (Smith *et al.*, 2007; Stout *et al.*, 2000; Moritani *et al.*, 1993). Creatine supplementation might reduce the reliance on anaerobic glycolysis and thus, reduce the metabolic perturbations by increasing the amount of ATP provided through the creatine-kinase reaction (Prevost *et al.*, 1997; Volek & Kraemer, 1996) and thus, delay alterations in motor unit recruitment patterns. However, the present study did not find differences between CRE and both, PLA and ISO. Both, Stout *et al.* (2000) and Smith *et al.* (2007) did not apply neurostimulation techniques and used different exercise protocols compared to the present study (supramaximal discontinuous protocol in Smith *et al.*, 2007; incremental test to task failure in Stout *et al.*, 2000). To the best of the authors' knowledge, this is the first study that combined creatine supplementation and neurostimulation techniques when exercising above CP. Thus, based on findings from EMG studies as mentioned previously, creatine appears to delay the time course of neuromuscular fatigue development, however, based on the present study, it does not affect the magnitude at task failure. Despite a potential reduction or delay in metabolic perturbations associated with anaerobic glycolysis, an increase in muscle metabolites linked to the creatine-kinase reaction may instead impair excitation-contraction coupling to a relatively greater extent and subsequently, lead to similar levels of metabolic perturbations at task failure between PLA and CRE. This may help to explain why similar levels of neuromuscular fatigue were found in PLA and CRE. Nevertheless, a larger amount of work was performed in CRE compared to PLA, likely due to an increase in total ATP turnover. Collectively, similar levels of neuromuscular fatigue observed across all three conditions in the present study provide support for a critical level of peripheral fatigue in the population tested.

Moreover, one may suggest that these findings contradict the positive correlations between the size of W' and the magnitude of neuromuscular fatigue reported in Study 2 (Chapter 5). However, the findings of Study 2 (Chapter 5) are related to between-participant comparisons, whereas the present study draws within-participant comparisons. It may be speculated, that improvements in performance induced by creatine supplementation in the present study were too small to reveal differences in neuromuscular fatigue at task failure and alternative interventions inducing greater performance improvements may be required to experimentally link W' and the level of neuromuscular fatigue, and to potentially disprove a critical level of peripheral fatigue.

7.5.3. Limitations

The authors decided against a double-blinded, fully-randomised, cross-over design due to the approximately 6-week wash-out period required following creatine supplementation (Hultman *et al.*, 1996). The duration of the study and the variations in individual fitness levels over time could have affected the performance trials. Therefore, all participants started with the placebo trial and only the second and third main trials (CRE and ISO) were randomised. Therefore, a possible order effect cannot be fully excluded. Nonetheless, adequate familiarisation prior to the main trials was ensured and participants were blind to the order of the supplements until all experimentation had been completed.

Full depletion of W' was not controlled and therefore, an earlier termination of the voluntary task before 'true' exhaustion during PLA and CRE could have confounded the results (i.e. behavioural effect). However, it must be noted that similar neuromuscular changes were reported in Study 2 (Chapter 5), where test termination was controlled for the full depletion of W' (MVC, −20 ± 10 vs. −20 ± 9%; Q_{pot}, −35 ± 13 vs. −32 ± 14%; PS10, −38 ± 13 vs. −36 ± 13%; PS100, −18 ± 9 vs. −18 ± 12%).

The delayed assessment of neuromuscular measures will have likely caused an underestimation of the magnitude of neuromuscular fatigue due to substantial recovery of neuromuscular function within the first 1-2 min post-exercise (Froyd *et al.*, 2013). To control and limit a potential recovery effect, the present study standardised timings within the neuromuscular assessment protocol, and the transition time between exercise termination and start of the neuromuscular assessment (60 s).

Although the muscle [TCr] was not measured in the present study, previous investigations using similar supplementation protocols reported an increase in muscle [TCr] by up to 20% and therefore, similar changes would be expected for the present study (Finn *et al.*, 2001; Casey *et al.*, 1996; Hultman *et al.*, 1996; Greenhaff *et al.*, 1994; Harris *et al.*, 1992).

Because of the duration of the study's implementation and the requirement for a high number of times to task failure to model the P-t relationship, the present study did not include the addition of four to five visits following creatine supplementation to re-assess W' and/or CP. Thus, it may be argued that alterations in performance could be due to changes in CP instead. However, previous research found that creatine supplementation did not result in changes in CP and therefore, improvements in performance due to an increased CP are unlikely (Smith *et al.*, 1998; Miura *et al.*, 1999; Eckerson *et al.*, 2005).

7.6. CONCLUSION

In conclusion, the present study confirmed a performance enhancing effect of creatine supplementation and indicates that the level of neuromuscular fatigue is not dependent on the amount of work done above CP. These findings challenge a direct causative link between utilisation of W' and neuromuscular fatigue and support a critical level of peripheral fatigue.

8. GENERAL DISCUSSION

8.1. Introduction

In general, substantial levels of peripheral fatigue have been evidenced within the severe intensity domain. The primary aim of the present thesis was to better understand the mechanism(s) of these exercise-induced neurophysiological alterations following severe intensity cycling exercise using the CP concept. Four studies were conducted to address this aim.

In Study 1 (Chapter 4), between-day reliability of key neuromuscular measures in the knee extensor muscles was investigated at rest and following cycling exercise above CP to ensure the application of a reliable and sensitive protocol for the assessment of neuromuscular changes in the remaining studies of this thesis.

In Study 2 (Chapter 5), neuromuscular fatigue was compared following cycling exercise at two different exercise intensities (near the upper and lower boundary of the severe intensity domain) and thus, different durations above CP, when W' was fully depleted. To our knowledge, this is the first study to investigate exercise-induced changes in neuromuscular function following locomotor exercise using the CP concept. This study further explored relationships between aerobic/anaerobic capacities and the observed exercise-induced changes in neuromuscular function.

Study 3 (Chapter 6) provided a comparison of short-term neuromuscular recovery following cycling exercise at these two different severe exercise intensities. In addition, relationships between aerobic capacity and neuromuscular recovery were explored.

Finally, based on the findings from Study 2, Study 4 (Chapter 7) aimed to experimentally challenge a potential causal relationship between an individual's anaerobic capacity and neuromuscular fatigue. Therefore, creatine supplementation was used to increase an individual's anaerobic capacity, to subsequently study its effect on performance and neuromuscular fatigue following severe intensity cycling exercise.

The present chapter will summarise and discuss the principal findings from the experimental chapters 4 - 7 (Section 8.2). This will be followed by a discussion of the progress within the research area from this thesis while focusing on a potential link between W' depletion and the magnitude of neuromuscular fatigue and relating the thesis' findings to the notion of a 'critical threshold of peripheral fatigue' (Section 8.3). Data across different studies within this thesis have been pooled together in the general discussion to strengthen some discussion points when appropriate. Finally, this chapter will conclude with some general consideration of limitations and assumptions of the present findings (Section 8.4) to then propose directions for future research (Section 8.5).

8.2. Principal findings

The aim of Study 1 (Chapter 4) was to examine the between-day reliability of key neuromuscular measures in the knee extensor muscles at rest, before and 1, 6, 15 and 30 min following severe intensity cycling exercise using femoral nerve stimulation. Moderate to good reliability was found for key neuromuscular measures at rest, with CVs ranging from ~4 to 6%. Between-day reliability was slightly reduced in most measures 1 min following severe intensity cycling exercise, with CVs ranging from ~3 to 9%, before approaching resting reliability values in most measures during neuromuscular recovery (Chapter 4, Table 4.5. and 4.6.). Within-twitch measures (i.e. CT, MRFD, MRR and HRT) showed moderate reliability at rest and 1 min following severe intensity cycling exercise, except for the rather poor reliability for MRFD (Chapter 4, Table 4.5. and 4.6.). M-wave PPA and area showed moderate to low reliability at rest and following severe intensity exercise (Chapter 4, Table 4.5. and 4.6.). In conclusion, commonly used markers of force generating capacity, muscle contractile properties and peripheral VA are highly repeatable between days at rest and following severe intensity cycling exercise, whereas the interpretation of changes associated with M-wave area should be done with caution.

A summary of each study's hypotheses is displayed in Table 8.1.

Table 8.1. Hypotheses for each study within the present thesis.

Hypotheses	Accept	Reject
Study 1: Reliability of neuromuscular measurements in the non-fatigued and fatigued knee extensors using femoral nerve stimulation.		
It was hypothesised that;		
1) Voluntary, evoked twitch forces and VA show good between-day reliability in the fresh knee extensors.	✓	
2) Voluntary, evoked twitch forces and VA show good between-day reliability in the fatigued knee extensors.	✓	
3) M-wave properties show moderate between-day reliability in the fresh and fatigued state based on poor reliability of surface EMG.	✓	
Study 2: The magnitude of neuromuscular fatigue is not intensity dependent once *W′* if fully depleted when cycling above CP, but relates to aerobic and anaerobic capacities.		
It was hypothesised that;		
1) Exercise above CP leads to a reduction in MVC without differences between exercise intensities (P-3 vs. P-12).	✓	
2) Exercise above CP induces similar levels of peripheral and central fatigue without differences between exercise intensities (P-3 vs. P-12)	✓	
Study 3: Neuromuscular recovery following cycling exercise above critical power.		
It was hypothesised that;		
1) No significant difference is observed in neuromuscular recovery between conditions.		✓
2) The ability to voluntary produce force recovers rapidly, but only partially within the first few minutes following exercise termination.	✓	
3) Both, central and peripheral fatigue recover substantially but not fully within the first few minutes following exercise.	✓	
4) Twitch forces evoked by low-frequency stimulations recover at a slower rate compared to twitch forces evoked by high-frequency stimulations.	✓	
5) A faster neuromuscular recovery is observed in individuals with greater aerobic capacities.		✓
Study 4: Creatine supplementation improves performance above critical power but does not influence the magnitude of neuromuscular fatigue at task failure.		
It was hypothesised that;		
1) Creatine supplementation improves performance by increasing the amount of work done above CP.	✓	
2) The same absolute amount of work completed above CP leads to the same magnitude of neuromuscular fatigue regardless of creatine supplementation.	✓	
3) A greater amount of work done above CP increases the magnitude of neuromuscular fatigue observed at task failure.		✓

The aim of Study 2 (Chapter 5) was to examine neuromuscular fatigue induced by supra-CP cycling exercise near the lower and upper boundaries of the severe intensity domain when the curvature constant of the power-duration relationship (*W′*) was fully depleted. Constant-load supra-CP trials set to deplete *W′* in 3 (P-3) and 12 min (P-12) caused a reduction in MVC and induced substantial levels of peripheral and central fatigue with no significant difference between conditions ($p > 0.05$) (Chapter 5, Table 5.1.). Further, smaller changes in the force-generating capacity were seen in individuals with greater aerobic capacities for the longer severe intensity exercise, but larger changes were found in individuals with greater anaerobic capacities for the shorter severe intensity cycling exercise (Chapter 5, Figure 5.4. and 5.5.). Thus, although the magnitude of neuromuscular fatigue is not intensity-dependent when *W′* is fully depleted during supra-CP cycling exercise, the inter-individual variations suggest that the mechanisms underpinning exercise tolerance within the severe intensity domain may differ between short- vs. long-duration exercise.

The aim of Study 3 (Chapter 6) was to examine the time course of neuromuscular recovery following supra-CP cycling exercise near the upper and lower boundaries of the severe intensity domain. Whereas MVC fully recovered within 15 min after test termination ($p > 0.05$), peripheral (i.e. Q_{pot}, PS10, PS100) and central fatigue (i.e. VA) remained after 30 min of rest ($p < 0.05$). No difference in neuromuscular recovery was found between conditions except for PS10, with greater values for P-3 throughout all time points ($p < 0.05$). Further, slower recoveries in Q_{pot} and PS10 were also observed in individuals with a larger CP, and therefore aerobic capacity, however, when controlling for the magnitude of neuromuscular fatigue induced by the exercise itself (since change in Q_{pot} and PS10 were related to $\dot{V}O_{2peak}$), the relationship between recovery of PS10 and CP disappeared. These results indicate that impairments in muscle function outlast the exercise bout irrespective of exercise intensity and duration above CP. Further, the magnitude of neuromuscular fatigue post-exercise and not aerobic capacity seem to predominantly determine the time course of neuromuscular recovery following severe intensity cycling exercise.

The aim of Study 4 (Chapter 7) was to examine the effect of creatine supplementation on neuromuscular fatigue and exercise tolerance when cycling above CP. Therefore, participants performed three constant-load supra-CP trials set to fully deplete *W′* in 3 min: 1) one trial to task failure (TTF) following placebo supplementation (PLA); 2) one TTF following creatine supplementation (CRE); and 3) one trial of equal duration to PLA following creatine supplementation (ISO). Creatine supplementation increased time to task failure (205 vs. 184 s) and accordingly, the amount of work done above CP compared to PLA (21.2 vs. 19.3 kJ). However, no differences in the reductions in MVC, evoked twitch forces and VA from pre-to post-exercise were found between the three trials ($p > 0.05$). In conclusion, similar levels of neuromuscular fatigue were found following supra-CP cycling exercise to task failure irrespective of performance time and the amount of work done above CP.

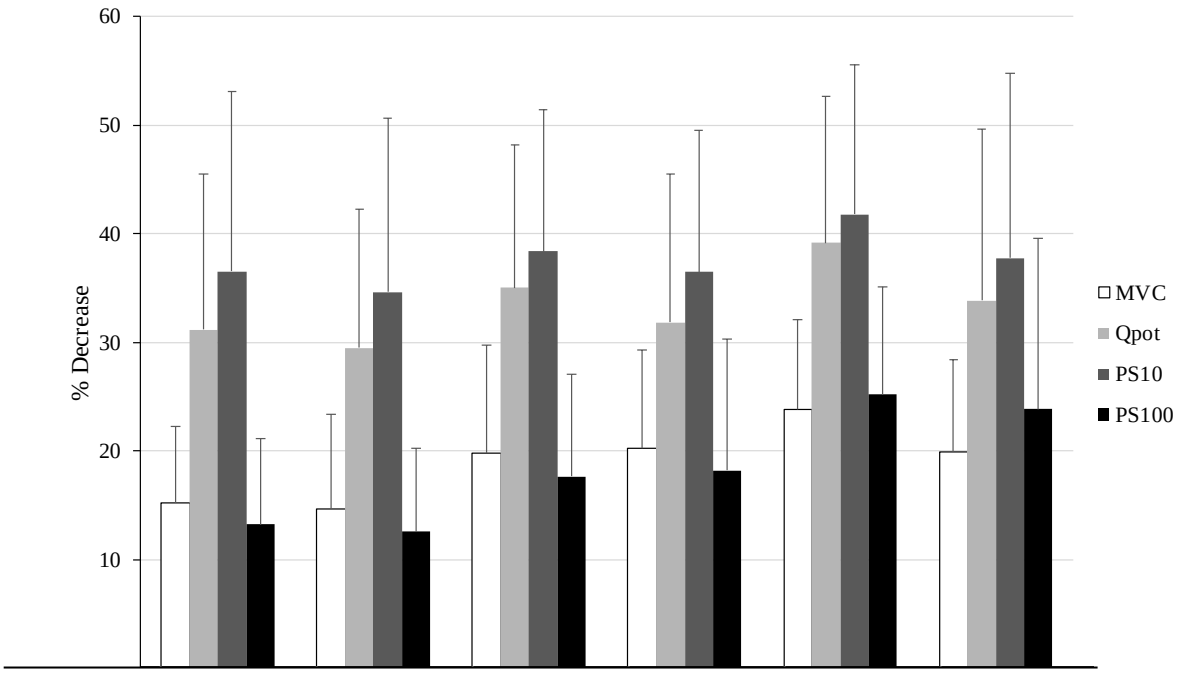
8.3. Progression of research area: CP concept – neuromuscular fatigue

The present thesis aimed to combine the CP concept and neurostimulation techniques to better understand the mechanism(s) underlying exercise tolerance during severe intensity cycling.

The CP concept, first described by Monod & Scherrer (1965), constitutes a potent framework for the investigation of exercise tolerance in the severe intensity domain (Burnley & Jones, 2016; Poole *et al.*, 2016; Poole & Barstow, 2015; Grassi *et al.*, 2015; Murgatroyd *et al.*, 2011). The depletion of *W′* has been associated with disturbances of muscle homeostasis, impairing muscle contractile function and ultimately, reducing the ability to produce force (Murgatroyd *et al.*, 2011). Indeed, exercise above CP has been demonstrated to substantially impair the ability to voluntary produce maximal force. Reductions within the range of −9 to −27% from pre-to post-exercise have been previously reported (Dominelli *et al.*, 2017; Goodall *et al.*, 2015; Johnson *et al.*, 2015; O'Leary *et al.*, 2016; Thomas *et al.*, 2015; Amann *et al.*, 2011). This is in line with the mean reductions in MVC observed across all studies within the present thesis (−15 to −24%; see Figure 8.1.). Impairments in the ability to produce force can be of central or peripheral origins, with the latter being acknowledged as the predominant factor altering force production when exercising above CP.

Peripheral fatigue following cycling exercise above CP

Substantial levels of peripheral fatigue have been reported following exercise within the severe intensity domain (Dominelli *et al.*, 2017; Goodall *et al.*, 2015; Johnson *et al.*, 2015; O'Leary *et al.*, 2016; Thomas *et al.*, 2015; Amann *et al.*, 2011) which is in accordance with the findings of the present thesis, observing pronounced reductions in Q_{pot}, across all studies (see Figure 8.1.). Moreover, pooled data from the present thesis shows that reductions in MVC were significantly correlated with reductions in Q_{pot} following severe intensity cycling, supporting the relevance of peripheral mechanism(s) in impairing the force-generating capacity (see Figure 8.2.).



	P-12.1	P-12.2	P-3	PLA	CRE	ISO
n	12*	12*	12	11	11	11
Time (s)	720	720	180	184 ± 46	205 ± 65	184 ± 46
% P_{peak}	73 ± 6	73 ± 6	98 ± 4	97 ± 7	97 ± 7	97 ± 7
Power (W)	248 ± 45	248 ± 45	329 ± 47	302 ± 38	302 ± 38	302 ± 38

Figure 8.1. The decrease (%) from pre- to 1 min post-exercise for maximal voluntary contraction (MVC, white), potentiated twitch force (Q_{pot}, light grey), low-frequency (10 Hz) doublet force (PS10, dark grey) and high-frequency (100 Hz) doublet force (PS100, black) for all trials in the present thesis. *for evoked twitch forces: n = 11.

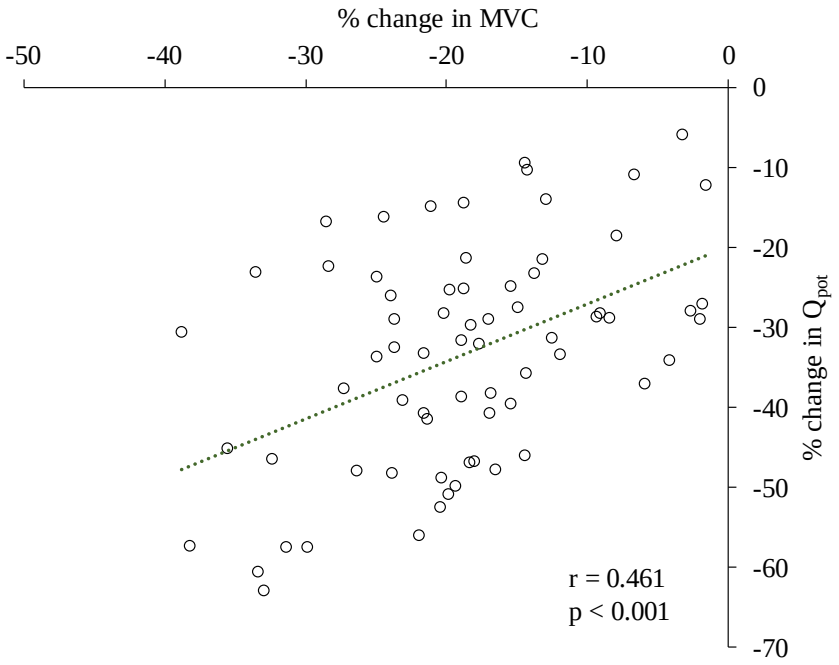


Figure 8.2. Correlations between the percentage change in maximal voluntary contraction (MVC) and the percentage change in potentiated twitch force (Q_{pot}). Pearson's correlation coefficients (r) are displayed for pooled data from P-12.1, P-12.2, P-3, PLA, CRE, ISO.

Reductions in Q_{pot} may be mediated by alterations in excitation-contraction coupling. Across all studies within the present thesis, changes in Q_{pot} were observed alongside substantial changes in PS100 (−13 to −24%) and to a greater extent in PS10 (−35 to −42%) (see Figure 8.1.). The proportionally greater changes in PS10 compared to PS100 indicate LFF (Verges *et al.*, 2009), which has been associated with a reduction in Ca^{2+} release from the SR (Balog, 2010; Allen *et al.*, 2008b; Keeton & Binder-Macleod, 2006; Rassier & MacIntosh, 2000). Similarly, Temesi *et al.* (2017) reported a reduction in PS10:PS100 of ~-30% following 6 min of severe intensity cycling exercise (80% P_{peak}). In the present thesis, reductions in Q_{pot} were significantly correlated with both reductions in PS10 ($r = 0.942$; $p < 0.001$) and PS100 ($r = 0.815$; $p < 0.001$; see Figure 8.3.). Collectively, these relationships, in combination with the distinct and prolonged reduction of Q_{pot} and PS10 post-exercise (Study 2, Chapter 5) alongside relatively smaller changes in PS100, indicate that excitation-contraction coupling failure, and the muscle contractile property in particular, is the predominant factor impairing the ability to produce force not only within the first few minutes, but also throughout 30 min of recovery following a severe intensity exercise.

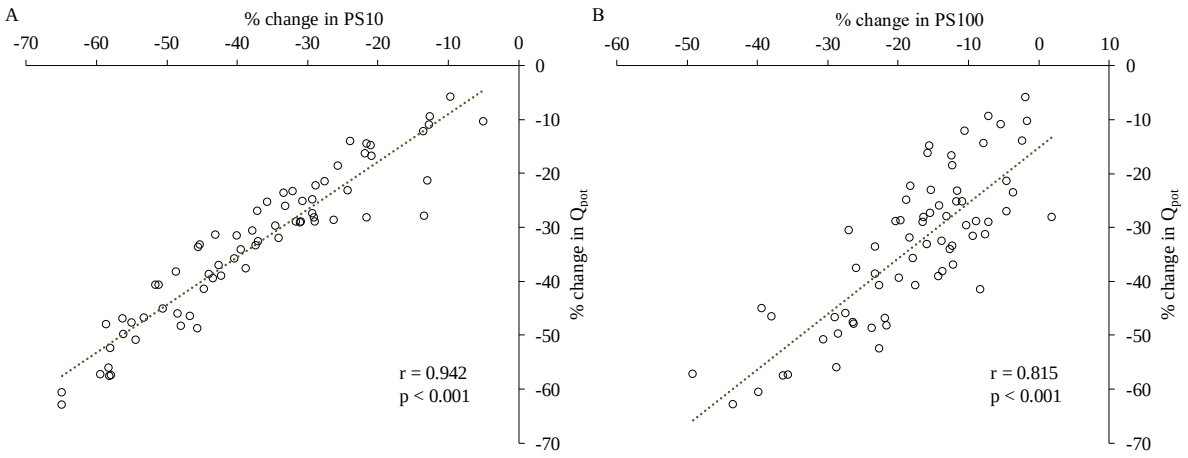


Figure 8.3. Correlations between the percentage change in potentiated twitch force (Q_{pot}) and the percentage change in low-frequency doublet force (PS10; A) and high-frequency doublet force (PS100; B). Pearson's correlation coefficients (r) are displayed for pooled data from Study 1, 2 and 4 (Chapter 4, 5 and 7; P-12.1, P-12.2, P-3, PLA, CRE, ISO).

Interestingly, similar levels of peripheral fatigue (i.e. reductions in evoked twitch forces of ~35%) have been reported across a wide range of severe exercise intensities (Dominelli *et al.*, 2017; O'Leary *et al.*, 2016; Thomas *et al.* 2015; Goodall *et al.*, 2015; Johnson *et al.* 2015; Amann *et al.* 2011; 2009; Romer *et al.* 2007; Amann & Dempsey, 2008). In agreement with these changes, the present thesis found similar range of reductions in Q_{pot} (−30 to −39%) across all studies (see Figure 8.1.). However, Thomas *et al.* (2016; 2015) also reported an intensity- and duration-dependent development of peripheral fatigue, with greater reductions in evoked twitch forces near the upper boundary and smaller reductions near the lower boundary of the severe intensity domain. To the best of our knowledge, Study 2 (Chapter 5) was the first to demonstrate similar reductions in Q_{pot} following cycling exercise at two 'extreme' intensities and thus, durations (3 vs. 12 min), within the severe intensity domain when controlling for *W′* depletion. Different findings between studies may be due to the design of the task (i.e. open-end vs. closed-end test): Study 2 (Chapter 5) removed the behavioural component of performance by terminating exercise when *W′* was fully depleted, in order to avoid a premature end of the task which may lead to an underestimation of the magnitude of neuromuscular fatigue. The reduction in Q_{pot} following P-12 (−31%) in Study 2 (Chapter 5) was substantially greater than those following a time to task failure of ~11 min (−16%) in Thomas *et al.* (2016). It may be speculated that open-end tests risk participants' disengagement with the task prior to full *W′* depletion in comparison to close-end tests and thus, may underestimate neuromuscular fatigue. In the present study, both, close-end tests (Study 1, 2 and 3) and open-end tests (Study 4) were used. An open-end test was felt the best choice of design in Study 4 (Chapter 7) in order to measure potential performance improvements following creatine supplementation while avoiding a possible confounding effect of pacing on the neuromuscular measures. Rather reassuring may be the

negligible difference between participants' mean performance time in PLA (184 ± 46 s) and the actual time predicted to induce full *W′* depletion (180 s). Despite a large range of TTF in PLA across participants (154 to 302 s) which may indicate that early task disengagement occurred in some participants in Study 4, TTF and reductions in MVC were not correlated for PLA (r = 0.237; p = 0.483).

Although Study 2 (Chapter 5) found similar levels of neuromuscular fatigue when *W′* was fully depleted, and thus, regardless of its depletion rate (3 vs. 12 min), bivariate correlations between *W′* and peripheral fatigue indicate that individuals with a larger *W′* may experience greater levels of peripheral fatigue. Figure 8.4. displays pooled data (n = 23) from Study 2 (Chapter 5) and Study 4 (Chapter 7) confirming a positive relationship between *W′* and reductions in Q_{pot} and PS10. Although bivariate correlations do not prove a causal relationship, they support a link between the depletion of *W′* and the development of peripheral fatigue, as suggested by Murgatroyd *et al.* (2011). Based on these findings, the present thesis aimed to further investigate a potential link between *W′* and neuromuscular fatigue by manipulating the size of *W′* in Study 4 (Chapter 7). *W′* was originally described as a fixed, anaerobic work capacity, mathematically equivalent to a given amount of work that can be performed above CP (see Section 2.1.1.4.). Although the anaerobic nature of *W′* has been questioned due to its sensitivity to interventions altering O_2 delivery (Dekerle *et al.*, 2012; Vanhatalo *et al.*, 2010), its primarily anaerobic nature is still widely accepted (Miura *et al.*, 2000; 1999; Smith *et al.*, 1998; Jenkins & Quigley, 1993). In contrast, the aerobic nature of CP is well evidenced through manipulation of O_2 delivery and/or utilisation (Parker Simpson *et al.*, 2015; Dekerle *et al.*, 2012; Vanhatalo *et al.*, 2010). Therefore, Study 4 (Chapter 7) used creatine supplementation to manipulate *W′*, without altering CP.

To the best of our knowledge, Study 4 (Chapter 7) was the first to find similar reductions in peripheral fatigue (i.e. Q_{pot}, PS10, PS100), irrespective of performance time and the amount of work done above CP within the severe intensity domain. Therefore, it may be concluded, that the magnitude of peripheral fatigue is neither dependent on the rate of *W′* depletion (Study 2, Chapter 5), nor does it depend on the size of *W′* (work done above CP) or performance time (Study 4, Chapter 7). The primary alternative hypothesis of Study 4 was rejected. Nonetheless, it may be worth highlighting here that the bivariate correlations found in Study 2 (Chapter 5) were based on between-participant comparisons with heterogeneity in the neuromuscular changes obtained (Section 5.4.8.; Figure 5.4.) whereas Study 4 (Chapter 7) observed similar levels of peripheral fatigue despite increases in *W′* for within-participant comparisons. The increase in performance due to creatine supplementation may have been too small to evoke significant changes in neuromuscular fatigue within individuals. This does not necessarily contradict between-individual differences found in a sample of individuals of large *W′* heterogeneity (CV ~30%). Either an alternative intervention inducing greater *W′* changes, potentially combined with the selection of a sample of individuals with a small *W′* prior to the

intervention, may have the potential to confirm the correlations reported in Study 2 (Chapter 5) for within-participant comparisons.

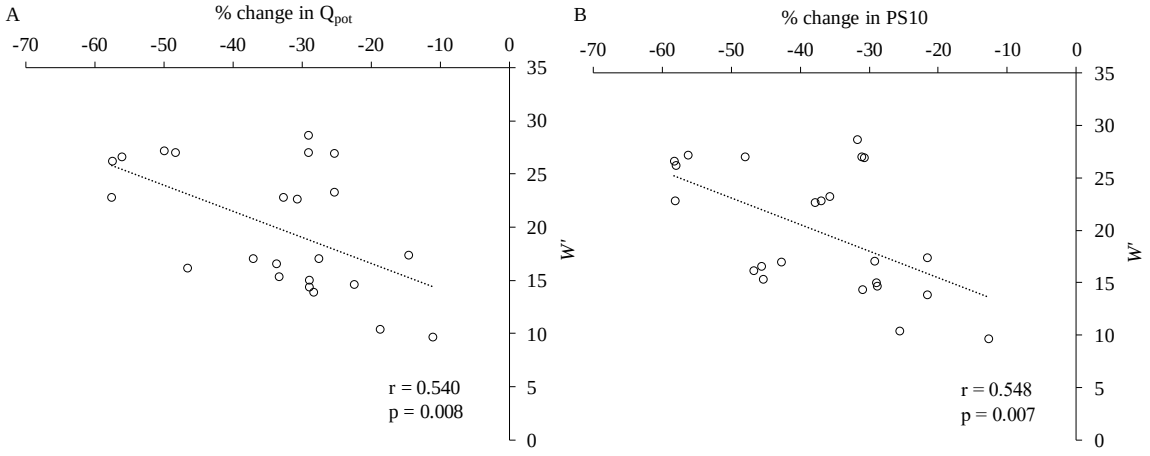


Figure 8.4. Correlations between the curvature constant (*W′*) of the power-duration relationship and the percentage change in potentiated twitch force (Q_{pot}; A) and between *W′* and the percentage change in low-frequency doublet force (PS10; B). Pearson's correlation coefficients (r) are displayed for pooled data from P-3 (Study 2, Chapter 5) and PLA (Study 4, Chapter 7).

Central fatigue following cycling exercise above CP

A moderate level of central fatigue, indicated by reductions in VA by −5 to −12%, was found across studies (Table 8.2.). These findings are in line with previously reported reductions of −5 to −10% following severe intensity exercise (Temesi *et al.*, 2017; Thomas *et al.*, 2016; 2015; Goodall *et al.*, 2015; Johnson *et al.*, 2015; Sidhu *et al.*, 2014). A reduction in VA suggests a suboptimal neural drive from the motor cortex (supra-spinal fatigue) and/or changes in the intrinsic properties of the motor neurons (spinal fatigue) (Taylor *et al.*, 2006; 2001; Gandevia, 1998). Similar to peripheral fatigue, exercise intensity dependent changes in VA have previously been described, with greater reductions in the lower part of the severe intensity domain (Thomas *et al.*, 2016; Burnley *et al.*, 2012). The present thesis did not find intensity-dependent alterations in VA when *W′* was fully depleted (P-3 vs. P-12; Chapter 5, Study 2) or when the amount of work done above CP and performance were increased following creatine supplementation (Chapter 7, Study 4), thus regardless of whether *W′* depletion was controlled (Chapter 5, Study 2) or manipulated (Chapter 7, Study 4). It remains unclear why changes in PLA are substantially smaller compared to P-3 (−11 ± 13%) since in both trials, participants performed cycling exercise at similar intensities (P-3: 98 ± 4% P_{peak} vs. PLA: 97 ± 7% P_{peak}) and for a similar duration (180 vs. 184 ± 46 s). However, as mentioned previously, it may be speculated that the open-end test performed in PLA risked a premature test termination due to

participants' disengagement with the task in comparison to P-3 (closed-end test), where *W′* depletion and thus, test termination were controlled.

Table 8.2. The mean decrease (%) ± standard deviation from pre- to 1 min post-exercise for voluntary activation (VA) for all trials in the present thesis.

	% decrease
P-12.1	−12 ± 8
P-12.2	−8 ± 5
P-3	−11 ± 13
PLA	−5 ± 7
CRE	−8 ± 8
ISO	−6 ± 9

Interaction between peripheral and central fatigue

Interestingly, peripheral fatigue above CP has been associated with intramuscular disturbances, i.e. H^+, P_i, ADP, La^- (Blain *et al.*, 2016; Burnley *et al.*, 2010; Allen *et al.*, 2008a; Jones *et al.*, 2008) and a similar magnitude of change in these muscle metabolites has been reported following exercise above CP (Black *et al.*, 2017; Chidnok *et al.*, 2013). Blain *et al.* (2016) found a positive relationship between exercise-induced increases in intramuscular metabolites (i.e. P_i, H^+) and decreases in evoked twitch forces (i.e. $Q_{pot.}$ PS10, PS100) following attenuation of group III/IV muscle afferent feedback via intrathecal fentanyl. The authors concluded that muscle afferents have a regulatory role to prevent muscle damage due to severe intramuscular metabolic perturbations. Consequently, it may be speculated that in the present thesis, the full depletion of *W′* across conditions (controlled in Study 2; assumed in Study 4) led to similar substantial muscle metabolic disturbances and thus, similar levels of peripheral fatigue at task failure. These findings are in accordance with the 'critical threshold of peripheral fatigue' proposed by Amann *et al.* (2006a) (see Section 2.2.5., Figure 2.11.). This theory describes that performance is determined by adjusting central motor output to the working muscles in order to not surpass a critical level of peripheral fatigue within the exercising muscles. Amann *et al.* (2006a) compared the magnitude of peripheral fatigue following a 5 km cycling TT performed under four different levels of arterial oxygen content (C_aO_2). It has previously been shown that the development of peripheral fatigue is sensitive to changes in C_aO_2 induced by varying fractions of inspired O_2 from hypoxia to hyperoxia (Amann *et al.*, 2006b). Although increasing the inspired O_2 fraction (0.15-1.0) increased the central neural output (measured as iEMG of VL) and power output,

and thus, improved cycling performance, the level of peripheral fatigue (i.e. reductions in Q_{pot}) post-exercise was not different between the four conditions (Amann *et al.*, 2006a). Similarly, increasing C_aO_2 improved TTF (at 80-100% $\dot{V}O_{2max}$) but had no effect on peripheral fatigue (Amann *et al.*, 2006b). It has been suggested that changes in C_aO_2 influence the metabolic milieu in the working muscle(s) (i.e. changes in SR Ca^{2+} (Duhamel *et al.*, 2004); Na^+-K^+-ATPase (Sandiford *et al.*, 2004); intramuscular P_i, PCr and H^+ levels (Hogan *et al.*, 1999); plasma lactate (Amann, *et al.*, 2006a)). These exercise-induced metabolic alterations are detected by group III/IV muscle afferents which indirectly reduce the central motor drive (i.e. central fatigue) via a negative feedback loop to limit the level of peripheral fatigue to a 'critical threshold' and ultimately, performance in order to prevent a catastrophic failure of muscle and overall homeostasis (Amann, 2011; see Section 2.2.5., Figure 2.11.).

These findings are supported by the above-discussed main results of the present thesis. Indeed, Study 2 (Chapter 5) in particular aimed to better understand whether peripheral fatigue was regulated to a 'critical threshold' following cycling exercise at different exercise intensities and therefore, durations above CP. The findings provided support for a 'critical threshold of peripheral fatigue'. A similar conclusion may be drawn from Study 4 (Chapter 7) during which creatine supplementation may have altered the muscular metabolic milieu which reduced the central motor drive via group III/IV muscle afferents, thereby, limiting the magnitude of peripheral fatigue at task failure.

Although creatine supplementation and therefore, manipulation of W' did not affect the level of peripheral fatigue as described in Study 4 of the present thesis (Chapter 7), the concept of an individual critical threshold of peripheral fatigue has recently been challenged. Other studies reported (1) smaller levels of peripheral fatigue and shorter performance times when cycling TTF (at 85% P_{peak}) was preceded by high-intensity arm-cranking (Johnson *et al.*, 2015), (2) greater levels of peripheral fatigue following the second of two consecutive bouts of repeated sets of 10 x 5 s isometric knee extension TTF (Froyd *et al.*, 2016b), and (3) greater levels of peripheral fatigue following single-leg vs. double-leg leg knee extensions (Rossman *et al.*, 2014). The authors suggested that exercise tolerance is not regulated to a critical threshold of peripheral fatigue, but mediated by the level of central fatigue and sensory perception (Johnson *et al.*, 2015), the rate of increase in RPE (Froyd, *et al.*, 2016b) and/or the active muscle mass involved (Rossman *et al.*, 2014), with the CNS tolerating greater levels of peripheral fatigue and associated metabolic disturbances in smaller muscle mass when strong afferent feedback is restricted to one muscle group and/or a smaller muscle mass in comparison to the sum of weaker afferent feedback originating from more than one muscle group and/or a larger muscles mass (Rossman *et al.*, 2014). According to Thomas *et al.* (2018), greater levels of peripheral fatigue (performance fatigability) following single- vs. both-leg exercise can be explained by greater recruitment of the active skeletal muscle and a more local signal of group III/IV

afferent feedback, while limiting the demand on other physiological systems and restricting alterations predominantly to one muscle group. This reduces the potential threat to overall homeostasis and therefore, allows a greater level of peripheral fatigue to be perceived as tolerable.

Based on the recent findings reporting differences in end-exercise levels of peripheral fatigue between different exercise modalities, Hureau *et al.* (2016b) modified the original theory of a 'critical threshold of peripheral fatigue' and re-introduced the 'sensory tolerance limit', initially described by Gandevia (2001). The 'sensory tolerance limit', a more global negative feedback loop, emphasises that in addition to inhibitory afferent feedback from the working muscle(s), other factors (e.g. respiratory muscles, muscles not directly involved in the exercise) limit peripheral fatigue and thus, performance and help explaining differences between end-exercise levels of peripheral fatigue (Hureau, *et al.*, 2016b).

Perceptual fatigue following cycling exercise above CP

In the present thesis, perceptual fatigue, measured as the score rated on the VAFS increased across all conditions in Study 2 (Chapter 5) and Study 4 (Chapter 7) by 5 to 8 scores from pre- to post-exercise (see Figure 8.5.). Further, Study 2 (Chapter 5) found no differences between P12.1 and P-3 ($p > 0.05$) and Study 4 (Chapter 7) found no differences between PLA, CRE and ISO ($p > 0.05$).

Consequently, when accepting the definition of fatigue as set out at the beginning of this thesis, it may be concluded that peripheral, central and perceptual measures describe similar levels between trials and that it remains unclear which factor predominantly regulates exercise tolerance. However, it must be acknowledged that this reductionist approach is not without its limitations and may not adequately take into account the complex nature of fatigue and the system as a whole (Lambert *et al.*, 2005). Barry & Enoka (2007) suggest that distinguishing between central and peripheral fatigue undermines the interplay between the nervous system and the muscles and therefore, should be avoided (Barry & Enoka, 2007). Instead, Enoka & Duchateau (2016) aimed to broaden this classic dichotomy approach and defined fatigue as a symptom, which is limited by the interaction between performance fatigability and perceived fatigability, a taxonomy first proposed by Kluger *et al.* (2013). Nonetheless, a holistic approach was not intended within this thesis and instead, the research conducted aimed to focus on what is understood as 'performance fatigability' according to the taxonomy proposed by Enoka & Duchateau (2016). However, the present thesis applied neurostimulation techniques to examine the origin of exercise-induced impairments in the force generating capacity according to their peripheral and central origin and therefore, fatigue was discussed according to the traditional taxonomy as peripheral and central fatigue.

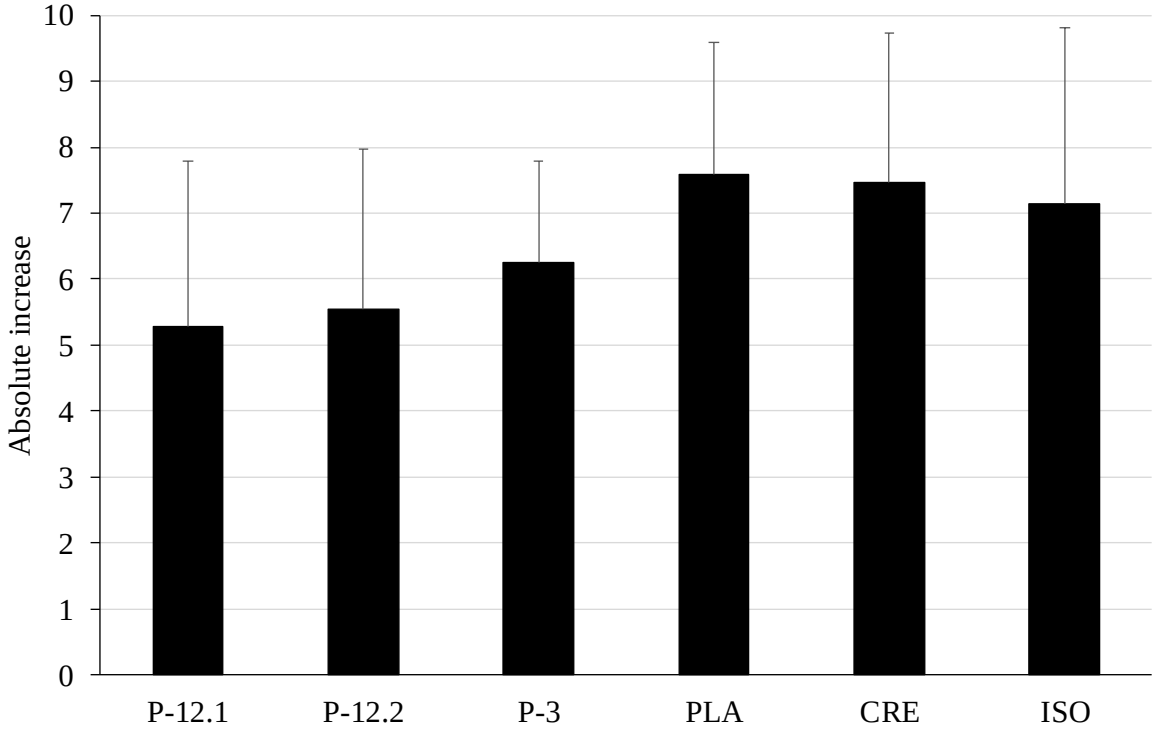


Figure 8.5. Absolute increase in the score rated on the Visual Analogue Fatigue Scale for all trials in the present thesis.

8.4. Assumptions and limitations

The present thesis was conducted under several assumptions and limitations. Findings are only applicable (1) to the severe intensity domain, (2) only for cycling exercise, and (3) only for healthy, recreationally trained males. The following section outlines further key points applicable to the present thesis:

Key assumptions of the two-parameter CP concept

The approach to terminate exercise based on the full depletion of W' (Study 2) is not without its limitations considering the physiological validity of thekey assumptions associated with the two-parameter CP model as outlined in Section 2.1.2.1.. Moreover, the error associated with the prediction of W' requires consideration when interpreting the results, in particular for Study 2 and 4.

Neuromuscular assessment

The time delay between exercise termination and neuromuscular assessment, due to the transition from the cycle ergometer to the dynamometer, represents a major methodological limitation in

studies investigating neuromuscular fatigue after whole-body exercise. This delayed assessment of neuromuscular changes likely allowed substantial neuromuscular recovery and thus, caused an underestimation of the magnitude of neuromuscular fatigue measured 1 min post-exercise. However, the present thesis standardised the time window for this transition to 60 s for each trial, and all neuromuscular assessments were completed within 100 s to both control and minimise a potential recovery effect.

Moreover, the muscular force-generating capacity was assessed during isometric contractions whereas neuromuscular fatigue was induced during cycling exercise. Therefore, the results should be interpreted with caution. Sidhu *et al.* (2012b) used a setup which allowed the delivery of transcranial magnetic stimulation of the motor cortex depending on the crank angle during cycling exercise. However, this setup allows the assessment of indices of excitability only. Further, Temesi *et al.* (2017) introduced a novel recumbent cycle ergometer with instantly lockable pedals which might have great potential for future studies because neuromuscular fatigue can be assessed at task failure with no time delay. Nonetheless, the results in Temesi *et al.* (2017) reveal an underestimation of evoked twitch forces and therefore, adjustments to the ergometer may be required.

Transferability of reliability data across studies

Study 1 (Chapter 4) assessed between-day reliability of the key neuromuscular assessment outcomes used in the present thesis for a 12 min severe intensity exercise bout only. It was assumed that reliability of these neuromuscular variables would be the same for a 3 min severe intensity exercise bout. When this thesis commenced, it was not intended to focus more predominantly on the shorter bout of exercise; the results from Study 2 and 3 influenced the direction of the subsequent study. However, Place *et al.* (2007) reported similar reliability findings for a short exercise duration (2 min sustained MVC).

Contribution of other muscle groups

The reasons for MVC to recover back to baseline within 15 min while both central and peripheral fatigue remained below baseline at 30 min post-exercise are unclear (Study 3; Chapter 6). It may be that other muscle groups along with the knee extensor muscles (i.e. hip extensors) have been activated and contributed to MVC force generation despite upper body and hip movement being minimised via two cross-shoulder straps.

Further, only EMG responses in the vastus lateralis were studied, although vastus medialis and rectus femoris also contribute to knee extension.

8.5. Directions of future research

The findings of the present thesis led to the following recommendations for future research:

Firstly, whether neuromuscular fatigue development follows a similar time course between different exercise intensities and durations above CP is unknown. Although no differences between P-3 and P-12 and between PLA and CRE were found at test termination in Study 2 (Chapter 5) and Study 4 (Chapter 7), their respective time course might have differed. To study the relationship between *W′* and neuromuscular fatigue further, exercise could be stopped when 25, 50 and 75% of *W′* is depleted during P-3 and P-12, so that the same amount of *W′* is depleted across conditions and comparisons are then made against the same amount of *W′*. A given fraction of *W′* utilisation may induce a set change in markers of neuromuscular fatigue.

Secondly, *W'* may be manipulated by alterations in $\dot{V}O_2$ kinetics while investigating changes in neuromuscular measures. A quicker $\dot{V}O_2$ response following exercise onset increases the contribution of the oxidative metabolism to the total energy turnover and thus, may delay the depletion of the anaerobic capacity. Prior heavy exercise increased the amplitude of the primary $\dot{V}O_2$ response and decreased the trajectory of the $\dot{V}O_{2sc}$ resulting in an increase in *W'* (Burnley *et al.* (2011). Temesi et al (2017) linked alterations in the $\dot{V}O_2$ response to neuromuscular measures by demonstrating a correlation between low-frequency fatigue and the time constant of the $\dot{V}O_2$ response, with greater levels of peripheral fatigue for a slower $\dot{V}O_2$ response. Investigating the effect of exercise intensity within the severe intensity domain on neuromuscular measures while also considering potential differences in $\dot{V}O_2$ kinetics or even manipulating the latter may provide further mechanistic insight.

Thirdly, similar to the experimental approach applied in Study 4 (Chapter 7), the negative relationship found between aerobic capacity and neuromuscular changes could be challenged using an experimental study design to either confirm or disprove a potential causative relationship. Moderate hypoxia systematically reduced (Deb *et al.*, 2017; Parker Simpson *et al.*, 2015; Dekerle *et al.*, 2012) and hyperoxia increased CP (Vanhatalo *et al*., 2010) compared to normoxia. Therefore, manipulating CP via changes in inspired O_2 fraction while investigating the effect on neuromuscular changes may reveal further insight into a potential relationship. However, the effect of alterations in inspired O_2 fraction on *W'* as demonstrated by Vanhatalo *et al.* (2010) require considerations when investigating this relationship.

Fourthly, selecting participants with large aerobic capacities and participants with large anaerobic capacities could help characterise further the relationship between *W′* and anaerobic/aerobic capacities observed in Study 2 (Chapter 5). A different study design cautiously pre-selecting two different groups (highly aerobically trained vs. highly anaerobically trained) or alternatively, a

training intervention which manipulates aerobic and/or anaerobic capacities while studying the effect on neuromuscular changes might be of interest for future research.

Finally, combining the two frameworks used within this study (CP concept and NMF) with the application of more advanced techniques, such as ^{31}PMRS or muscle biopsies may offer promising outcomes to associate or dissociate the neuromuscular changes studied in the present thesis with metabolic perturbations within the muscle or muscle cells, to provide a further understanding of the mechanism(s) underlying neuromuscular fatigue.

9. CONCLUSION

The present thesis investigated the exercise-induced neurophysiological responses to fatiguing exercise above critical power in healthy humans and explored a potential link between W' depletion and the magnitude of neuromuscular fatigue during cycling exercise.

Substantial levels of peripheral fatigue have been evidenced within the severe intensity domain across all studies within the present thesis. These changes were accompanied by pronounced reductions in neuromuscular variables (i.e. Q_{pot}, PS10 and to a smaller extent, reductions in PS100), indicating impairments in excitation-contraction coupling, and predominantly in the muscular contractile ability, within the severe intensity domain.

Moreover, the present thesis was the first to demonstrate that neuromuscular fatigue observed after full depletion of W' is of similar magnitude whether supra-CP cycling exercise is performed close to the lower boundary or the upper boundary of the severe intensity domain. Exploratory analysis revealed greater changes in measures of peripheral fatigue in individuals with greater anaerobic capacities (W') following the shorter exercise bout. This between-participant comparison suggests an association between the size of W' and the magnitude of neuromuscular fatigue at task failure. However, even though increasing an individual's W' through short-term creatine supplementation improved short-duration severe intensity cycling performance, the increase in W' did not lead to a greater level of neuromuscular fatigue at task failure. Therefore, it may be concluded that the magnitude of neuromuscular fatigue is neither dependent on the rate of W' depletion, nor does it depend on the size of W'.

The CP concept and its integration with electromyographic and mechanical measures of neuromuscular fatigue offers great potential for a better understanding of the limits of tolerance within the severe intensity domain and future studies should consider the combination of these frameworks to further explore a potential link between W' and neuromuscular fatigue and ultimately, understand better exercise tolerance within the severe intensity domain.

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APPENDICES

Appendix A – Medical questionnaire

(Study 1-4)

University of Brighton

SCHOOL OF SPORT AND SERVICE MANAGEMENT

MEDICAL QUESTIONNAIRE

Name: .....
Date of birth: .....

Are you in good health? Yes / No
If no, please explain:

How would you describe your present level of activity?
Vigorous:

< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

Have you suffered from a serious illness or accident? Yes / No
If yes, please give particulars:

Do you suffer, or have you ever suffered from:

Asthma	Yes	No
Diabetes	Yes	No
Bronchitis	Yes	No
Epilepsy /Convulsion/Seizure	Yes	No
High blood pressure	Yes	No
Fainting/Syncope	Yes	No

Are you currently taking medication? Yes / No
If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? Yes / No
If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment? Yes / No

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to participate in the study if they:

- are unsure of the test protocol and the possible risks and discomforts designated on the subject information sheet;
- the answers given on the medical questionnaire or informed consent form do not meet the required criteria;
- have not been regularly exercising over the last 2 years;
- are pregnant;
- have suspended training due to joint or muscle injury;
- have been verified, or documented as having any blood carried infections (Hepatitis, HIV), are diabetic or obese (Body Mass Index>30), have a known history of haematological, cardiac, respiratory, or renal disease; or had a head injury, brain infection (meningitis) or brain tumour;
- have a known history of severe headaches, fainting, dizziness, heat stroke, other heat induced illness, or mental health problems;
- have symptoms of nausea or light-headedness to needles, probes or other medical-type equipment;
- Smoke

To the best of your knowledge, please indicate **Yes** or **No** in answer to the questions below:

1. Have you ever been diagnosed as having heart disease or a heart condition?
   Yes / No
2. Do you suffer from chest pains, heart palpitations or tightness of the chest?
   Yes / No
3. Do you feel pain in your chest when you undertake physical activity?
   Yes / No
4. Have you had a neurosurgical procedure?
   Yes / No
5. Do you have known high blood pressure?
   Yes / No

6. Do you have known low blood pressure or do you often feel faint?
   Yes / No
7. Do you suffer from epilepsy?
   Yes / No
8. Have you ever had any type of a seizure?
   Yes / No
9. Have you suffered an upper respiratory tract infection in the last month?
   Yes / No
10. Have you had anaphylactic shock symptoms to needles, probes or other medical-type equipment?
    Yes / No
11. Have you had a history of infectious diseases (e.g. HIV, Hepatitis B)?
    Yes / No
12. Have you had a history of kidney disease?
    Yes / No
13. Do you suffer from diabetes?
    Yes / No

If the answer to any of the above details is yes, please give further details below:

. . . . . .
. . . . . .
. . . . . .
.. . .
. . . . . .

If you feel at all unwell because of temporary illness such as cold or fever please inform the investigator. If your health status changes so that you would subsequently answer **Yes** to any of the above questions, please notify the investigator immediately.

DECLARATION

I ..... have read and fully understand this health questionnaire. I confirm that to the best of my knowledge, the answers are correct and accurate and I understand that they will be treated with the strictest confidence. I know of no reason why I should not participate in this study, and I understand that I will be taking part at my own risk.

Furthermore, I hereby volunteer to be a participant in experiments/investigations during the period commencing .....2018.

The experimenter has fully informed me of, and I have understood, the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Participant name (please PRINT) . . . . . .
Participant signature . . . . . .
Date . . . . . .

Witnessed by researcher (please PRINT) . . . . . .
Researcher signature . . . . . .
Date . . . . . .

Appendix B – Informed consent form (Study 1-3)

University of Brighton

SCHOOL OF SPORT AND SERVICE MANAGEMENT
INFORMED CONSENT FORM

Title of Study: Key neuromuscular determinants of exercise tolerance during high-intensity exercise

Name of Researchers: Lisa Schäfer, Dr Jeanne Dekerle, Dr Mark Hayes

DECLARATION

I ..... hereby volunteer to take part in this research, which is to investigate the key neuromuscular determinants of exercise tolerance during high-intensity exercise.

The principal investigator has explained to my satisfaction the purpose of the experiment and the possible risks involved.

I have had the principles and the procedure explained to me, outlining any possible risks and I have also read the participant information sheet. I understand the principles and procedures fully and have had the opportunity to ask questions.

I am aware that for participation in this study I am required to provide fingertip blood samples and exercise to task failure as outlined in the participant information sheet.

I understand how the data collected will be used and that it will be kept confidential and anonymous and that the confidential information will be seen only by the researchers and will not be revealed to anyone else.

I understand that I am free to withdraw from the investigation at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

I agree that should I withdraw from the study, the data collected up to that point might be used by the researcher for the purposes described in the participant information sheet.

I understand that the results of the study can be made known to me.

Furthermore, if I am a student, I am aware that taking part, or not taking part in this experiment, will neither be detrimental to, nor further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the investigators regarding safety, subject only to my right to withdraw declared above.

_____	_____	_____
Name of Participant	*Date*	*Signature*
_____	_____	_____
Name of Researcher	*Date*	*Signature*

Appendix C – Informed consent form (Study 4)

SCHOOL OF SPORT AND SERVICE MANAGEMENT
INFORMED CONSENT FORM

Title of Study: Effect of creatine supplementation on neuromuscular fatigue during high-intensity exercise

Name of Researchers: Lisa Schäfer, Dr Jeanne Dekerle, Dr Mark Hayes

DECLARATION

I ..... hereby volunteer to take part in this research, which is to investigate the key neuromuscular determinants of exercise tolerance during high-intensity exercise.

The principal investigator has explained to my satisfaction the purpose of the experiment and the possible risks involved.

I have had the principles and the procedure explained to me, outlining any possible risks and I have also read the participant information sheet. I understand the principles and procedures fully and have had the opportunity to ask questions.

I am aware that for participation in this study I am required to provide fingertip blood samples, creatine supplementation and exercise to task failure as outlined in the participant information sheet.

I understand how the data collected will be used and that it will be kept confidential and anonymous and that the confidential information will be seen only by the researchers and will not be revealed to anyone else.

I understand that I am free to withdraw from the investigation at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

I agree that should I withdraw from the study, the data collected up to that point might be used by the researcher for the purposes described in the participant information sheet.

I understand that the results of the study can be made known to me.

Furthermore, if I am a student, I am aware that taking part, or not taking part in this experiment, will neither be detrimental to, nor further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the investigators regarding safety, subject only to my right to withdraw declared above.

_____	_____	_____
Name of Participant	***Date***	***Signature***
_____	_____	_____
Name of Researcher	***Date***	***Signature***